JURNAL KEDOKTERAN DIPONEGORO
(*DIPONEGORO MEDICAL JOURNAL*)
Online : http://ejournal3.undip.ac.id/index.php/medico
E-ISSN : 2540-8844
DOI : 10.14710/dmj.v13i3.43904
JKD (DMJ), Volume 13, Number 3, May 2024 : 147-151

THE EFFECT OF MORINGA LEAVES EXTRACT ON SPERM MOTILITY IN MALE MICE EXPOSED TO ELECTROMAGNETIC RADIATION FROM MOBILE PHONE

Hasnaa Alyasi Sabrina[1], Trianggoro Budisulistyo[2], Achmad Zulfa Juniarto[3], Tun Paksi Sareharto[3]
[1]Undergraduate Program, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia
[2]Department of Neurology, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia
[3] Department of Medical Biology, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia
* Corresponding Author : E-mail : paksi@fk.undip.ac.id

ABSTRACT

Background: Electromagnetic radiation can induce an increase in oxidative stress. The presence of oxidative stress can impact the structure of the plasma membrane of sperm cells, damage the structure of Deoxyribonucleic Acid (DNA), and accelerate the process of apoptosis, ultimately leading to a decline in sperm quality. This increase in oxidative stress can be prevented by substances that serve as antioxidants. Moringa plants, particularly the leaves, contain various substances that can act as antioxidants for the body. These include flavonoids, alkaloids, saponins, tannins, and terpenoids. The antioxidant content in Moringa leaves is believed to safeguard the process of spermatogenesis. **Aim:** To determine the impact of Moringa (Moringa oleifera) leaf extract on the motility of spermatozoa in BALB/C mice subjected to exposure to electromagnetic waves. **Methods:** This study utilized design featuring a post-test-only control group design, dividing participants into four randomly assigned groups, namely a negative control group, a positive control group (exposure to electromagnetic waves), and 2 treatment groups (moringa leaf extract at dose of 100 mg/kg BW and 400mg/kg BW). Each group compromised 5 experimental animals, and the treatments were administered for duration of 30 days. Spermatozoa preparations were made for each group and observed under a microscope (400x). Data were analyzed by initially testing for normality using the Shapiro-Wilk test. If the data distribution was normal, the hypothesis examination involved conducting a One Way ANOVA followed by subsequent Post Hoc testing. **Results:** The results of the one-way ANOVA test indicated differences between groups of mice ($p<0.05$). The post hoc test results further demonstrated that a dose of 400 mg/kg BW was the most effective in preventing a decrease in spermatozoa motility. This effect is believed to be atttributed to the presence of vitamin C, beta carotene, beta-sitosterol, flavonoids, and polyphenols. **Conclusion:** There is an improvement in spermatozoa motility in BALB/C mice exposed to electromagnetic waves after the administration of Moringa leaf extract.

Keywords: *Electromagnetic waves, Moringa leaf extract, Spermatozoa motility*

INTRODUCTION

Among the 40 million couples in the reproductive age group in Indonesia, the occurance of infertility ranges between 10-15% .[1] Infertility can occur due to factors from both women and men. In particular, men contribute significantly to 30-40% of infertility cases caused by factors that decrease sperm quality.[2] Electromagnetic waves can affect sperm cells, which are crucial for the fertilization process, influencing factors such as motility, morphology, and sperm cell count.

In daily life, exposure to electromagnetic waves, particularly from cell phones, is widespread.[3] Electromagnetic wave radiation can induce an increase in oxidative stress, describing an imbalance between pro-oxidants or free radicals and antioxidants responsible for maintaining conditions against tissue damage. Oxidative stress occurs when the generation of ROS or free radicals exceeds the existing antioxidants as an intrinsic defense. The presence of oxidative stress can impact the structure of the plasma membrane of sperm cells, the structure of Deoxyribonucleic Acid (DNA), and accelerate the process of apoptosis, ultimately leading to a decrease in sperm quality.[4] Antioxidants have been demonstrated to prevent the formation of ROS.[5]

Antioxidants are present in various foods containing vitamin C, vitamin E, beta-carotene, flavonoids, such as tomatoes, Dutch eggplant, and Moringa leaves.[6,7] Moringa leaves contain a variety of vitamins (A, C, E, K, B1, B2). , B3, B6), flavonoids, alkaloids, saponins, tannins, and terpenoids, serving as active substances that can be a source of antioxidants.[8] The antioxidant content in Moringa leaves is believed to safeguard the process of spermatogenesis.

METHODS

The research was conducted at the Biology Laboratory, Faculty of Mathematics and Natural Sciences (FMIPA), Semarang State University. The

JURNAL KEDOKTERAN DIPONEGORO
(*DIPONEGORO MEDICAL JOURNAL*)
Online : http://ejournal3.undip.ac.id/index.php/medico
E-ISSN : 2540-8844
DOI : 10.14710/dmj.v13i3.43904
JKD (DMJ), Volume 13, Number 3, May 2024 : 147-151

research method used was a true experimental laboratory study with a post-test-only control group design plan, using BALB/C mice as experimental animals. The study aimed to determine the impact of Moringa (Moringa oleifera) leaf extract on the motility of spermatozoa in BALB/C mice subjected to exposure to electromagnetic waves. The samples used in this study comprised 24 male mice, divided into 4 groups. The were 6 male mice in the negative control group, 6 BALB/C mice in the positive control group, exposed solely to electromagnetic waves. Additionally, 6 BALB/C mice were assigned to the treatment group P, receiving a dose of 100 mg/kg BW of Moringa leaves and exposure to electromagnetic waves. Lastly, the remaining 6 BALB/C mice in group P2 were given a dose of 400 mg/kg BW of Moringa leaves and exposed to electromagnetic waves. An additional 1 BALB/C mouse was included in each group to anticipate the potential death of the samples during the study.

Samples were selected through simple random sampling with termination on the 31st day. The terminated mice were then taken from the cauda epididymis and massaged to remove sperm. The sperm that has come out is diluted with NaCl, placed on an object-glass, and covered with a glass deck. Observations were made under a microscope in 5 fields of view for 200 spermatozoa with a light microscope using a magnification of 400x. The obtained data were analyzed using univariate analysis. Subsequently, to determine the relationship between the two variables, the data underwent bivariate analysis, namely the One Way ANOVA test followed by the Post Hoc test. This research was conducted after obtaining ethical clearance from the Faculty of Health Research Ethics Commission (KEPK).

RESULTS

Descriptive Analysis

The general description of the research results can be obtained using descriptive analysis. Descriptive analysis shows the mean, standard deviation, median, and percentile of the dependent variable, namely spermatozoa motility, which is categorized into 3 criteria, namely progressive, non-progressive and immotile.

Analytical Analysis

Based on the results, after confirming the normal distribution of the data through the normality test, the subsequent analysis employed the one-way ANOVA test.

From the results of the one-way ANOVA test, where the p-value = < 0.001 and Levene test = 0.443, it can be concluded that there is a significant difference with homogeneous data variance. Further testing will be conducted using the Post Hoc LSD test.

Table 1. Descriptive Analysis of Spermatozoa Motility Progressive Criteria

Groups	Mean ± SD	Median (min – max)	p£
Negative Control	55,56 ± 10,04	55 (43,33 – 70)	0,901*
Positive Control	13,33 ± 8,50	13,33 (3,33 – 26,67)	0,537*
Treatment I	23,33 ± 12,65	23,33 (10 – 36,67)	0,113*
Treatment II	51,11 ± 11,67	46,67 (40 – 73,33)	0,055*

Table 2. Descriptive Analysis of Spermatozoa Motility Criteria for Non-Progressive

Groups	Mean ± SD	Median (min – max)	p£
Negative Control	17,22 ± 6,47	15 (10 – 26,67)	0,452*
Positive Control	34,67 ± 16,77	40 (10 – 53,33)	0,795*
Treatment I	41,67 ± 9,37	46,67 (26,67 – 50)	0,060*
Treatment II	30,00 ± 10,95	33,33 (10 – 40)	0,181*

Table 3. Descriptive Analysis of Spermatozoa Motility Criteria for Immotil

Groups	Mean ± SD	Median (min – max)	p£
Negative Control	27,22 ± 9,53	28,33 (13,33 – 40)	0,987*
Positive Control	52,00 ± 24,79	46,67 (20 – 86,67)	0,947*
Treatment I	31,67 ± 10,70	31,67 (16,67 – 43,33)	0,571*
Treatment II	18,33 ± 4,08	16,67 (13,33 – 23,33)	0,101*

Table 4. One way ANOVA test for Progressive Criteria

Groups	Mean ± SD	p	Levene
Negative Control	55,56 ± 10,04	<0,001*	0,443**
Positive Control	13,33 ± 8,50		
Treatment I	23,33 ± 12,65		
Treatment II	51,11 ± 11,67		

Table 5. LSD Post Hoc Test Progressive Criteria

Groups		p	
I	II		
Negatif Control	Positif Control	<0,001	Significant
	Treatment I	<0,001	Significant
	Treatment II	0,490	Not significant
Positive Control	Treatment I	0,148	Not significant
	Treatment II	<0,001	Significant
Treatment I	Treatment II	<0,001	Significant

JURNAL KEDOKTERAN DIPONEGORO
(*DIPONEGORO MEDICAL JOURNAL*)
Online : http://ejournal3.undip.ac.id/index.php/medico
E-ISSN : 2540-8844
DOI : 10.14710/dmj.v13i3.43904
JKD (DMJ), Volume 13, Number 3, May 2024 : 147-151

Table 6. One Way ANOVA Test Non-Progressive Criteria

Groups	Mean ± SD	p	Levene
Negative Control	17,22 ± 6,47	0,010*	0,190**
Positive Control	34,67 ± 16,77		
Treatment I	41,67 ± 9,37		
Treatment II	30,00 ± 10,95		

From the results of the Post Hoc LSD test, it was observed that there was a significant difference between the negative control and treatment I, whereas there was no significant difference between treatment II and the negative control. Moreover, no significant difference was found between the negative control group and treatment I, but a significant difference was observed between the negative control group and treatment II. Additionally, a significant difference was identified between the treatment group I and treatment II.

From the results of the one-way ANOVA test, p value = 0.010 and Levene test = 0.190, it can be concluded that there is a significant difference with the variance of homogeneous data, for further testing using the Post Hoc LSD test.

From the results of the Post Hoc LSD test, it was found that the negative control against the negative control and treatment I was significant while treatment II was not significant. The negative control group against treatment I and treatment II was not significant. And the treatment group I to treatment II was not significant.

From the results of the one-way ANOVA test, p-value = 0.006 and Levene test = 0.026, so it can be concluded that there is a significant difference with the variance of the data is not homogeneous, for further tests using the Post Hoc Games-Howell test.

Table 7. Post Hoc LSD Test Non-Progressive Criteria

Groups		p	
I	II		
Negative Control	Positive Control	0,018	Significant
	Treatment I	0,001	Significant
	Treatment II	0,062	Not significant
Positive Control	Treatment I	0,314	Not significant
	Treatment II	0,499	Not significant
Treatment I	Treatment II	0,086	Not significant

Table 8. One Way ANOVA Test for Motile Criteria

Groups	Mean ± SD	p	Levene
Negative Control	27,22 ± 9,53	0,006*	0,026
Positive Control	52,00 ± 24,79		
Treatment I	31,67 ± 10,70		
Treatment II	18,33 ± 4,08		

Table 9. Post Hoc LSD Test with Immotil Criteria

Groups		p	
I	II		
Negative Control	Positive Control	0,267	Not significant
	Treatment I	0,870	Not significant
	Treatment II	0,243	Not significant
Positive Control	Treatment I	0,405	Not significant
	Treatment II	0,119	Not significant
Treatment I	Treatment II	0,097	Not significant

From the results of the Post Hoc Games-Howel test, it was found that there was no significant difference between groups.

DISCUSSION

Exposure to electromagnetic waves can lead to damage to the structure and function of Leydig cells and an increase in the amount of ROS in the body.[9] Moringa leaves possess a radioprotective function by inhibiting radiation-induced lipid peroxidation, increasing GSH and acts as an antioxidant that inhibits free radicals. With this, the imbalance of ROS and antioxidants in the body that can cause oxidative stress can be prevented so as not to cause massive damage to DNA and lipid peroxidation in male reproductive organs.[10]

Progressive Criteria

The results of data analysis showed significant differences in the comparison of each treatment group I with the negative control group and the second treatment group with the positive control group. When looking at the average spermatozoa motility of the two groups (Treatment I = 23.33 P2 = 51.11), it exceeded the average motility of spermatozoa in the positive control group (+) (Mean = 13.33). However, it did not surpass the mean spermatozoa motility of the negative control group (-) (Mean = 55.56). From these data results, it can be concluded that the progressive criteria of spermatozoa motility in the group exposed to electromagnetic waves improved with Moringa leaf extract therapy but did not achieve results that exceeded the negative control.

Moringa leaves have a radioprotective function by inhibiting radiation-indiced lipid peroxidation, increasing GSH, and acting as an antioxidant that inhibits free radicals. This helps prevent the imbalance of ROS and antioxidants in the body, which can cause oxidative stress, from causing massive damage to DNA and lipid peroxidation in male reproductive organs.[4]

JURNAL KEDOKTERAN DIPONEGORO
(*DIPONEGORO MEDICAL JOURNAL*)
Online : http://ejournal3.undip.ac.id/index.php/medico
E-ISSN : 2540-8844
DOI : 10.14710/dmj.v13i3.43904
JKD (DMJ), Volume 13, Number 3, May 2024 : 147-151

Non-Progressive Criteria

In the results of the non-progressive criteria of spermatozoa motility data analysis, a significant difference was observed between treatment group I and the negative control group. However, no significant difference was found in the other comparison groups. When considering the non-progressive criteria of spermatozoa motility average, the negative control group had the lowest score (Mean = 17.22). This is because the negative control group did not receive exposure to electromagnetic waves from cell phones.

Subsequent data analysis revealed that the mean spermatozoa motility of the treatment group II (Mean = 30.00) was lower than the positive control group (Mean = 34.67). Regarding the non-progressive criteria, Moringa leaf extract therapy with a dose of 100 mg/kg BW given to mice yieldedthe highest data yield on this criterion (Mean = 41.67). However, it is important to note that only spermatozoa meeting progressive criteria can effectively move for fertilization. These results indicate that Moringa leaf extract therapy at a dose of 400 mg/kg BW can reduce the increase in spermatozoa with non-progressive motility. On the other hand, therapy with Moringa leaf extract at a dose of 100 mg/kg BW given to mice, the data yielded the highest on this criterion (Mean = 41.67). Further research is necessary to explore the effect of Moringa leaf extract on spermatozoa quality variables to determine its protective effect on male fertility.

Immotile Criteria

In the immotile criteria of spermatozoa motility, the results of data analysis no significant differences in all groups. However, when considering the average motility of spermatozoa with immotile criteria, it was observed that the positive control group had the highest mean (Mean = 52.00), while the treatment group II (dosed with Moringa leaf extract 400 mg/kg BW) had the lowest average (Mean = 18.33). From the results of the data analysis, it can be concluded that the high mean in the positive control group was a result of the treatment solely involving exposure to electromagnetic waves without the presence of Moringa leaf extract, leading to a reduction in spermatozoa motility. Conversely, treatment II had the lowest average due to the administration of Moringa leaf extract therapy with a dose of 400 mg/kg body weight.

In the results of the study, several data indicated that there were no significant differences (not significant) between groups in several categories of spermatozoa motility. This is because the motility of spermatozoa is preceded by DNA fragmentation at any stage in spermatogenesis. A positive correlation between poor spermatozoa parameters and DNA damage in mature spermatozoa indicates spermatogenesis problems in certain individuals. DNA fragmentation of spermatozoa can arise from both internal and external factors. Internally, issues such as abnormalities in the maturation process of spermatozoa, oxidative stress, and unsuccessful apoptosis contribute to this phenomenon.[11,12] Therefore, spermatozoa motility is also influenced by the degree of DNA fragmentation occuring in spermatogenesis.

CONCLUSION

The findings from this study indicated that there was an improvement in spermatozoa motility in BALB/C mice exposed to electromagnetic waves after administration of Moringa leaf extract.

REFERENCES

1. Noveriyanti N. Faktor risiko infertilitas pada wanita usia subur (Studi di klinik fertilitas Rumah Sakit Islam Sultan Agung Semarang). Universitas Muhammadiyah Semarang; 2017. Google Scholar [Internet]. [cited 2021 Dec 28]. Available from: http://repository.unimus.ac.id/397/3/05.%20bab%202.pdf
2. Hendarto H, Wiweko B, Santoso B, Harzif AK. Konsensus penanganan infertilitas. Himpunan endokrinologi reproduksi dan fertilitas Indonesia, Perkumpulan Obstetri dan Ginekologi Indonesia. 2019.
3. Hamada AJ, Singh A, Agarwal A. Cell phones and their impact on male fertility: Fact or fiction. The Open Reproductive Science Journal. 2011;5:125-37.
4. Walczak–Jedrzejowska R, Wolski JK, Slowikowska–Hilczer J. The role of oxidative stress and antioxidants in male fertility. Cent Eur J Urol. 2013;66(1):60.

JURNAL KEDOKTERAN DIPONEGORO
(*DIPONEGORO MEDICAL JOURNAL*)
Online : http://ejournal3.undip.ac.id/index.php/medico
E-ISSN : 2540-8844
DOI : 10.14710/dmj.v13i3.43904
JKD (DMJ), Volume 13, Number 3, May 2024 : 147-151

5. Dja'afara AL, Wantouw B, Tendean L. Pengaruh pemberian kopi terhadap kualitas spermatozoa tikus wistar jantan (Rattus norvegicus) yang diberi paparan asap rokok. e-Biomedik. 2015;3(2):676-680.
6. Eveline E, Siregar TM, Sanny. Studi aktivitas antioksidan pada tomat (Lycopersicon esculentum) konvensional dan organik selama penyimpanan. Prosiding Seminar Nasional Sains dan Teknologi. 2014;1(1):22-8.
7. Asih IARA, Sudiarta IW, Suci AAW. Aktivitas antioksidan senyawa golongan flavonoid ekstrak etanol daging buah terong belanda (Solanum betaceum cav.). Jurnal Kimia. 2015;9(1):35-40.
8. Kurniasih E. Khasiat dan manfaat daun kelor : untuk penyembuhan berbagai penyakit. Penerbit Pustaka Baru Press Yogyakarta. 2020.
9. Kesari KK, Kumar S, Behari J. Effects of radiofrequency electromagnetic wave exposure from cellular phones on the reproductive pattern in male Wistar rats. Appl Biochem Biotechnol. 2011;164(4):546-59.
10. Houston BJ, Nixon B, King BV, De Iuliis GN, Aitken RJ. The effects of radiofrequency electromagnetic radiation on sperm function. Reproduction. 2016;152:R263-76.
11. Singh A, Agarwal A. The role of sperm chromatin integrity and DNA damage on male infertility. The Open Reproductive Science Journal. 2011;3:65-71.
12. Aitken RJ, Koppers AJ. Apoptosis and DNA damage in human spermatozoa. Asian J Androl. 2011;13(1):36-42.