

Original article

**The effect of watermelon (*Citrullus lanatus*) rind ethanolic extract on the number of Leydig, Sertoli, and spermatogenic cells of rat (*Rattus norvegicus*) exposed to heat**

**Pengaruh pemberian ekstrak etanol kulit semangka (*Citrullus lanatus*) terhadap jumlah sel Leydig, sel Sertoli dan sel spermatogenik tikus (*Rattus norvegicus*) yang dipapar panas**

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### ABSTRACT

High temperatures lead to oxidative stress, which can disturb spermatogenesis process. Watermelon (*Citrullus lanatus*) peel contain antioxidant expected to compensate oxidative stress due to heat stress exposure. This study aimed to determine the effect of watermelon rind ethanolic extract on the number of Leydig, Sertoli, and spermatogenic cells of rats exposed to heat (40°C). Twenty rats (*Rattus norvegicus*) were divided randomly into five groups. In the control group (T0) rats were not exposed to heat nor given the watermelon rind extract. T1, T2, T3, and T4 groups were exposed to heat for an hour daily and orally given placebo (1% Na CMC), 100, 200, and 400 mg/kg BW of watermelon rind extract (in 1% Na CMC). Rats were treated for 52 days, and sacrificed for the testicle collection. Hematoxylin-eosin stained histological slides were prepared for the examination of Leydig, Sertoli and spermatogenic cells. The results showed no significant difference ( $p > 0.05$ ) in the average number of Leydig cells in rats among groups. The number of Sertoli cells and spermatogenic cells of rats exposed to heat (T1) was lower than those of the normal rats (T0 group). The dose of watermelon rind ethanolic extract at 200 mg/kg BW (T3 group) and 400 mg/kg BW (T4) increased ( $p < 0.05$ ) the number of Sertoli and spermatogenic cells. It could be concluded that a dose of 400mg/kg BW of watermelon rind ethanolic extract maintained the number of Leydig cells, Sertoli cells, and spermatogenic cells of rats exposed to heat.

**Keywords:** heat, watermelon rind ethanolic extract, Leydig cell, Sertoli cell, spermatogenic cell

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### INTRODUCTION

Spermatogenesis is a temperature-sensitive process that needs a temperature of 2-7°C below core body temperature to ensure the production of normal spermatozoa. Heat stress exposure to mammals' testicles impairs spermatogenesis and

leads to low spermatozoa quality in the ejaculate (Aldahhan and Stanton, 2021). The spermatogenesis process occurs in the lumen of the seminiferous tubules. Spermatogenesis involves Sertoli cells (nourishing spermatogonia cells) to develop into primary spermatocytes and Leydig cells (produce testosterone) for the

maturation of spermatozoa cells. The process of spermatozoa formation is influenced by several factors such as hormones, nutrition, and temperature. Failure to maintain testicular temperature will cause spermatozoa damage. Exposure to hot temperatures can lead to heat stress, a condition where there is an increase in the environment's unfavorable temperatures. Continuous heat stress conditions will lead to oxidative stress. There is an imbalance in the number of antioxidants and free radicals in the body (Werdhasari, 2014).

Free radicals can be neutralized physiologically by an endogenous antioxidant; unfortunately, environmental factors, such as heat stress, can decrease this capacity. Therefore, antioxidant treatment is expected to fight free radical-induced oxidative stress and improve spermatogenesis (Reynertson, 2007; Asadi *et al.*, 2017). One of the exogenous antioxidants is Lycopene. Lycopene is one of the best antioxidants found in natural foods. Lycopene's mechanism in preventing free radicals is by donating electrons to free radical compounds to become stable (Durairajanayagam *et al.*, 2014). Watermelon peel was reported to contain 48.8% of lycopene. Lycopene is commonly found in the white part of watermelon rind (Erhirhie and Ekene, 2013; Rahman, 2013). Many people avoid the white part of watermelon rind because it has an unpleasant taste (Filla, 2013). In this study, the watermelon rind extract was used to see its effect on the number of Leydig cells, Sertoli cells, and spermatogenic cells of rats exposed to hot temperatures.

## MATERIAL AND METHODS

This study used 20 rats (*Rattus novergicus*) aged 3 to 4 months weighing about 20 grams obtained from the Veterinaria Farma Center Surabaya. The study was carried out with the approval of the Study Ethics commission No. 247/HRECC.FORM/IX/2018. Heat exposure was performed in a 50 x 30 x 40 cm chamber with heating lamp and thermostat to keep the temperature at a steady 40°C as earlier study (Rohmah *et al.*, 2018).

The watermelon rind extraction in ethanol was carried out at the Pharmacology Laboratory,

Division of Basic Veterinary Medicine, Faculty of Veterinary Medicine, Universitas Airlangga.

## Treatment of rats

Twenty rats were divided randomly into five treatment groups equally. The control group (T0) rats were not exposed to heat nor given the watermelon rind ethanolic extract. The T1, T2, T3, and T4 groups were exposed to heat for an hour daily and administered orally through a stomach tube with placebo (1% Na CMC), 100, 200, and 400 mg/kg BW of watermelon rind ethanolic extract dissolved in 1% Na CMC. Rats were treated for 52 days, and sacrificed the next day. Testicles were collected for the preparation of hematoxylin-eosin stained histological slides.

## Measurement of variables

Measurement of the number of Leydig, Sertoli, and spermatogenic cells was performed on a 400x magnification light microscope (Nikon E200) equipped with Optilab Viewer Software Version 2.2. The number of Leydig cells was counted randomly on inter seminiferous tubules space. Meanwhile, the number of Sertoli cells and spermatogenic cells were counted in seminiferous tubules. The observations were conducted randomly with five replicates for the averaged of those variables' values.

## Data analysis

Data on the number of Leydig cells, Sertoli cells, and white rat spermatogenic cells were then analyzed statistically using the Analysis of Variance (ANOVA) test and if there were significant differences ( $p < 0.05$ ) followed by multiple distance tests (Duncan) using Statistics Product and Service Solution (SPSS) version 21.

## RESULTS

There were no significant difference ( $p > 0.05$ ) on the average number of Leydig cells in rats among groups. However, the average number of Sertoli cells and spermatogenic cells of rats exposed to heat (T1) was lower than those of the normal rats (T0 group). Administration of watermelon rind extract at the dose of 100 mg/kg BW (T2 group) resulted in a non significantly different ( $p > 0.05$ ) average number of Sertoli

cells and spermatogenic cells to the T1 group. Meanwhile, the dose of watermelon rind extract starting from 200 mg/kg BW (T3 group) increased ( $p < 0.05$ ) the average number of Sertoli cells and spermatogenic cells than those

of the T1 and T2 group. The dose of 400 mg/kg BW (T4) resulted in a higher average number of Sertoli and spermatogenic cells than those of the T3 group but not significantly different ( $p > 0.05$ ) to those of the normal rats (Table 1).

**Table 1** The number of Leydig cells, Sertoli cells, and spermatogenic cells of rats exposed to heat and administered with ethanolic watermelon rind extract

	Leydig cells	Sertoli cells	spermatogenic cells
T0	12,50 ± 5,07 <sup>a</sup>	10,00 ± 0,82 <sup>bc</sup>	73,00 ± 5,35 <sup>c</sup>
T1	8,75 ± 1,89 <sup>a</sup>	6,50 ± 0,58 <sup>a</sup>	51,00 ± 5,35 <sup>a</sup>
T2	7,75 ± 1,50 <sup>a</sup>	7,25 ± 0,50 <sup>a</sup>	54,25 ± 3,86 <sup>a</sup>
T3	14,00 ± 5,60 <sup>a</sup>	9,25 ± 0,50 <sup>b</sup>	65,50 ± 3,42 <sup>b</sup>
T4	13,75 ± 1,70 <sup>a</sup>	10,50 ± 0,58 <sup>c</sup>	75,00 ± 1,41 <sup>c</sup>

Different superscripts in the same column were significantly different ( $p < 0.05$ ); T0: control group rats, received placebo; T1-T4: rats were exposed to heat for an hour daily for 52 days; T2, T3, and T4: rats were administered 100, 200, and 400 mg/kg BW of watermelon rind ethanolic extract orally for 52 days; heat exposure was performed in a heating chamber at 40 °C

## DISCUSSION

Testis is a temperature-sensitive organ that needs to maintain a temperature of 2-7°C below core body temperature to ensure the production of normal spermatozoa. Failure to maintain the testicular temperature in mammals impairs spermatogenesis and leads to low spermatozoa counts, poor spermatozoa motility, and abnormal spermatozoa morphology in the ejaculate (Aldahhan and Stanton, 2021).

Exposure to heat only (T1), or its combination with different watermelon rind extract doses (T2-T4), did not affect Leydig cells' number. In the testicle, Leydig cells are not susceptible to heat stress. The cells that are susceptible to heat stress are spermatocytes and spermatids (Hansen, 2009). The effect of heat stress varies on each cell. The Leydig cells receive the effects of heat stress indirectly (Setchel, 1998). This finding was consistent with previous reports that Scrotal hyperthermia (43°C for 30 min once daily for six consecutive days) induces Leydig cell damage (Kanter *et al.*, 2009). Meanwhile, the other study reported that testicular exposure to 43°C for 30min leads to Leydig cell hyperplasia and a reduction in testosterone biosynthesis in adult rat testes (Li *et al.*, 2015). The Leydig cell response to heat stress likely occurs after seminiferous tubule damage (Aldahhan and Stanton, 2021).

The number of Sertoli cells and spermatogenic cells of rats exposed to heat (T1) was lower than those of the normal rats (T0 group). Heat stress is a condition where there is an increase in environmental temperature to affect the body directly. Heat stress increased animal's body temperature above 38 °C (Bunglavan and Chaturvedi, 2013). In the T1 group, rats were exposed to heat without watermelon rind ethanolic extract administration. The watermelon rind contains antioxidants (Erhirhie and Ekene, 2013; Fila *et al.*, 2013). The lowest number of Sertoli cells and spermatogenic cells of rats in the T1 group was due to the absence of exogenous antioxidants to protect the Sertoli cells from heat stress-free radicals. In the process of spermatogenesis, Sertoli cells function to nourish spermatogonia cells to develop into primary spermatocytes (Wang *et al.*, 2019). Heat stress exposure to the number of Sertoli cells will cause oxidative stress, a condition where free radicals exceed the body's number of antioxidants (Umar *et al.*, 2015). The low amount of antioxidants in the body cannot protect Sertoli cells from heat stress in the form of free radicals. Oxidative stress will cause a decrease in the synthesis of androgen binding protein (ABP). This protein functions to bind testosterone to the process of spermatozoa cell maturation. The decline in the ABP synthesis process causing this protein is to fail to

bind testosterone, interfering with spermatogenesis. In addition to decreasing protein synthesis, oxidative stress also causes cell membrane damage. Oxidative stress damages cell membranes by deactivating cell membrane bonds with receptors or enzymes to interfere with the normal function of Sertoli cells (Murray *et al.*, 2003). The disruption of the Sertoli cell's normal function can affect the spermatogenesis process. Spermatogenesis depends on the normal function of Sertoli cells (Min Chen *et al.*, 2008). Sertoli cell damage can be seen in the positive controls treatment that has the lowest number of values. However, no clear evidence has yet emerged to confirm whether Sertoli cells are directly affected by testicular heating or germ cell damage (Aldahhan and Stanton, 2021).

Treatment using watermelon rind extract at the dose of 100 mg/kg BW (T2 group) resulted in a non-significant difference ( $p > 0.05$ ) average number of Sertoli cells and spermatogenic cells to the T1 group. Meanwhile, the dose of 200 mg/kg BW (T3 group) increased ( $p < 0.05$ ) the average number of Sertoli cells and spermatogenic cells than those of the T1 and T2 group. The watermelon rind ethanolic extract administration as an exogenous antioxidant was proved to be able to maintain the number of Sertoli cells. The Lycopene contained in watermelon rind ethanolic extract protects heat stress by reducing single oxygen and free radical deactivators (Sayuti and Yenrina, 2015).

The cells that are susceptible to heat stress are spermatocytes (Hansen, 2009). In the process of spermatogenesis, the mitotic phase, such as spermatocyte cells, is more susceptible to high temperatures (Shiraishi *et al.*, 2012). Heat stress exposure to spermatogenic cells will result in the same oxidative stress occurring in Sertoli cells. Oxidative stress causes damage to the spermatogenic cell membrane. Damage to the spermatogenic cell membrane caused by the number of antioxidants in the body cannot protect and maintain the bond between cell membranes. The spermatogenic cell structure is rich in polyunsaturated fatty acids. This structure makes spermatogenic cells very susceptible to oxidative stress damage. In normal situations, the mechanism of antioxidants present in reproductive tissue tends to reduce oxidative

stress and protect spermatogenic cells from oxidative damage (Sikka, 2001). Oxidative stress in spermatogenic cells will decrease enzyme bonds in spermatogenic cells so that it damages cell membranes, damage to cell membranes will interfere with physiology, and spermatozoa motility to interfere with fertilization (Rivlin *et al.*, 2004).

The dose of 400 mg/kg BW (T4) resulted in a higher average number of Sertoli cells and spermatogenic cells than those of the T3 group and not significantly different ( $p > 0.05$ ) to those of normal rats (Table 1). The testis is a temperature-sensitive organ that needs to be maintained 2-7°C below core body temperature to ensure the production of normal spermatozoa. Failure to maintain the testicular temperature in mammals impairs spermatogenesis and leads to low spermatozoa counts, poor spermatozoa motility, and abnormal spermatozoa morphology in the ejaculate. Therefore, there was a need for further studies to explore the role of watermelon rind ethanolic extract on spermatozoa viability, motility, and abnormality, also the levels of testosterone of rats exposed to heat stress.

## CONCLUSION

The administration of watermelon rind ethanolic extract maintained the number of Sertoli and spermatogenic cells of rats exposed to heat but did not affect Leydig cells' number.

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