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### THE EFFECT OF MORINGA LEAVES EXTRACTS ON MDA LEVEL IN MALE MICE EXPOSED TO ELECTROMAGNETIC RADIATION FROM MOBILE PHONES

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#### ABSTRACT

Background: The use of cell phones causes anxiety about the effects of electromagnetic wave radiation emitted because of the production of free radicals in the body is increased by cell phone electromagnetic wave radiation. This escalation results in an elevated production of Reactive Oxygen Species (ROS), resulting in elevated Malondialdehyde (MDA) levels due to lipid peroxidation, which is toxic to the body. One way to reduce MDA levels is to introduce antioxidants into the body. Previous studies have found that Moringa leaf extract can be used as a good source of antioxidants. Objective: The aim of this research was to assess the impact of Moringa leaf extract administration on the MDA levels in BALB/C mice subjected to electromagnetic waves emitted by cell phones. Methods: This study employed an experimental study with a post-test only control group design. The research involved 23 male BALB/c mice, divided into four groups. The healthy control group received no treatment, while the negative control group was exposed to cell phone electromagnetic waves for 2 hours/day. Groups P1 and P2 received Moringa leaf extract at doses of 100 mg/kgBW and 400 mg/kgBW, respectively, in addition to being exposed to cellular phone electromagnetic waves for 2 hours/day. The treatment was administered for 30 days following a 7-day adaptation period. On the 31st day, blood plasma samples were taken from all mice and examined for MDA levels. The data underwent analysis through non-parametric One-Way ANOVA and subsequent Pos Hoc LSD tests. Significance is acknowledged for p-values less than 0.05. **Results:** The highest MDA levels were found in the K (-) group, namely  $0.43 \pm 0.18$  ppm, and the lowest MDA levels were observed in the P1 group, with values of  $0.19 \pm 0.08$  ppm. A significant difference was noted between the K (-) group and the P1 group (p = 0.05), while no significant difference were observed in the other groups. Conclusion: Administering Moringa leaf extract at a dose of 100 mg/kgBW/day can reduce MDA levels in mice exposed to electromagnetic waves from cell phones. Keywords: cell phone electromagnetic waves, Moringa leaves, Malondialdehyde

#### BACKGROUND

The use of electronic devices in the modern era, as it is today, has become an essential part of human life, especially the use of cell phones. GSMA Intelligence reported in 2017 that cellular phone users increased by five million users every year.<sup>1</sup> Additionally, with the present condition of the COVID-19 pandemic, the use of cellular phones, especially the internet, has increased by around 7% or approximately  $\pm 875,000$  new users every day worldwide.<sup>2</sup> With the rising prevalence of cell phones use, there is growing concern about the effects of electromagnetic wave radiation emitted by cell phones on the body's tissues of users.<sup>3</sup>

In 2019, it was demonstrated by a study that showed that degeneration, necrosis, apoptosis and fibrosis in liver cells can be induced by prolonged exposure to electromagnetic waves from cell phones.<sup>1</sup> In a 2018 study discussing radiation and male infertility by Kavindra Kumar Kesari et al. laptops, and Wi-Fi were found to cause damage to the testicles affecting sperm count, morphology, motility, and causing DNA damage, disturbances in protein kinase, hormones, and antioxidant enzymes.<sup>4</sup>

Cell phone electromagnetic wave radiation leads to an increase in free radicals in the body beyond its capacity to produce protection against these free radicals, namely antioxidants. Imbalances in the body resulting from exposure to cell phone electromagnetic waves can have adverse effects by elevating Reactive Oxygen Species (ROS), leading to an increase in Malondialdehyde (MDA) levels and a reduction in antioxidant activity, resulting in oxidative stress.<sup>5</sup>

Malondialdehyde (MDA) is an active aldehyde compound that results from the lipid peroxidation process, specifically the degradation of Poly Unsaturated Fatty Acids (PUFA) with Reactive Oxygen Species (ROS) which are toxic to cells by causing toxic stress and forming protein plugs.<sup>6</sup> Malondialdehyde (MDA) is frequently employed as a marker or lipid peroxidation to indirectly measure free radicals in the body.<sup>7</sup>



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One way to mitigate the negative impact of exposure to electromagnetic waves is by reducing the resulting oxidative stress. This can be achieved by combating free radicals using antioxidants, including those produced by the body and external sources such as consuming foods rich in antioxidants. In 2016, a study conducted by Jahan et al. determined the antioxidant activity of Moringa leaf extract, the results indicated that Moringa leaves can serve as a valuable source of antioxidants.<sup>8</sup> The flavonoids content in Moringa leaves, a class of polyphenolic compounds, is known for its ability to capture free radicals, exhibit anti-inflammatory properties, and inhibit hydrolysis and oxidative enzymes.<sup>9</sup>

With this background description, researchers are interested in conducting research on the impact of using Moringa leaf extract as an antioxidant on MDA levels in BALB/C mice exposed to electromagnetic waves from cell phones.

#### **METHODS**

The research employed an experimental design with a post-test only control group. Twentyfour male BALB/C mice, weighing 20-25 grams and aged 6-8 weeks, were selected for the study. These mice were active and without defects, divided randomly into four groups, each consisting of 6 mice. The healthy control group received no treatment, while the negative control group was exposed to cell phone electromagnetic waves 2 hours per day. Group P1 received Moringa leaf extract at a dose of 200 mg/kgBW and exposure to electromagnetic waves on a cell phone 2 hours per day. Group P2 was administered garlic extract at a dose of 400 mg/kgBW and was exposed to cell phone electromagnetic waves 2 hours per day. The treatment were conducted in the Biology Laboratory, Faculty of Mathematics and Natural Sciences, State University of Semarang, for 30 days according to the group division after a 7- day adaptation period. All groups were provided with ad libitum access to food and water for the duration of the study.

#### **MDA Level Check**

On day 31, blood samples were collected from all mice through retroorbital veins, placed into anticoagulant tubes, and then centrifuged at 3000 rpm for 10 minutes to obtain plasma. Subsequently, the plasma samples were sent to the Integrated IBL Laboratory, Sultan Agung University, Semarang, for the examination of the MDA levels.

#### **Statistic Analysis**

The analysis of the data was conducted utilizing SPSS version 26.0, checking for the normality of the data using the Shapiro-Wilk test and assessing the homogeneity of the data using the Levene test. Subsequently, the data underwent the non-parametric Anova test followed by the Pos Hoc test. The difference was considered significant if the p value <0.05.

#### **Ethical Clearance**

Ethical clearance for the research was sought from the Health Research Ethics Commission (KEPK) of the Faculty of Medicine, Diponegoro University, under the reference number no. 82/EC/H/FK-UNDIP/VIII/2021.

#### RESULTS

Throughout the study, one participant droped out from the negative control group (-), resulting in a total research sample of 23 participants. A spectrophotometer with a wavelength of 532 nm measured the concentration of MDA in the research sample. The results of the average measurement of MDA levels for each group in this study can be seen in table 1.

Group	Absorbance (OD)	MDA Concentration Level (ppm)
Ks	$0,14 \pm 0,05$	$0,30 \pm 0,10$
K (-)	$0,21 \pm 0,10$	$0,\!43 \pm 0,\!18$
P1	$0,09 \pm 0,04$	$0,\!19\pm0,\!08$
P2	$0,14 \pm 0,07$	$0,29 \pm 0,13$

The normality test, conduted using the *Saphiro-Wilk* test to assess data distribution, revealed that the data exhibited a normal distribution, with a significance value exceeding 0.05. Additionally, a test for data homogeneity test was carried out using *Levene's Test*, resulting in homogeneous data with a significance value exceeding 0.05, namely p = 0.369. Subsequently, hypothesis testing with the *One-Way ANOVA* test revealed significant differences in MDA levels between groups, with a significant value less than 0.05, namely p = 0.044. The results of data analysis can be seen in table 2.



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Table 2. One Way ANOVA Test Results						
Group	Mean ± SD	р	Levene			
Ks	$0,\!30\pm0,\!10$	0,044*	0,369**			
K(-)	$0,\!43 \pm 0,\!18$					
P1	$0,19 \pm 0,08$					
P2	$0,29 \pm 0,13$					
Note: * Significant ( $p < 0.05$ ); ** Homogeneous ( $p > 0.05$ )						

The test was further continued with Pos Hoc LSD test to assess whether a significant difference existed between the two groups. Based on the Pos Hoc test, the results showed a significant difference between groups K (-) and P1 (<0.005). Meanwhile, in other groups, there was no significant difference observed between the two groups. Data analysis can be seen in table 3.

Table 3. LSD Post Hoc Test Results								
G	froup	Ks	K (-)	P1	P2			
	Ks	-	0.098	0.160	0.960			
]	K (-)	0.098	-	0.005*	0.089			
	P1	0.160	0.005*	-	0.175			
	P2	0.960	0.089	0.175	-			

Note: \* Significant

#### DISCUSSION

The sample used in this study compromised 24 BALB/C mice, categorized into four groups, namely the healthy control group, the negative control group, the first and second treatment groups. However, during the study, one sample dropped out from the negative control group, resulting in a total of 23 BALB/C mice. The aim of this research was to assess the impact of Moringa leaf extract administration on the MDA levels in BALB/C mice subjected to electromagnetic waves emitted by cell phones.

Cell phones serve as source of free radicals that surround us constantly. Exposure to electromagnetic waves or radiation generated by cell phones increases the production of ROS in the body. The elevation in ROS levels results in an upsurge in MDA levels and a reduction in the activity of antioxidants. This imbalance can lead to a state of oxidative stress when the levels of free radicals in the body, such as ROS, surpass antioxidant levels. Consequently, the antioxidants in the body may become insufficient to protect the body from oxidative damage.<sup>10,11</sup> MDA itself is an aldehyde compound formed through the lipid peroxidation in cell membranes, which is a reaction between free radicals and *poly unsaturated fatty acids* (PUFA) which are toxic to cells by causing toxic stress and forming protein blockages.<sup>6</sup> Administering Moringa leaf extract functions as a natural antioxidant, aiming to prevent the formation of ROS and, consequently, minimizing the lipid peroxidation process in the body, including reducing MDA levels.<sup>12</sup> MDA itself is often utilized as a marker for measuring oxidative stress in the body because it is a more stable and accurate component.<sup>13</sup>

The findings from this study suggest that the negative control group ( $0.43 \pm 0.18$  ppm) exhibited the highest levels of MDA, which was exposed solely to cellular telephone electromagnetic waves 2 hours/day over 30 days. This is attributed to the fact that the control group (-) was not provided with antioxidants, leading to an imbalance between antioxidant and oxidant levels in the body. While there was no statistically significant difference between the healthy control group and the control group (-), the MDA levels in the healthy control (0.30) $\pm 0.10$  ppm) remained lower than those in the control group (-)  $(0.43 \pm 0.18 \text{ ppm})$ . These finding align with previous research by Dharmawan et al., which indicates that exposure to cell phone electromagnetic waves can increase MDA levels.<sup>14</sup>

The administration of Moringa leaf extract at a dose of 100 mg/kgBW/day to mice exposed to cellular phone electromagnetic waves for 2 hours/day revealed a significant difference in MDA levels compared to the control group (-), which was solely exposed to cellular phone electromagnetic waves for 2 hours/day over 30 days (Post Hoc = 0.005). This substantiates that Moringa leaf extract exhibits effective antioxidant activity. The flavonoids in Moringa leaves, acting as antioxidants, function by capturing free radicals, thereby suppressing the production of ROS and lipid peroxidation in the body.<sup>8,15</sup>

The MDA level of the P2 group also decreased compared to the control group (-), although it was not statistically significant (Pos Hoc = 0.089), precisely at the level of  $0.29 \pm 0.13$  ppm, while the MDA level of the control group (-) was  $0.43 \pm 0.18$  ppm. However, the lack of significance in this study could be attributed to the high dose of Moringa leaf extract in the P2 group, potentially causing an



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overdose that, in turn, resulted in an insignificant decrease in MDA levels. This aligns with a previous study by Elinda Yuniarti Rahayu in 2020, which stated that the administration of Moringa leaf extract at a dose of 75 mg/kgBW was more effective than other doses (150 mg/kgBW and 300 mg/kgBW) in reducing blood MDA levels.<sup>16</sup>

The data analysis in this study indicated that there was no significant difference in MDA levels between the healthy control group and the P1 (Pos Hoc = 0.160) and P2 (Pos Hoc = 0.960) groups. However, the average MDA level in the treatment group (P1 =  $0.19 \pm 0.08$  ppm, P2 =  $0.29 \pm 0.13$  ppm) was still lower than the average MDA level in the healthy control group, which was  $0.30 \pm 0.10$ . ppm.

The lowest MDA levels were observed in the P1 group with the additional administration of Moringa leaves at a dose of 100 mg/kgBW/day compared to other groups. The research data also indicated that MDA levels in the treatment group did not decrease with increasing doses, as evidenced by the lack of significant differences between the P1 and P2 groups (Pos Hoc = 0.175). It is possible that this study was influenced by depressive or stressful conditions in experimental animals. Previous research by Liu T et al in 2015 stated that MDA levels tend to be higher in patients with depression.<sup>17</sup>

### CONCLUSION

Drawing conclusions from the outcomes of this study, it can be inferred that exposure to electromagnetic waves 2 hours/day over 30 days affects the levels of MDA of mice. The MDA level of the control group (-)  $(0.43 \pm 0.18)$  was higher than the healthy control group  $(0.30 \pm 0.10)$ , which was only given standard feed for 30 days, although statistically not significant (p = 0.098). There was a significant reduction in MDA levels among mice in the P1 group, given a dose of 100 mg/kgBW of Moringa leaf extract and exposure to cell phone electromagnetic waves for 2 hours/day over 30 days, compared to the negative control group. However, this reduction was not significant in the other groups.

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