

Melatonin prevented the increased serum lactate levels and the reduced hemoglobin levels in burn-induced Wistar rats



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ABSTRACT

Background: Burns are the most common and devastating form of trauma yet constitute a common cause of morbidity worldwide. Complications of burns such as sepsis and tissue hypoperfusion may increase serum lactate and reduce hemoglobin. Measuring lactate levels has been proven to be a useful predictive marker of hypoperfusion and sepsis in patients with burns. Melatonin which acts as an anti-inflammatory and antioxidant, has been proposed to be used as pharmacological adjuvant in patients with burns, reducing oxidative damage. The study aims to investigate the effects of melatonin supplementation on serum lactate and hemoglobin levels in burn-induced male Wistar rats.

Method: This was an experimental animal study with randomized control group design. All samples (n=12 male Wistar rats) were randomized and divided into two groups. Each rat was exposed to a hot metal rod for 10 seconds to induce 30% burns injury. Rats in the control group were administered with placebo (aquadest), while rats in the experimental group were administered with melatonin intraperitoneally at 0, 8, and 16 hours after burns injury. Blood samples were collected from the retro-orbital sinuses at 0, 3, and 24 hours after treatment. Data were statistically analyzed using Paired *t*-Test, Independent *t*-Test Mann-Whitney Rank Test and Wilcoxon test.

Results: There were no significant differences in lactate and hemoglobin levels between melatonin group and control group 0 hours after treatment (T0), 3 hours after treatment (T3), and 24 hours after treatment (T24) ($p > 0.05$). There were increased lactate levels and decreased hemoglobin levels in both melatonin and control groups, however the increment of lactate and the decrement of hemoglobin were steeper in control group compared to melatonin group. There were higher serum lactate increments and higher hemoglobin decrement at 24 hours ($\Delta T0-T24$) in control compared to melatonin group ($p < 0.05$).

Conclusion: Melatonin might prevent dramatic rise of serum lactate levels and decrease of hemoglobin levels until 24 hours after treatment in burns injury. If confirmed by further studies, melatonin might have a role as adjuvant therapy in burns.

Keywords: burns injury, serum lactate level, hemoglobin, melatonin.

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INTRODUCTION

Burn is one of the most common and devastating forms of trauma and is often encountered in everyday life.¹ Compared to other wounds, the morbidity and degree of disability caused by burns was quite serious, as well as its expensive management.¹ There were approximately 2.5 million people affected by burns in the United States each year, in whom 200,000 patients required outpatient care, 100,000 patients were hospitalized, and about 12,000 died each year.² In Indonesia, the prevalence of burns were 0.7%, in which the most injury occurred was in 1-4 years

age group.³

Burns can be caused by high temperatures, chemicals, electric shock, and radiation. Burns can be classified based on the degree of severity, namely: first degree burns, second-degree burns, and third-degree burns. In burns, damage to the epidermis, dermis and subcutaneous tissue can occur, depending on the causative factor and the length of time the skin is exposed to heat sources. The longer the skin is exposed to the heat source, the deeper or heavier the burn will be.² Severe burns can trigger an immunocompromised state, increasing the occurrence of complications such as sepsis

infection and dehydration.¹ Therefore burns managements care including fluid therapy to prevent dehydration, maintenance of average body temperature, infection and sepsis prevention, and pain relieve.^{1,2}

Lactate is the product of cellular anaerobic metabolism through the anaerobic glycolysis pathway caused by oxygen deprivation or tissue hypoxia. Lactate is the final product of glucose metabolism that is normally produced 1400 mmol/L daily.^{4,5} Lactate acid levels can be used as a biomarker to determine the prognosis of burns.⁶ Lactate was recognized as an early predictor of

morbidity and mortality in patients with severe burns.^{7,8} Hyperlactatemia, the increase in serum lactate level, can occur in patients with burns due to lack of tissue perfusion caused by hypovolemia or sepsis.⁹ Clinicians were using serum lactate levels greater than 4 mmol/L to determine which patients were at high risk for complications such as sepsis or death.^{1,6,9,10}

Anemia in burn patients can be contributed by acute blood loss during the first 1 to 2 weeks after a burn injury. Blood is lost directly from the thermal injury or the wounds, red blood cell (RBC) sequestration, and direct erythrocyte damage.¹¹

Melatonin, a 5-methoxy-N-acetyltryptamine, is a hormone secreted by the pineal gland with anti-inflammatory, antioxidant, analgesic, and sedating effects.¹² Melatonin has been proposed to be used as pharmacological support in burn patients where it reduced oxidative damage caused by hypoxia or sepsis occurred in burns.^{13,14} Hypoxia and sepsis can cause hyperlactatemia, whereas melatonin may prevent excess damage that occurs during the inflammatory and oxidative response and may eliminate free radicals that are harmful to the body, thus preventing an increase in serum lactate levels in these situations.^{4,6,12-14} Studies in the inflammatory mechanisms of melatonin are still developing. However, the exact roles of melatonin in pathological conditions including burns remain unclear. This study investigates the effects of melatonin supplementation on serum lactate and hemoglobin levels in burn-induced male Wistar rats.

METHODS

Animals

This was an experimental animal study with a randomized control group design. Study samples were twelve healthy male Wistar rats with age range of 2–3 months and body weight range of 200–250 g. All animals were kept in standard plastic cages and fed with standard pellet diet and water *ad libitum*. They were lived in a 22±2°C under controlled environmental conditions with 12-hours light/dark cycles for 7 days. All animals were fasting for 12 hours before experimental burn-injury

Table 1. Comparison of lactate levels between melatonin group and control group

Lactate Levels (mmol/L)	Group		p
	Control	Melatonin	
T0	1.7 ± 1.03; 1.5 (0.8 – 2.9)	1.7 ± 1.09; 1.2 (0.8 – 3.4)	0.932 ^a
T3	3.8 ± 0.56; 3.6 (3.2 – 4.7)	3.4 ± 0.96; 3.5 (1.4 – 4.3)	0.065 ^b
T24	5.7 ± 0.68; 5.7 (5.0 – 8.8)	5.1 ± 0.41; 5.1 (3.1 – 5.5)	0.054 ^b
ΔT0–T3	2.05 ± 0.97	1.7 ± 0.91	0.699 ^b
ΔT0–T24	4.1 ± 0.35	3.4 ± 0.68	0.047 ^{b*}
ΔT3–T24	1.9 ± 0.12	1.7 ± 0.55	0.576 ^b
T0–T3	p=0.027 ^{d*}	p=0.029 ^{c*}	
T0–T24	p=0.017 ^{d*}	p=0.023 ^{c*}	
T3–T24	p=0.032 ^{c*}	p=0.039 ^{c*}	

Abbreviations: Data was shown as mean ± SD; median (min–max) value; Time 0 hour since treatment (T0), Time 3 hours since; treatment (T3), Time 24 hours since treatment (T24); *Significantly different if p<0.05; ^aMann-Whitney Rank Test; ^bIndependent t-Test; ^cPaired t-Test; ^dWilcoxon test.

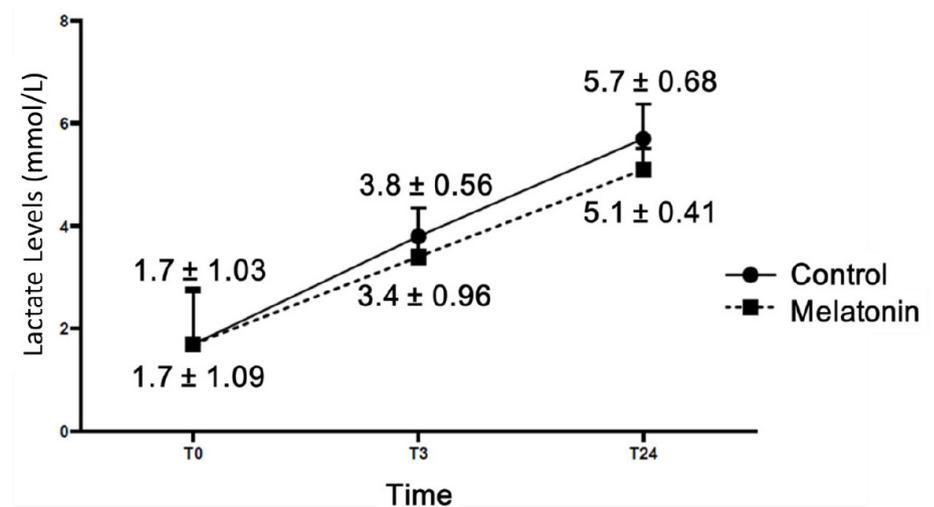


Figure 1. Lactate levels in melatonin group and control group at 0 hours after treatment (T0), three hours after treatment (T3), and twenty-four hours after treatment (T24).

but were still allowed to drink water.

Burn-induced injury

Wistar rats were divided into two (2) groups, namely (1) melatonin group (X_M) and (2) the control group (X_C). General anesthesia was performed using thiopental intraperitoneally. Thiopental dose was 0.54 mg/200 g Wistar rat or 30

mg/kg body weight in humans after dose conversion. The backs of all Wistar rats were shaved. To obtain 30% burns of body surface area or third-degree burns, Meeh's formula was used to count rats' total body surface area. The hot metal rod heated in a hot boiling water (90°C), was applied on the backs of all Wistar rats for 10 seconds. Immediate resuscitation

with physiological saline (0.18 mg / 200g Wistar rat or 10 mg/kg body weight in human after dose conversion of 0.018) was performed to all rats.

Melatonin or placebo administration

Melatonin (Melatonin M5250, Sigma-Aldrich, Darmstadt, Germany) dissolved in distilled water was administered intraperitoneally to Wistar rats in melatonin group at 0, 8, and 16 hours after burn injury, while the control group was administered with placebo (aquadest Otsu-WI) in the same method. Melatonin dose was 0.18 mg/200 g Wistar rat or 10 mg/kg body weight in human after dose conversion of 0.018.

Blood was drawn from retroorbital sinuses of each rat at 0, 3, and 24 hours after treatment with melatonin or placebo. Measurement of serum lactate levels was carried out enzymatically with blood lactate analyzer using lactate strip test (Accutrend® Plus-Lactate strips, Roche Diagnostics Limited, Switzerland) (in mmol/L). Meanwhile hemoglobin was examined with automatic hematologic analyzer.

Statistical Analysis

Data were analyzed with descriptive test to determine mean, standard deviation, and median. Shapiro-Wilk was used to test data distribution normality. Statistical analysis was performed with Paired *t*-Test and Independent *t*-Test if data were normally distributed and with Mann-Whitney Rank Test or Wilcoxon test if data were not normally distributed. The *p*-value of less than 0.05 was considered to be statistically significant.

RESULTS

Effect of Melatonin in Lactate Levels

Table 1 showed that there were no significant differences in lactate levels between melatonin group and control group in 0 hours after treatment (T0) ($p=0.932$), 3 hours after treatment (T3) ($p=0.065$), and 24 hours after treatment (T24) ($p=0.054$). We found that there were increased lactate levels in both melatonin and control groups, however the increment was steeper in control group compared to melatonin group (Table 1 and Figure 1).

Table 2. Comparison of hemoglobin levels between melatonin group and control group

Hemoglobin Levels (g/dL)	Group		<i>p</i>
	Control	Melatonin	
T0	11.7 ± 1.72; 10.7 (10.3 – 13.6)	11.7 ± 1.40; 11.3 (10.3 – 14.0)	0.690 ^a
T3	10.1 ± 2.26; 10.9 (6.1 – 11.7)	10.5 ± 2.59; 11.4 (5.9 – 12.1)	0.310 ^a
T24	9.3 ± 1.39; 9.7 (7.0 – 10.7)	10.0 ± 2.42; 10.9 (5.8 – 11.6)	0.151 ^a
Δ T0–T3	1.6 ± 1.05	1.2 ± 1.04	0.094 ^b
Δ T0–T24	2.4 ± 0.35	1.7 ± 0.97	0.042 ^{b*}
Δ T3–T24	1.0 ± 0.74	0.7 ± 0.95	0.098 ^b
T0–T3	$p=0.223^d$	$p=0.684^d$	
T0–T24	$p=0.080^d$	$p=0.500^d$	
T3–T24	$p=0.138^d$	$p=0.138^d$	

Abbreviation: Data was shown as mean ± SD; median (min–max) value; Time 0 hour since treatment (T0); Time 3 hours since treatment (T3); Time 24 hours since treatment (T24); *Significantly different if $p<0.05$; ^aMann-Whitney Rank Test; ^bIndependent *t*-Test; ^cPaired *t*-Test; ^dWilcoxon test.

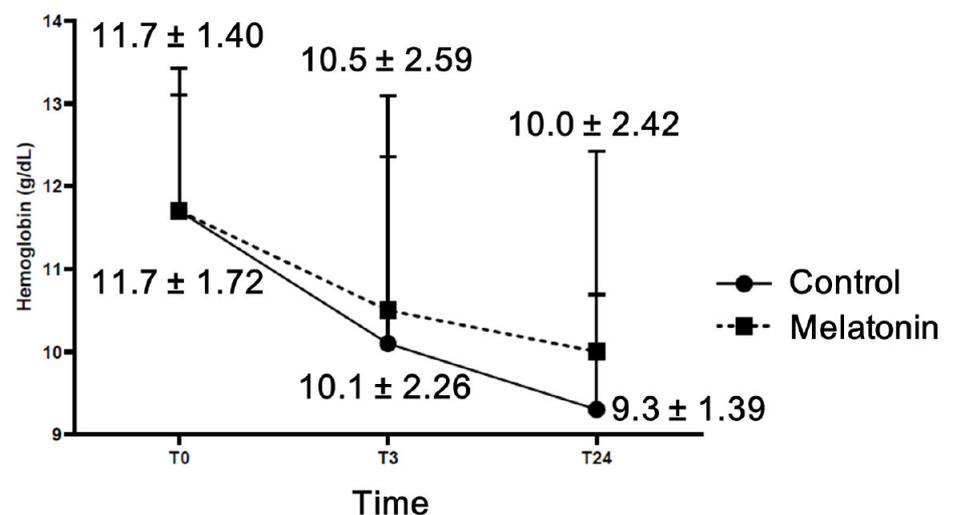


Figure 2. Hemoglobin levels in melatonin group and control group at 0 hours after treatment (T0), three hours after treatment (T3), and twenty-four hours after treatment (T24).

There was a significant difference of serum lactate increment in T0 to T24 ($\Delta T0-T24$) between control (4.1 ± 0.35 mmol/L) and melatonin group (3.4 ± 0.68 mmol/L) ($p=0.047$), but not in $\Delta T0-T3$ and $\Delta T3-T24$ (Table 1 and Figure 1). It seemed that melatonin could prevent a dramatic rise of lactate levels until 24 hours after treatment compared to control.

Effect of Melatonin in Hemoglobin Levels

Table 2 showed that there were no significant differences in hemoglobin levels between melatonin group and control group in 0 hours after treatment (T0) ($p=0.690$), 3 hours after treatment (T3) ($p=0.310$), and 24 hours after treatment (T24) ($p=0.151$). We found that there were decreased hemoglobin levels

in both melatonin and control groups. However the decrease was steeper in control group compared to melatonin group, although they were not statistically significant (Table 2 and Figure 2).

There was a significant difference of hemoglobin decrement in T0 to T24 ($\Delta T0-T24$) between control (2.4 ± 0.35 g/dL) and melatonin group (1.7 ± 0.97 g/dL) ($p=0.042$), but not in $\Delta T0-T3$ and $\Delta T3-T24$ (Table 2 and Figure 2). It seemed that melatonin could prevent a decrease of hemoglobin levels until 24 hours after treatment compared to control.

DISCUSSION

Burns are thermal injuries caused by hot fluids (scalding), contact with cold or hot objects, chemicals, electric currents, and flames.² Extensive burns can lead to hypovolemic conditions,² in which fluid deficiency can cause tissue hypoperfusion and increased lactate production.^{4,5} Lactate levels are considered as high if the levels are more than 4 mmol/L.^{9,10} Tissue hypoperfusion is the most frequent cause of elevated lactate levels, but it can be increased due to another etiology and other contributing factors.⁵ Lactate has been shown to predict the occurrence of sepsis and mortality in burn patients, its levels can be measured easily, sensitive, specific, and reliable.⁶⁻⁹

The phases of burns can be described as phases of hemostasis and inflammation, proliferation, and remodeling interrelated and overlapping phases. In acute burns, the inflammatory phase lasts for the first 5–7 days, however, severe burns may display chronic, persistent inflammation long after the initial tissue damage and may even result in multiple organ failure (MOF) due to systemic inflammatory response syndrome (SIRS).¹⁵

As the inflammatory response to burns progresses, pro-inflammatory cytokines such as TNF- α and nitric oxide are produced during the local immune response that promotes the vascular permeability needed for immune cell infiltration. When pro-inflammatory cytokines are released into the circulation, they may attack the integrity of distant blood vessels, allowing blood to flood end organs leading to organ failure.¹⁵ Excess evaporation of fluid happens in severe

burn injuries, disruption of intravascular volume can lead to impaired tissue perfusion and increased lactate levels.⁵ Within hours, the increased capillary permeability can lead to hypovolemic shock due to massive fluid loss and requires immediate fluid resuscitation to prevent death.¹⁵

Tissue damage caused by thermal injury might be protected with melatonin, which acts as a supportive therapy in burns patients by working as a potent antioxidant that prevents oxidative damage.^{12,16} The role of melatonin in burns therapy is as an anti-inflammatory agent which can reduce the production of TNF- α , induce Nitric Oxide Synthase (iNOS) enzyme, prevent tissue hypoperfusion, and thus prevent excess lactate levels.^{12,13}

We have succeeded in making burns model from male Wistar rats. The treatment model of 30% burned could cause trauma and acute stress to the rats, triggering an increase in lactate levels.^{2,15} Apart from the stress and tissue hypoperfusion in burn patients, other factors could increase lactate levels such as mitochondrial dysfunction (lack of cofactor enzymes), hypermetabolic state, and hepatic dysfunction that could also cause increased production and decreased clearance lactate in burns.^{5,10}

Our study showed tendencies of lower lactate levels in the melatonin group compared to control group at 0, 3, and 24 hours after treatment, although there were no significant differences between melatonin and control groups in each timeline. Moreover, we also found that there were continuous increases of lactate levels in both melatonin and control groups, however the increment was steeper in control group compared to the melatonin group. There was a larger increment of serum lactate in T0 to T24 ($\Delta T0-T24$) in control compared to melatonin group. It seemed that melatonin could prevent a dramatic rise of lactate levels until 24 hours after treatment compared to control.

Our present study was similar to our previous studies which showed that melatonin could prevent excess increase of lactate and random blood glucose levels in Wistar rats endotoxemia model.¹⁷ We also previously showed that an adjuvant

treatment of melatonin could prevent the elevation of leukocyte count and the decrease of platelet count in Wistar rats endotoxemia model.¹⁸

Kaya et al. showed that melatonin supplementation could reduce lactate levels in rats subjected to acute swimming exercise.¹⁹ Kaya et al. stated that the length of exposure and the dose of melatonin might affect on amount of lactate released. The longer the exposure time and the higher the melatonin dose given, the more visible the effect of melatonin will be.²⁰ This finding might explain why melatonin was only limited to preventing the increased lactate levels in our study due to short-term treatment.

Moreover, we also showed lower hemoglobin levels in the control group than the melatonin group in each timeline, although they were not significant. We found that there were decreased hemoglobin levels in both control and melatonin groups, however the decrease was steeper in control group compared to melatonin group. There was also a larger hemoglobin decrement at T0 to T24 ($\Delta T0-T24$) in control compared to melatonin group. It seemed that melatonin could prevent a decrease of hemoglobin levels until 24 hours after treatment compared to control. We hypothesized that melatonin's effect in regulating inflammatory response could also prevent acute loss of red blood cell from intravascular.

However, we still not certain whether therapy with melatonin might be sufficient to inhibit increased lactate levels and anemia in the longer term during burns injury progression, in which several complications might usually appear. Furthermore, we still cannot elucidate whether we will need a higher dose of melatonin to inhibit the increase of lactate and the decrease of hemoglobin in the longer term.

Further studies are needed to examine the effect of melatonin administration in multilevel doses, with varying length of exposure, and with larger study subjects and conducting other blood chemical analysis to determine the level of organ damage or stress due to burns. Epidemiological studies are also needed regarding the safe dosage of melatonin in its role as an antioxidant and anti-

inflammatory.

CONCLUSIONS

Melatonin might prevent dramatic rise of serum lactate levels and decrease hemoglobin levels until 24 hours after treatment in burns-induced Wistar rats. If confirmed by further studies, melatonin might have a role as adjuvant therapy in burns

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This study doesn't receive any specific grant from government or any private sectors.

CONFLICT OF INTEREST

The author declares there is no conflict of interest regarding publication of this article.

ETHICAL STATEMENT

This study has been approved by Ethical Committee Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia with ethical clearance reference number no. 39/EC/H/FK-UNDIP/V/2020.

AUTHOR CONTRIBUTION

Graciela Dhea and Satrio Adi Wicaksono contribute equally in this study.

REFERENCES

- Church D, Elsayed S, Reid O, Winston B, Lindsay R. Burn wound infections. *Clin Microbiol Rev.* 2006;19(2):403-34.
- Lim JJ, Rehmar SG, Elmore P. Rapid response: Care of burn victims. *AAOHN Journal.* 1998;46(4):169-78.
- Ministry of health Republic of Indonesia. Indonesian basic health research. 2018. Jakarta: Health research and development agency. Ministry of Health, Republic of Indonesia; 2018.
- Garcia-Alvarez M, Marik P, Bellomo R. Sepsis-associated hyperlactatemia. *Crit Care* 2014;18(5):503.
- Andersen LW, Mackenhauer J, Roberts JC, Berg KM, Cocchi MN, Donnino MW. Etiology and therapeutic approach to elevated lactate. *Mayo Clin Proc.* 2013;88(10):1127-40.
- Mokline A, Abdenneji A, Rahmani I, Gharsallah L, Tlaili S, Harzallah I, et al. Lactate: Prognostic biomarker in severely burned patients. *Ann Burns Fire Disasters.* 2017;30(1):35-8.
- Kamolz LP, Andel H, Schramm W, Meissl G, Herndon DN, Frey M. Lactate: Early predictor of morbidity and mortality in patients with severe burns. *Burns.* 2005;31(8):986-90.
- Cochran A, Edelman LS, Saffle JR, Morris SE. The relationship of serum lactate and base deficit in burn patients to mortality. *J Burn Care Res.* 2007;28(2):231-40.
- Faix JD. Biomarkers of sepsis. *Crit Rev Clin Lab Sci.* 2013;50(1):23-36.
- Broder G, Weil MH. Excess lactate: An index of reversibility of shock in human patients. *Science.* 1964;143(3613):1457-9.
- Posluszny JA, Gamelli RL. Anemia of thermal injury: Combined acute blood loss anemia and anemia of critical illness. *J Burn Care Res.* 2010;31(2):229-42.
- Kurdi MS, Patel T. The role of melatonin in anesthesia and critical care. *Indian J Anaesth.* 2013;57(2):137-44.
- Şener G, Şehirli AÖ, Şatroğlu H, Keyer-Uysal M, Yeğen BÇ. Melatonin improves oxidative organ damage in a rat model of thermal injury. *Burns.* 2002;28(5):419-25.
- Maldonado M-D, Murillo-Cabezas F, Calvo J-R, Lardone P-J, Tan D-X, Guerrero J-M, et al. Melatonin as pharmacologic support in burn patients: A proposed solution to thermal injury-related lymphocytopenia and oxidative damage. *Crit Care Med.* 2007;35(4):1177-85.
- Strudwick XL, Cowin AJ. The role of the inflammatory response in burn injury. *Hot topics in burn injuries.* Adelaide, South Australia, Australia: Intechopen; 2018:37-57.
- Reiter RJ, Tan D-X, Fuentes-Broto L. Melatonin: A multitasking molecule. *Progress Brain Res.* 2010;181:127-51.
- Wicaksono SA, Kumalasari D, Valeria A, Utami SB. Melatonin and polymyxin b administration, alone or in combination, prevent the elevation of random blood glucose and lactate levels in wistar rats endotoxemia model. *Pakistan J Med Health Sci.* 2020;14(2):1082-90.
- Wicaksono SA, Harisiani DA, Riani RI, Utami SB. An adjuvant treatment of melatonin prevented the elevation of leukocyte count and the decrease of platelet count in wistar rats endotoxemia model. *Pakistan J Med Health Sci.* 2020;14(2):705-12.
- Kaya O, Gokdemir K, Kilic M, Baltaci A. Melatonin supplementation to rats subjected to acute swimming exercise: Its effect on plasma lactate levels and relation with zinc. *Neuro Endocrinol Lett.* 2006;27(1-2):263-66.
- Dalton KM. Effects of melatonin on hemolymph glucose and lactate concentrations in the fiddler crab, *uca pugnator*. Department of Chemistry. 2002; In Partial Fulfillment of the Requirements for Graduation With Honors in Chemistry.



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