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ETHANOL EXTRACT OF *Moringa oleifera* INCREASED THE NUMBER OF SPERMATOZOA AND IMPROVED SPERM MORPHOLOGY OF OLD *Rattus norvegicus*

Pemberian Ekstrak Etanol *Moringa oleifera* Meningkatkan Jumlah serta Memperbaiki Morfologi Spermatozoa *Rattus norvegicus* Tua

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ABSTRACT
*Aging in men can lead to decreased semen volume, abnormal sperm morphology, and decreased sperm motility. This study aims to determine the effect of ethanol extract of moringa (*Moringa oleifera*) leaf on the number and morphology of spermatozoa in old Wistar rats (*Rattus norvegicus*). This study used healthy old rats, 18–19 months old, with a bodyweight of 200–250 g and no physical disabilities. The 36 rats were divided into 2 groups, i.e. the treatment group (fed with ethanol extract of moringa leaves 50 mg/kgBW/0.5 mL CMC 0.5% every day) and the control group (0.5 mL CMC 0.5% every day) for 30 days. The results showed that moringa leaf ethanol extract increased the number of spermatozoa (p-value 0.000) and improved spermatozoa morphology (p-value 0.000). It can be concluded that the ethanol extract of moringa leaves significantly increased the number and improved the morphology of the spermatozoa in the aged rats.*

Keywords: *ethanol extract, moringa leaf, number of spermatozoa, spermatozoa morphology, aged* Rattus norvegicus

ABSTRAK
Penuaan pada pria dapat menyebabkan penurunan volume semen, morfologi yang abnormal dan penurunan motilitas sperma. Tujuan dari penelitian ini adalah untuk mengetahui pengaruh pemberian ekstrak etanol daun kelor (*Moringa oleifera*) terhadap jumlah serta morfologi spermatozoa tikus putih (*Rattus norvegicus*) galur Wistar usia tua. Penelitian ini menggunakan tikus tua yang berusia 18–19 bulan dengan berat badan 200–250 g, kondisi sehat dan tidak cacat fisik. Sebanyak 36 ekor tikus dibagi menjadi 2 kelompok, yaitu kelompok perlakuan (ekstrak etanol daun kelor 50 mg/kgBB/0,5 mL CMC 0,5% per hari) dan kelompok kontrol (CMC 0,5% 0,5 mL per hari) selama 30 hari. Hasil penelitian menunjukkan bahwa ekstrak etanol daun kelor meningkatkan jumlah spermatozoa (nilai p 0,000) dan memperbaiki morfologi spermatozoa (nilai p 0,000). Dapat disimpulkan bahwa pemberian ekstrak etanol daun kelor secara signifikan meningkatkan jumlah dan kualitas morfologi spermatozoa tikus putih usia tua.

Kata Kunci: daun kelor, ekstrak etanol, jumlah spermatozoa, morfologi spermatozoa, *Rattus norvegicus* usia tua

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INTRODUCTION

Indonesia's population growth rate increased by 1.25 percent per year in the last ten years. There was a slowdown in the population growth rate of 0.24 percent compared to 2000–2010, which was 1.49 percent (BPS 2021). One of the reasons for this decline was the birth rate. The birth rate may decline due to the decrease of infertility.

According to the World Health Organization (WHO), infertility fails to achieve pregnancy after one year of active sexual intercourse without contraception (Aziz and Agarwal 2017, Majzoub and Sabanegh 2017). Causes of infertility in married couples can be classified into three proportions: 45% is caused by female factors, 40% by male factors, and 15% by idiopathic factors (Lestari and Sari 2015). According to Kumar and Singh (2018), the malefactor is responsible for 40–50% of infertility cases in couples (Aziz and Agarwal, 2017).

The underlying causes of male infertility are grouped into three factors (1) pretest, (2) testicular, and (3) post-aesthetic (Dimitriadis et al. 2017). Four main categories of sperm damage lead to the diagnosis of male infertility, including the absence of sperm in the semen (azoospermia), low sperm count (oligozoospermia), malformed sperm morphology (teratozoospermia), and problems with sperm motility (asthenozoospermia) (Majzoub and Sabanegh 2017). Spermatozoa are very susceptible to oxidative stress because the antioxidant enzymes in spermatozoa are low. Spermatozoa spend a long time in the reproductive tract (Sabeti et al. 2016), and spermatozoa plasma membranes contain high polyunsaturated fatty acids (PUFAs). This high proportion of PUFA causes Spermatozoa to undergo lipid peroxidation (Ayala et al. 2014, Lee et al. 2017, Dias et al. 2020, Ali et al. 2021). According to Henkel et al. (2018), oxidative stress is closely related to various pathologies such as infertility and aging (Ko et al. 2014, Bisht et al. 2017, Kumar and Singh 2018).

The reproductive aging process is characterized by a progressive decrease in physiological integrity that triggers damage to the testes, epididymis, and other reproductive organs. Aging in men can cause a reduction in semen volume, abnormal morphology, and a decrease in normal sperm motility (Lucio et al. 2013, Morielli and O'Flaherty 2015). Aging closely relates to free radicals in the body, inducing oxidative stress. Oxidative stress is an imbalance between reactive oxygen species (ROS) and antioxidant production (Luceri et al. 2018). ROS production has a central role in age-related decline in male fertility, influencing aging biomarkers (López-otín et al. 2013). Previous studies revealed that spermatozoa from older animals produced more free radicals than younger ones and lower antioxidant activity (Sabeti et al. 2016).

The body needs antioxidants that function to protect the destruction of cells in the body due to free radical attacks, ward off free radicals and prevent chain reactions so that more significant damage does not occur, and repair damaged cells and tissues. The body synthesizes antioxidants called endogenous antioxidants, while antioxidants that come from outside the body or from food and drinks are called exogenous antioxidants (Widiastini et al. 2022). High-quality spermatozoa require ascorbic acid, amino acids, sterols, isoquartsetin glucoside, carotene, ramentin, kaempferol, kaemferitin, and vitamin E. One of the plants that contain all these elements is moringa leaves (Singh et al. 2012, Syarifuddin et al. 2017). The high nutritional value, properties, and benefits content has caused moringa to be nicknamed the Miracle tree and Mother's Best Friend. Moringa contains more than 90 nutrients in essential vitamins, minerals, amino acids, anti-aging, and anti-inflammatory (Aminah et al. 2015). Moringa contains 539 compounds known in traditional African and Indian medicine and has been used in traditional medicine to prevent more than 300 diseases (Toripah et al. 2014). The phytochemical tests on moringa leaves carried out in the South Denpasar area, Bali, revealed that the ethanolic extract of moringa leaves has an antioxidant capacity, including phenolics and flavonoids, tannins, vitamin C, alkaloids, and saponins (Widiastini et al. 2021). The Wistar rat strain was chosen as the subject of the study because it had a higher percentage of live spermatozoa (92%) compared to the Sprague-Dawley (90.7%) (Simbolon et al. 2013).

Based on this background, this study aimed to determine the effect of moringa leaf ethanol extract on the number and morphology of spermatozoa in the aged Wistar rat.

MATERIALS AND METHODS

Location and time

This research was conducted from January to March 2021. The study took place in the Integrated Biomedical Laboratory, Faculty of Medicine, Udayana University.

Experimental animals

Thirty-six healthy male Wistar rats met the eligibility criteria, age 18–19 months, with a bodyweight of 200–250 g and a body length of circa 300-400 mm. Specifically, the average body weight of the mice used was 229.35 ± 11.4 g. The dose of 50 mg/kg BW moringa leaf ethanol extract was given per day for 30 days. The other materials used in this study were 0.5% CMC, ketamine-xylazine, 1 set of surgical instruments, surgical board, 0.9% NaCl solution, 1% Eosin dye, and 10% Nigrosin, dropper, petri dish, object-glass, light microscope (Olympus brand).

Design of experiment

This study was experimental research using the randomized post-test only control group design. The samples were male Wistar rats aged 18–19 months, with a bodyweight of 200–250 g. Rats that looked sick and did not move actively were excluded. Samples which died during the study or suffered a weight loss of more than 10% after the acclimatization period in the laboratory were also excluded. As many as 36 rats were divided into two groups: 18 rats each for the treatment and control groups. The random sampling technique was used to determine the sample.

Moringa leaf ethanol extract preparation

Dry moringa leaf (50 g) was macerated, crushed using a blender, added with 96% ethanol solvent, placed into a closed container for 2 days (protected from sunlight). This mixture was filtered to obtain the macerate solution, whereas the pulp was macerated with 96% ethanol using the same procedure. Repeated maceration procedure was carried out until a clear macerate solution was obtained. The macerate solution was evaporated using a rotary vacuum evaporator at 40 ºC (Widiastini et al. 2021).

Feeding procedure

The weight of all the experimental animals (Figure 1) were initially determined. The animals were all fed a common rat commercial diet of BRI CP511B manufactured by Charoen Pokphand Indonesia Tbk (containing 21–23% protein, 40% carbohydrate, 5% fat, 5% fibre, and 27-29% other components). The daily dose of ethanol extract of moringa leaves was fed to the treatment group was 50 mg/kg BW (dissolved in 0.5 mL of 0.5% CMC). The control group was fed 0.5 mL of 0.5% CMC

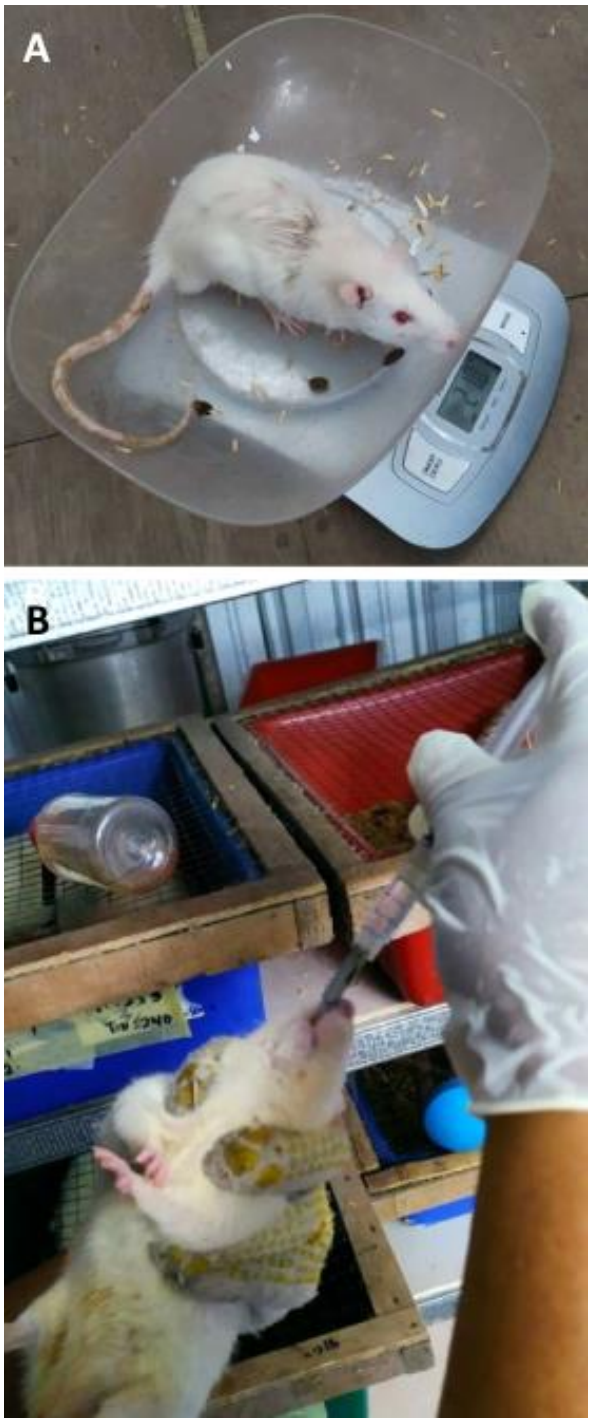

Figure 1. The male Wistar rats (*R. norvegicus*) weighed on a balance (A), and fed moringa leaf ethanol extract (B)

per day. The feeding was carried out every morning for 30 days. CMC was used as it mixes well the moringa ethanol extract, is safe, and does not damage the antioxidant contents of the moringa extract.

On the 30th day, the rats were euthanized (Figure 2). The rats were anesthetized using ketamine-xylazine at a dose of 100:10 mg/kg intramuscularly (IM), then sacrificed using the cervical dislocation method. The testes were separated from the cauda epididymis and then put into a petri dish containing 5 mL of 0.9% NaCl. The cauda epididymis was cut finely in a petri dish (120 × 20 mm) and stirred until homogeneous.

Counting spermatozoa

The spermatozoa were enumerated using an improved Neubauer counting chamber (hemocytometer; each chamber: 1.0 × 1.0 × 0.1 mm). After diluted with 5 mL of physiological saline solution (0.9% NaCl), 10 µL of the spermatozoa suspension was pipetted into a, covered with a cover-glass so as to prevent air bubbles formation. The spermatozoa were counted under a light microscope (400× magnification) in 6 different views: central part, top edge, and left edge of the chamber. The average number of spermatozoa (n) was calculated as the total number of spermatozoa present in all 6 chambers divided by 6.

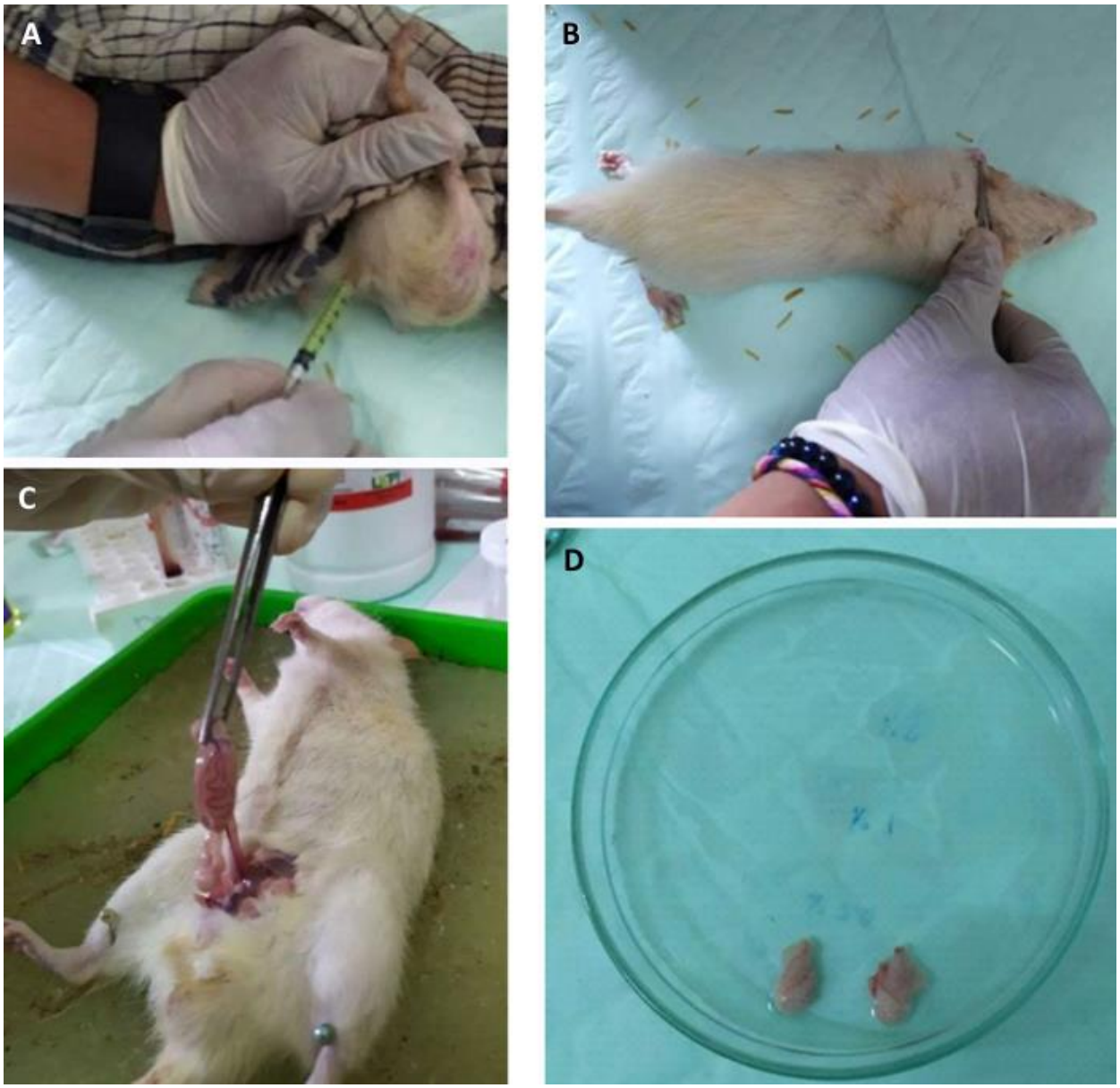

Figure 2. Following 30-day moringa ethanol extract feeding, the male Wistar rats (*R. norvegicus*) were anesthetized (A), cervically dislocated (B), and subjected to orchidectomy (C) to isolate the cauda epididymis (D)

Spermatozoa morphological observations

Spermatozoa morphology was observed using the Eosin 1% and Nigrosin 10% staining method by smearing the spermatozoa on an object-glass. Then one drop of Eosin 1% and one drop of Nigrosin 10% were given onto the sample, homogenized, and air-dried for 5 minutes. The observation was done under a light microscope at 400× magnification on 100 spermatozoa cells, each sample being repeated six times. Typical spermatozoa morphology is intact head and neck parts with a straight tail. Spermatozoa are normal if the head is curved like a hook, the neck is straightforward, and the tail is single and free. It is considered abnormal if the head is small or too large, the neck is broken or branched, the tail is branched, curled, and damaged, and there are cytoplasmic droplets on the head and neck or tail (Majzoub and Sabanegh 2017).

Data analysis was carried out descriptively by displaying the distribution of the frequency and the mean of spermatozoa and its morphology. Data normality was analyzed using the Shapiro-Wilk test (considered normally distributed when $p > 0.05$), followed by a comparative analysis of the independent sample t-test at a significance level of 0.05 (for $p > 0.05$) or the Mann-Whitney test (for $p < 0.05$).

RESULTS AND DISCUSSION

Number of sperm

Live spermatozoa were characterized by no color absorption (transparent), while dead spermatozoa were indicated by the absorption of 1% Eosin and 10% Nigrosine dyes (Figure 3). Table 1 shows that the treatment group has an average value of 87.70% with a 95% CI value of 87.11–88.29, while that of the control group is 74.56% with a 95% CI value of 71.66–77.46. Sperm count data in the treatment and control groups were normally distributed with a p-value of 0.632 for the treatment group and 0.172 for the control group. The subsequent independent t-test analysis gave a p-value 0.000 (< 0.05), indicating significant difference in sperm count between the treatment and the control group. Thus, the moringa leaf ethanol extract significantly increased sperm count (Figure 4).

The antioxidant content in moringa leaves may work by neutralizing free radicals, preventing oxidative damage to most biomolecules and providing significant protection against oxidative damage. It can protect lipoproteins from peroxyl radicals, thereby increasing the number of spermatozoa. This is in line with the research conducted by Bebas et al. (2015), which showed that adding vitamin C at 0.1 mg mL^{-1} could maintain the viability of spermatozoa and increase the motility of spermatozoa. The experiment conducted by Diao et al. (2019) showed that supplementation with quercetin (flavonoid) could protect spermatozoa from sperm damage mediated by H_2O_2 (hydrogen peroxide). A study by Dafaalla et al. (2016) found a significant difference in sperm count in white rats between the treatment group given moringa leaf ethanol extract and the control group, so it could be concluded that moringa leaf ethanol extract could have a significant effect on sperm count. The study by Priyadarshani and Varma (2014) also showed the same results, in which the sperm count increased significantly in hyperglycemic rats given 200 mg/kg body weight of moringa powder ($p < 0.05$). Research by Fatoba et al. (2013) obtained a p-value < 0.05, indicating that *M. oleifera* significantly increased mass activity, progressive motility, and sperm concentration in mice.

Sperm morphology

Spermatozoa morphology observation (Figure 5, Table 2) shows that the treatment group had good sperm morphology with an average value of 82.28% with a 95% CI value of 78.97–85.58, while that of the control group is the standard was 61.89% with a 95% CI value of 57.02–66.75. The data on the number of sperm in the treatment and control groups were not normally distributed, with a p-value of 0.023 for the treatment group and 0.015 for the control group. The following Mann-Whitney test gave a p-value of 0.000 (< 0.05), indicating significant difference in sperm morphology between the treatment and the control group. Therefore, the moringa leaf extract significantly affected the sperm morphology.

This result is in line with previous research (Wahjuningsih et al. 2019), which showed that adding moringa leaf extract improved semen quality. Carrera-Chávez et al. (2020) revealed that semen supplemented with *M. oleifera* seeds increased antioxidant activity, sperm membrane integrity, viability,

and progressive motility. Moringa leaf ethanol extract containing ascorbic acid had a much more potent antioxidant than others by supplying up to 65% of seminal plasma (Sahu 2016). Other research showed that moringa leaf extract, which contains antioxidants,

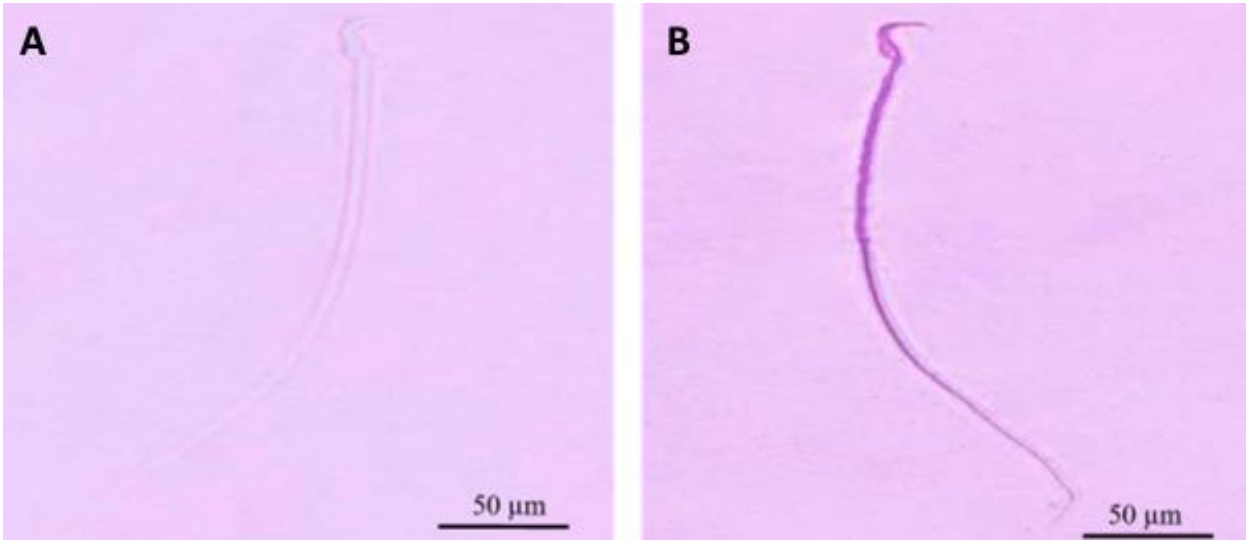

Figure 3. Microscopic views of single live (A) and dead (B) spermatozoa of the male Wistar rats (*R. norvegicus*) after treatment using moringa leaf ethanol extract (400× magnification)

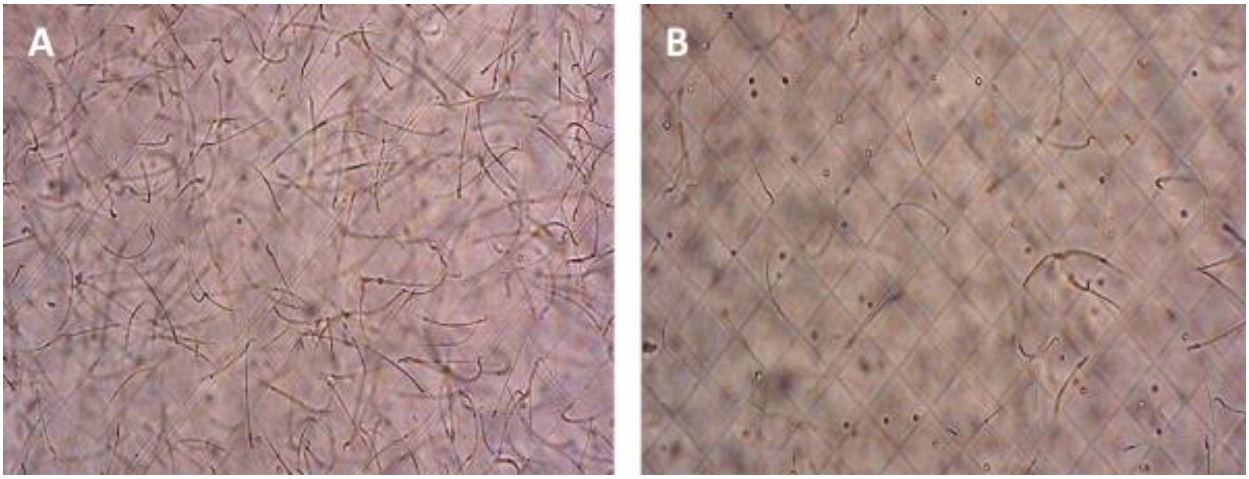

Figure 4. Microscopic views of spermatozoa suspension of the male Wistar rats (*R. norvegicus*) after treatment using moringa leaf ethanol extract: the treatment group (dense) (A) and control group (less dense) (B) (400× magnification)

Table 1. Number of spermatozoa in the male Wistar rats (*R. norvegicus*) after treatment using moringa leaf ethanol extract

Group	Mean ± SD	95% CI	Minimum	Maximum	Normality Test	p
Treatment	87.70 ± 5.82%	87.11–88.29	86%	89.67%	0.632	0.000
Control	74.56 ± 1.18%	71.66–77.46	65.67%	85.17%	0.172	

Note: $p < 0.05$ indicates significant effect based on the independent t-test

Table 2. Spermatozoa morphology in the male Wistar rats (*R. norvegicus*) after treatment using moringa leaf ethanol extract

Group	Mean ± SD	95% CI	Minimum	Maximum	Normality Test	p
Treatment	82.28 ± 6.64%	78.97–85.58	68.83%	89.33%	0.023	0.000
Control	61.89 ± 9.78%	57.02–66.75	38.83%	78%	0.015	

Note: $p < 0.05$ indicates significant effect based on the Mann Whitney test

maintained spermatozoa membranes and protect against sperm damage caused by free radicals, thereby increasing the density and improving spermatozoa morphology (Wahjuningsih et al. 2019, Carrera-Chávez et al. 2020). The addition of antioxidant compounds increase sperm parameters, such as morphology, motility, concentration, and reduce DNA damage by $p < 0.05$ (Martin-Hidalgo et al. 2019, Dias et al. 2020). However, it should be noted that the excessive use of antioxidants can cause pro-oxidants called antioxidant paradoxes. This can reduce endogenous oxidants that are important for the induction of physiological pathways, thereby inhibiting sperm capacitation, hyperactivation, and acrosomal reactions of spermatozoa which can increase pathological conditions such as aging and infertility in men (Henkel et al. 2018, Ali et al. 2021).

CONCLUSION

This study showed that the spermatozoa number of old Wistar rats given moringa leaf ethanol extract (50 mg/kgBW/0.5 mL CMC 0.5% every day) was higher than that of the control group. The treatment also showed more normal spermatozoa morphology than the control group. There was a significant effect of giving moringa leaf ethanol extract in increasing the number of spermatozoa and improving the sperm morphology in old white Wistar rats.

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REFERENCES

Ali M, Martinez M, Parekh N (2021) Are antioxidants a viable treatment option for male infertility? Andrologia 53:e13644. doi: 10.1111/and.13644

Aminah S, Ramdhan T, Yanis M (2015) Nutritional content and functional properties of *Moringa oleifera*. Bul

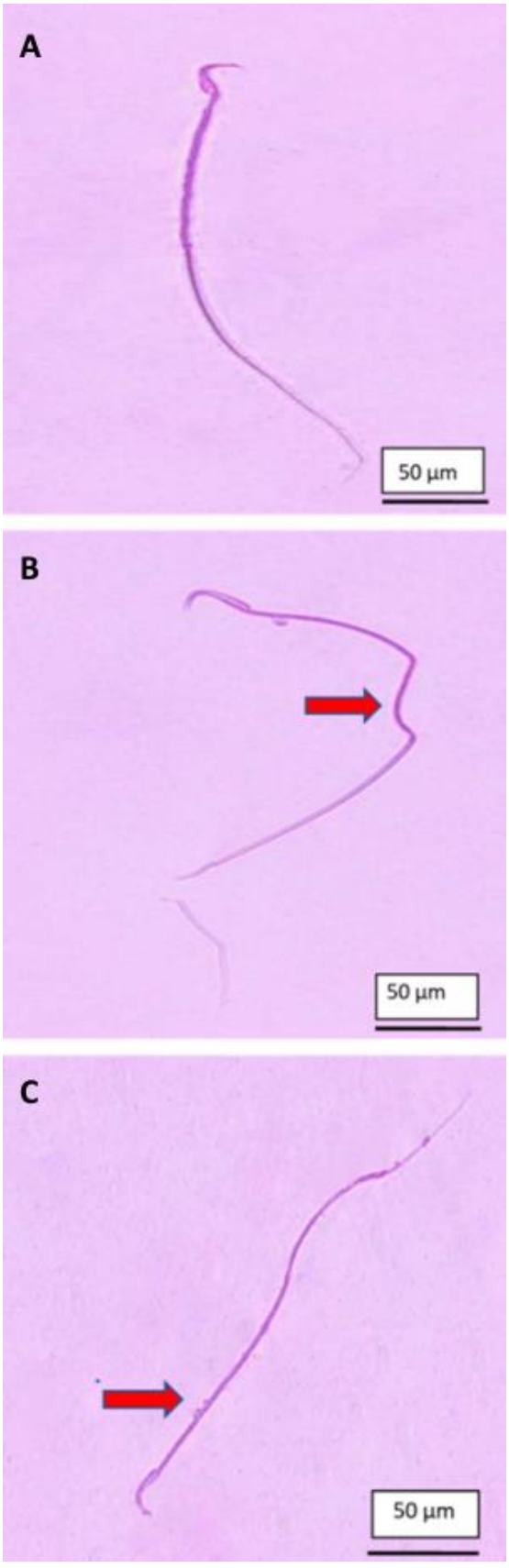

Figure 5. Spermatozoa the male Wistar rats (*R. norvegicus*) with normal morphology (A), abnormal tail (B), and cytoplasmic droplets (C)

Pertan Perkota 5:35–44

Ayala A, Muñoz MF, Argüelle S (2014) Lipid peroxidation: Production, metabolism, and signalling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev 2014:360438. doi: 10.1155/2014/360438

Aziz N, Agarwal A (2017) The Diagnosis and Treatment of Male Infertility. Springer, Switzerland. doi: 10.1007/978-3-319-56547-7

Bebas W, Budiasa MK, Astutik IY (2015) Addition of vitamin C in diluent of landrace sperm stored in temperature of 15ºC. Bul Vet Udayana 7:179–185

Bisht S, Faiq M, Tolahunase M, Dada R (2017) Oxidative stress and male infertility. Nat Rev Urol 14:470–485. doi: 10.1038/nrurol.2017.69

BPS (2021) Population Census Results 2020 (Hasil Sensus Penduduk 2020), BPS Statistics Indonesia, Jakarta

Carrera-Chávez JM, Jiménez-Aguilar EE, Acosta-Pérez TP, Núñez-Gastélum JA, Quezada-Casasola A, Escárcega-Ávila AM, Itza-Ortiz MF, Orozco-Lucero E (2020) Effect of *Moringa oleifera* seed extract on antioxidant activity and sperm characteristics in cryopreserved ram semen. J Appl Anim Res 48:114–120. doi: 10.1080/09712119.2020.1741374

Dafaalla MM, Hassan AW, Idris OF, Abdoun S, Modawe GA, Kabbashi AS (2016) Effect of ethanol extract of *Moringa oleifera* leaves on fertility hormone and sperm quality of male albino rats. World J Pharm Res 5:1–11. Corpus ID: 212555904

Diao R, Gan H, Tian F, Cai X, Zhen W, Song X, Duan YG (2019) *In vitro* antioxidation effect of Quercetin on sperm function from the infertile patients with leukocytospermia. Am J Reprod Immunol 82:e13155. doi: 10.1111/aji.13155

Dias TR, Martin-Hidalgo D, Silva BM, F. Oliveira PF, Alves MG (2020) Endogenous and exogenous antioxidants as a tool to ameliorate male infertility induced by reactive oxygen species. Antioxid Redox Signal 33:767–785. doi: 10.1089/ars.2019.7977

Dimitriadis F, Adonakis G, Kaponis A, Mamoulakis C, Takenaka A, Sofikitis N (2017) Pre-testicular, testicular, and post-testicular causes of male infertility. In Simoni M, Huhtaniemi IT (Eds). Endocrinology of the Testis and Male Reproduction. Pp 1–47. Springer, Cham. doi: 10.1007/978-3-319-29456-8_33-1

Fatoba TA, Faleyimu OI, Adebayo AJ (2013) Effects of increasing aqueous root extract of *Moringa oleifera* on sperm production of albino rats. Agrosearch 13:29–36. doi: 10.4314/agrosh.v13i1.3

Henkel R, Sandhu IS, Agarwal A (2018) The excessive use of antioxidant therapy: A possible cause of male infertility? Andrologia 51:e13162. doi: 10.1111/and.13162

Ko EY, Sabanegh Jr ES, Agarwal A (2014) Male infertility testing : reactive oxygen species and antioxidant capacity. Fertil Steril 102:1518–1527. doi: 10.1016/j.fertnstert.2014.10.020

Kumar N, Singh AK (2018) Reactive oxygen species in seminal plasma as a cause of male infertility. J Gynecol Obstet Hum Reprod 47:565–572. doi: 10.1016/j.jogoh.2018.06.008

Lee D, Moawad AR, Morielli T, Fernandez MC, O'Flaherty C (2017) Peroxiredoxins prevent oxidative stress during human sperm capacitation. Mol Hum Reprod 23:106–115. doi: 10.1093/molehr/gaw081

Lestari SW, Sari T (2015) Fragmentasi DNA spermatozoa: Penyebab, deteksi, dan implikasinya pada infertilitas laki-laki. eJ Kedokt Indones 3:152–160. doi: 10.23886/ejki.3.5050

López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer (2013) The hallmarks of aging. Cell 153:1194–1217. doi: 10.1016/j.cell.2013.05.039

Luceri C, Bigagli E, Femia AP, Caderni G, Giovannelli L, Lodovici M (2018) Ageing-related changes in circulating reactive oxygen species (ROS) and protein carbonyls are indicative of liver oxidative injury. Toxicol Rep 5:141–145. doi: 10.1016/j.toxrep.2017.12.017

Lucio RA, Tlachi-López JL, Eguibar JR, Ågmo A (2013) Sperm count and sperm motility decrease in old rats. Physiol Behav 110–111:73–79. doi:

10.1016/j.physbeh.2012.12.015
Majzoub A, Sabanegh Jr E (2017) Making a diagnosis. In: Aziz N, Agarwal A (Eds). The Diagnosis and Treatment of Male Infertility. Pp 1–18. doi: 10.1007/978-3-319-56547-7_1
Martin-Hidalgo D, Bragado MJ, Batista AR, Oliveira PF, Alves MG (2019) Antioxidants and male fertility: From molecular studies to clinical evidence. Antioxidants 8:89. doi: 10.3390/antiox8040089
Morielli T, O'Flaherty C (2015) Oxidative stress impairs function and increases redox protein modifications in human spermatozoa. Reproduction 149:113–123. doi: 10.1530/REP-14-0240
Priyadarshani N, Varma MC (2014) Effect of *Moringa oleifera* leaf powder on sperm count, histology of testis and epididymis of hyperglycaemic mice *Mus musculus*. Am Int J Res Form Appl Nat Sci 7:7–13. Corpus ID: 3154813
Sabeti P, Pourmasumi S, Rahiminia T, Akyash F, Talebi AR (2016) Etiologies of sperm oxidative stress. Int J Reprod BioMed 14:231–240. doi: 10.29252/ijrm.14.4.231
Sahu CR (2016) Reactive oxygen species turns men infertile but vitamin C prohibits - Behind the scene. Austin Androl 1:1009
Simbolon IS, Lubis TM, Adam M (2013) Persentase spermatozoa hidup pada tikus Wistar dan Sprague-Dawley. J Med Vet 7:79–83. doi: 10.21157/j.med.vet..v7i2.2935
Singh GP, Garg R, Bhardwaj S, Sharma SK (2012) Anti inflammatory evaluation of leaf extract of *Moringa oleifera*. J Pharm Sci Innov 1:22–24
Syarifuddin NA, Toleng AL, Rahardja DP, Ismartoyo, Yusuf M (2017) Improving libido and sperm quality of Bali bulls by supplementation of *Moringa oleifera* leaves. Media Peternak 40:88–93. doi: 10.5398/medpet.2017.40.2.88
Toripah SS, Abidjulu J, Wehantouw F (2014) Antioxidant activity and total phenolic content of moringa leaf extract (*Moringa oleifera* Lam). Pharmacon 3:37–43. doi: 10.35799/pha.3.2014.6043
Wahjuningsih S, Ihsan MN, Ciptadi G, Isnaini N, Rahayu S, Afifah DD (2019) Effect of *Moringa oleifera* leaves extract on post-thawed semen quality of Senduro Goat. IOP Conf Ser Earth Environ Sci 247:012055. doi: 10.1088/1755-1315/247/1/012055
Widiastini LP, Karuniadi IGAM, Karmaya INM, Widhiantara IG (2022) The potential of a Balinese traditional medicine kelor leaves (*Moringa oleifera*) for male infertility treatment: a mini review. Indian J Forensic Med Toxicol 16:734–742. doi: 10.37506/ijfmt.v16i1.17579
Widiastini LP, Karuniadi IGAM, Tangkas M (2021) Antioxidant compounds ethanol extract of kelor leaves (*Moringa oleifera*) in Denpasar Selatan Bali. Media Kesehatan Politekmik Kesehatan Makassar 16:135–139. doi: 10.32382/medkes.v16i1.2038