



THE EFFECT OF MORINGA LEAF EXTRACT ADMINISTRATION ON SPERM MORPHOLOGY AND BLOOD GLUCOSE REDUCTION IN ALLOXAN-INDUCED SPRAGUE DAWLEY RATS

PENGARUH PEMBERIAN EKSTRAK DAUN KELOR TERHADAP MORFOLOGI SPERMA DAN PENURUNAN KADAR GULA DARAH TIKUS GALUR SPRAGUE DAWLEY YANG DIINDUKSI ALOKSAN

Dinda Nuraini Hanifah Wahab¹, Uswatun Hasanah^{2*}, Erna Harfiani³,
Maria Selvester Thadeus⁴

¹Faculty of Medicine, UPN "Veteran" Jakarta, Jl RS. Fatmawati Raya, Pd. Labu, 12450 Depok, West Java

²Department of Molecular Biology, UPN "Veteran" Jakarta, Jl RS. Fatmawati Raya, Pd. Labu, Depok, West Java

³Department of Pharmacology, UPN "Veteran" Jakarta, Jl RS. Fatmawati Raya, Pd. Labu, Depok, West Java

⁴Department of Pathology Anatomy UPN "Veteran" Jakarta, Jl RS. Fatmawati Raya, Pd. Labu, Depok, West Java

*Corresponding author: Uswatun@upnvj.ac.id

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Abstract

Increased blood sugar levels can be triggered by increased intake or insulin resistance, leading to increased oxidative stress that affects sperm quality during spermatogenesis. *Moringa oleifera*, rich in antioxidants, has been proven effective in improving sperm quality through several studies. This study aims to evaluate the influence of moringa leaf extract on sperm morphology and the reduction of blood sugar levels in Sprague Dawley white rats (*Rattus norvegicus*) induced by alloxan. Fasting blood sugar tests showed that rats induced with alloxan without moringa leaf extract had the highest blood sugar levels among the groups. In contrast, the negative control and treatment groups with moringa leaf extract successfully maintained blood sugar levels at normal levels. Normal sperm morphology reached 94.5% in the treatment group with the highest dose of moringa leaf extract. The results of the study indicate a significant relationship between blood sugar levels and sperm morphology in alloxan-induced white rats after the administration of moringa leaf extract. *Moringa oleifera* has the potential to be a therapeutic intervention to improve sperm quality and control blood sugar levels in hyperglycemic conditions.

Keywords: Blood glucose; Hyperglycemic; *Moringa oleifera*; *Rattus norvegicus*; Sperm morphology

Abstrak

Peningkatan kadar gula darah dapat dipicu oleh peningkatan asupan atau resistensi insulin menyebabkan peningkatan stres oksidatif yang mempengaruhi kualitas sperma selama spermatogenesis. Tanaman kelor (*Moringa oleifera*), tumbuhan yang kaya akan antioksidan, telah terbukti efektif dalam meningkatkan kualitas sperma melalui beberapa penelitian. Penelitian ini bertujuan untuk mengevaluasi pengaruh ekstrak daun kelor terhadap morfologi sperma dan pengurangan kadar gula darah pada tikus putih Sprague Dawley (*Rattus norvegicus*) yang diinduksi aloksan. Uji gula darah puasa menunjukkan bahwa tikus yang diinduksi aloksan tanpa perlakuan ekstrak daun kelor memiliki kadar gula darah tertinggi di antara kelompok-kelompok lainnya. Sebaliknya, kelompok kontrol negatif dan kelompok perlakuan dengan ekstrak daun kelor berhasil menjaga kadar gula darah pada tingkat normal. Morfologi sperma normal mencapai 94,5% pada kelompok perlakuan dengan dosis 400 mg/kgBB ekstrak daun kelor. Kesimpulan yang didapatkan pada penelitian ini berkaitan dengan temuan hubungan signifikan antara kadar gula darah dan morfologi sperma pada tikus putih yang diinduksi aloksan setelah pemberian ekstrak daun kelor. Tanaman kelor (*Moringa oleifera*) memiliki potensi sebagai intervensi terapeutik untuk meningkatkan kualitas sperma dan mengendalikan kadar gula darah dalam kondisi hiperglikemia.

Kata Kunci: Glukosa darah; Hiperglikemia; *Moringa oleifera*; *Rattus norvegicus*; Morfologi sperma

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INTRODUCTION

Elevated blood glucose levels, stemming from insulin resistance or excessive sugar intake, significantly impact the body's metabolic processes, leading to the production of free radicals. These free radicals, in turn, exert a pronounced effect on spermatogenesis, a complex process occurring within the seminiferous tubules that culminates in the formation of spermatozoa. This impact is closely associated with compromised hypothalamic organ function, resulting in the disruption of FSH and LH secretion by the anterior pituitary, or a direct decrease in reproductive organ function. The elevated levels of free radicals, specifically Reactive Oxygen Species (ROS), contribute to the diminished axonemal protein and immobilization of sperm, as outlined by Mannucci et al. (2021). This oxidative stress, influencing the quality of specific cells, is characterized by heightened free radical levels that trigger apoptosis, a programmed cell death mechanism essential for regulating cell populations and responding to pathological conditions, as discussed by Lu et al. (2021). The apoptotic process encompasses intrinsic (mitochondrial) and extrinsic (receptor) pathways, involving signal transduction activation, mitochondrial dysfunction, and cell degradation, as elucidated by Kashyap et al. (2022).

At the cellular level, a discernible manifestation of programmed cell death unfolds within the reproductive system, particularly in spermatozoa, a vital component for fertilization. These spermatozoa exhibit a distinctive morphology, featuring a head containing DNA, a midpiece housing mitochondria as an energy source, and a tail that facilitates sperm motility for uninhibited movement, as delineated by Franken and Oehninger (2012). Simultaneously, the intricate process of spermatogenesis transpires in the seminiferous tubules, involving the proliferation and differentiation of germ cells to yield mature spermatozoa through successive stages of mitosis, meiosis, and cell differentiation, as elucidated by Neto et al. (2021). *Moringa oleifera*, a traditional Indonesian plant rich in nutrients and β -carotene, functions as a powerful antioxidant, with its compounds, particularly flavonoids, especially quercetin, effectively neutralizing Reactive Oxygen Species (ROS). Empirical evidence from research conducted at Chiang Mai University in Thailand substantiates the beneficial influence of these flavonoids on spermatogenesis, leading to an increase in sperm count and a comprehensive enhancement of the male reproductive system, as demonstrated by the findings of Laoung-On et al. (2021).

Utilizing white rats as test subjects due to their endocrine system similarity to humans and a brief reproductive cycle, this study facilitates the observation of hyperglycemia effects or the collection of semen analysis samples across different generations (Husna et al., 2019). Given this context, the researcher aims to explore the effects of moringa leaf extract on sperm morphology and the reduction of blood glucose levels in white rats induced with alloxan.

MATERIALS AND METHODS

The research materials employed include *Moringa oleifera* leaf extract, white rats (*Rattus norvegicus*), alloxan, blood glucose test strips, distilled water (NaCl 0.9%), ejaculate semen, immersion oil, Giemsa solution, and methanol. This research utilizes male Sprague-Dawley white rats (*Rattus norvegicus*) weighing between 150 to 250 g and approximately 2 months old.

The research instruments used in this study include a variety of essential tools such as filter paper, which aids in the separation and analysis of substances. Then, a rotary vacuum evaporator, is employed for the efficient removal of solvents. Syringes for precise administration and extraction of fluids. For measuring glucose levels in this research, a glucometer and glucose strips were used. A microscope for the magnified examination of samples, with slides and cover glasses for preparing specimens for microscopic observation. The last instrument is a staining rack, which is used to hold slides during the staining process to ensure clear and accurate visualization of sample structures.

The research was conducted from February to December 2023. The determination of moringa leaf extract (*Moringa oleifera*), extraction, and phytochemical analysis took place at the Testing Center for Spice, Medicinal, and Aromatic Plant Instrument Standards (BPSI TROA) in Bogor. The study encompassed multiple locations, including the Animal Laboratory at the Medical Education and Research Center Faculty of Medicine (UPNVJ MERce) in Limo and the Molecular Biology

Laboratory at the Faculty of Medicine University of Pembangunan Nasional “Veteran” Jakarta. This research received ethical approval with letter number 333/VII/2023/KEPK from the authorized institution.

Methods

The conducted study is experimental research with a true experimental design. The chosen experimental design is a post-test-only control group design, where observations are made after the application of the treatment. The research focuses on examining the sperm morphology and reduction in blood glucose levels of white rats induced with alloxan after being treated with *Moringa oleifera* leaf extract. The results are then compared with a control group.

Research Variable

The independent or predictor variable in this study is the *Moringa oleifera* leaf extract with doses of 200, 300, and 400 mg/kg body weight. The dependent variables in this research are the reduction in blood glucose levels and sperm morphology of hyperglycemic-induced Sprague-Dawley white rats (*Rattus norvegicus*) induced by 125 mg/kg body weight of alloxan.

Sampling Technique and Intervention

Sample collection is carried out using a form of probability sampling known as purposive random sampling, wherein samples are randomly selected from a homogeneous population without considering strata. The control group is left untreated, receiving no intervention, while the negative control is induced with alloxan without being given *Moringa oleifera* leaf extract. The treatment group is administered three different doses of *Moringa oleifera* leaf extract, namely 200, 300, and 400 mg/kg body weight.

Moringa Leaf Extraction

A quantity of 1,000 g of moringa leaves undergoes extraction through the maceration method, commencing with the transformation into powder, amounting to 307.2 g. Subsequently, the moringa leaf powder is combined with 70% ethanol in a ratio of 1:20 (weight per volume) at room temperature for 4 days. Following this, the mixture is filtered using filter paper. Additional solvent is introduced, and the extraction process is reiterated until the final extract is devoid of color. All produced extracts are amalgamated and evaporated under a pressure of 75 mbar at a temperature of 40 °C utilizing a rotary vacuum evaporator. The concentrated extract undergoes further evaporation using a container with boiling water at a temperature of 70 °C until the extract's weight reaches a constant value, specifically a dense extract weight of 46.88 g.

Rats Procedure

A total of 30 male rats were subjected to a one-week acclimation period within the research environment to facilitate their adjustment and reduce stress in the unfamiliar setting. Throughout this adaptation phase, the rat's feed and water were provided ad libitum. The procedures were carried out in a standard facility equipped with individually ventilated cages, maintaining a lighting schedule synchronize with the animal's active periods.

The testing included a 7-day acclimatization period and a 26 day treatment period. In the negative control group (K-), white rats were not administered alloxan or moringa leaf extract. In the positive control group (K+), white rats received only alloxan induction. Group K1 was a treatment group where white rats were induced with alloxan and given 200 mg/kg body weight of moringa leaf extract. Group K2 was a treatment group where white rats were induced with alloxan and given 300 mg/kg body weight of moringa leaf extract. Group K3 was a treatment group where white rats were given alloxan and moringa leaf extract at a dose of 400 mg/kg body weight. Alloxan was administered once as a single dose using the intraperitoneal induction method, while moringa leaf extract was given daily using a gastric tube for 26 days. The total amount of moringa leaf extract used in the study over 26 days was 21.06 g.

Blood Sugar Levels Examination

The fasting blood sugar levels were examined before and after the 26-day treatment period. The initial examination conducted before administering alloxan aimed to ensure inclusion criteria, confirming that the test animals were in a normal state (not experiencing inherent hyperglycemia). White rats were considered hyperglycemic if their fasting blood sugar range was between 140–160 mg/dL (Corwin, 2009).

Collecting Sperm Samples

Subsequently, the rats were terminated, and a surgical procedure was conducted to collect experimental samples. Samples for the examination of sperm concentration and morphology were obtained from the reproductive organs of white rats, specifically the epididymis, through a surgical procedure. Following the surgery, sperm suspensions were prepared, involving mixing the epididymis with a 0.9% NaCl solution. A brief dip method in 0.9% NaCl solution for the first form of suspension was carried out to reduce blood contamination post-surgery. The prepared suspensions were then used to create slides, which were subsequently examined under a microscope to assess sperm morphology.

Staining Procedure for Sperm Samples

After preparing the samples, the object glass was prepared. One drop of sperm sample was dispensed, and a thin smear was made, followed by air-drying. Methanol was applied and allowed to dry to fix the sample on the object glass. Once dried, the Giemsa solution was dropped and left for 15–20 minutes. Rinse with distilled water until the stain disappears completely. Allow the object glass to dry, cover it with a cover glass, apply immersion oil, and observe under a microscope.

RESULTS

Analysis of White Rats Blood Sugar Levels

The assessment of fasting blood sugar levels before the intervention indicated that the experimental subjects met the inclusion criteria, as none of them fell into the hyperglycemia category before the commencement of the study. Subsequently, the treatment groups received Moringa leaf extract for 26 days, following the prescribed dosages for each group. The final phase of the experimental procedure involved a reevaluation of fasting blood sugar levels in rats before the study concluded, aiming to identify any improvements in hyperglycemic conditions corresponding to the specific extract doses administered. The results of the fasting blood sugar measurements following the Moringa leaf extract treatment can be found in Table 1.

Table 1. Fasting blood sugar data of white rats before & after treatment

White rats group	Before (mean ± sd)	After (mean ± sd)
K (-)	109 mg/dL ± 36.04	92 mg/dL ± 8.38
K (+)	117 mg/dL ± 30.79	292 mg/dL ± 181.73
K1 (200 mg/kg body weight)	121 mg/dL ± 15.50	140 mg/dL ± 36.66
K2 (300 mg/kg body weight)	125 mg/dL ± 34.51	100 mg/dL ± 26.73
K3 (400 mg/kg body weight)	131 mg/dL ± 41.09	137 mg/dL ± 44.52

Descriptive findings reveal changes in fasting blood sugar levels before and after the treatment. The negative control shows a significant increase in average fasting blood sugar, indicating the presence of hyperglycemia in rats. The negative control group experiences a decrease in fasting blood sugar levels without any intervention. On the other hand, the treatment group shows an increase in fasting blood sugar levels post-intervention. The K2 group, the treatment group with a dose of 300 mg/kg body weight moringa leaf extract, has the lowest average fasting blood sugar level, at 100 mg/dL. The other treatment groups, K1 and K3, exhibit average fasting blood sugar levels that are not significantly different, measuring 140 mg/dL and 137 mg/dL, respectively. The rats are terminated, and surgery is performed to collect epididymis for sperm samples.

Analysis of White Rats' Sperm Morphology

The mean percentage of sperm morphology in white rats (*Rattus norvegicus*) in each group is presented in Table 2. Descriptively, the data in Table 2 illustrates the mean values of the percentage

of normal and abnormal sperm morphology in a count of 200 cells. The positive control group, which only received alloxan, had a lower percentage of normal morphology at 43.5% compared to the negative control group, which received no treatment and had a normal morphology percentage of 63.5%. The K3 treatment group, receiving a dose of 400 mg/kg body weight *Moringa oleifera* leaf extract, emerged as the group with the highest mean percentage of normal sperm morphology, reaching 94.5%.

Table 2. Mean results of sperm morphology percentage in white rats

White rats group	Mean		SD
	Normal (%)	Abnormal (%)	
K (-)	63.5	36.5%	91.21
K (+)	43.5	56.5%	37.21
K1 (200 mg/kg body weight)	85	15%	13.99
K2 (300 mg/kg body weight)	82	18%	7.05
K3 (400 mg/kg body weight)	94.5	5.5%	4.27

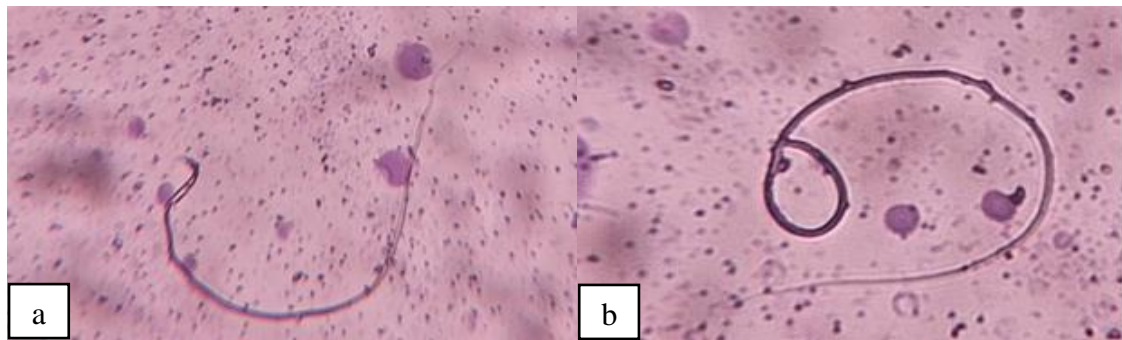


Figure 1. The morphological description was obtained using a light microscope with the first magnification is 10×100 until sperm was visible, then changed to 40×100 of the negative of the negative control group; normal morphology with a hook-shaped head, long tail, abundant normal morphology (a) and abnormal morphology with tangled head in a considerable quantity (b)

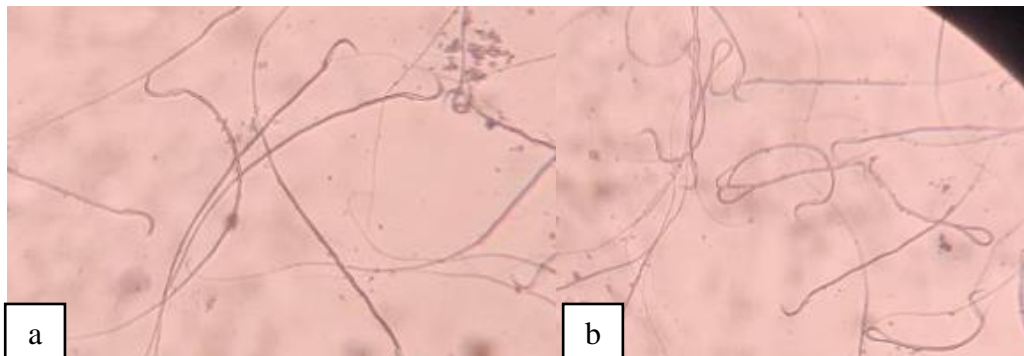


Figure 2. Morphological descriptions were obtained using a light microscope with the first magnification is 10×100 until sperm was visible, then changed to 40×100 of the positive control group; normal morphology with a hook-shaped head and a tail, which is straight and somewhat rare (a) and abnormal morphology with tangled tails in a very large quantity (b)

The morphological description was obtained using a light microscope with an initial magnification of 10×100 until the sperm became visible, then changed to 40×100 for a clearer view (Figure 1–5). The normal morphology of white rat sperm observed in the study includes a head shaped like a fishing hook and a straight long tail. The abnormal morphology of white rat sperm obtained in the study includes abnormalities in the tail resembling a knot and abnormalities in the head resembling a pin.

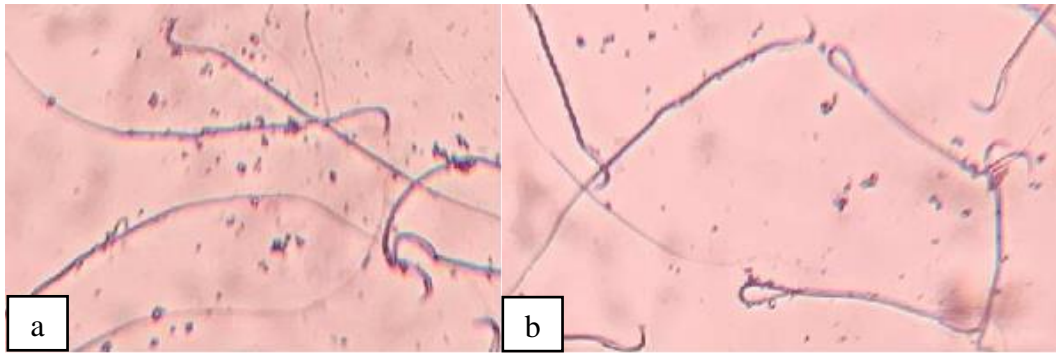


Figure 3. Morphological descriptions were obtained using a light microscope with the first magnification is 10×100 until sperm was visible, then changed to 40×100 of the *Moringa oleifera* leaf extract treatment group at 200 mg/kg body weight; few instances of normal morphology with a hook-shaped head and a straight, long tail (a) and abundant instances of abnormal morphology with tangled tails (b)

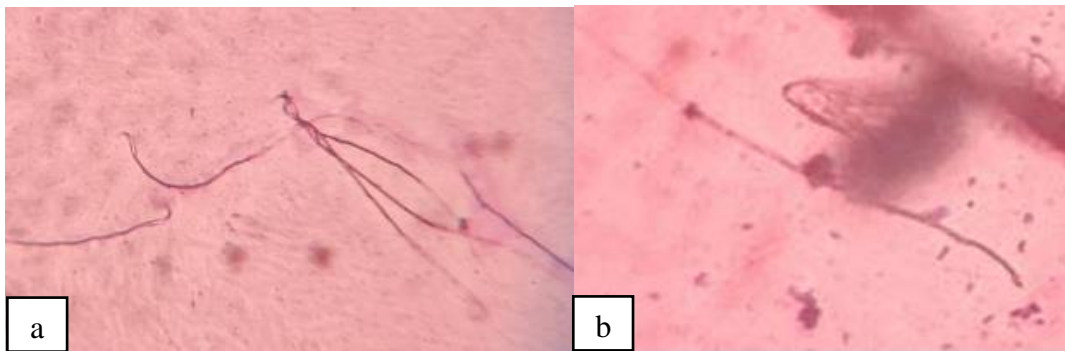


Figure 4. Morphological descriptions were obtained using a light microscope with the first magnification is 10×100 until sperm is visible, then changed to 40×100 of the *Moringa oleifera* leaf extract treatment group at 300 mg/kg body weight; abundant instances of normal morphology with a hook-shaped head and a straight, long tail (a) and abnormalities in the head resembling a pin in a considerable number (b)

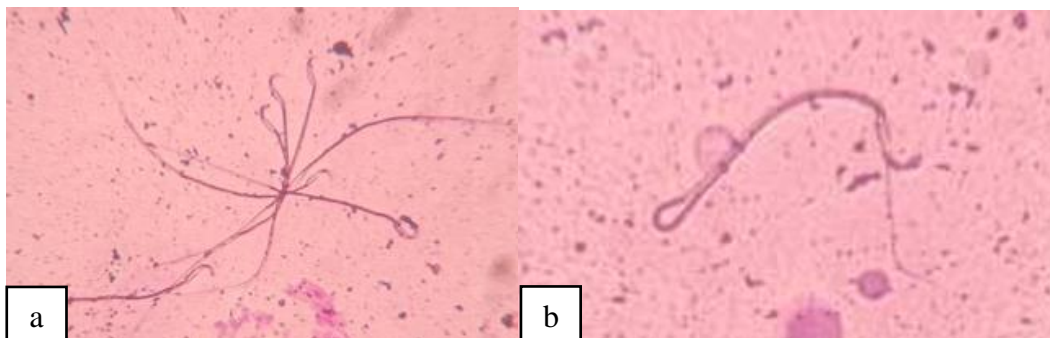


Figure 5. Morphological descriptions were obtained using a light microscope with the first magnification is 10×100 until sperm is visible, then changed to 40×100 of the *Moringa oleifera* leaf extract treatment group with a dose of 400 mg/kg body weight; abundant normal morphology with heads forming hooks and long straight tails (a) and scarce abnormal morphology with tails forming knots (b)

DISCUSSION

This research indicates that the administration of different doses of *Moringa oleifera* leaf extract has a significant and distinct impact on the quality of white rat (*Rattus norvegicus*) sperm, as evidenced by the increased mean values of sperm quality, especially morphology. *Moringa* leaf extract also had an impact on the fasting blood sugar levels in white rats induced with alloxan and subjected to treatment. The differences observed between the control group and the treatment group are presumed to be due to the constituents present in the *Moringa oleifera* leaf extract itself.

Phytochemical analysis conducted on *Moringa oleifera* leaves has identified the presence of quercetin, a plant pigment known as a type of flavonoid compound with antioxidant and anti-inflammatory properties.

One crucial role of quercetin is its involvement in enhancing the process of spermatogenesis, which refers to the formation and development of sperm in the male reproductive organ, this theory is proven by Zhang et al. (2023). This compound has been proven to possess robust antioxidant properties, capable of combating reactive oxygen species (ROS) that contribute to cell damage and hinder spermatogenesis. Its ability to protect testicular cells from oxidative stress and stimulate the proliferation of spermatogonia cells constitutes a key mechanism in supporting optimal spermatogenesis.

In the fasting blood sugar examination, data were obtained before and after treatment to compare the effectiveness of moringa leaf extract in altering blood sugar levels. White rats, initially within the normal blood sugar range, were later induced with alloxan. Subsequently, they underwent a 26-day *Moringa oleifera* leaf extract treatment period. The group subjected to moringa leaf extract treatment experienced a significant change, maintaining normal blood sugar levels. In contrast, the positive control group exhibited a substantial increase in blood sugar levels, indicating hyperglycemia. This can be interpreted as the contribution of moringa leaf extract in maintaining blood sugar levels through its role as an antioxidant that combats free radicals under hyperglycemic conditions. This is in line with research conducted by Salsabila et al. (2021) regarding phytochemicals in the form of flavonoids which are potent antioxidants and cytoprotectants that can lower blood sugar levels.

In the morphological assessment, the positive control group displayed the highest average percentage of abnormal sperm morphology, reaching 56.5%, while recording the lowest average percentage of normal morphology at 43.5%. The prevailing abnormality observed in the positive control involved sperm with a bound tail, impeding normal and swift movement. This indicates disturbances in the spermatogenesis process within the positive control group, affecting both the formation and maturation phases, leading to a normal sperm count but with defects in morphological quality.

These disruptions in spermatogenesis are associated with heightened oxidative stress under hyperglycemic conditions. Consequently, the findings from the treatment group, administered *Moringa oleifera* leaf extract to enhance spermatogenesis quality, yielded satisfactory results. In treatment group K1, receiving 200 mg/kg body weight of *Moringa oleifera* leaf extract, the average normal morphology reached 85%, with abnormal morphology averaging 15%. The prevalent abnormality in K1 was sperm tail coiling or binding. Compared to the negative control, K1 showed a notable 21.5% increase in normal morphologies.

For treatment group K2, administered a dose of 300 mg/kg body weight of *Moringa oleifera* leaf extract, the average normal morphology was 82%, with abnormal morphology averaging at 18%. Common abnormalities observed in K2 samples included sperm heads resembling a pin, possibly hindering the effective transport of genetic material. This average value was lower than that of treatment group K1, suggesting that not all bioactive compounds may work effectively, even with a larger dose.

Treatment group K3, receiving 400 mg/kg body weight of *Moringa oleifera* leaf extract, achieved the highest average value of normal morphology at 94.5%, with abnormal morphology averaging 5.5%. This represents the peak average value of normal morphology compared to all control and treatment groups. The 400 mg/kg body weight dose of *Moringa oleifera* leaf extract can be considered the most effective and significant dose for improving sperm quality in terms of morphology. This finding aligns with the research conducted by Laoung-On et al. (2021), indicating an increase in normal sperm morphology in the treatment group given *Moringa oleifera* leaf extract.

CONCLUSION

The administration of *Moringa oleifera* leaf extract at doses of 200, 300, and 400 mg/kg body weight can reduce blood sugar levels in alloxan-induced white rats. The administration of *Moringa*

oleifera leaf extract also enhances or improves the quality of sperm count with normal morphology in white rats (*Rattus norvegicus*) based on the respective doses given. For future research suggestions, researchers may conduct further studies examining the impact of administering *Moringa oleifera* leaf extract at higher doses or using the same dose over an extended period on the sperm quality of white rats (*Rattus norvegicus*).

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