



**Research Article**

**The effect of black cummin oil (*Nigella sativa*)  
on the reproduction of male Wistar rats**

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**ABSTRACT**

*Nigella sativa* oil is antioxidant compound has the effect that serves to prevent cellular damage. The effect of *Nigella sativa* oil in the motility and total count of spermatozoa wistar rats. Experimental research with the design of post test only control group design. Wistar rats consists of 24 head and divided into 4 groups consist of control and treatment group. The control group was distilled. The first treatment was of *Nigella sativa* oil everyday as much with dose 150 mg/kgbw, 250 mg/kgbw, and 350 mg/kgbw, for 16 days. . Statistic test for motility and count of sperm wistar rats use the Kruskal-Wallis followed by Mann Whitney test. Avarage value of motility in the control ( $21.67 \pm 9.832$ ) its lower than treatment. In dose 350 mg/kgbw its highest ( $52.33 \pm 13.292$ ) compare in the treatment 250 mg/kgbw ( $40.67 \pm 17.512$ ) and 150 mg/kgbw ( $30.67 \pm 8.165$ ). avarage value of count sperm in the control ( $130.83 \pm 41.877$ ) its lower than treatments. In dose 350 mg/kgbw its highest ( $199.67 \pm 23.480$ ) compare in the treatment 250 mg/kgbw ( $187.50 \pm 74.538$ ) and 150 mg/kgbw ( $140.83 \pm 32.568$ ). Administration of *Nigella sativa* oil occur to enhancement motility and number of spermatozoa wistar rats.

**Keywords:** *Nigella sativa*, motility, count of spermatozoa

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**INTRODUCTION**

The male reproductive system consists of the hypothalamus, anterior pituitary and testes. Disorders of each of these organs can cause infertility and reduce secondary sexual characteristics. Disorders of the testes can affect the Leydig cells and the Sertoli cells which play a role in the production of testosterone and the maturation of spermatozoa. Damage to the male reproductive system can lead to infertility (Roychoudhury et al. 2021). Infertility is a condition in which the wife does not get pregnant after more than one year of regular sexual intercourse without using a contraceptive method. The main cause of male infertility in developing countries is spermatogenesis (Anshuka et al. 2021). Male infertility is caused by low sperm motility (asthenozoospermia), the number of spermatozoa (oligoszoospermia), sperm morphological abnormalities (teratozoospermia) (Zhang et al. 2021). *Nigella sativa* has compounds that have antioxidant effects. The physiological function of antioxidants is

to prevent damage to cellular components due to free radicals (Salima et al. 2021). The production of free radicals occurs continuously in all cells as part of normal cell function. If there is excessive free radical production, it will cause oxidative stress (Marija et al. 2021). Oxidative stress plays an important role in the pathophysiology of various diseases (Zhang et al. 2021). Based on the results of research, *Nigella sativa* is useful as an antioxidant, anticancer, anti-inflammatory, anti-cholesterol, antihistamine, antibiotic, immunomodulator and so on. The oil content of *nigella sativa* consists of alpha pinen (13.75%), limonene (2.55%), p-cymen (43.58%), carvacrol (2.53%), tyoquinone (1.65%), Linoleic acid (25%), oleic acid (12%), palmitic acid (2.84%), stearic acid (0.34%), linoleic acid (0.35%) and meristid acid (0.35%), carbohydrates, fats, vitamins, mineral elements, protein, unsaturated fatty acids such as linoleic, oleic and essential fatty acids such as phospholipids, phosphatidylcholine, phosphatidyletholamin, phosphatidyl serine, calcium, iron and potassium (Sanaz et al. 2021).

Research of *Nigella sativa* was able to improve the quality of spermatozoa in several experimental animals. This is evidenced by research which states that there is an increase in testosterone levels, sperm motility, seminal vesicle weight, and spermatozoa quality in rats with hyperlipedemia (Kristian et al. 2021). This study aims to determine the distribution of *nigella sativa* oil on the motility and number of spermatozoa in male Wistar rats.

## METHODS

This research is a laboratory experimental study. The research design used experimental design. Randomized posttest only control group design using experimental animals as research objects. The research process lasted for 40 days starting January 27 to March 6, 2021 using 24 Wistar rats. Motility and sperm count examination were carried out at the laboratory Biology Universitas Pattimura. The experimental animals were adapted to cages for 1 week and divided into 4 groups consisting of a control group of 6 male wistar rats given distilled water and a treatment group of 3 groups which were given oil *nigella sativa* for 16 days. Consisting of group 1 with a dose of 150 mg/kgbw, group 2 dose of 250 mg/kgbw, and group 3 dose of 350 mg/kgbw. The results of the examination and calculation of research data were analyzed using a Program SPSS 20 for windows. The significance value in this study is that the analyzed variables have a p value <0.05.

## RESULTS AND DISCUSSION

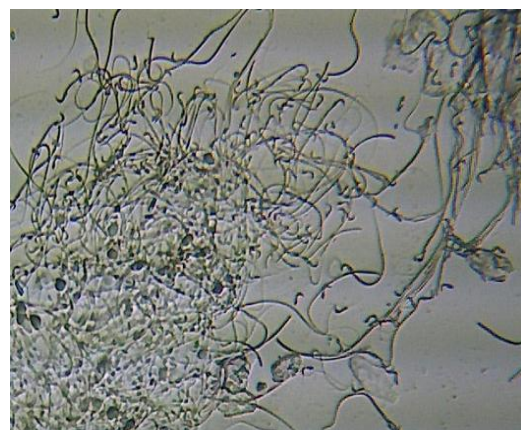
### Observation of motility and number of spermatozoa

After the treatment of *nigella sativa* oil for 16 days, male wistar rats were take analysis of motility and number of spermatozoa. Wistar rat were anesthetized with ether then their defferent ducts were taken by clamping the ampulla of the defferent ducts and cauda epididymis, then cut approximately 1 cm from the cauda epididymis clamp and defferent ducts, after being cut they were sorted and collected for sperm fluid then mixed with EBSS as much as 2 milli microns. examination of spermatozoa with 100 X magnification at 18-24<sup>o</sup> C room temperature, the examination was carried out with 3 fields of view. Observation of Wistar rat spermatozoa can be seen in Figure 1.

**Figure 1.** Observation of Wistar rat spermatozoa (100 x Magnification)



Motility of spermatozoa



the number of spermatozoa

The results showed of the nigella sativa oil to male Wistar rats was able to increase the motility and number of spermatozoa. The results of the analysis can be seen in table 1.

**Table 1.** Results of analysis motility of spermatozoa

Treatment	Mean ± Std. Deviation
Control	21.67±9.832
150 mg/kgbw	30.67±8.165
250 mg/ kgbw	40.67±17.512
350 mg/ kgbw	52.33±13.292

Average value of motility spermatozoa in the control group was 21.67±9.832) lower than the three treatments of nigella sativa oil. In treatment 350 mg/kgbw is much higher (52.33±13,292) compared to treatment 250 mg/kgbw (40.67±17,512) and treatment 150 mg/kgbw (30.67±8,165). This study shows that giving negella sativa oil at a dose of 350 mg/kgbw for 16 days can increase motility of spermatozoa in male Wistar rats. To determine the difference in motility of spermatozoa in each treatment of nigella sativa oil for 16 days, a Mann-Whitney test was performed. The results of the Mann-Whitney test analysis can be seen in table 2.

**Table 2.** Mann-Whitney of motility Spermatozoa

Group	Treatment	P value
Control	150 mg/ kgbw	0.115
	250 mg/ kgbw	0.137
	350 mg/kgbw*	0.021
150 mg/kgbw	250 mg/ kgbw	0.104
	350 mg/ kgbw*	0.002
250 mg/ kgbw	350 mg/ kgbw*	0.005

Significant P < 0.05

Based on the table, it can be seen that in the control with a dose of 350 mg/kgbw (p = 0.021) there was a significant difference (p < 0.05), while the control was with a dose of 150 mg/kgbw (p = 0.115) and 250 mg/kgbw (0.137). ) there is no significant difference. In dose 150 mg/kgbw with the dose 350 mg/kgbw (p = 0.002) and the dose 250 mg/kgbw with a dose of 350 mg/kgbw (p = 0.005) there was a significant difference.

**Table 3.** Results of analysis number of spermatozoa

Treatment	Rata-Rata ± Std. Deviasi
Control	130.83±41.877
150 mg/kgbw	140.83±32.568
250 mg/ kgbw	187.50±74.538
350 mg/ kgbw	199.67±23.480

Average number of spermatozoa in the control (130.83±41.877) was much lower than that in the treatment of nigella sativa oil. The average higher number of spermatozoa was found in the dose 350 mg/kgbw (199.67±23.480), followed by the dose 250 mg/kgbw (187.50±74.538) and the dose 150 mg/kgbw (140.83±32.568). This study indicated that the provision of nigella sativa oil increased the number of spermatozoa. The treatment of nigella sativa oil to rats for 16 days was then analyzed using the Mann-Whitney test. The results of the Mann-Whitney test analysis can be seen in table 4.

**Table 4.** Mann-Whitney test for the number of spermatozoa

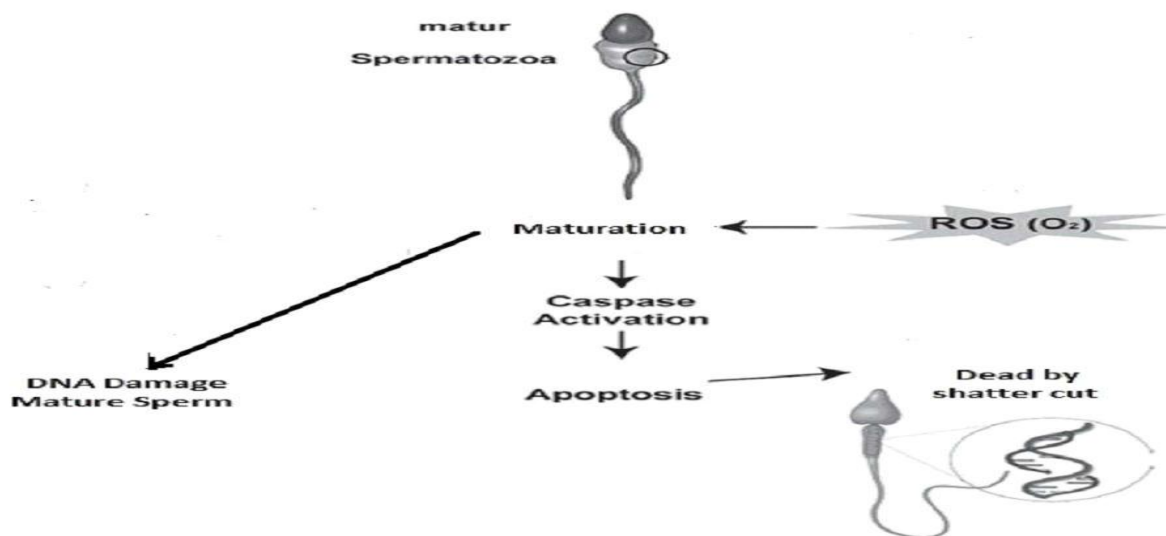
Group	Dose	P value
Control	150 mg/ kgbw	0.215
	250 mg/ kgbw	0.337
	350 mg/ kgbw*	0.019
150 mg/kgbw	250 mg/ kgbw	0.404
	350 mg/ kgbw*	0.009
250 mg/kgbw	350 mg/ kgbw*	0.012

Based on the table above, it can be seen that in the control with a dose of 350 mg/kgbw ( $p = 0.019$ ) there was a significant difference ( $p < 0.05$ ), control with a dose of 150 mg/kgbw ( $p = 0.215$ ) and a dose 250 mg/kgbw (0.337).) there is not significant difference. In the dose 150 mg/kgBB with the dose 350 mg/kgbw ( $p = 0.009$ ) and the dose 250 mg/kgbw with a dose 350 mg/kgbw ( $p = 0.012$ ) there was a significant difference.

## DISCUSSION

The low number of motility and number of spermatozoa in the control group compared to the treatment of nigella sativa oil was due to the cytochrome P450s enzymes present in the animal body biologically causing the production of ROS (Seungjin et al. 2020). Excessive production of ROS causes oxidative stress which affects antioxidant enzymes which consist of enzymatic components of the body's defense system, such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione-S-transferase (GST) (Alugoju et al. 2015). In wistar rats that were not given nigella sativa oil caused an increase in ROS compounds. ROS plays a role in oxidative stress such as superoxide ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ), peroxy ( $ROO^-$ ), hydroxyl ( $OH^-$ ) and nitrogen oxide derivatives such as ( $NO^-$ ), peroxynitrate ( $ONOO^-$ ) (Elisa et al. 2021). Increased ROS in the tissue that produces spermatozoa can cause abnormal spermatozoa. In addition, free radicals also stimulate apoptosis by involving a series of events that occur both in the cytoplasm and in the cell nucleus (Ismail et al. 2020). In the cytoplasm it activates caspase and in the cell nucleus chromatin condensation occurs, the nuclear sheath breaks and DNA fragmentation occurs for subsequent apoptosis (Ralf & Kristian, 2020). Oxidative stress and apoptosis are involved in mediating the DNA damage of spermatozoa. Intact spermatozoa DNA is an important condition for fertilization. Spermatozoa DNA damage is closely related to sperm function and male infertility. Spermatozoa in infertile men have been shown to have abnormal sperm (Ralf & Kristian, 2020).

**Figure 2.** Role of ROS on Spermatozoa DNA Damage (Ralf & Kristian, 2020).



The body has its own mechanism that can neutralize the dangers of free radicals with an antioxidant system, but if free radicals exceed the amount of antioxidants in the body it will cause cell damage (Zhang et al. 2021). The results of nigella sativa oil to male wistar rats in this study were higher than that of the control group. This is because Nigella sativa oil has antioxidant and immunomodulatory effects where the physiological function is to prevent cellular damage due to free radicals and can repair cellular damage. Antioxidants play a role by catalyzing free radicals by the enzymes SOD catalase and peroxidase, binding pro-oxidants and cleaning ROS in the body's metabolism. Nigella sativa oil has thymoquinone compounds that act as immunomodulators and antioxidants by activating antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione-S-transferase (GST) (Mehdi et al. 2020).

## CONCLUSION

The administration of *Nigella sativa* oil at a dose of 350 mg/kgbw for 16 days increased the motility and the number of spermatozoa was higher than the dose of 250 mg/kgbw and 150 mg/kgbw in male Wistar rats.

## ACKNOWLEDGMENT

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