

## The Effect of Moringa Leaf Extract on Malondialdehyde Levels in Male Wistar Rats

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**Abstract:** Excessive physical activity will result in body fatigue. This causes oxidative stress that occurs due to an imbalance between oxidants and antioxidants which can then cause damage characterized by MDA. Moringa leaf extract is an antioxidant from outside the body that is useful in reducing fatigue. This study aimed to decrease MDA levels in male Wistar rats by Moringa leaf extract. We gave swimming exercises to tire the mice. It will increase the levels of MDA in their blood. Fatigue can be characterized by a variety of biological signs. The difference in our research lies in the biological marker used as an indicator of fatigue, namely MDA levels. The sample used in this study consisted of 4 groups to be given treatment, with each group consisting of 5 rats. MDA levels were tested using the competitive ELISA (enzyme-linked immunosorbent assay) method. One-way ANOVA test was used to find out where the difference was, with 95% correct confidence for this test, and if  $p < 0.05$ , a significant difference was obtained. The lowest mean MDA levels were in the treatment of Moringa extract at 300 mg/kg in the pre-test, the first week, and the second week,  $1.82 \pm 0.20$ ,  $1.24 \pm 0.03$ , and  $0.86 \pm 0.12$ , respectively. The results of the one-way ANOVA test in this study showed differences in each treatment group with  $p = 0.000$ . The results of the examination of MDA levels at each treatment time showed a difference between before the treatment, the first week, and the second week.

**Keywords:** exercise, fatigue, male Wistar rats, malondialdehyde levels, Moringa leaf extract.

### 辣木叶提取物对雄性维斯塔大鼠丙二醛水平的影响

**摘要:** 过度的体力活动会导致身体疲劳。这会导致由于氧化剂和抗氧化剂之间的不平衡而发生的氧化应激, 然后会导致以 MDA 为特征的损害。辣木叶提取物是一种来自体外的抗氧化剂, 有助于减轻疲劳。本研究旨在通过辣木叶提取物降低雄性维斯塔大鼠的 MDA 水平。我们进行游泳练习以使老鼠疲倦。它将增加他们血液中的 MDA 水平。疲劳可以通过多种生物体征来表征。我们研究的不同之处在于用作疲劳指标的生物标志物, 即 MDA 水平。本研究使用的样本由 4 组进行治疗, 每组由 5 只大鼠组成。使用竞争性酶联免疫吸附试验 (酶联免疫吸附测定) 方法测试 MDA 水平。单因素方差分析用于找出差异在哪里, 该测试的正确置信度为 95%, 如果  $p < 0.05$ , 则获得显著差异。在预测试、第一周和第二周, 300 毫克/公斤辣木提取物的最低平均 MDA 水平分别为  $1.82 \pm 0.20$ 、 $1.24 \pm 0.03$  和  $0.86 \pm 0.12$ 。本研究中单因素方差分析的结果显示每个治疗组之间存在差异,  $p = 0.000$ 。各治疗时间 MDA 水平检测结果显示治疗前、第一周、第二周存在差异。

**关键词:** 运动、疲劳、雄性维斯塔大鼠、丙二醛水平、辣木叶提取物。

## 1. Introduction

Fatigue is caused by too much physical activity.

This excessive activity will result in increased levels of free radicals in the body so that endogenous

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antioxidants cannot neutralize free radicals, and exogenous antioxidants are needed in greater quantities to counteract the effects of free radicals [1], [2]. MDA is an indicator with the highest reaction sensitivity when there are free radicals in tissue. Strenuous exercise will produce excess ROS, thereby lowering antioxidant defenses in the body, resulting in oxidative stress [3]. Oxidative stress is a phenomenon caused by an imbalance between the production and accumulation of reactive oxygen species (ROS) in cells and tissues and the ability of a biological system to detoxify these reactive products [4]. Moringa leaves have a high flavonoid function as antioxidants. Flavonoids are the main phenolic compounds found in Moringa leaves [5]–[9]. For this reason, the purpose of this study was to give Moringa leaf extract to decrease MDA levels in male Wistar rats.

## 2. Materials

### 2.1. Ingredients and Preparation of Moringa Leaf Extract

Moringa fresh leaves come from Blora Regency, Central Java. After the leaves are harvested, then the clean leaves are dried. Moringa leaf extract is made using leaves dried and mashed as much as 3 kg, then mixed into 75% ethanol for 72 hours. The ethanol was evaporated at a temperature of  $35 \pm 20$  °C for 48 hours, and a net residue of 25.7 g/bb was obtained and stored at  $-40$  °C; this is liquid extraction. Moringa leaf extract is made at Unimus Food and Beverage Chemistry and Biochemistry Laboratory.

### 2.2. Animals and Grouping

#### 2.2.1. Treatment of Experimental Animals

The animal used in this experiment was a male Wistar rat from the Experimental Animal Laboratory of the Muhammadiyah University of Muhammadiyah Semarang. Rat body weight is 150-200 g. Mice were fed a basal diet and water ad libitum with standard mouse chow feed.

#### 2.2.2. Animal Group

The samples used in this study were 20 rats adapted for seven days. The treatment of rats consisted of 4 groups, with each group consisting of 5 rats: positive control group K (swimming for 45 minutes and given 0.5 ml of distilled water orally), P1 (swimming for 45 minutes, given Moringa leaf extract at a dose of 100 mg/kg in 0.5 ml of distilled water orally), P2 (swimming for 45 minutes, given Moringa leaf extract at a dose of 200 mg/kg in 0.5 ml of distilled water orally), and P3 (swimming for 45 minutes, given Moringa leaf extract at a dose of 300 mg/kg in 0.5 ml of distilled water orally). Moringa leaf extract was administered orally (oesophageal intubation) for 14 days.

## 3. Methods

### 3.1. Swimming Treatment

Each animal was placed individually in a swimming pool (90 cm × 45 cm × 45 cm), filled with water to a depth of 35 cm, and maintained at  $25 \pm 1$  °C. Swimming in mice was carried out for 45 minutes. At the end of the swim, the mice were rested for one hour and then sacrificed (under ether anesthesia) by cutting through the jugular vein.

### 3.2. MDA Check

MDA was taken in mice to obtain blood serum taken from the orbital sinuses of the eyes and heart of mice using a syringe. The blood is placed in a centrifuge tube that has been anticoagulated and labeled. Then, the blood was centrifuged at 3000 rpm for 10 minutes. The centrifuge result used in MDA measurement is yellowish plasma on the top layer. Then, the serum is labeled on the sample holder. Serum was stored at  $\pm -200$  C.

After the treatment, the mice's malondialdehyde (MDA) levels were tested using the competitive ELISA (enzyme-linked immunosorbent assay) method. The examination is carried out by preparing serum and allowing it to reach room temperature and no clots. Standard HRP wash buffers were prepared and diluted as required. Then, samples of 20 serums were processed, which were read in ELISA Reader A 450.

### 3.3. Statistic Analysis

The data obtained were processed and analyzed using SPSS. The one-way ANOVA test determines where the difference lies, with 95% true confidence for this test, and if  $P < 0.05$ , a significant difference is obtained.

### 3.4. Ethical Approval

This research has received proper ethics from the KEPK FK UNIMUS number 071/EC/FK/2021.

## 4. Results

The study results in Table 1 explain that the lowest mean MDA levels were in the treatment of *M. oleifera* extract at 300 mg/kg in the pre-test, the first week, and the second week,  $1.82 \pm 0.20$ ,  $1.24 \pm 0.03$ , and  $0.86 \pm 0.12$ , respectively. Compared with other treatment groups, the control group showed high levels of MDA. Meanwhile, MDA levels decreased in the second week compared to those of the first week and before the treatment.

Table 1 The results of the average measurement of MDA levels in rats in each group

	Control	<i>M. oleifera</i> (100 mg/kg)	<i>M. oleifera</i> (200 mg/kg)	<i>M. oleifera</i> (300 mg/kg)
Pre test	$3.04 \pm 0.19$	$1.86 \pm 0.08$	$1.95 \pm 0.17$	$1.82 \pm 0.20$
Week 1	$2.27 \pm 0.12$	$1.58 \pm 0.11$	$1.65 \pm 0.09$	$1.24 \pm 0.03$
Week 2	$2.52 \pm 0.07$	$1.41 \pm 0.07$	$1.44 \pm 0.06$	$0.86 \pm 0.12$

The results in Table 2 of the one-way ANOVA test in this study showed that there were differences in each treatment group with  $p = 0.000$ .

Table 2 Differences in MDA levels by treatment group

MDA Levels	p-value
Pre-test	0,000
Week 1	0,000
Week 2	0,000

## 5. Discussion

Excessive and intense physical activity can cause oxidative stress [2]. Oxidative stress occurs due to an imbalance between oxidants and antioxidants, which can cause cell damage. In this situation, ROS will be formed. Strenuous physical exercise can lead to excess ROS in skeletal muscle, leading to peripheral fatigue [10]. Damage to body cells occurs due to free radicals that cause oxidative stress conditions, which have positive and negative physiological effects that result in disease [11]–[13].

Cell damage caused by free radicals is characterized by MDA [14]. MDA results from lipid peroxidation [15] or producing unsaturated fatty acids by free radicals in dialdehyde compounds with high toxicity. In addition, it can react with DNA to form mutagenic substances. Free radicals increase lipid peroxidation, which decomposes into malondialdehyde (MDA) in the blood.

The average MDA in this study was the highest in the control group, while the lowest MDA value was in the P3 group with the administration of Moringa leaf extract at 300 mg/kg. This indicates that the greater the concentration of Moringa leaf extract given, the more effective it reduces MDA in rats. The swimming treatment given to the rats was intended as a physical activity to show the fatigue that occurred [16].

The results of the one-way ANOVA test in this study explained that there were differences in MDA levels in rats with the treatment group given with  $P < 0.05$ . Oxidative stress can be effectively neutralized by increasing defenses in the form of antioxidants [11]. Antioxidants can counteract free radicals in the body. Antioxidant compounds react in one-electron reactions with free radicals in vitro and prevent oxidative damage. Previous studies in rats have shown that high antioxidants can reduce various oxygen free radicals and prevent oxidative stress [17].

Moringa leaves have flavonoid compounds that function as antioxidants [18]–[20] that can prevent cell damage due to oxidative stress. In addition, Moringa leaves contain vitamins, nutrients, and amino acids that can ward off free radicals, with vitamin C being the most dominant vitamin in Moringa plants [21]. Vitamin C and flavonoids in Moringa leaves play a major role in fat metabolism, increasing the rate of cholesterol excretion in the form of bile acids, increasing HDL levels, and decreasing the reabsorption of bile acids

playing a role in forming collagen to inhibit lipid peroxidation [22].

Previous research explained that Moringa leaf extract could reduce MDA levels in hypercholesterolemic rats [23]. The antifatigue effect of Moringa leaf extract may occur through the protection of cell membranes by preventing lipid oxidation by modifying several enzymes' activity. These results agree with previous studies, which showed the same effect of the course of red betel leaf and purple passion fruit travel on decreasing MDA levels [24], [25].

## 6. Conclusion

The decrease in MDA levels occurred in the second week compared to the first week before the treatment. The results of the examination of MDA levels at each treatment time showed a difference between before the treatment, the first week, and the second week. The results showed that, of the three treatments of *M. oleifera* extract, namely 100 mg/kg, 200 mg/kg, and 300 mg/kg, administration of *M. oleifera* extract at a concentration of 300 mg/kg experienced an accelerated decrease in MDA levels compared to other treatments. The administration of Moringa leaf extract at 300 mg/kg in the P3 treatment group showed the lowest mean MDA results. *M. oleifera* extract at 300 mg/kg showed a significant effect on MDA levels in rat blood. Further research is needed on the effects of other fatigue factors with MDA. There are still many biological signs of fatigue that *M. oleifera* is likely to influence. Further research will answer this possibility. The limitation of the study lies in the treatment of rats, which was only carried out for 14 days. This is likely to reduce the wealth of research data.

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