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Preventive Effect of *Moringa oleifera* L. and *Solanum lycopersicum* Fruit Against Toxicity of Induced Paracetamol Rat Spermatozoa

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Abstract

The aim of the research was to determine the preventive effect of *Moringa oleifera* L. extract and *Solanum lycopersicum* L. fruit on rat spermatozoa after being induced by toxic doses of paracetamol. This type of experimental research used 30 mice divided into 6 treatments, namely K- (paracetamol induction 500mg/kg BW); K+ (*M. oleifera* extract 600 mg and S. *lycopersicum* fruit extract 60 mg/kg BW); P1 (*M. oleifera* extract 600 mg/kgBW); P2 (*S. lycopersicum* fruit extract 60 mg/kg BW) and P3 (M. *oleifera* extract 600 mg and S. lycorpesicum fruit extract 60 mg/kg BW). Test animals at P1, P2, and P3 were previously induced by paracetamol. Treatment was given orally for 15 days, while paracetamol induction was given on day 11. On the 16th day, mice were sacrificed using chloroform and dissected to obtain spermatozoa from the cauda epididymis. Observation variables include motility, viability, and morphology of spermatozoa. Data were analyzed using the Analysis of Variance (ANOVA) test and the Honest Significant Difference further test ($P<0.05$). The results showed that K+ treatment had a better preventive effect. The percentage of normal motility was 79.33%, the percentage of live spermatozoa was 76.16%, and the percentage of normal morphology was 77.67%. The conclusion of the research is that the combination of M. *oleifera* extract and S. *lycopersicum* fruit is preventive in preventing spermatozoa damage caused by the effects of excessive use of paracetamol.

Keywords: *Moringa oleifera*; *Rattus norvegicus*; *Solanum lycopersicum*; spermatozoa.

INTRODUCTION

Reproductive health is an optimal condition that reflects an individual's ability to lead a proper sexual and reproductive life (Leekuan *et al.*, 2022). Reproductive health problems in men are currently receiving increasing attention at the global level (Roudsari and Goudarzi, 2023). Based on data from the World Health Organization (WHO) in 2010, male reproductive health consists of various aspects, namely sexual function, fertility, and prevention and treatment of sexually transmitted infections (STIs) (Ansari *et al.*, 2023). Factors that influence reproductive health are unhealthy lifestyles, exposure to dangerous chemicals, and lack of access to reproductive health information and services, which also worsen this condition (Choudhary *et al*., 2025). Preventive strategies play an important role in efforts to maintain reproductive health.

Preventive is an effort made to prevent the emergence of disease or health problems before they occur (Horsch *et al.*, 2024), so in its implementation, it is necessary to be wary of the use of certain drugs, such as paracetamol in toxic doses, because it can affect reproductive function. Paracetamol is a drug that is commonly consumed as a mild analgesic or even an antipyretic in several regions such as the United States, Europe, and Indonesia (Setiawan *et al.*, 2025). The increase in the amount of paracetamol consumption by humans is uncontrolled and has a negative impact on reproductive health (Husseina *et al.*, 2025). Paracetamol consumption in big cities will be difficult to reduce because people still rely heavily on paracetamol to relieve dizziness and pain (Petrides *et al.*, 2024).

Excessive consumption of paracetamol will form the compound N-acetyl-p-benzoquinone (NAPQI), which is toxic and produces free radicals in the body (Barasker *et al.*, 2024). These free radicals can trigger lipid peroxidation in spermatozoa membranes and damage sperm cell DNA through oxidative mechanisms (Setiawan *et al.*, 2024). Efforts to deal with free radicals in the body can be done by utilizing natural compounds from plants that have antioxidant properties. Antioxidants are needed to ward off and protect the body from oxidative stress due to excessive formation of free radicals in response to exposure to excessive doses of paracetamol.

Plants that have the potential to be a source of antioxidants are Moringa leaves (Shalaby *et al.*, 2022). Moringa leaves (*Moringa oleifera* L.) are known to contain flavonoids, phenolics, vitamin C, and beta-carotene, which play an important role in neutralizing free radicals (Qadir *et al.*, 2022). Moringa leaf extract is able to improve the number, motility, and morphology of rat spermatozoa and normalize testosterone hormone levels (Ismail *et al.*, 2025). Tomato fruit (*Solanum lycopersicum* L.) is a natural compound that has antioxidant properties and is known as the main source of lycopene, a lipophilic carotenoid that is effective in preventing lipid peroxidation and sperm DNA damage (Paengkoum and Lu, 2024). Epididymal sperm counts are generally used in toxicology studies to assess the damage to the male reproductive system, or vice versa, to determine the therapeutic effect of a substance (Luthfi, 2015). Therefore, this study aimed to determine the potential of Moringa leaf and tomato extracts as a source of natural antioxidants in counteracting free radicals on the quality of sperm and testicular organs of Wistar rats induced by toxic doses of paracetamol. It is hoped that the results of this research can become an alternative herbal ingredient to improve sperm quality due to exposure to toxic ingredients from synthetic drugs after long-term use.

MATERIALS AND METHODS

The materials used include: *Moringa oleifera* leaf powder and tomato fruit, paracetamol powder, 30 male rats, wood husks, and rat food in the form of commercial pellets (Broiler 1) produced by PT. Japfa Comfeed Indonesia, drinking water, Na CMC 0.5%, Chloroform, NaCL 0.9%, distilled water, Eosin-nigrosin, Methanol 96%, Ethanol 96%, and Giemsa dye 20%. The tools used in this research include: a light microscope, a set of surgical tools, glass objects, petri dishes, dropper pipettes, blenders, analytical scales, basins, digital cameras, rang-rangs, oral syringes, and rat drinking bottles.

Procedures

Preparation of test animals

The male rats (*Rattus norvegicus* L.) used in the research were healthy and showed clear eyes, hair, cleanliness, and normal behavior. 2-3 months old, with an average body weight of 190-200 g, a total of 30 individuals were obtained from laboratory animal breeders. Rats were acclimated for 14 days in cages made from plastic basins measuring 50×40×20 cm^3 with a density of 6 mice per cage. The top of the cage is given a wire frame, and the base is covered with sawdust with a thickness of ± 2 cm, which is replaced every 3 days. During acclimation, the cage is placed in a cage house equipped with ventilation for air circulation with an average room temperature of 25-29°C and a light:dark cycle of 12:12 hours (Eskandarani *et al*., 2023). Rats were fed in the form of commercial pellets (Broiler 1) produced by PT. Japfa Comfeed Indonesia provides as much as 6 grams/head/day and given drinking water from drilled wells ad libitum. Feed is given by placing the pellets in a container made from the bottom of a 600 ml plastic bottle of bottled drinking water, which is assembled hanging in 3 cages.

Extraction and treatment of test materials

Moringa oleifera leaves and *Solanum lycopersicum* fruit were washed thoroughly and dried using an oven at 70°C, then powdered and macerated with 96% ethanol for 48 hours. The filtrate was then filtered and evaporated with a rotary evaporator at a temperature of 45° and a speed of 100 rpm to obtain an extract in the form of semisolid macerate (Ncama *et al*., 2025). The extract was given orally for 15 days to mice according to the treatment unit, namely *M. oleifera* extract at a dose of 600 mg/kg BW and *S. lycopersicum* fruit extract at a dose of 60 mg/kg BW. A toxic dose of paracetamol solution, namely 500 mg/kg BW, was given on the 11th day.

Observation

Spermatozoa samples were taken by chopping the cauda epididymis with scissors until fine in 2 ml of 0.9% NaCl, which had been heated using a hot plate to a temperature of 32-34^{o}C, and stirring until a homogeneous spermatozoa suspension was obtained. The temperature of the NaCl solution used was adjusted to match the temperature within the rat epididymis. This aligns with the findings of (Morrell, 2020), who stated that the optimal temperature for spermatozoa within the epididymis is 32–34°C, as higher temperatures can cause sperm denaturation. An NaCl solution helps maintain the pH stability of spermatozoa due to its isotonic nature relative to cellular fluid, thereby reducing sperm density and preserving viability during a specific storage period (Ncama *et al*., 2025). Sperm suspension stored in 0.9% NaCl remains viable for up to 60 minutes (Zhang *et al.*, 2021).

Spermatozoa motility

Observation of spermatozoa motility was aimed at determining the ability of spermatozoa to move due to zinc supplementation. The observations followed the procedure carried out by (Hozyen *et al.*, 2023), the spermatozoa suspension was drawn up to the 0.5 mark using a hemocytometer pipette, followed by the addition of 0.9% NaCl solution up to the 1.01 mark. The mixture was then shaken for 2–3 minutes and loaded into an improved Neubauer counting chamber. Calculations were carried out on 5 boxes while the counting chamber was in a diagonal direction using a 400x magnification microscope connected to a computer. The field of view was systematically examined, and motility was

calculated for 100 spermatozoa cells using a hand counter. Spermatozoa motility is divided into 4 categories based on the type of movement, namely fast progressive, slow progressive, nonprogressive and nonmotile. The motility percentage is calculated based on the calculation formula of (Luthfi and Noor 2021) as follows:

$$Abnormal\ spermatozoa\ motility = \frac{B + A}{A+B+C+D} \text{ x } 100\ \%$$

Description:
A = Fast progressive
B = Slow progressive
C = Nonprogressive
D = Nonmotile

Spermatozoa viability
Observation of spermatozoa viability is aimed at determining the ability of spermatozoa to survive after leaving the reproductive organs. 50 µl of spermatozoa suspension was dropped on a glass slide and 25 µl of Eosin-nigrosin dye was added, and then covered with a cover glass. Spermatozoa viability was observed using a microscope with 400x magnification connected to a computer. The visual field was checked systematically, and viability was calculated for 100 spermatozoa cells using a hand counter. Eosin dye is used because it has acidic properties, so it can detect alkaline or dead spermatozoa. If eosin is met with live sperm, eosin will not enter because the sperm membrane is the same acid as eosin, so they repel each other (Haugen *et al.*, 2023). The percentage of spermatozoa viability is calculated using the Harlis *et al*., (2026) formula, as follows:

$$Dead\ spermatozoa = \frac{B}{A+B} \text{ x } 100\ \%$$

Description:
A = Live spermatozoa
B = Dead spermatozoa

Spermatozoa morphology
One drop of spermatozoa suspension is placed on a glass object, then a smear is made by sliding another glass object over it at a 450 angle and only sliding once. After that, it was dried for 15 minutes, then fixed with 96% methanol for 5 minutes and stained with 20% Giemsa solution for 30 minutes, rinsed with distilled water. Calculations were carried out under a microscope with 1,000X magnification connected to a computer. The field of view was examined systematically, and the morphology was counted on 100 spermatozoa cells using a hand counter. The percentage of spermatozoa morphology is calculated based on the formula according to Harlis *et al*., (2026) as follows:

$$Abnormal\ spermatozoa\ morphology = \frac{B}{A+B} \text{ x } 100\ \%$$

Description:
A = Normal morphology
B = Abnormal morphology

Ethical Consideration: This ethical permission was obtained from the Chair of the Health Research Ethics Commission, Institute for Research and Community Service, Halu Oleo University, with Number 2545b/UN.29.20.1.2/PG/2025

Data analysis
Data analysis in this study used the Statistical Product Solution (SPSS) version 26.0 application. Data on abnormal spermatozoa parameters were analyzed using one-way Analysis of Variance (ANOVA) and the Honest Significant Difference (HSD) further test ($P<0.05$) confidence level to see which treatment gave different effects.

RESULTS AND DISCUSSION

The quality of spermatozoa is an indicator used to determine the fertility level of a male individual. Spermatozoa quality parameters include motility, viability and morphology of spermatozoa.

Spermatozoa motility:
Motility observations in this study were aimed at seeing the motility of normal and abnormal spermatozoa. The results of observations of spermatozoa motility can be seen in Table 1

Table 1. Average percentage of mouse spermatozoa motility between treatments.

Treatment	Spermatozoa Motility (%)	
	Normal ± SD	Abnormal ± SD
K+	79.33±4.55[a]	20.17±2.48[a]
K-	36.17±2.93[b]	66.00±2.45[b]
P1	65.67±7.28[c]	38.67±3.08[c]
P2	56.67±3.72[d]	45.00±1.67[d]
P3	69.50±3.02[c]	38.00±3.63[c]

Note: Numbers followed by different letters indicate significant differences based on honest real difference test with a 95% confidence level.

Based on Table 1, it can be seen that administering a single extract of Moringa and tomato fruit to each treatment had an effect on spermatozoa motility. Based on analysis using the ANOVA test at a 95% confidence level, administration of Moringa leaf extract and tomato fruit has an effect on normal spermatozoa motility. Based on the results of the HSD follow-up test at a confidence level of 95%, it shows that there is a significant difference between treatments regarding the motility of normal and abnormal spermatozoa. In normal motility,

K+ treatment had the highest mean (79.33), followed by P1 (65.67), P2 (56.67), and P3 (69.50), while K- had the lowest mean (36.16). Abnormal motility, K- has the highest mean (66.00), followed by P2 (45), P1 (38.67), P3 (38.00), and K+ has the lowest mean (20.16).

Treatment of *M. oleifera* leaf extract and *S. lycopersicum* fruit at all doses had a normal motility percentage above 50%. According to Hoek *et al*. (2022), quality spermatozoa are spermatozoa that have normal spermatozoa motility above 50%. Based on these values, it shows that administration of *M. oleifera* leaf extract and *S. lycopersicum* fruit reduces abnormal spermatozoa. The treatment that had the highest preventive effect was a combination of *M. oleifera* leaf extract 600 mg/kg BW and *S. lycopersicum* fruit 60 mg/kg BW (P3) with a normal motility percentage of 79.33%.

Spermatozoa viability

This spermatozoa viability parameter measures the integrity of the spermatozoa plasma membrane, which is maintained by the antioxidant activity of bioactive compounds, so that the number of live cells increases in the preventive treatment compared to the negative control. Dead spermatozoa have red heads after staining with eosin, while the heads of live spermatozoa are colorless (transparent). The results of the percentage of live spermatozoa and dead spermatozoa, which is the viability of spermatozoa, can be seen in Table 2

Table 2. Average percentage of rat spermatozoa viability between treatments.

Treatment	Spermatozoa Viability (%)	
	Life ± SD	Dead ± SD
K+	76.16±2.82[a]	23.50±1.87[a]
K-	36.00±2.78[b]	63.30±2.42[b]
P1	59.50±1.87[c]	40.33±2.80[c]
P2	56.00±3.40[d]	35.16±2.85[d]
P3	65.30±65.33[c]	31.67±2.85[d]

Note: Numbers followed by different letters indicate significant differences based on honest real difference test with a 95% confidence level.

Table 2 shows that administration of M. oleifera leaf extract and S. lycopersicum fruit has a significant effect on spermatozoa viability. The percentage of live spermatozoa in the K+ treatment had the highest mean (76.16), followed by P3 (65.30), P2 (56.00), and P1 (59.50), while K- had the lowest mean (36.00). Masae et al (2018) stated that spermatozoa viability is said to be normal if the percentage of live spermatozoa is more than 50%.

Spermatozoa morphology

The average percentage of spermatozoa morphology is shown in Table 3.

Table 3. Average percentage of mouse spermatozoa morphology between treatments.

Treatment	Spermatozoa morphology (%)	
	Normal ± SD	Abnormal ± SD
K+	77,67±2,58[a]	22,33±2,58[a]
K-	40,83±2,31[b]	59,16±2,31[b]
P1	61,67±2,16[c]	38,33±2,16[c]
P2	55,50±1,87[d]	44,50±1,87[d]
P3	67,67±2,16[e]	32,20±2,38[e]

Note: Numbers followed by different letters indicate significant differences based on honest real difference test with a 95% confidence level

The spermatozoa abnormalities found in this study can be seen in Figure 2.

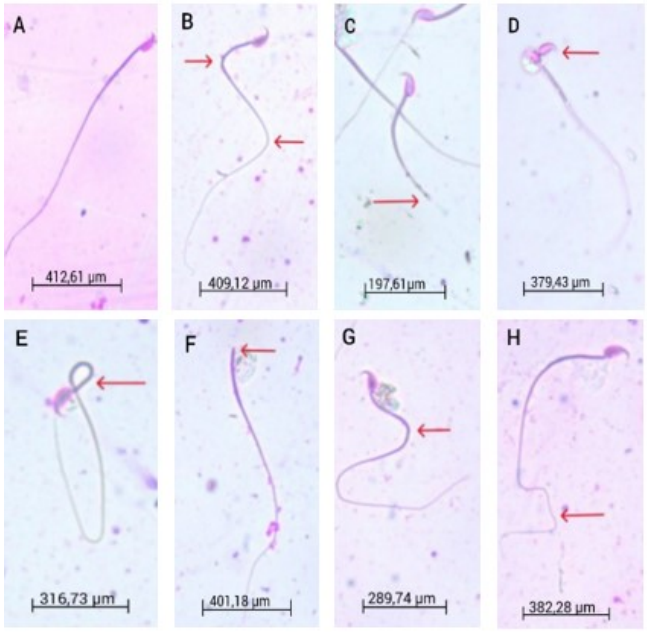


Figure 2. Various forms of abnormal spermatozoa were found after treatment. A Normal spermatozoa; B. Zigzag body spermatozoa; C. Spermatozoa without tails; D. Double-headed spermatozoa. E.Spermatozoa body rolled up wavy tail; F. Head body spermatozoa; G. Wavy spermatozoa; H. Wavy tail spermatozoa.

Based on Figure 2, the morphological abnormalities of spermatozoa that were most often found in this study were secondary abnormalities, which were characterized by changes in the middle and tail parts of the spermatozoa. These abnormalities include a curved or circular middle part, as well as a tail that is bent, coiled, or at an angle. According to Boguenet *et al.*, (2021), secondary abnormalities are related to disturbances in the activity of the ATPase enzyme in the spermatozoa cell membrane, especially in the middle part and tail. Decreased ATPase activity can inhibit ATP production, causing damage to the middle piece structure and increasing the risk of tail abnormalities, such as bent or severed tails. Antioxidant compounds in the form of polyphenols and lycopene contained in extracts of *M. oleifera* leaves and tomatoes (*S. lycopersicum*) play a role in protecting spermatozoa cell membranes from oxidative stress, so that ATPase activity can be

maintained and the incidence of secondary abnormalities in the middle pieces and tails of spermatozoa can be minimized (Caroppo *et al.*, 2022). The preventive effect of polyphenol & flavonoid compounds from Moringa leaf extract (*M. oleifera*) and lycopene & vitamin C compounds from S. lycopersiccum fruit, which complement each other in warding off ROS (Reactive Oxygen Species), provides a protective effect on mitochondria and stabilizes spermatozoa cell membranes.

Discussion

Antioxidant compounds such as polyphenols, flavonoids, and vitamin C in extracts of Moringa leaves (*M. oleifera*) and tomatoes (*S.lycopersicum*) protect sperm cells, especially those that require high energy to move. Sperm cells require a lot of energy through mitochondrial respiration in the middle, but oxidative stress can damage the osmotic balance of mitochondria and sperm tails. This compound keeps the mitochondrial osmotic balance stable, so that energy production runs smoothly and sperm motility increases (Haugen *et al.*, 2023). Kowalczyk (2022), giving antioxidants in the form of ethanol extract of green spinach leaves at a dose of 147–588 mg/Kg BW, was proven to be able to reduce oxidative stress due to an increase in Reactive Oxygen Species (ROS), thereby contributing to improving the quality of spermatozoa, especially increasing sperm cell motility.

The percentage of normal motility of spermatozoa showed an increase along with the treatment dose, with the highest value found at the highest treatment dose, namely at P3, followed by P1, P2, K+, and the lowest K-. This increase in spermatozoa quality indicates a synergistic preventive effect from bioactive compounds such as polyphenols, flavonoids, lycopene, and vitamin C which, act as antioxidants.

Based on Table 2, it shows that administration of M. oleifera leaf extract and S. lycopersicum fruit is effective in increasing the percentage of live spermatozoa and reducing the percentage of dead spermatozoa. The most effective treatment was P3 with a percentage of live spermatozoa of 67.35%. This increase in percentage is caused by the protection of epididymis function by antioxidant compounds and compounds such as polyphenols and lycopene suppress oxidative stress in *M.oleifera* leaf extract and *S.lycopersicum* fruit. so that spermatozoa maturation in the epididymis runs optimally, membrane integrity is maintained, and cell viability increases (Capra *et al.*, 2023).

Polyphenols and lycopene inhibit paracetamol-induced apoptosis of testosterone-producing cells, so that spermatozoa maturation in the epididymis runs smoothly and viability increases (Santi *et al*., 2023). Induction of a toxic dose of paracetamol of 500 mg/kgBW causes an increase in free radicals through the formation of NAPQI metabolites, which have an impact on damage to reproductive organs (Shyam and Sabina, 2024). The percentage value of normal spermatozoa motility increases with the administration of preventive treatment doses, the highest at P3, followed by P1, P2, K+, and the lowest at K-. Increasing the optimal dose at P3 shows that the combination of antioxidant compounds works in mutual support in maintaining the integrity of the sperm membrane. This effect occurs without causing resistance, resulting in viability Sperm reach, maximum levels by protecting cells from damage and preventing cell death.

Observation of the percentage of spermatozoa viability shows that live spermatozoa have an intact plasma membrane so they have the ability to prevent dye molecules from entering the cell because they are considered foreign substances by the cell's selectively permeable properties. Live spermatozoa have heads that are colorless or transparent (Tavs, 2020). Live spermatozoa have an intact plasma membrane and function optimally, so that eosin-Y dye molecules cannot penetrate into the cell. The sodium-potassium pump mechanism (Na+/K+-ATPase) in live spermatozoa actively regulates ion gradients and maintains osmotic homeostasis, preventing the entry of dye, so that live spermatozoa appear colorless under a microscope when staining with eosin-nigrosine, especially in the head and tail regions. The integrity of this membrane is maintained by the antioxidant effect of Moringa leaf extract (*M. oleifera*) and tomato fruit (*S. lycopersicum*), which prevents lipid peroxidation due to paracetamol (Abdelhameed *et al*., 2025)

Based on Table 3, it shows that the K− treatment had the highest percentage of abnormal spermatozoa morphology, namely 59.16%. These results indicate that without preventive treatment, paracetamol induction can cause increased damage to spermatozoa structure. This condition is related to oxidative stress which increases the formation of free radicals, thereby damaging cell membranes, heads, and tails of spermatozoa. According to Setiawan *et al.* (2024) paracetamol at a toxic dose of 500 mg/kg BW can increase the formation of NAPQI which triggers oxidative stress and an increase in free radicals, thus potentially reducing the function and quality of the reproductive system. K+ treatment showed the lowest percentage of ab normal spermatozoa morphology, namely 22.33%. These results indicate that the combination of the two extracts provided provides an optimal preventive effect on spermatozoa structure. This effect comes from antioxidant activity, which can suppress lipid peroxidation and protect the integrity of spermatozoa cell membranes. According to Abdelhameed *et al*. (2025), Moringa leaf extract is known to contain high antioxidant content, such as flavonoids, polyphenols, and tannins, which play a role in suppressing oxidative stress, so that it can protect spermatozoa cell membranes and improve the quality and motility of spermatozoa through a mechanism of reducing the formation of Reactive Oxygen Species (ROS).

Administration of M. oleifera leaf extract and S lycopersicum fruit has an effect on increasing the normal morphology of spermatozoa. This is indicated by the percentage of normal morphology in the treatment which is above 50%. According to the World Health Organization (WHO), the percentage of normal spermatozoa morphology ≥ 40% is still categorized as within the normal range (Notari *et al.*, 2024). The results showed that all extract and control treatments had a percentage of normal spermatozoa morphology that was above the WHO reference value. Each treatment has a different percentage of normal spermatozoa morphology. The negative control showed a lower percentage of normal morphology compared to treatments P1, P2, and P3. The most dominant treatment that increased normal spermatozoa morphology was P3 treatment, with a percentage of normal morphology of 67.77%.

The increase in normal morphology of spermatozoa is related to the content of bioactive compounds, namely flavonoids, polyphenols and lycopene contained in the extract of *M. oleifera* leaves at a dose of 600 mg/kg BW and *S. lycopersicum* fruit at a dose of 60 mg/kg BW. Setiawan *et al.* (2024) reported that induction of toxic doses of paracetamol caused an increase in free radicals through the formation of NAPQI metabolites, which had an impact on reproductive organ damage. Polyphenolic compounds and lycopene in M. oleifera leaf extract and *S. lycopersicum* fruit protect proteins and ions in the spermatozoa membrane, so that the energy phosphorylation process runs smoothly and prevents morphological abnormalities (Sadek *et al.*, 2021). Stimulation of FSH (Follicle Stimulating Hormone) secretion, namely optimal FSH production, increases the resistance of spermatogonia, accelerates spermatogenesis, and produces more spermatozoa with normal morphology (Wang *et al.*, 2022).

Abnormal morphology of spermatozoa consists of primary abnormalities and secondary abnormalities. Primary abnormalities occur due to impaired spermatogenesis in the seminiferous tubules, while secondary abnormalities occur when spermatozoa have left the seminiferous tubules and during their journey to the epididymis (Barrachina *et al.*, 2022). Primary abnormalities in spermatozoa can include large heads because they still contain residual cytoplasmic fluid, double heads, folded middle part of the spermatozoa and double tails (Kleshchev *et al*., 2023). Secondary abnormalities can include a head that is separated from the tail, a body and tail without a head, a thickened body and a curved tail.

CONCLUSIONS

The conclusion of the research is that *Moringa oleifera* leaf extract and *Solanum lycopersicum* fruit have a preventive effect on spermatozoa because they can increase the percentage of motility, survival ability, and normal morphology of spermatozoa in male rats after being induced by toxic substances, namely, toxic doses of paracetamol. It is hoped that the results of this research will provide a scientific contribution to society in improving sperm quality against free radicals, which can disrupt reproductive health.

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