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ADMINISTRATION OF MORINGA LEAF EXTRACT TO REDUCE OXIDATIVE STRESS AND INCREASE FERTILITY OF DIABETES MELITUS MICE (MUS MUCULUS)

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ABSTRACT
The impact of diabetes mellitus on decreased male fertility or infertility will greatly reduce the quality of life. Therefore, it is necessary to conduct research on the prevention and treatment of decreased fertility experienced by diabetes mellitus patients, including by utilizing local naturalingredients. Objective: The aim of this study is to determine the effect of moringa to reduce Oxidative Stress and increase Fertility of Diabetes Mellitus Mice. Method: Randomized Posttest Control Group design with 35 mice (Mus Musculus) as respondents which were divided into 5 groups randomly. Results: Moringa leaf extract can significantly reduce MDA levels in diabetic mice given moringa extract at doses of 200 and 300 mg/kgbb Conclusions: Moringa leaf extract can reduce Oxidative Stress and increase Fertility of Diabetes Mellitus Mice.

Keywords: fertility; mda; moringa; spermatozoa

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INTRODUCTION

Diabetes mellitus (DM) is a disease in which blood glucose levels increase above normal, either because the body cannot produce enough insulin or because of cell resistance. It is estimated that there were 451 million people with diabetes worldwide in 2017 and will increase to 693 million by 2045(Cho et al., 2018). Diabetes causes various complications including in the reproductive system such as decreased sexual response (Hendry et al., 2023) and impaired fertility. In men, fertility problems have been reported in around 51% of patients (Ma et al., 2020). Diabetes mellitus induces oxidative stress through increased ROS levels which are considered the main cause of reproductive deficiency. Insulin and glucose levels in diabetics affect the maturation of sperm cells in the acrosome and plasma membrane which then affects the concentration, motility, viability, and morphology of spermatozoa(Alves et al., 2013). Sperm concentration, motility, morphology and viability are very important in relation to the fertilization process. If the concentration and motility are inadequate, the ability of spermatozoa to penetrate cervical mucus will be lost so that fertilization of the egg is likely to fail (Hendry & Fahmi, 2023). Hyperglycemic conditions are also correlated with a decrease in the population of germ cells, epithelial cell groups, reduced stereocilia and lipid vacuolization in the testes (Jangir & Jain, 2022). The Moringa oleifera plant contains various bioactive compounds that have protective effects against various diseases and oxidative stress(Vergara-Jimenez et al., 2017;Mohlala et al., 2023).

Various parts of the tree such as leaves, roots, flowers, fruits, and seeds are traditionally used to treat various diseases because they contain many compounds in the form of nutrients and phytonutrients such as vitamins (E, C, beta-carotene, B6), various minerals, and fatty acids.

Intrinsic phytonutrients include flavonoids, phenolic acids, saponins, alkaloids, tannins, isothiocyanates and glucosinolates(Susanto et al., 2018). The flavonoid group found in Moringa leaves includes quercetin and kaempferol with concentrations reaching 137.81 and 106.75 mg/g . Moringa leaves have a significant effect on improving spermatozoa characteristics such as in buffalo fed Moringa leaves with concentrations of 4% and 8%, reducing spermatozoa abnormalities in male Swiss mice (Zeng et al., 2019), increasing viability, membrane integrity and motility of spermatozoa in rabbits, and increasing sperm motility in bulls fed 15% Moringa leaves. Moringa plant is a phytonutrient-rich material that has been proven to have benefits to increase fertility, especially the leaves that have high antioxidant content. Moringa leaves have the potential to be developed as a drug for the prevention and treatment of male fertility disorders caused by diabetes mellitus, so it is necessary to prove the effect of moringa leaf extract on MDA levels, spermatozoa concentration, motility, morphology, and viability of spermatozoa. The specific objectives of this study to prove the effect of moringa leaf extract on spermatozoa concentration in DM mice with Streptozotocin induction, proving the effect of moringa leaf extract on spermatozoa motility in DM mice with Streptozotocin induction, proving the effect of moringa leaf extract on spermatozoa morphology in DM mice with Streptozotocin induction, proving the effect of moringa leaf extract on spermatozoa viability in DM mice with Streptozotocin induction, proving the effect of moringa leaf extract on serum MDA levels in DM mice with Streptozotocin induction(Wafa et al., 2017).

The urgency of this research is the increasing prevalence of diabetes mellitus and its distribution which is starting to shift to a younger age and is still in the active reproductive period. The impact of diabetes mellitus on decreased male fertility or infertility will greatly reduce the quality of life. Therefore, it is necessary to conduct research on the prevention and treatment of decreased fertility experienced by diabetes mellitus patients, including by utilizing local natural materials. One alternative local plant that has proven to have high potential, is widely cultivated, easy and cheap is the moringa plant. Diabetes mellitus causes various short-term and long-term impacts including dysfunction and disorders of various organ systems, one of which is the reproductive organs. In men, glucose metabolism greatly affects the spermatogenesis process and affects specific functions such as motility and fertilization ability (Ding et al., 2015). Increased oxidative stress that occurs in diabetes mellitus can trigger enzyme inactivation, susceptibility to proteolysis, fragmentation (Asadi et al., 2017), disruption of steroidogenesis enzymes (Baskaran et al., 2021), and post-translational protein damage facilitated by ROS (Kehm et al., 2021). MDA is one of the markers often used to identify lipid peroxidation and oxidative stress. In spermatozoa, MDA molecules can penetrate the cell membrane structure and disrupt lipid distribution in the middle of sperm cells and cause loss of acrosome capacity (Asadi et al., 2017).

The problem-solving approach in this study is an effort to deal with high oxidative stress that has an impact on decreasing male fertility in diabetes mellitus by utilizing natural ingredients, namely Moringa leaves. Moringa leaves have high active metabolite and antioxidant content that have great potential to be beneficial and developed into standardized herbal ingredients. In addition, this plant is easy to cultivate, relatively cheap and already widely known in Indonesia. The novelty of this study is the use of moringa leaf extract to reduce high free radicals that trigger oxidative stress which in the next stage will theoretically increase fertility parameters, in this study the subjects were in diabetes mellitus. Several previous studies have also identified the effect of moringa extract on fertility, such as the effect of moringa extract on the morphology and motility of spermatozoa in mice exposed to cigarette smoke (Zubir et al., 2023), increased libido and quality of Balinese cattle spermatozoa through moringa leaf

supplementation , moringa leaf water extract reduces free radicals in spermatozoa , and spermatozoa quality in male white mice after being given moringa leaf extract (Suaskara et al., 2019). Until now, researchers have not found any research using Moringa leaf extract in an animal model of streptozotocin-induced diabetes mellitus that measures oxidative stress with Malondialdehyde (MDA) levels, and its effects on fertility with comprehensive parameters including spermatozoa concentration, motility, morphology and viability. The objective of this study is to evaluate the effects of Moringa oleifera leaf extract in reducing oxidative stress and improving fertility in diabetic mice (Mus musculus) to support the development of natural-based alternative therapies for addressing reproductive disorders caused by diabetes.

METHOD

This study used an experimental design with a Randomized Posttest Control Group design approach with 35 mice (Mus Musculus) as respondents which were randomly divided into 5 groups. and can be seen in the following diagram:

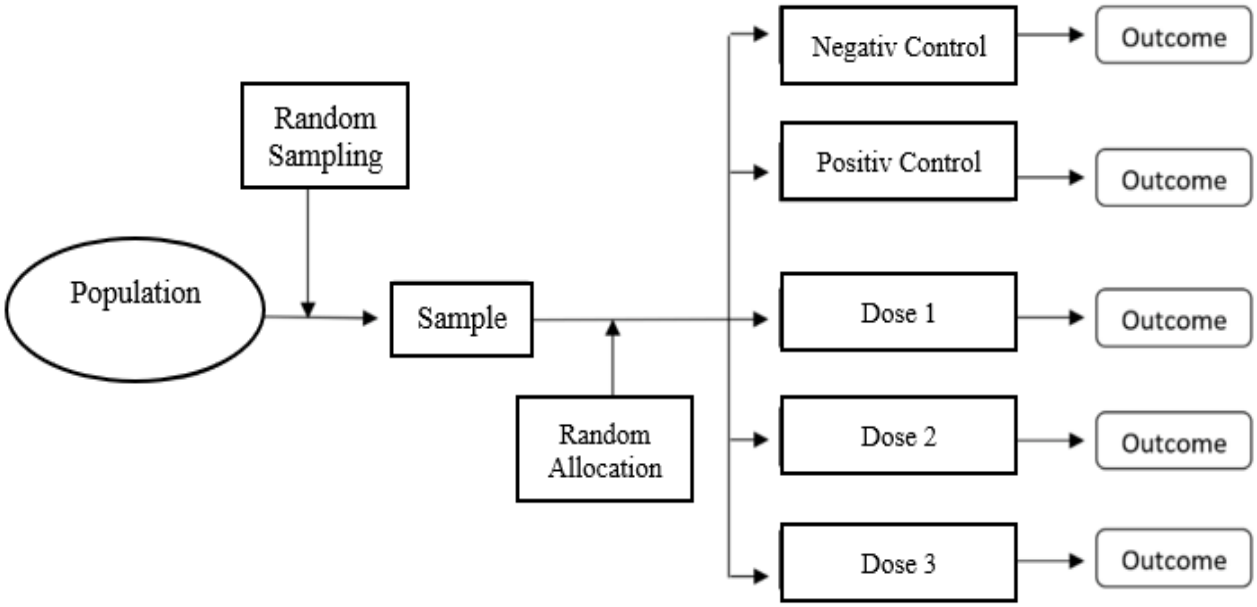

Figure 1. Randomized Posttest Control Group design

The research was conducted in the integrated laboratory of the Faculty of Medicine, University of Mataram with the following stages:

Preparation Stage

a. Making Moringa leaf extract Fresh Moringa leaves were purchased from local farmers around Mataram which will be identified and validated in the Advanced Biology Laboratory of the Faculty of Mathematics and Natural Sciences, University of Mataram. After wet sorting to separate the leaves and twigs/stems, they were chopped and dried using an oven at a temperature of 30-40 degrees Celsius. The extract was made using the maceration method using ethanol solvent in the Integrated Pharmacy Laboratory of the Faculty of Medicine, University of Mataram.
b. Experimental Animals The animals to be used in this study were 35 male mice (mus musculus) aged 10-12 weeks with a body weight of 30-40 grams, obtained from the Pharmacy Laboratory of the Faculty of Medicine, University of Mataram. The mice

were placed in a closed plastic cage measuring 40x30x15 cm with a base covered with 1-2 cm thick rice husks which were replaced every 3 days. Food and drink were given ad libitum using commercially available mouse feed. Before being given treatment, acclimatization was carried out for two weeks under standard laboratory conditions. Lighting used room lights with a duration of 12 hours of light and 12 hours of darkness. While the temperature and humidity of the room were left in the natural range.

Implementation Stage

a. Experimental Protocol Mice were randomly divided into five groups, each group consisting of 7 mice. Group 1 was a negative control group that did not receive any treatment. Group 2 was a positive control, namely mice that were induced with DM without being given treatment with Moringa extract, while groups 3, 4, and 5 respectively received DM induction treatment by administering Moringa leaf extract equivalent to doses of 100, 200, and 300 mg/kgbb. Moringa leaf extract was given ad libitum for 36 days or equivalent to 1 cycle of mouse spermatogenesis. Moringa leaf extract was given once a day between 10:00 - 12:00 WITA every day.
b. Blood and spermatozoa sampling Mice were sacrificed one day after the last dose of Moringa leaf extract. Blood samples were taken from mice through the lateral vein at the tip of the tail and then inserted into an Eppendorf tube to examine MDA levels. The euthanasia process is carried out using the cervical dislocation method after previously being given light anesthesia using intra-peritoneal ketamine hydrochloride. Surgery is performed to remove the epididymis, the remaining organs and unused tissues are inserted into the hole and then burned.
c. Measurement of MDA Levels and Sperm Parameters Measurement of MDA Levels Measurement of Malondialdehyde (MDA) levels is carried out by reacting blood plasma with Thiobarbituric Acid (TBA) to form a red MDA-TBA complex. The first step in this procedure is the preparation of blood samples followed by reaction with TBA. Furthermore, after the reaction is complete, the products formed are measured using a spectrophotometer at a wavelength of 532 nm. Measurement of Sperm Concentration Measurement of spermatozoa concentration by diluting sperm samples, filling the counting chamber, and microscopic observation. After counting spermatozoa in several fields of view, the average number is calculated. The results are reported in units of spermatozoa per milliliter (sperm/mL). Measurement of Sperm Motility Measurement of spermatozoa motility is carried out by diluting and applying sperm samples to the counting chamber. Under a microscope, sperm movement is observed and classified based on the level of motility. Motility is determined by calculating the concentration of spermatozoa in the field of view minus the number of immotile spermatozoa then multiplied by 100%. Measurement of Sperm Viability Measurement of spermatozoa viability is carried out by staining sperm samples with vital dye solutions such as eosin. Live sperm will maintain the integrity of the cell membrane, while dead sperm will absorb the dye. Viability is calculated as the percentage of live sperm from the total sperm. Measurement of Sperm Morphology Measurement of spermatozoa morphology is carried out by staining sperm samples and microscopic observation to evaluate the structure and shape of sperm. Sperm are evaluated based on established morphological criteria, such as head shape, tail, and length. The results are reported as the percentage of sperm with normal morphology. d. Data Analysis To analyze differences in MDA levels, concentration, motility, morphology, and viability of spermatozoa, One Way ANOVA multiple comparison

was used. If a difference in the average between groups is found, then the LSD (Least Significant Difference) test is continued.

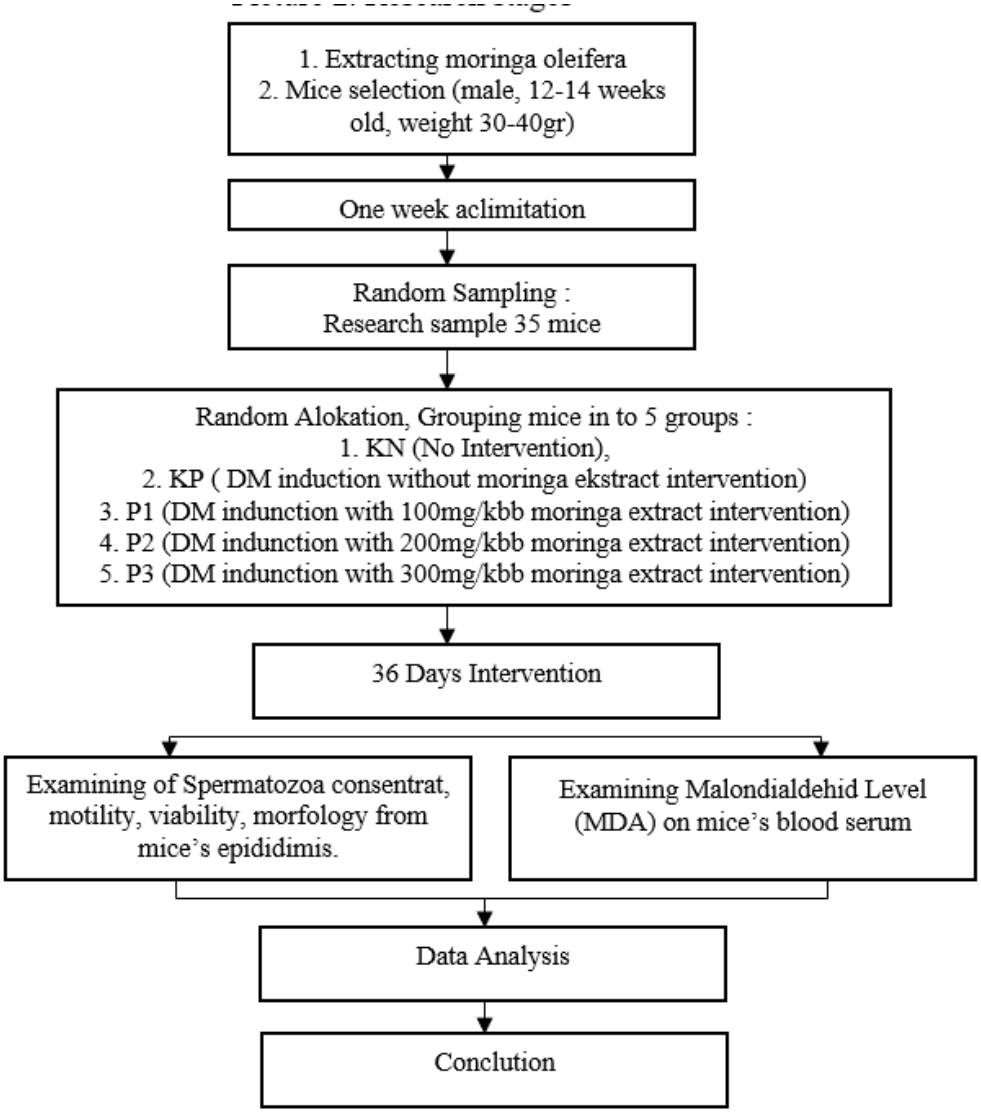

Figure 2. Research stages

RESULT

Extract Preparation, Determination, and Vitochemical Testing

The process of making ethanol extract of moringa leaves, determination, and phytochemical testing was carried out in the Advanced Biology Lab of the Faculty of Mathematics and Natural Sciences, University of Mataram. Moringa leaves were purchased from farmers around Mataram, collected, then sorted and separated from the stems and twigs. Furthermore, washing and drying were carried out.In this study, the extraction process was carried out using the maceration method using 70% ethanol solvent. Moringa leaves were soaked for 2 days, then filtered and the dregs were macerated again with the same treatment. After maceration, the evaporation and drying processes were carried out using a dry freeze machine.

Table 1.
extraction results process

No	Parameter	Result	Method
1	Saponin	Negative	Qualitative test
2	Flavonoid	Positive	Qualitative test
3	Tanin	Positive	Qualitative test
4	Quinon	Positive	Qualitative test
5	Alkanoid	Positive	Qualitative test
6	Tarpenoid	Positive	Qualitative test

Spermatozoa Parameter and MDA level test

The result of spermatozoa parameter covering of concentrate, motility, morfology and viability also MDA level of mice shown in table 2 below :

Table 2.
Spermatozoa Parameter and MDA level test

Parameter	Negatif Control (K-)	Positif Control (K+)	100mg/kbpp Dose (P1)	200mg/kbpp Dose (P2)	300mg/kbpp Dose (P3)
Spernatozoa Concentrate ($x10^{6)}$)	65.33 ±5.86	30.66 ± 3.90	38.90 ± 2.91[a,b]	45.71±1.49[a,b]	50.09±1.86[a,b]
Spermatozoa motiliy (%)	80.95±3.09	45.19±3.09	52.81±3.74[a,b]	60.00±5.02[a,b]	60.52±5.53[a,b]
Spermatozoa viability (%)	76,47±1.69	45.94±3.90	56.28±1.06[a,b]	63.09±5.58[a,b]	73.76±6.14[a.b]
Morfologi Spermatozoa (%)	80.61±1.32	72.19±1.95	73.09±2.51[a]	73.52±3.59[a]	75.09 ± 1.63[a]
MDA Serum level (ng/ml)	487.87±81.40	743.00±105.72	639.61±67.16[a]	528.34±59.32[b]	516.70±69.26[b]

Note : [a]Significant differences level on p<o.05 with K-, [b] Significant differences level on p<o.05 with K+.

Spermatozoa concentrated

In the treatment group, the spermatozoa concentration data had the highest average in group P3 with a dose of 300 mg/kgbb. In addition, there was a significant difference in the average spermatozoa concentration parameters between the control group and the three treatment groups given moringa extract, where it was also seen that the higher the dose, the higher the spermatozoa concentration. The research data showed that giving moringa extract had a positive effect on increasing low spermatozoa concentration due to hyperglycemia. This increase in concentration is directly proportional to the increase in the dose given from P1 to P3 as shown in Figure 3 below:

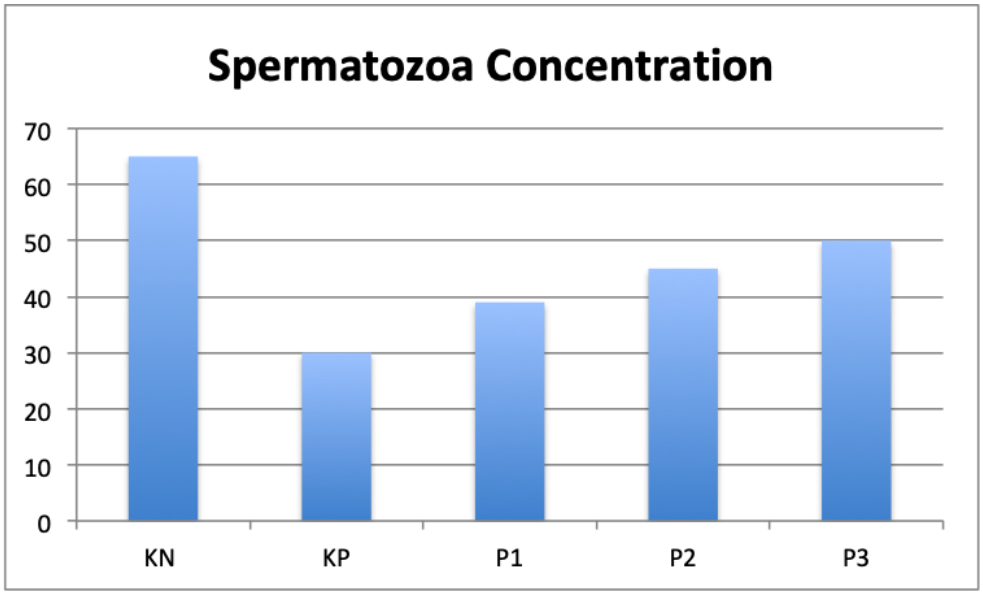

Figure 3. Spermatozoa Concentrated

In statistical analysis using One Way Anova on the sperm concentration parameter showed a significant difference p = 0.00. In further LSD tests, it was found that the (K +) group was significantly different from groups P1, P2, and P3. Meanwhile, between the treatment groups, the group with a dose of 100 mg / kg (P1) showed a significant difference with doses of 200 mg / kg (P2) and 300 mg / kg (P3). On the other hand, there was no significant difference in the sperm concentration parameter between the 200 mg / kg (P2) and 300 mg / kg (P3) dose groups.

Spermatozoa Motility

In the motility parameter, it was found that there was a significant difference between the Control group and the three treatment groups. The tabulation results showed that the highest average was obtained in the treatment group with a dose of 300 mg/kgbb (P3). The research data showed that the administration of Moringa extract had a positive effect on increasing spermatozoa motility in hyperglycemic mice. The increase in concentration was directly proportional to the increase in the dose given from P1 to P3, although the average between groups P2 and P3 only increased slightly as shown in diagram 2 below:

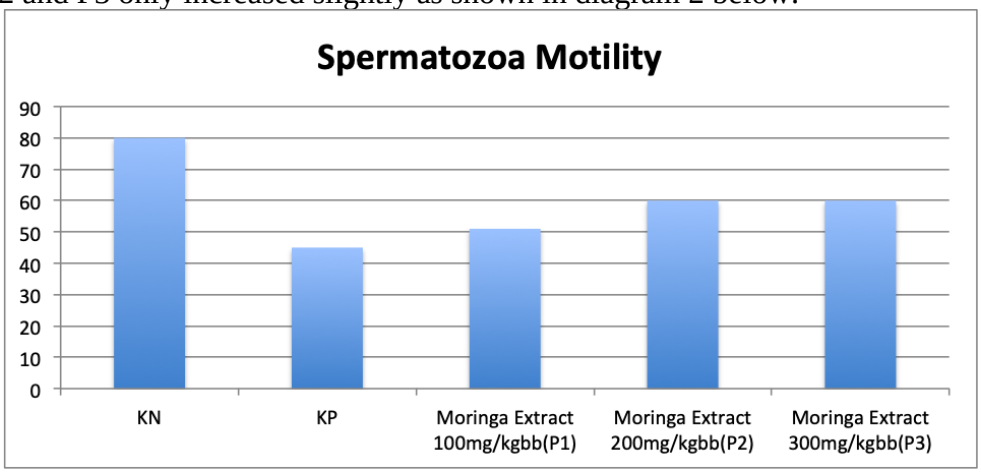

Figure 4. Spermatozoa Motility

Based on data analysis using One Way Anova, a significant difference was found in spermatozoa motility p = 0.000. The results of the LSD follow-up test showed that the

spermatozoa motility of the negative control group (K-) and Positive Control (K +) was significantly different from the treatment groups P1, P2, and P3. Meanwhile, between the treatment groups, the dose of 100 mg / kgbb (P1) was significantly different from the dose of 200 mg / kgbb (P2) and the dose of 300 mg / kgbb (P3). While between P2 and P3, no significant difference was found (p = 0.069).

Morphology

In the morphology parameters, it was found that there was no significant difference between the Positive Control group (K+) and the three treatment groups. On the contrary, the Negative Control group (K-) showed a significant difference with the three treatment groups. The distribution of mouse spermatozoa morphology is depicted in diagram 5 below:

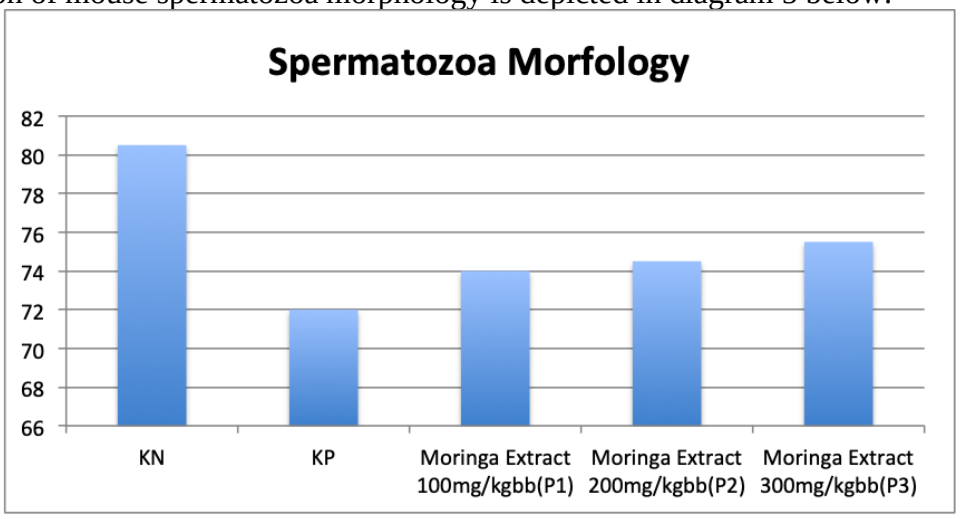

Figure 5. Spermatozoa Morphology

The diagram shows that the percentage of spermatozoa with normal morphology increases in treatment groups P1, P2 and P3 which are given moringa extract according to the increase in dose. In the statistical test using One Way Anova, there is a difference between the Negative control group (P-) and the other groups (p <0.05). However, between the positive control group (K +) there was no significant difference either with the treatment group (P1) p = 0.939, Treatment Group 2 (P2) p = 0.904 and treatment group 3 (P3) p = 0.068.

MDA Levels

The results of the serum MDA level examination showed that there was a significant difference in the average MDA levels between the Control group and the three treatment groups. The tabulation results showed that the lowest average MDA levels were obtained in the treatment group with a dose of 300 mg/kgbb (P3) while the highest average MDA levels were obtained in the positive control group (K+). The research data showed that the administration of Moringa extract had a positive effect on reducing MDA levels as a marker of Oxidative Stress in hyperglycemic mice. The distribution of MDA levels in each group can

be seen in diagram 6 below:

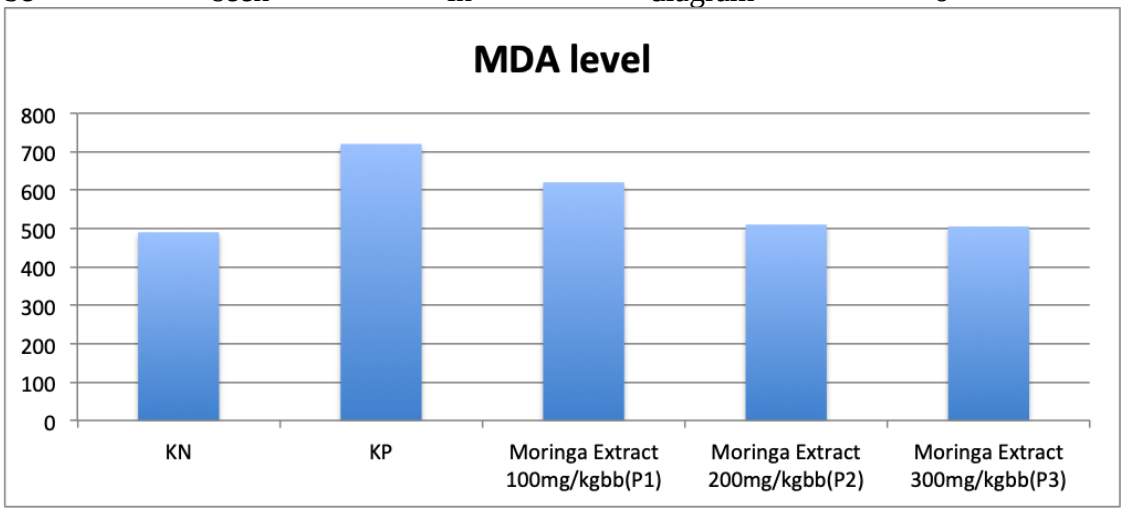

Figure 6 MDA levels

Based on the results of statistical tests using One Way Anova, it was found that MDA levels differed significantly in the treatment group and the control group. The results of further LSD tests to measure differences between groups showed that the MDA levels of the positive control group (K+) were significantly different from the 200 mg/kgbb dose treatment group (P2) and the 300 mg/kgbb dose treatment group (P3), while the 100 mg/kgbb dose treatment group (P1) did not show a significant difference ($p = 0.194$). In contrast, the average MDA levels of the negative control group (K-) showed a significant difference from the 100 mg/kgbb dose treatment group (P1) but did not show a significant difference from the 200 mg/kgbb dose treatment group (P2) and 300 mg/kgbb dose treatment group (P3). The results of this analysis indicate that the administration of moringa leaf extract is effective in reducing MDA levels at doses of 200 and 300 mg/kgbb.

DISCUSSION

Spermatozoa concentrated

Based on the data above, it can be seen that in this study the most effective dose in improving sperm concentration was 400 mg / kg. In hyperglycemic conditions, there is a decrease in fertility quality in various parameters, including sperm concentration (Ma et al., 2020). Insulin levels and high blood glucose in diabetes will affect the maturation of germ cells which has an impact on decreasing sperm concentration. On a broader scale, another impact that diabetes also causes is changes in the structure of the testes(Wang et al., 2021). In this study, an increase in spermatozoa concentration was obtained after administration of moringa leaf extract which was linear with the increase in the dose given. This could be due to the high phytonutrient content found in moringa leaves such as various vitamins, minerals, and fatty acids (Priyadarshani & Varma, 2014). Administration of moringa leaf extract This study investigates the effect of Moringa oleifera leaf extract on spermatozoa concentration in diabetic mice (Mus musculus). One important parameter in assessing sperm quality is spermatozoa concentration, which reflects the number of sperm in the ejaculate. Low spermatozoa concentration can be an indicator of reproductive dysfunction, particularly in individuals with medical conditions such as diabetes mellitus(Frenkel et al., 1978).The results show that administering Moringa leaf extract to diabetic mice can increase spermatozoa concentration compared to the control group that did not receive the extract. This suggests that Moringa leaf extract may have the potential to improve reproductive health in diabetic mice, which typically experience a reduction in sperm quantity and quality due to the effects of chronic hyperglycemia (Mohamed et al., 2019).

One possible mechanism underlying the increased spermatozoa concentration is the ability of Moringa leaf extract to reduce oxidative stress. High oxidative stress in diabetes can damage sperm structure and disrupt spermatogenesis, leading to a decrease in sperm count . By lowering oxidative stress levels, Moringa leaf extract can protect sperm cells from damage and enhance the production of healthy sperm(Alahmar, 2019).

Furthermore, Moringa leaf extract contains bioactive compounds such as vitamin C, vitamin E, and flavonoids, which are known to have antioxidant properties. These compounds may play a role in protecting the testes from damage caused by free radicals, which are common in individuals with diabetes. Reducing oxidative damage can improve sperm quality and accelerate the recovery of reproductive function(Zeng et al., 2019).Moringa also affects the levels of reproductive hormones such as testosterone, FSH, and LH. where Moringa leaf extract significantly increases FSH and LH levels. These reproductive hormones play a direct role in the spermatogenesis process starting from germ cell proliferation, mitosis, meiosis, to the process of spermatozoa maturation in the epididymis(Dafaalla et al., 2017;Ajuogu et al., 2019).The administration of Moringa leaf extract has been shown to positively affect spermatozoa concentration and semen quality overall in various studies. Moringa oleifera, rich in antioxidants, improves sperm motility, viability, and concentration, making it a valuable supplement for reproductive health. The addition of 9% methanolic Moringa leaf extract significantly enhanced post-thaw quality and metabolic activity of buffalo spermatozoa, demonstrating increased sperm concentration and fertility rates(El-Hawary et al., 2024). Daily administration of ethanolic Moringa seed extract increased sperm cell concentration and total sperm output, with optimal results observed at 900 mg/buck (Agwa et al., 2020).

Moringa leaf extract improves sperm density and motility in diabetic rats, showing its potential to counteract diabetes-induced reproductive issues (Meldawati et al., 2024). Moringa extract reduces oxidative stress, which is crucial for maintaining sperm integrity and function. For instance, supplementation with Moringa leaf extract decreased malondialdehyde levels and increased glutathione peroxidase activity, correlating with better sperm characteristics. While Moringa extract shows promising benefits for spermatozoa concentration and quality, further research is needed to fully understand the mechanisms and long-term effects on fertility across(Moichela et al., 2021)

Spermatozoa Motility

Based on data analysis using One Way Anova, a significant difference was found in spermatozoa motility p = 0.000. The results of the LSD follow-up test showed that the spermatozoa motility of the negative control group (K-) and Positive Control (K +) was significantly different from the treatment groups P1, P2, and P3. Meanwhile, between the treatment groups, the dose of 100 mg / kgbb (P1) was significantly different from the dose of 200 mg / kgbb (P2) and the dose of 300 mg / kgbb (P3). While between P2 and P3, no significant difference was found (p = 0.069). The results of this analysis indicate that moringa leaf extract has a significant effect on increasing spermatozoa motility in mice experiencing hyperglycemia significantly, although this increase has not reached the normal point as in mice that were not given induction (K-). In addition, this data analysis also shows that in this study the addition of a dose of 100 mg / kgbb did not show a significant increase in effect. Motility is the ability of sperm to move progressively, especially to reach and penetrate the oocyte. Sperm motility is one of the important parameters of fertility during conception (Dcunha et al., 2022). In diabetes, there is a significant decrease in spermatozoa motility (Lotti & Maggi, 2023). Administration of moringa leaf extract in this study was shown to

significantly increase spermatozoa motility. This effect may be due to the fact that moringa extract is very rich in antioxidants and phenolic compounds that help protect the testes (Iliyasu et al., 2020). A series of bioactive phenolic compounds such as terpenoids, flavonoids, phenolic acids, and essential oils can reduce damage to reproductive tissue and increase reproductive capacity and quality, including spermatozoa motility (Ghadimi et al., 2024).

The administration of Moringa leaf extract has been shown to have significant effects on spermatozoa mortality, particularly in the context of oxidative stress and exposure to harmful agents. Moringa oleifera, rich in antioxidants, appears to mitigate negative impacts on sperm quality caused by factors such as electromagnetic radiation and pharmaceutical agents like indomethacin. The following section details findings from various studies regarding the protective effects of Moringa extract on spermatozoa.Moringa leaves contain flavonoids, alkaloids, and polyphenols, which act as antioxidants, reducing oxidative stress that can damage sperm cells (Sabrina et al., 2024). In one study, Moringa extract improved sperm motility in rats exposed to electromagnetic radiation, demonstrating its protective role against oxidative damage (Sudha & Deepa, 2024).Moringa extract exhibited dose-dependent protective effects on testicular and sperm quality in rats treated with indomethacin, a drug known to cause spermatozoa degeneration . Histological improvements in the testes were observed, suggesting that Moringa can counteract the toxic effects of certain medications (Ngizzah et al., 2023). While Moringa leaves showed beneficial effects, the methanolic fraction of Moringa seeds was found to induce reproductive toxicity, decreasing sperm motility and testosterone levels. This highlights the importance of the specific parts of the Moringa plant used, as different fractions may have varying effects on sperm health. Conversely, although Moringa extract generally exhibits protective effects, the methanolic fraction of Moringa seeds raises concerns about potential toxicity, emphasizing the need for further research to fully understand the implications of different Moringa components on male reproductive health(Obembe, 2019).

Morphology

Based on the data from the table and the results of this statistical analysis, it shows that the administration of moringa extract increases the average viability of normal spermatozoa, but is not statistically significant. Morphology is the shape, size, and appearance of spermatozoa and is one of the factors examined to evaluate fertility (Gatimel et al., 2017). Diabetes can cause disorders in sperm morphology due to disruption of glucose metabolism during the spermatogenesis process (Zhong et al., 2021). In this study, there was an improvement in spermatozoa morphology, although it was not statistically significant. Moringa oleifera extract in previous studies has been shown to help restore testicular morphology and semen quality. This morphological improvement includes spermatozoa parts such as head, neck, and tail defects(Ogunlade et al., 2022).The administration of Moringa oleifera extract has been shown to positively affect spermatozoa morphology and overall reproductive health in various studies. Moringa's rich antioxidant properties help reduce oxidative stress, which compromises sperm quality. The following section details the observed effects in different experimental contexts.In a study involving alloxan-induced rats, the administration of Moringa extract resulted in an impressive 94.5% normal sperm morphology, demonstrating a strong correlation between blood sugar control and sperm quality (Wahab et al., 2025). Another study found that Moringa extract significantly restored sperm parameters, including morphology, in rats exposed to 4G electromagnetic radiation, which typically induces sperm damage (Sudha & Deepa, 2024)

Moringa leaves contain antioxidants such as flavonoids and polyphenols, which protect sperm cells from oxidative damage caused by environmental stressors like electromagnetic radiation and nanoparticles (Sabrina et al., 2024;Mostafa-Hedeab et al., 2023). The extract also enhances the number of spermatogenic cells and Leydig cells, both crucial for testosterone production and spermatogenesis, thereby improving overall sperm health (Ngizzah et al., 2023).While Moringa oleifera shows promise in improving sperm morphology and quality, it is essential to consider that over-reliance on herbal supplements may overlook other critical factors influencing male fertility, such as lifestyle and environmental influences. Further research is needed to establish comprehensive guidelines for its use in reproductive health.

MDA Levels

The results of the serum MDA level examination showed that there was a significant difference in the average MDA levels between the Control group and the three treatment groups. The tabulation results showed that the lowest average MDA levels were obtained in the treatment group with a dose of 300 mg/kgbb (P3) while the highest average MDA levels were obtained in the positive control group (K+). The research data showed that the administration of Moringa. Based on the results of statistical tests using One Way Anova, it was found that MDA levels differed significantly in the treatment group and the control group. The results of further LSD tests to measure differences between groups showed that the MDA levels of the positive control group (K+) were significantly different from the 200 mg/kgbb dose treatment group (P2) and the 300 mg/kgbb dose treatment group (P3), while the 100 mg/kgbb dose treatment group (P1) did not show a significant difference ($p = 0.194$). In contrast, the average MDA levels of the negative control group (K-) showed a significant difference from the 100 mg/kgbb dose treatment group (P1) but did not show a significant difference from the 200 mg/kgbb dose treatment group (P2) and 300 mg/kgbb dose treatment group (P3). The results of this analysis indicate that the administration of moringa leaf extract is effective in reducing MDA levels at doses of 200 and 300 mg/kgbb.

Malondialdehyde (MDA) is a compound produced from lipid peroxidation which is an indicator of oxidative stress and is often used as a biomarker to measure oxidative stress in various conditions including Diabetes (Angie et al., 2024). Hyperglycemia in diabetes is thought to play a role in increasing free radicals and decreasing blood antioxidants. Increased free radicals trigger blood lipid peroxidation which is characterized by increased malondialdehyde (MDA) levels(Subandrate, 2016). In this study, there was a significant decrease in MDA levels in diabetic mice given moringa extract at doses of 200 and 300 mg/kgbb. The high total phenolic content in moringa leaves is thought to be a substance that plays a role in reducing free radical(Owoade et al., 2017). In addition to phenolics, the content of flavonoids and flavonols as antioxidants can protect normal and diabetic patients from oxidative damage (Jaiswal et al., 2013).Oxidative stress is an important factor affecting spermatozoa fertility through lipid peroxidation. The decrease in total antioxidant capacity in diabetes will cause low concentration, motility, and morphology of spermatozoa (Colagar et al., 2013). When given moringa leaf extract, there was a decrease in oxidative stress indicated by a decrease in MDA levels and an increase in antioxidants which had an impact on increasing spermatozoa fertility parameters including concentration, motility, viability, and morphology of spermatozoa.

CONCLUSION

Based on the results and discussion of this study, it is known that the administration of moringa extract can reduce oxidative stress on spermatozoa, in addition, the administration of moringa extract can also increase the concentration and morphology of spermatozoa which

are indicators of spermatozoa fertility. The synthesis of this study can contribute to the development of science in the form of the administration of moringa extract as an improvement in spermatozoa quality in diabetes mellitus.

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