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MELATONIN INTERVENTION EFFECT ON BLOOD SUGAR LEVELS

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ABSTRACT

Background: Burn is trauma that can cause local and systemic damage including hypermetabolism, inflammation, and hyperglycemia. Stress-Induced Hyperglycemia (SIH) occurs by increasing gluconeogenesis and insulin resistance. Both of these are mediated through an increase in regulated stress hormones and proinflammatory cytokines. Melatonin has been proposed as a burn supportive therapy that may prevent oxidative damage and inhibit excessive inflammatory responses. **Aim:** Proving the effects of melatonin supplementation on blood glucose level in male Wistar rats with burn injury. **Methods:** This research was experimental with a randomized control group pre-post test design. Twelve healthy male Wistar rats were included and divided into two groups, control, and experimental group. Each rat has induced 30% burn injury under anesthesia. Rats in the control group were given a placebo, while rats in the experimental group were treated with melatonin i.p 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 male Wistar rats with third-degree burns given a placebo, blood sugar levels significantly increase in 0-3 hours (p=0.006) and 0-24 hours (p=0.037). Meanwhile, in male Wistar rats with third-degree burns given melatonin, blood sugar levels did not change significantly in 0-3 hours (p=0.470), 0-24 hours (p=0.286), dan 3-24 hours (p=0.833). **Conclusions:** Melatonin administration can not significantly reduce blood sugar levels.
Keywords: *Burn, burn injury, blood glucose level, melatonin.*

INTRODUCTION

Burns is trauma that can cause local and systemic damage and can interfere with homeostasis. Based on the cause, burns can be classified as burns caused by flames, chemicals, high-voltage electricity (> 1000 mV), or low-voltage electricity (<200 mV).[1] Burn victims can experience potentially fatal complications such as shock, infection, electrolyte imbalance, and respiratory failure, depending on the location affected and the depth of the burn. Morbidity and mortality tend to increase with increasing burn surface area.[2]

Burns is an injury that is often found in emergency departments around 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.[3] Approximately 450,000 patients receive treatment for burns annually and around 30,000 are treated in hospital.[4] In Indonesia, based on data from patients treated at the RSCM burns unit in 2012-2016, the most common causes of burns in adults are fire (53.1%), hot water (19.1%), and electricity. (14%), while in children it was caused by hot water (52%), fire (26%), and electricity (6%).[5]

In severe burns that cover more than 30% of the total body surface area, followed by periods of hyper inflammation and stress, proinflammatory cytokines can indirectly stimulate the secretion of counter-regulatory hormones. It is characterized by a sustained increase in catecholamines, glucocorticoids, and cytokine secretion.[6,7] Increases in dopamine, cortisol, norepinephrine, and epinephrine levels are subsequently associated with reduced insulin-mediated glucose uptake into skeletal muscle and additional tissue and increased hepatic gluconeogenesis.[8] Levels blood glucose can be increased while the insulin concentration can be normal or increased, this clinical state is defined as insulin resistance.[9 10]

The pineal gland produces the hormone melatonin, whose production follows a circular rhythm, low during the day and high at night. Melatonin can also be digested in the form of food. Only a small amount of melatonin is excreted into the

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urine. Melatonin has minimal toxicity, this is seen from several in vivo studies in animals involving large doses of melatonin showing that melatonin toxicity is very low.[11,12]

Based on previous research, melatonin has the potential as a pharmacological agent in burn patients which can be seen from its function as a scavenger agent for oxygen and nitrogen-based reactants, stimulation of the activity of various antioxidant enzymes, decreased proinflammatory cytokines, chronobiotic effects, and reduced toxicity of the drugs used in the protocol to treat patients. thermal injury. This combined action of melatonin, together with its low toxicity and ability to penetrate all morphophysiological membranes, can make it a ubiquitous molecule and is very useful in burn patients.[13-14] In previous studies, the anti-hyperglycemic effect by increasing secretion and action insulin, as well as increasing adiponectin levels and reducing free fatty acids.20 Hence, melatonin with its efficiency for reconstituting glucose homeostasis and as an anti-inflammatory can be considered as a potential therapy as an agent for reversing organ damage and insulin resistance after burn trauma.

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 and blood sugar levels 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 90^0C, amounting to 30% of the dorsal and ventral body surface area which was calculated through Meeh's Formula simultaneously. Both groups were dead at the end of the study period. The examination of the blood sugar levels used a strip-test. Blood was taken from retroorbital blood vessels, then blood sugar levels were measured 3 times, namely; before giving melatonin (0 hours), after giving melatonin 1 (3 hours), after giving melatonin 2 (24 hours).

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 and blood sugar levels 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 Saphiro-Wilk test. All data were normally distributed, so different tests were performed using an independent t-test.

RESULT

The mean blood sugar levels in treatment group before giving melatonin were 118.17 mg / dL (SD = 8.33 mg / dL), 3 hours 110.50 mg / dL (SD = 16.29 mg / dL), and 24 hours 121,67 mg/dL (SD= 32,18 mg/dL), and the control group increased every hour, at 0 hours was 76.33 mg / dL (SD = 18.65 mg / dL), 3 hours was 108, 33 mg / dL (SD = 11.61 mg / dL), and 24 hours was 130.33 mg / dL (SD = 43.33 mg / dL).

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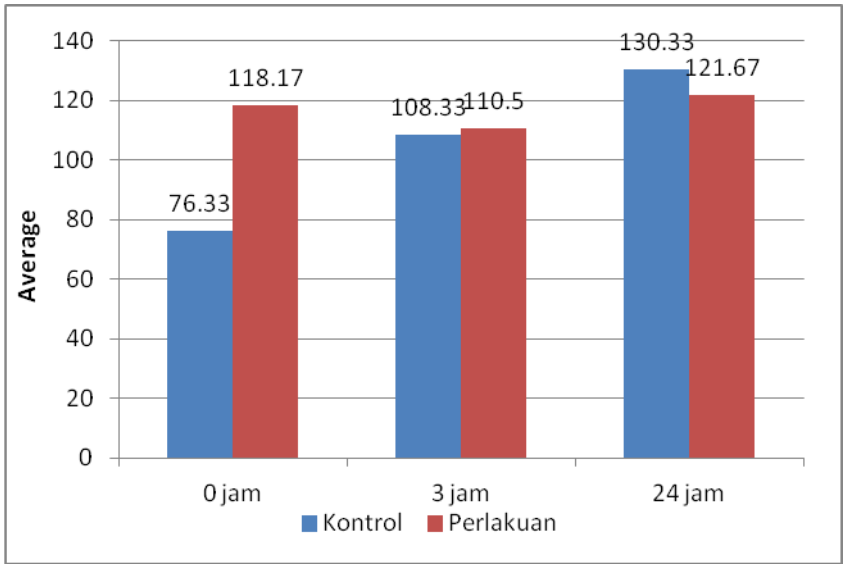

Picture 1. Graph of average blood sugar levels

Table 1. Results of the Parametric Paired t-test of blood sugar levels

Group	Blood Sugar Levels		
	0-3 hour	**3-24 hours**	**0-24 hours**
Control	0,006*	0,301	0,037*
Treatment	0,470	0,286	0,833

Notes: * Significant (p <0.05)

Based on table 2, there was a significant difference in the blood sugar levels of the control group 0-3 hours (p = 0.006) and 0-24 hours (p = 0.037) and there was no significant difference for blood sugar levels 3-24 hours (p = 0.301) . Meanwhile, there was no significant difference in blood sugar levels in the treatment group 0-3 hours (p = 0.470), 0-24 hours (p = 0.833) and 3-24 hours (p = 0.286).

Table 2. Results of the Parametric Independent t-test of the number of blood sugar levels

Group	Blood Sugar Levels		
	0 hour	**3 hours**	**24 hours**
Control -Treatment	0,001*	0,796	0,702

Notes: * Significant (p <0.05)

Based on table 2, the Parametric Independent t-Test, the comparison of blood sugar levels between the control and treatment groups before giving melatonin obtained significant results (p = 0.001). blood sugar levels showed no significant difference in blood sugar levels at 3 hours (p = 0.796) and 24 hours (p = 0.124).

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

Groups	Blood Sugar Levels	
	Δ 0-3 hours	**Δ 0-24 hours**
Control-Treatment	0,008*	0,069

Notes: * Significant (p <0.05)

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Based on table 3, the difference between the control and treatment groups on blood sugar levels at Δ 0-3 hours showed significant results (p = 0.008) and Δ 0-24 hours not showed significant results (p = 0.069).

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.

Based on the paired test, the treatment group experienced insignificant changes and was relatively stable. Meanwhile, the control group's blood sugar levels increased significantly at 0-3 hours and 0-24 hours, but the increase between 3-24 hours was not significant. The results of the analysis of blood sugar levels showed that there was no significant difference between blood sugar levels in the control group and the treatment group after being given melatonin therapy, the calculated blood sugar levels in 0-3 hours and 0-24 hours were not following the hypothesis. However, in the 0 hours, there were significant differences between the control and treatment groups.

Metabolism after a burn injury has two phases. The first phase, the ebb phase occurs within the first 48 hours of injury, is characterized by decreased cardiac output, increased oxygen demand, and impaired metabolism associated with hyperglycemia. This phase activates the hypothalamic-pituitary-adrenal axis thereby increasing stress hormones such as catecholamines, glucocorticoids, and cytokines, causing hyperglycemia. In the first five days after a burn injury, this state will gradually change to a flow phase, due to the hyperdynamic circulation and hyper-molecular states mentioned above. In this phase, insulin released doubles in response to an increase in glucose, and plasma glucose levels increase, indicating insulin resistance. These metabolic changes resolve immediately after total wound closure.[7,8]

Stress-Induced Hyperglycemia (SIH) occurs by increasing gluconeogenesis and insulin resistance. Both of these mechanisms are mediated through an increase in unregulated stress hormones (i.e., epinephrine, norepinephrine, glucagon, cortisol, growth hormone) and the proinflammatory cytokine tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), interleukin- 6 (IL-6). Where in the first 1 hour after burns were a peak increase of the early phase pro-inflammatory response in the form of TNF-α and IL-6. Meanwhile, IL-1β peaks in the first 4 hours after burns.[15] This proinflammatory cytokine can directly inhibit insulin secretion by pancreatic β cells through stimulation of α adrenergic receptors which can cause blood sugar levels to rise.[16] So that significant results can be seen on blood sugar levels. a difference of 0-3 hours.

The increase in stress hormones during severe inflammation is counter-regulatory to insulin, which aims to meet the energy needs of vital organs. Proinflammatory cytokines can indirectly stimulate the secretion of these counter-regulatory hormones.[16] Thus, the processes of glycogenolysis, gluconeogenesis, and insulin resistance are stimulated.[17] Stress-Induced Hyperglycemia has been determined if the plasma blood sugar level is> 200 mg/ml in non-diabetic patients that occurs as a result of the injury.[18]

Melatonin (N-acetyl-5-methoxytryptamine) functions to regulate the circadian rhythm, produced by the pineal gland.25 Synthesis and release of melatonin by the pineal gland are tightly controlled by the suprachiasmatic nucleus (SCN) and inhibited by bright conditions.[19]

In previous studies, melatonin is an anti-inflammatory agent by activating antioxidant enzymes and reducing inflammatory mediators.[13] Melatonin has an antioxidant effect that can bind free radicals due to oxidation processes so that ROS and NO are reduced. This allows glucose levels to below. Melatonin decreases gene expression and synthesis of proinflammatory cytokines such as TNF-α, IL-1β, IL-6, and prostaglandins.[14] So that melatonin can prevent the effects of these inflammatory conditions from getting worse. Melatonin activates the use of glucose in muscles, the transformation of intracellularly with GLUT4 through activation of IP3 kinase which inhibits cAMP thereby increasing insulin receptor function and decreasing glucose levels.[20] Glucose homeostasis in

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mice is modulated by melatonin MT1 receptors, thereby stimulating insulin for glucose uptake.[21] Melatonin increases calcium metabolism and inhibits the activity of the alpha-glucosidase enzyme which will cause an increase in insulin secretion.[22] This can prevent an increase in blood sugar at any time.

Younis Ahmad Hajam stated that the length of exposure and the dose of melatonin given affect the effect of the hormone on blood sugar, where the longer the exposure time and the higher the melatonin dose is given, the melatonin effect will be more visible. Research conducted by Younis Ahmad Hajam stated that a significant reduction in blood sugar levels occurred at 1 mg/kg BW of melatonin for 4 weeks,[23] whereas in this study the rats were burnt and given melatonin at a dose of 2 mg / 200 mg of rats at 0, 8, and 16 hours after burns. The short treatment in this study caused melatonin to not significantly reduce blood sugar levels.

Alice Valeria states that melatonin cannot significantly reduce blood sugar levels or cure sepsis, but melatonin can inhibit excess blood sugar levels due to sepsis.[24] Chung-Cheng Lo et al also stated that oral administration of melatonin cannot lower blood sugar, however, melatonin can delay the increase in blood sugar and increase glucose tolerance.[25] Tilden A et al. explained that melatonin causes the increase in glucose to be delayed along with this, the increase in lactic acid becomes lower.[26] Rosana F. Dantas Ferreira et al stated that melatonin alone or in combination with metformin did not significantly change glucose tolerance in the high-fat diet of Sprague-Dawley and Zucker fatty rats.[27] Emilia Kasturi Lukito stated that giving melatonin did not significantly decrease fasting blood sugar but was significant in decreasing glycated albumin and increasing β cells. pancreas.[28] This supports the results of the study that has been done. After giving burns, blood sugar levels during the control group continued to increase, but in the treatment group after giving melatonin, the blood sugar levels decreased which could be said to be slight but could prevent the increase in blood sugar levels due to burns so that they were not too high. It can also be seen that in the first 3 hours the control group had a progressive increase in blood sugar which was more significant than the treatment group, whereas at the next hour of observation there was no significant difference.

Blood sugar levels at 0 hours (before giving melatonin), there were significant differences in the treatment group and the control group, this was due to limitations in this study, namely the researchers had difficulty controlling several factors such as the condition of the rats' blood sugar before the study, the diet before fasting, environmental conditions, other diseases, the resistance of each rat, and stress factors. Where in this study the experimental animal was kept for 7 days so that it was not monitored all the time. This allows stress to the mice due to contact with other mice in the same cage. For food intake before fasting, it is also not monitored every day whether it is following standard needs or not. Where feeding was ad libitum, the success of food intake was seen from the remaining food that is still there.[29] When fasting 12 hours, the rats may have fasted for more than 12 hours. This condition (fasting for more than 12 hours) causes hypoglycemia and low insulin concentrations in the blood so that it stimulates the metabolic effects of glucagon and other catabolic hormones and the process of breaking down carbohydrates, proteins and fats will occur. This process also causes the breakdown of glycogen in the liver so that peripheral blood sugar will increase (gluconeogenesis).[22] So further research is needed with a longer study time and more confounding factors.

CONCLUSION AND SUGGESTIONS

Conclusion

In male Wistar rats, the third-degree burns model that was given placebo, blood sugar levels at 0-3 hours ($p = 0.006$) and 0-24 hours ($p = 0.037$) experienced a significant increase. Meanwhile, in male Wistar rats with third-degree burns given melatonin, blood sugar levels at 0-3 hours ($p = 0.470$), 0-24 hours ($p = 0.286$), and 3-24 hours ($p = 0.833$) were not underwent significant changes. Giving melatonin can not significantly reduce blood sugar levels but can prevent excess blood sugar from increasing.

Suggestions

Further research needs to be done with a longer study time. Also, it is necessary to carry out further research on the effect of melatonin

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administration and varying lengths of exposure by conducting other blood chemical analyses to determine the level of organ damage or stress due to burns.

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