

## Oral administration of melatonin increased superoxide dismutase and reduced malondialdehyde in male wistar rats (*rattus norvegicus*) induced by cigarette

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**Introduction:** Cigarette smoke is one of the most common sources of free radicals that accelerate aging process. This process can be countered by avoiding cigarette and consuming anti-oxidants. Melatonin is one of well-known anti-oxidant which can be safely consumed. Therefore, the aim of this study was to assess the effect of oral melatonin supplementation toward the level of superoxide dismutase and malondialdehyde in male Wistar rats exposed by cigarette smoke.

**Methods:** An experimental pretest-posttest control group study was conducted using 18 male Wistar rats, 12 weeks old, and 180-200 gr in weight. The subjects were divided into two groups ( $n=9$  rats) randomly. Both groups were exposed by cigarette smoke but treatment group (P1) was treated with 2

mg of melatonin per 200 gr BW rats for 14 days. The levels of SOD and MDA were measured using the ELISA method.

**Results:** The baseline level of SOD and MDA in both groups was homogenous. At the end of the experiment, there was significant increase of SOD level (baseline:  $1.13 \pm 0.45$ ; post-treatment:  $2.57 \pm 0.08$  ng/mL;  $p < 0.001$ ) and decreased in MDA level (baseline:  $9.53 \pm 1.25$ ; post-treatment:  $1.44 \pm 0.46$ ;  $p < 0.001$ ) in treatment group while no change was observed in both SOD and MDA in control group.

**Conclusion:** The oral consumption of melatonin countered the adverse effect of smoking by increasing the level of SOD and lowers MDA level.

**Keywords:** moringa leaves, fibroblasts, neovascularization, epithelialization, wound

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Submitted: 1 January 2020

Accepted: 23 May 2020

Published: 1 June 2020

### INTRODUCTION

Excessive free radicals are one of important factors of aging. With advancing age, the production of antioxidants is decreasing and insufficient to neutralized free radicals which then cause oxidative stress. In the concept of Anti-Aging Medicine, aging process can be prevented, delayed, and even reversed by several approaches; one of them is antioxidant consumption.<sup>1</sup>

The human body has natural mechanisms to neutralize free radicals either enzymatically or non-enzymatically. The major enzymatic antioxidants include Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPx). Non-enzymatic exogenous antioxidants often obtained from food stuffs such as vitamin A, beta carotene, Vitamin C, Vitamin E, Flavonoids, selenium, and zinc.

The reaction between biological molecules with free radicals produces various by product that can be used to indirectly measure systemic free radical levels. For instance, free radicals reaction with cell lipid membrane will produce peroxidation product such as malondialdehyde (MDA). Plasma MDA

levels are directly correlated with the level of cell membranes oxidative damage. Usually, sufficient antioxidant supplementation resulted in decrease in MDA levels.<sup>2</sup>

Melatonin is a neurohormon produced by the pineal gland and stimulated by darkness and is inhibited by bright light; this pattern is known as the circadian rhythm cycle. Melatonin can pass through the blood-brain barrier and placenta barrier due to its solubility in water and fat which enable melatonin to reach almost all cells of the body. Melatonin is also considered as a powerful antioxidant as demonstrated in several studies which showed that melatonin supplementation increased plasma SOD and glutathione levels.<sup>3</sup>

Cigarette smoke is one source of free radicals that can impair and overwhelm natural antioxidants in the body and triggers oxidative stress. Cigarettes cause Reactive Oxygen Species (ROS), oxidative stress, and cells membrane damage-related cell death as shown by increased MDA level in smokers.<sup>4</sup> However, the research on the effects of melatonin as an antioxidant has been carried out with various subjects, indicators, assessments and treatments. In

this study, the effect of melatonin administration was assessed regarding its effect toward plasma SOD and MDA levels in cigarette smoke-exposed Wistar male rats.

## MATERIALS AND METHODS

An experimental study using a pretest-posttest control group design was conducted using 18 male Wistar rats, 12 weeks old, and weighed 180-200 gr. The rats were divided into two groups ( $n=9$  rats). Both groups were exposed to cigarette smoke for 14 days but only treatment group (P1) received oral supplementation of melatonin at 2 mg of melatonin per 200 gr BW rats. The control group (P0) was only treated with placebo.

The level of plasma SOD and MDA level were examined twice, at the beginning of the experiment and at the 14<sup>th</sup> days of treatment. SOD level was assessed using ELISA method while MDA level was assessed using TBARS (*Thiobarbituric Acid Reactive Substance*) technique. The melatonin was obtained from Natural Organic Laboratories/Nature Plus (New York, USA) imported by PT. Radiant Sentral Nutrindo, Jakarta.

## RESULTS

Both groups were considered homogenous according to the result of first SOD and MDA measurement. Baseline SOD value of the control group was found at  $1.19 \pm 0.41$  ng/mL while in treatment group, it was  $1.13 \pm 0.45$  ng/mL. Baseline MDA level in control group was  $9.66 \pm 1.47$   $\mu$ M while in treatment group, it was  $9.53 \pm 1.25$   $\mu$ M. Bivariate comparison showed that there was no significant difference of SOD and MDA between both groups (SOD:  $p=0.737$ ; MDA:  $p=0.828$ ) (Figure 1).

After 14 days of melatonin supplementation, the SOD and MDA level were measured for the second time to assess the effect of the treatment. Significant

changes were observed in treatment group both in SOD and MDA levels. The SOD level of treatment group was increased to  $2.57 \pm 0.08$  ng/mL ( $p < 0.001$ ) while MDA level was dropped to  $1.44 \pm 0.46$   $\mu$ M ( $p < 0.001$ ). Meanwhile, no difference was observed in control group both in SOD ( $1.12 \pm 0.12$  ng/mL;  $p = 0.629$ ) or MDA level ( $8.91 \pm 0.61$   $\mu$ M;  $p = 0.203$ ). Comparison analysis between the group also revealed that the mean difference of SOD and MDA level were significant (Figure 1).

## DISCUSSION

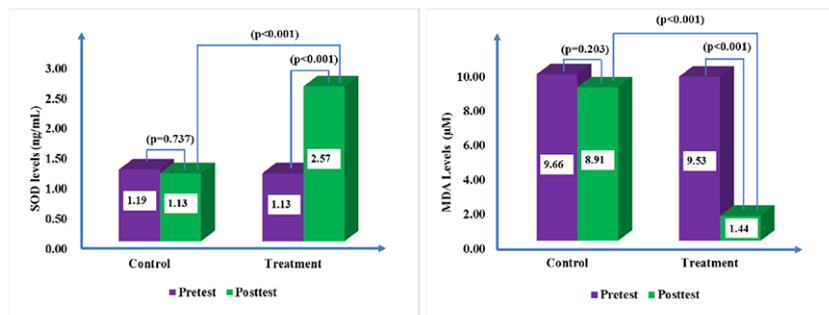
Cigarette smoke is one of common sources of free radicals. There is evidence that showed that cigarette smoke exposure significantly reduced SOD levels in saliva of active smokers.<sup>6</sup> In addition, another study also reported that cigarette smoke exposure decreased total antioxidant capacity in saliva in active smokers.<sup>5</sup> However, this effect in not only localized but also systemic.<sup>7</sup>

The results of this study indicated that the oxidative effect of cigarette smoke exposure can be prevented. Administration of 2 mg melatonin per 200 gr BW rats doubled the level of SOD while sharply reduced MDA levels. Theoretically, this effect was the result of signaling activation by melatonin that activates SOD-encoding gene as well as its inherent antioxidant property.<sup>8</sup>

Melatonin is known to have antioxidant anti-inflammatory capacity. Inflammation is also important aspect of aging as it is also the source of free radicals and oxidative stress, but also the consequences of cellular damage due to oxidative stress. Thus, inflammation creates positive feedback loop which could accelerate aging. Recent research showed that melatonin enhanced internal anti-oxidant system by increasing plasma SOD, increasing antioxidant enzymes and glutathione levels<sup>3</sup>. It also soluble in lipid and water so it easily passes through the blood brain barrier and counteract hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), nitric oxide (NO), peroxynitrite anion, peroxynitrous acid and hypochlorous acid which are common oxidant in cigarette smoke.<sup>9,10</sup>

Melatonin is considered as a terminal antioxidant. Unlike other antioxidants, melatonin cannot reduce repeatedly in oxidation and reduction reactions. Once reduced, melatonin cannot return to its original form and forms stable metabolites.<sup>9</sup>

In addition to its direct antioxidant effect, melatonin can also increase the expression of enzymatic endogenous antioxidants, such as SOD. Previous



**Figure 1.** The intra- and intergroup comparison of SOD and MDA level in male Wistar rats exposed to cigarette smoke

research has shown that melatonin induces PKC $\alpha$  activation, thus inducing phosphorylation and translocation of the two transcription factors, and activating the transcription of enzymatic antioxidants NAD (P) H-quinon oxidoreductase 1, heme oxygenase-1, superoxide dismutase, glutathione and catalase.<sup>11</sup>

### CONCLUSION

In conclusion, the results of this study indicated that oral administration of melatonin increased SOD level while reduced MDA level in the male Wistar rats exposed by cigarette smoke.

### CONFLICT OF INTEREST

All authors declared that there is no conflict of interest regarding this publication

### AUTHOR CONTRIBUTION

All authors contributed equally in the writing of this article

### FUNDING

This study was self-funded without any contribution from third party.

### ETHIC APPROVAL

This study had been ethically approved by ethical commission of Faculty of Medicine Udayana University with approval letter number 423/KE-PH-Lit-2/VII/2019

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