

## EFEK IMUNOPROTEKTIF DARI MELATONIN ORAL PADA TIKUS PUTIH JANTAN (*RATTUS NORVEGICUS*) YANG DIBERIKAN METILPREDNISOLON

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### ABSTRAK

**Latar Belakang:** Fungsi imun akan menurun seiring dengan penuaan. Penggunaan steroid dapat mempercepat proses penuaan imun, khususnya pada orang dewasa di Indonesia. Melatonin merupakan hormone alami yang memiliki efek antioksidan dan proteksi imun. Pemberian melatonin diharapkan dapat memperbaiki dampak negatif dari penuaan dan steroid eksogen pada fungsi imun.

**Tujuan:** Eksperimen ini dilakukan untuk mengukur efek dari melatonin yang diberikan pada tikus Wistar dengan imunosupresi menggunakan metilprednisolon, khususnya pada jumlah leukosit dan limfosit.

**Metode:** Penelitian ini adalah eksperimen murni dengan post-test dan kelompok kontrol pada 32 tikus Wistar jantan, yang dibagi menjadi dua kelompok, yaitu kontrol dan intervensi. Kedua kelompok diberikan metilprednisolon 0,144 mg/200 g tiga kali sehari, dan tetapi hanya kelompok intervensi yang diberikan satu dosis melatonin sebesar 0,054/200 g. Perlakuan diberikan selama 14 hari dan darah diambil di hari ke-14.

**Hasil:** Kelompok intervensi menunjukkan jumlah limfosit ( $4.95 \pm 1.58 \times 10^3 / \mu\text{L}$ ) yang lebih tinggi dibanding kelompok kontrol ( $3.09 \pm 1.33 \times 10^3 / \mu\text{L}$ ), dengan  $p < 0.01$ . Kelompok intervensi juga memiliki jumlah leukosit ( $8.37 \pm 2.02 \times 10^3 / \mu\text{L}$ ) yang lebih tinggi dibanding kelompok kontrol ( $6.70 \pm 1.96 \times 10^3 / \mu\text{L}$ ), dengan  $p < 0.05$ .

**Kesimpulan:** Pada penelitian ini ditemukan bahwa melatonin oral dapat menghambat penurunan jumlah limfosit dan leukosit pada tikus yang diberi metilprednisolon.

**Kata Kunci:** melatonin, leukosit, limfosit, metilprednisolon, tikus Wistar

## **IMMUNOPROTECTIVE EFFECT OF MELATONIN ADMINISTRATION ON METHYLPREDNISOLONE-TREATED WISTAR MALE RAT (*RATTUS NORVEGICUS*)**

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### **ABSTRACT**

**Background:** Immune system function decreases with age. Steroid use might accelerate this process, especially in adults in Indonesia. Melatonin is a natural hormone which show antioxidant effect and immunoprotective effect. Melatonin administration might help in reviving humane immune system due to aging and exogenous steroid exposure.

**Objective:** This experiment is aimed to determine the effect of melatonin on leukocyte and lymphocyte count of methylprednisolone-induced immunosuppression on Wistar rat.

**Method:** This study was true experimental with post-test only control group design using 32 male rats. Rats were divided into control and the intervention group. Control group was given methylprednisolone 0.144 mg/200 g rat 3 times a day and distilled water. The treatment group was given methylprednisolone 0.144/200 g rat 3 times a day and 0.054 mg/200 g melatonin once daily. The blood was taken after 14 days of treatment.

**Results:** Intervention group shows higher lymphocytes count ( $4.95 \pm 1.58 \times 10^3/\mu\text{L}$ ) compared to the control group ( $3.09 \pm 1.33 \times 10^3/\mu\text{L}$ ), with  $p < 0.01$ . The intervention group also has higher leukocyte count ( $8.37 \pm 2.02 \times 10^3/\mu\text{L}$ ) compared to the control group ( $6.70 \pm 1.96 \times 10^3/\mu\text{L}$ ), with  $p < 0.05$ .

**Conclusion:** This study concluded that oral melatonin inhibited the decrease number of lymphocytes and leukocytes in rats given methylprednisolone.

**Keywords:** melatonin, leukocytes, lymphocytes, methylprednisolone, Wistar rats

## BACKGROUND

Immunosenescence is a part of aging process, featuring changes in micro-cytokine environment and decline in innate and acquired immunity, which increase the risk of infection and neoplasm development.<sup>1</sup> Exogenous steroid consumption, either prescribed for inflammation or other purpose, might accelerates this process.<sup>2</sup> In concordant with this degeneration, deterioration of hormone levels may worsen the negative effect of aging, through direct and indirect effects on the immune system. Growth hormone, sex hormone, and melatonin has been shown decreasing by age.<sup>3</sup> Melatonin is particularly important, where its antioxidant property and protective effect on the immune system might delay the aging process, especially immunosenescence.<sup>4</sup> This experiment is aimed to determine the effect of melatonin on steroid-induced immunosuppression on Wistar rat, which is important before clinical trial on human.

## MATERIAL AND METHOD

### Study Design

This study used post-test only true experiment design with control group. We randomly allocated 32 male Wistar rats (*Rattus norvegicus*) to intervention and control groups, equally. All experiment was performed from March to May 2016 at Animal Laboratory Unit (ALU), Department of Pharmacology, Universitas Udayana, Bali. The examination of blood specimens was conducted at Health Laboratory Unit, Department of Health, Bali. This study was approved by ethical committee of Faculty of Medicine, of Universitas Udayana (No. 381/UN/14/2/Litbang/2016).

### Experimental Animals

The experiments were carried out on 32 male Wistar rats (*Rattus norvegicus*), aged 12 weeks, and weighing 150-170 g. They were caged

in groups, with sufficient living area, weekly cleaning, warm room temperature (28 to 32°C), and water and food ad libitum. They were feed with pellet composed by 20-25% protein, 5% lipid, 5% fibre, 4-5% ash, and additional vitamin and mineral. The rats were given 7 days acclimatisation before treatment. The light was switched of at 6 to 7 PM for as long as 12 hours till the morning.

### Experimental Procedures

Both control (n=16) and intervention (n=16) group were given three doses of methylprednisolone (Medrol®, Pfizer – 0.144 mg/200 mg body mass/dose) orally (8 AM, 12 PM, 4 PM). In addition, the control group were given one dose of distilled water, and the intervention group were given one dose melatonin (Melatonin M5250, Sigma Aldrich Co – 0.054 mg/200 g body mass/dose), one hour before the light was switched off. The treatment was conducted for 14 days.

### Outcome Measurement

On the fourteenth day of treatment, the vein blood specimens were drawn from orbital sinus through medial canthus under anaesthesia (ketamine 40-50 mg/kg and xylazine 5-10 mg/kg). The specimens were stored in EDTA tube, and further analysed with haematology analyser (XS-800i, Sysmex) to measure the blood levels of lymphocyte and leukocyte.

### Statistical Analysis

Results were presented descriptively and the comparisons of leukocyte and lymphocyte level between control and intervention group were done with independent Student's t-test. All analysis was performed with SPSS Statistics V.23.

## RESULT

The leukocyte and lymphocyte count of each group after two-weeks experiment was shown in Table 1.

Table 1. Effect of melatonin on leukocyte and lymphocyte level on methylprednisolone-treated rat ( $n = 32$ )

Outcome	Control ( $n = 16$ )	Intervention ( $n = 16$ )	t (df)	p-value	95% CI of difference
Leukocyte count per $\mu\text{L}$ (mean $\pm$ SD)	6708 $\pm$ 1965	8368 $\pm$ 2016	-2.359 (30)	0.025	223 to 2098
Lymphocyte count per $\mu\text{L}$ (mean $\pm$ SD)	3093 $\pm$ 1334	4950 $\pm$ 1577	-3.594 (30)	0.001	801 to 2910

There is a higher level of blood lymphocyte and leukocyte on intervention group compared to the control group. As shown in Figure 1 (a and b), there is significant difference of mean lymphocyte and leukocyte count between two groups.

## DISCUSSION

### Effect Of Methylprednisolone On White Blood Cells

In this study, methylprednisolone administration decreases the leukocyte and lymphocyte count in control group compared to reference range of leukocyte (6000 to 17000 per  $\mu\text{L}$ ) and lymphocyte count (65-85% of leukocyte

count, or 3900 to 14450 per  $\mu\text{L}$ ).<sup>5</sup> Generally, exogenous steroids could inhibit leukocyte production through several mechanisms, including halting the production of NF- $\kappa\text{B}$  and leukocyte-attracting cytokines,<sup>6,7</sup> inhibiting macrophage adhesion through IL-10 induction,<sup>8</sup> suppressing T-lymphocyte and B-lymphocyte production by generating TGF- $\beta$ ,<sup>9</sup> inducing cell apoptosis with caspase-9 activation<sup>10</sup>, and generating oxidative stress.<sup>11</sup> Specifically, lymphopenia might occurs on exogenous steroid consumption, as a result of IL-1 and IL-2 inhibition,<sup>12</sup> and NF- $\kappa\text{B}$  and AP-1 inhibition through inactivation of MAPK kinases and ERK signals.<sup>13</sup>

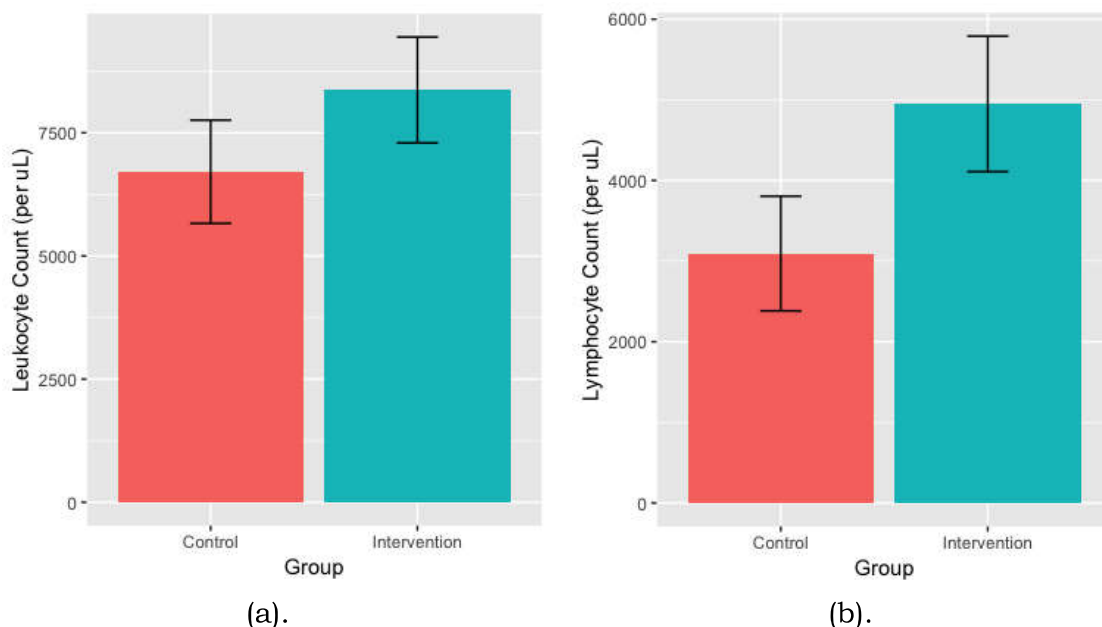


Figure 1. The mean count and 95% confidence interval for leukocyte (a) and lymphocyte (b) between control ( $n=16$ ) and intervention ( $n=16$ ) group

### **Protective Effect Of Melatonin On Steroid-Related Immunosuppression**

Both leukocyte count and lymphocyte count in intervention group are significantly higher than the control group. In addition, the lymphocyte count decrement (1857 per  $\mu\text{L}$ , 95% CI 223 to 2098  $\mu\text{L}$ ) is slightly higher than leukocyte count (1660 per  $\mu\text{L}$ , 95% CI 801 to 2910 per  $\mu\text{L}$ ), which explains that melatonin mostly protects the lymphocyte production.

Melatonin influence lymphocyte proliferation and differentiation by activating IL-2<sup>14</sup>, reversing oxidative stress by its antioxidant property<sup>15</sup>, and increasing chemotactic capacity through interferon-pathway.<sup>14</sup> Melatonin might affect other white blood cells, such as macrophage and monocyte. It increases macrophage phagocytosis activity, MHC II expression, and IL-1 and TNF  $\alpha$  production.<sup>14</sup> Melatonin receptor on monocytes induces IL-6 and IL-12 production, NF- $\kappa\text{B}$ , AP-1, and other pro-inflammatory cytokines.<sup>11</sup>

### **Limitation Of The Study**

This finding must be cautiously interpreted as immune status might be influenced by other factors, such as food, nutrition status, underlying disease and environmental factors.<sup>16</sup>

### **CONCLUSION**

This study shows that melatonin protects the leukocyte and lymphocyte from damages by exogenous corticosteroid ingestion. Blood melatonin levels is known to decrease over time, related to aging process. In addition, pre-elderly population might be exposed to steroid from multimorbidity or unintentional exposure. In the future, there is a need to study melatonin replacement therapy for high-risk population, such as exogenous steroid exposure.

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