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Original Research Article

Effect of Zinc (Zn) and Rhizome Extract of Red Ginger (*Zingiber officinale* var. Rubrum) on Testicular Function of Mice Induced with Depot-Medroxyprogesterone Acetate (DMPA)

Sutyarso[1], Nida Nurhanifah[1], Mohammad Kanedi[1]*

[1]Department of Biology, Faculty of Maths and Sciences, University of Lampung, Bandar Lampung, Indonesia

***Corresponding Author:** Mohammad Kanedi
Department of Biology, Faculty of Maths and Sciences, University of Lampung, Bandar Lampung, Indonesia

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Abstract: Zinc (Zn) and red ginger rhizome (*Zingiber officinale* var. Rubrum) extract have long been known to play a vital role in maintaining reproductive function and sexual health in males. However, their combined use in animals with DMPA-induced testicular dysfunction has not been widely studied. In this study twenty male Swiss Webster mice were grouped into four consisted of 5 mice each. Group 1 was given only distilled water (Control). Group 2 was given DMPA 1.25 mg/kg BW and distilled water (DMPA). Group 3 treated with DMPA 1.25 mg/kg BW and red ginger extract 400 mg/kg BW and Zn 1 mg/kg BW (DZE 400). Group 4 treated with DMPA 1.25 mg/kg BW and red ginger extract 600 mg/kg BW and Zn 1 mg/kg BW (DZE 600). After 35 days of treatment all animal were dissected for their testis to assess the intratesticular hormone level, to count the number of spermatozoa, to see the motility, morphology, and viability of the sperms. The results showed that red ginger extract at 400 mg/kg BW and zinc 1 mg/kg BW significantly increase testosterone levels, sperm count, motility, morphology, dan viability. In conclusion, zinc elements and red ginger extract, at a certain dose, have the potential as ingredients to overcome testicular dysfunction.

Keywords: Red Ginger, *Zingiber Officinale*, DMPA, Testicular Function, Intratesticular Testosterone.

1. INTRODUCTION

Many previous studies have shown that the active ingredients in red ginger (Zingiber officinale) have positive effects on sexual and reproductive health in males. In human red ginger is known to be able to restore male fertility because it is known to reduce sperm DNA fragmentation (SDF) in infertile men (Hosseini *et al*., 2016).

Experiments on rats show that ginger extract increased serum total testosterones, sperm viability and motility Khaki *et al*., 2009). Another experiment done by Ebeye *et al.*, (2025) found that ginger extract increase in the testicular and epididymal organ weights, sperm count, motility, viability, epididymal volume, luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone (Ebeye *et al*, 2025). In the white rat exposed to monosodium glutamate ginger extract maintained the epitelial and diameter of seminiferous tubules (Renald *et al*., 2024).

Apart from its effect on fertility, red ginger extract is also known to have a positive effect on male sexual behaviour. On the albino rats ginger oil microemulsion (G-ME) at a dose of 37.5 mg/kg BW significantly improve sexual behaviour, relative testes weight, and serum testosterone (Aziz *et al*., 2025) In a diabetic rat models red ginger ethanol extract at a dose of 400mg/kg BW found to increase libido (Hadibrata *et al*, 2025).

Next, regarding zinc. This element is also known to have a positive influence on male reproduction and sexual function. The zinc deficiency is impaired spermatogenesis because of reduced testosterone production, increased oxidative stress and apoptosis. (Omu *et al.*, 2025). It was also found that zinc deficiency can affects the testicular function directly

through its effect on the testicular steroidogenesis (Hafiez *et al*., 2016). Zinc sulfate treatment also revealed to improve sperm motility and survival indices (Mousavi Esfiokhi *et al*., 2023).

Similar to red ginger, zinc is also known to influence male sexual behaviour. On rat zinc therapy improves sexual competence by increasing the testosterone levels (Dissanayake *et al*., 2009). Co-administration of zinc on rats improves lead-induced sexual and erectile dysfunction by suppressing oxidative stress and upregulating testosterone (Besong *et al.*, 2023)

Separately research on the effect of red ginger and zinc on male reproduction has been carried out, but mixing these two ingredients as one treatment is still relatively rarely done. Our previous study showed that a combination of ginger extract and zinc given to mice increase serum testosterone levels, sperm count, percentage of sperm viability and motility (Sutyarso *et al.*, 2016).

In this study, we used zinc (Zn) and red ginger extract in male mice induced with DMPA (Depot Medroxyprogesterone Acetate) to determine its effect on testicular function especially on intratesticular testosterone levels, spermatozoa quantity and quality.

2. MATERIALS AND METHODS

2.1. Zinc and Plant Material Extraction

The zinc elements used in this study is a zinc sulphate heptahydrate ($ZnSO_4.7H_20$) from Merck. Rhizome of red ginger (Zingiber officinale var rubrum.) was obtained from local farmers in Sub Burb of Bandar Lampung, Lampung Province, Indonesia. The rhizomes were cut into thin slices and then sun-dried at noon. The dried rhizomes then milled into powder and macerated in ethanol 95% hours at room temperature and filtered. The macerate finally evaporated to obtain thick extract of red ginger for further use.

2.2. Experimental Animals and Treatment

Twenty male Swiss Webster mice weighing 25-30g, aged 10-12 weeks, obtained from Lampung Veterinary Centre, were placed in cages (one animal per cage) at room temperature, 12/12 hours light/dark cycle, and given water and food ad libitum. By using a completely randomized design, the animals were grouped into four groups consisted of 5 mice each. Each mouse in Group 1 was given only distilled water (Control). Group 2 was given DMPA 1.25 mg/kg BW and distilled water (DMPA). Group 3 treated with DMPA 1.25 mg/kg BW and red ginger extract 400 mg/kg BW and Zn 1 mg/kg BW (DZE 400). Group 4 treated with DMPA 1.25 mg/kg BW and red ginger extract 600 mg/kg BW and Zn 1 mg/kg BW (DZE 600). The distilled water was administered for 35 days, while red ginger extract and zinc were given for 33 days. The DMPA was given once in a week for two weeks.

2.3 Mouse Surgery

On the 36th day after treatment, the mice were anesthetized in jar containing cotton that has been soaked in chloroform. After the animals became lethargic, they were dissected to remove their testes and cauda epididymis.

2.4 Intratesticular Testosterone Assay

The testicular samples were weighed and then added to a 1g:1ml PBS (Phosphate Buffer Saline) solution. The testicular samples then crushed manually and crushed further using an ultrasonic device. The samples were centrifuged at 6688 rpm and RCF of 5000 x g for 15 minutes at 100°C. The supernatant was collected and stored in an Eppendorf at -80°C until the testosterone levels measurements were taken. The measurement of intratesticular testosterone levels were made by thawing the samples that are then treated using an ELISA kit. The sample that has been prepared with the ELISA kit is then read with an ELISA reader.

2.5 Spermatozoa Assay

To determine the quantity and quality of mouse spermatozoa, the cauda epididymis was placed in a Petri dish containing 1 ml of 0.9% physiological saline at 37-40°C. It was then cut, sorted with small scissors, and stirred with a glass stirrer to obtain a homogeneous spermatozoa suspension. The resulting spermatozoa suspension was used for sperm quantity and quality analysis.

Sperm Count:

Spermatozoa suspension of 10 µL diluted with 1 mL of physiological saline (0.9% NaCl) was taken and placed in a counting chamber (improved Neubauer haemocytometer) then observed under a light microscope at 20x10 magnification. The number of spermatozoa was calculated as number of cells/mL = number of spermatozoa (in 5 boxes) x 10^6.

Spermatozoa Motility:

Sperm motility was examined using a microscope at 20x10 magnification. Sperm motility was categorized into (A) motile and (B) non-motile spermatozoa. The percentage of motility is calculated based on the formula:

$$\frac{A}{A+B}x100\%$$

Spermatozoa Morphology:

One drop of spermatozoa suspension is placed on a glass slide, a smear is prepared by sliding another glass slide over it, air-dried, fixed with 70% methanol for 5 minutes, stained with Giemsa solution for 30 minutes, and rinsed with running water. Observation performed under a microscope at 40x10 magnification. A (abnormal spermatozoa), B (normal spermatozoa). Percentage of spermatozoa morphology determined using formula:

$$\frac{A}{A+B}x100\%$$

Spermatozoa Viability:

One drop of spermatozoa suspension is placed on a glass slide and mixed with eosin-nigrosin solution. Then, it is covered with a coverslip. Sperm viability is observed under a microscope at 40x10 magnification to determine the clear spermatozoa (A) and the coloured spermatozoa (B). The percent of viability was counted using formula:

$$\frac{A}{A+B}x100\% .$$

2.6. Statistical Analysis

The data, presented as Mean ± SE, was analysed with a one-way ANOVA. Means were separated using Least Significance Difference (LSD) test. All of the statistics that applied are programmed in IBM SPSS (Version 22).

3. RESULT AND DISCUSSION

The effects of the treatments given to mice in this study are summarized in Table 1. Based on the data in the table it is clear that DMPA was significantly reduce all testicular parameters of the mice including intratesticular testosterone, sperm count, sperm motility, sperm morphology, and sperm viability.

Table 1: Effects of zinc and red ginger extract on intratesticular testosterone levels and sperm quantity and quality of mice

Treatments	Testosterone (ng/ml)	Sperm quantity and quality			
		Count (10^6/ml)	Motility (%)	Morphology (%)	Viability (%)
Control	64,8 ± 9,95^{a}	33,4±5,25^{a}	73,02±1,79^{a}	90,3±4,51^{a}	79,02±5,74^{a}
DMPA	8,07±0,52^{c}	12,92±3,23^{b}	13,63±3,77^{b}	33,58±3,42^{c}	23,88±2,05^{b}
DZE 400	26,07±3,46^{b}	24,48±3,29^{a}	63,84±2,97^{a}	59,4±2,94^{b}	65,24±4,87^{a}
DZE 600	13,45±1,96^{bc}	16,52±2,41^{ab}	22,18±3,41^{b}	36,34±2,52^{c}	31,36±6,70^{b}
Values are presented as Mean ± SE; Values followed by the same superscript are not different statistically by LSD post hoc test.					

The types of spermatozoa abnormalities that we observed in this study included small heads, no heads, broken necks, no tails, broken tails, and curved tails (Figure 1). The type of spermatozoa damage in this study is consistent with the description of sperm damage in animals suffering from reproductive and fertility dysfunction (Pelzman and Sandlow, 2024). This result is also in line with the research results of Ilyas (2014) which stated that administering DMPA and *Momordica charantia* extract can reduce sperm count, motility, viability, and sperm morphology (Ilyas, 2014).

Administration of red ginger extract and zinc has been proven to be able to repair the damage caused by DMPA. The most effective dose of red ginger extract and zinc to improve testicular function was 400 mg/kg BW of extract and 1 mg/kg BW of zinc. Extract doses of 600 mg/kg BW and zinc 1 mg/kg BW have not been able to overcome all testicular function parameters damages due to the effect of DMPA.

In general, the results of this study are in line with our previous research that administering red ginger water extract and zinc can improve testicular function, especially in reducing sperm morphological abnormalities (Sutyarso *et al*., 2016). These results are also in line with the research findings of Xue *et al*., (2022) that administration of ginger extract combined with zinc can prevent reproductive dysfunction caused by lead by inhibiting apoptosis due to oxidative damage and inflammation (Xue *et al*., 2022).

One of the factors that causes zinc and red ginger extract to have protective properties for testicular function is their antioxidant properties. Zinc as mentioned by Powell (2000) has strong antioxidative properties that make its capable of cellular injury (Powell, 2000; Raiz *et al*., 2011).

Zinc exerts its antioxidant effect via two acute mechanisms, one of which is the stabilization of protein sulfhydryl against oxidation. Therefore, zinc also plays a role in activating antioxidant proteins and enzymes. (Jarosz, 2017). The protective effect of zinc on testicular function is also due to the role of this element in increasing sulfhydryl levels (Sankako *et al.,* 2012).

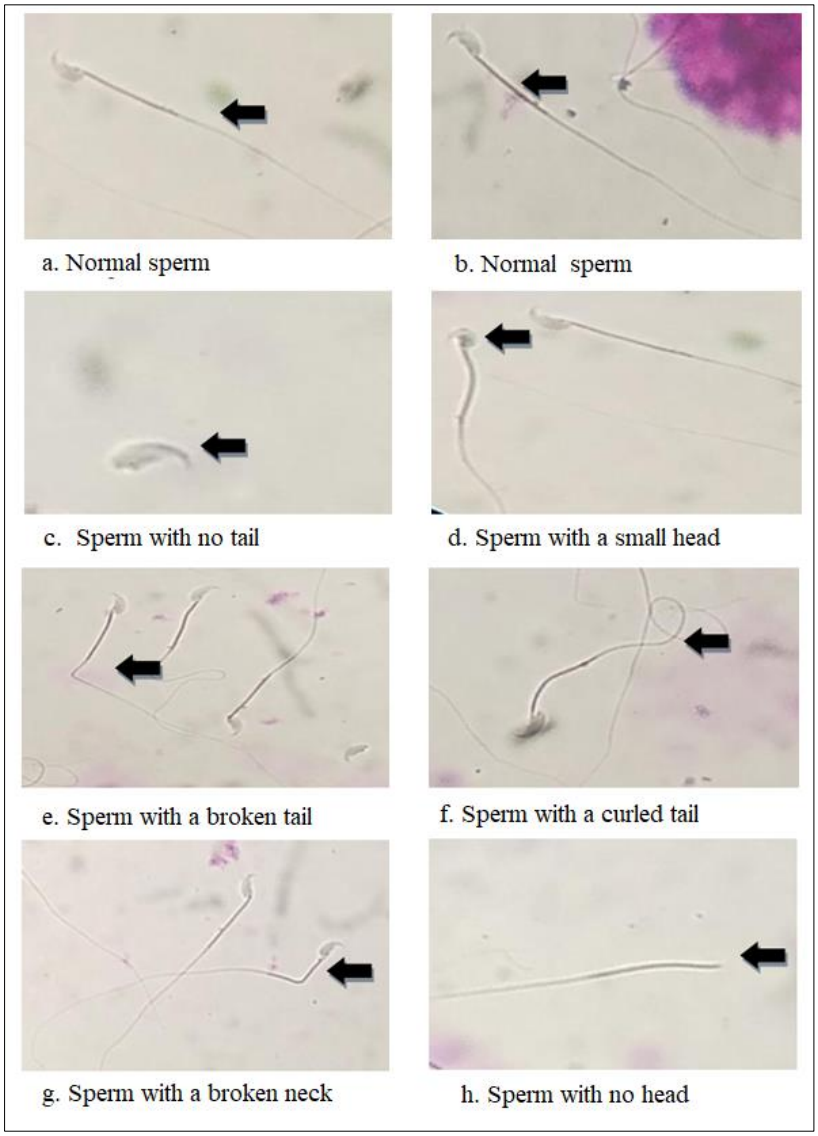

Figure 1: Photograph of normal and abnormal spermatozoa observed in this study

Furthermore, ginger and its active components have been shown to have strong antioxidant properties and the potential to reduce inflammation (Ayustaningwarno *et al*., 2024). Because of its antioxidant properties, ginger extract can prevent and treat factors that cause oxidative stress in the testes and impairing the latter's ability to produce viable spermatozoa (Aitken *et al.,* 2013)

The mechanism of ginger extract in protecting testicular function mainly by enhancing luteinizing hormone (LH) production, increasing cholesterol, reducing oxidative stress and lipid peroxidation in the testes, enhancing activity of the antioxidant enzymes, and recycling testosterone receptors [Saber *et al.,* 2011).

4. CONCLUSION

Administration of 400 mg/kg BW red ginger extract and 1 mg/kg BW zinc has been shown to increase intratesticular hormone levels, increase the number, motility, normal morphology, and viability of spermatozoa in DMPA-

induced mice. Meanwhile, at a dose of 600 mg/kg BW ginger extract and 1 mg/kg BW zinc failed to repair the damage caused by DMPA. Therefore, it can be concluded that red ginger extract and zinc elements have the potential as ingredients to overcome testicular dysfunction at certain doses. However, the most effective dose of red ginger extract and zinc still needs further research.

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Conflict of Interest: Authors declare there is no conflict of interest.

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