Abstract

Obesity is an accumulation of excessive fat tissue in the body. Excessive fat tissue in the body lead to infertility by increased Reactive Oxygen Species (ROS) and decrease the hormone balance regulation, those things can be affected the process of spermatogenesis, especially sperm morphology. Rambutan peel was known as a source of antioxidants because it has phenolic compounds, so it has a protective effect on free radicals. The research purpose knew the effect of Rambutan Peel Extract (RPE) (*Nephelium lappaceum*) on abnormal sperm morphology of Wistar rats (*Rattus norvegicus*) percentage induced with a High-Fat Diet (HFD). This study uses True experimental post control group design for this research on 30 male Wistar rats. Samples were divided into 5 groups. Group 1: induced HFD only, Group 2: only given RPE at a dosage of 30 mg/kilogram Body Weight (kg BW), Group 3, 4, and 5: induced HFD and RPE at dosage of 15 mg/kg BW, 30 mg/kg BW, and 60 mg/kg BW feed using sonde. The data were analysed using ANOVA One Way. The result showed that RPE has decreased the abnormal sperm morphology of male Wistar rats at dose of 15 mg/kg BW. This is the first study that observe the effect of RPE administration to sperm morphology of obese and non-obese group of rats, with larger rats’ population, several doses of the RPE extract, and longer time to complete one cycle of rat spermatogenesis.

**Keywords:** Abnormal sperm morphology; High-fat diet; *Nephelium lappaceum*; Obesity; Rambutan peel extract; *Rattus norvegicus*

Abstrak

Obesitas diartikan sebagai akumulasi jaringan lemak berlebihan yang ada di dalam tubuh. Jaringan lemak yang berlebihan dapat menyebabkan ketidakseimbangan regulasi hormonal dan terbentuknya Reactive Oxygen Species (ROS). Kedua hal tersebut dapat mempengaruhi proses spermatogenesis sehingga dapat menyebabkan infertilitas, terutama pada morfologi sperma. Ekstrak Kulit Rambutan (EKR) diketahui memiliki efek sebagai antioksidan dikarenakan memiliki senyawa fenolik, senyawa tersebut dapat menangkal radikal bebas. Penelitian ini dilakukan untuk mengetahui pengaruh pemberian EKR (*Nephelium lappaceum*) terhadap persentase morfologi abnormal sperma tikus Wistar (*Rattus norvegicus*) yang diinduksi dengan Pakan Tinggi Lemak (PTL). Penelitian ini menggunakan desain True experimental post control group design pada 30 ekor tikus. Sampel dibagi menjadi 5 kelompok. Kelompok 1: hanya diberi PTL, Kelompok 2: hanya diberi EKR dengan dosis 30 mg/kilogram Berat Badan (kgBB), Kelompok 3, 4 dan 5: diinduksi dengan PTL dan EKR dengan dosis 15 mg/kgBB, 30 mg/kgBB, dan 60 mg/kgBB. Pemberian PTL dan EKR dilakukan menggunakan sonde. Data dianalisis menggunakan uji ANOVA One Way. Hasil penelitian menunjukkan bahwa EKR dapat menurunkan jumlah morfologi abnormal pada kelompok dengan induksi PTL, dengan dosis yang paling efektif 15 mg/kgBB. Studi ini adalah yang pertama dalam mencari tahu efek pemberian RPE tehadap morfologi spermatozoa pada kelompok tikus obesitas dan tidak obesitas, dengan populasi tikus yang lebih besar, beberapa dosis ekstrak RPE, dan waktu yang lebih lama agar dapat menyelesaikan satu siklus spermatogenesis tikus.

**Kata Kunci:** Ekstrak kulit rambutan; Morfologi abnormal sperma; *Nephelium lappaceum*; Obesitas; Pakan tinggi lemak; *Rattus norvegicus*
INTRODUCTION
Infertility is the condition of a couple inability with at least one year married, had regular sexual intercourse, without using contraception, but failed to achieve clinical pregnancy. Prawirohardjo states that 25% of infertility cases were caused by male factors, one of the factors is having a Body Mass Index (BMI) of more than 29 (Prawirohardjo, 2011). The normal range of BMI is 18.5–24.9 (Centers for Disease Control and Prevention (CDC), 2022). One of the major health problems around the world is obesity. A Person who is obese will have abnormal or excessive fat tissue and which led to health problems. World Health Organization (WHO) states that 13% of adults over the age of 18 are obese worldwide, and the prevalence of obesity has increased 3 times higher from 1975 to 2016 (World Health Organization (WHO), 2018). In Indonesia, obesity prevalence is getting higher every year, in 2018 it was 21.8% with the highest prevalence in North Sulawesi Province (Ministry of Health Republic of Indonesia, 2018).

In obese men, abnormal semen parameters are often found, including lack of semen concentration, reduced motility, and abnormal spermatozoa morphology. This happens because adipose tissue will release adipokines. The release of adipokines will increase free radicals in the body, while antioxidants in the body are unable to reduce them all. Obesity can increase the production of body free radical levels. The major type of free radicals stored in the body is Reactive Oxygen Species (ROS) (Dambal & Kumari, 2012). Free radicals are considered an important factors of spermatozoa DNA damage and affect spermatozoa parameters (Du Plessis et al., 2010). Men with obesity have abnormal hormone regulation caused by an increase in estrogen and a decrease in testosterone, which will disrupt the process of spermatogenesis (Bullen & Judge, 2015). A study by Vignera, stated that obese men had spermatozoa with less motility and abnormal morphology than men with normal weight (Vignera et al., 2012). The preliminary study has shown that obese rats have higher semen parameters compared to normal group rats, it shows lower abnormal semen parameters (Rompis et al., 2018).

Along with advances in science and technology, many plants have proven to be herbal medicines or alternative medicines that can cure various diseases. One of the plants that is believed to be efficacious and can be found throughout Indonesia is the rambutan. Several researchers found the fact that rambutan fruit peel which had been considered useless, turned out to have a strong antioxidant content (Thitilertdecha et al., 2008). This peel has phenolic which consisted of geraniin, corilagin, and ellagic acid especially geraniin which is very effective in counteracting free radicals. The methanol extract of rambutan fruit peel has antioxidant activity with radical scavenger mechanism (Thitilertdecha & Rakariyatham, 2011; Thitilertdecha et al., 2010). The Rambutan Peel Extract (RPE) contains polyphenolic compounds which are classified as strong antioxidants and are included as anti-obesity agents through the mechanism of increasing leptin efficiency in visceral fat of obese rats (Atho'illah et al., 2011). The experimental research was conducted to know abnormal sperm morphology as the parameter of quality of sperm in Wistar rats induced with a High-Fat diet (HFD) to determine the potential of RPE as an alternative to reduce ROS.

MATERIALS AND METHODS
Sample Preparation
The samples used in this study were red and ripe rambutan fruit peel strain Nephelium lappaceum obtained from Binjai, Medan, North Sumatra, Indonesia and the compound was identified using Phytochemical Test.

Extract Preparation
The process of extraction conducted by the Bogor Province Research on Spice and Medicinal Crops (BALITRO). An extraction method of this research used 10 kg rambutan fruit for obtaining 4 kg of rambutan peel. The rambutan peel that was obtained will be cleaned, then slice into small pieces. The sample was dried out under the sunlight. The dried rambutan peel is crushed by using a grinder to form a dry powder (simplicia). Out of 4 kg of rambutan peel, 1 kg is simplicial. One kg of simplicia was put into a stainless steel container to macerate with 70% ethanol in comparison to 1 kg of simplicia and 6 L of ethanol solvent. The maceration process begins with stirring for 3 hours
and is deposited for at least 24 hours. The obtained liquid is filtered using filter paper until becomes a filtrate. Then put the filtrate into evaporator glass to be evaporated using a rotatory evaporator at a temperature of 40 °C for 1.5 hours until thick extract is obtained.

**Extract Dosage**

Extracts processed at the Bogor Province Research on Spice and Medicinal Crops (BALITRO) were used separately depending on the dose group. RPE divided into 3 doses, such as 15 mg/kilogram Body Weight (kg BW), 30 mg/kg BW, and 60 mg/kg BW. The optimum dose in the previous study was 30 mg/kg BW because it was able to reduce leptin levels in visceral fat, body weight, and nutritional intake of obese rats (Lestari et al., 2014; Atho’illah et al., 2011). The extract was given to the rats through a feeding tube. A total of 30 rats were classified into 5 groups with different treatments: Group 1 (Negative Control Group): induced with HFD only, Group 2 (Treatment Control Group): only given RPE with a dosage of 30 mg/kg BW, Group 3, 4, 5 (Treatment Group), Group 3: Induced HFD + RPE with a dosage of 15 mg/kg BW, Group 4: Induced with HFD + RPE with a dosage of 30 mg/kg BW, Group 5: Induced with HFD + RPE with a dosage of 60 mg/kg BW.

**Phytochemical Test**

The phytochemical test aims to determine the potential metabolite compounds as an antioxidant. Table 1. shows the RPE contains tannins, alkaloids, saponins, flavonoids, and triterpenoids with the highest content of tannins and saponins, but does not contain a steroid. The RPE contains phenols and flavonoids compound, this component has a potential effect as an antioxidant. The phytochemical test of Rambutan Peel Extract can be seen in Table 1 below:

**Table 1. Phytochemical test of rambutan peel extract (Kusumaningrum, 2012)**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Rambutan peel extract phytochemical test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>+++</td>
</tr>
<tr>
<td>Tannins</td>
<td>+++</td>
</tr>
<tr>
<td>Saponins</td>
<td>+++</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>+</td>
</tr>
<tr>
<td>Phenolic</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>++</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
</tr>
</tbody>
</table>

Description: -= no active substance; += low content of active substances; +++= moderate active substance content; +++= high content of active substances

**Animal Experiment**

All the experiment animal protocols have been approved by The Health Research Ethics Committee of UPN Veteran Jakarta with ethical approval number: B/2216/X/2019/KEPK. Outbreeds of 30 male Wistar rats (Rattus novergicus) were obtained of the School of Life Sciences and Technology, Institut Teknologi Bandung (SITH-ITB). The Rats used in this research age between 9–12 weeks, weighing 150–250 g, and in good condition, obtained from the same breeding grounds. Maintained at the same place and time. The animals were kept at room temperature (23 ± 2 °C), with controlled light and dark cycles for 12 hours.

**Procedure**

True experimental post control group design was used in this study on 30 male Wistar rats. Rats were divided into 5 groups. Group 1 was only given HFD. Group 2 was only given RPE with a dosage 30 mg/kg BW. Groups 3, 4 and 5 were given HFD and RPE with a dosage of 15mg/kg BW, 30mg/kg BW and 60mg/kg BW.

The group of rats with the induction of an HFD, will be given an HFD every day for 21 days until the group of rats were obese, and for 52 days according to the time of spermatogenesis in rats and given water were provided ad libitum. An HFD consists of goat fat, sugar, pork belly,
margarine, egg yolk, and flour, given as much as 50 g/rat/day and 5 mL of cow’s brain. An HFD was given orally using gastric sonde every day during the study period. Rats were considered obese using the Lee Index calculation formula: $\text{Lee~Index} = \frac{3 \times \text{body weight (gram)}}{\text{naso-anal length (cm)}} \times 10^3$.

Rats were obese when the Lee Obesity index more than 300. An HFD were given every morning at 9 for 73 days, feed through a sonde (feeding tube). The group of rats that were not induced by HFD, will be given a standard diet, such as CP551 made by PT Charoen Pokphand Indonesia, the diet consists of 14% of water, 21–23% of protein, 5–8% of fat, 0.9–1.2% of calcium, 0.7–1% of phosphorus and gives water were provided ad libitum.

The study process started from rats acclimatization, grouping, and given treatment was secured at the Therapeutics and Pharmacology Laboratory, Faculty of Medicine, University of Padjadjaran, Bandung, West Java, Indonesia. All of the rats were adapted in laboratories for the first 7 days with a standard diet and ad libitum drinking water. After acclimatization, the rats were divided into 5 different groups. The HFD group was given an HFD from day 8 to day 80 to achieve obesity according to the Lee Index. On day 28, the treatment was continued by giving RPE with a dosage of 15 mg/kg BW, 30 mg/kg BW, 60 mg/kg BW. The RPE was given every day at 10 AM for 52 days to complete one cycle of the rats spermatogenesis.

The rats were terminated surgically to remove cauda epididymis after 81 days. Pieces of cauda epididymis were inserted into a watch glass used in chemistry dripped with 0,5 cc of 0,9% NaCl solution and stirred homogeneously. Liquid containing sperm is maintained at a temperature of around 20–33.5 °C. The morphology of Spermatozoa was identified using Giemsa staining and counted under a microscope to find 100 morphologies of spermatozoa, then divided into normal and abnormal morphological groups.

**Sperm Morphology Identification**
Morphology of spermatozoa was observed by taking 2 drops of liquid from arloji glass containing spermatozoa onto the object glass. Flatten using another glass object, and fixed with methanol to the entire surface of the specimen. The preparate was stained using Giemsa. According to the World Health Organization in calculating the abnormal morphology of spermatozoa can be seen under a microscope, then counting the number of sperm up to 100, and the group is separated between abnormal and normal spermatozoa (WHO, 2010).

**Statistical Test**
Statistical analysis was used to determine whether there is an effect of giving RPE on the morphology of rat spermatozoa induced by an HFD. This research were using international Business Machines-Statistical Product and Service Solutions version 24 (IBM-SPSS) Software for statistical analysis. For multiple comparisons, One-way ANOVA was used and post hoc analysis. P values 0.05 was considered significant.

**RESULTS**
The average body weight of rats before and after given HDF and normal diet for 21 days (Figure 1). The group of rats that had been given HFD was shown to reach a level of obesity compared to the body weight of the rats that were only given a normal diet. The data was collected after 81 days period of time. Figure 2 shows the effects of rambutan peel (*Nephelium lappaceum*) extract on the percentage of abnormal sperm morphology of Wistar rats (*Rattus novergicus*), it showed that the negative control group, a group who was only given HFD had the highest abnormal sperm morphology compared to the other groups with an average amount of 50.33%. Group 2 had the lowest abnormal sperm morphology compared to the others. The result from this study was the same as the previous study that obesity can lead to abnormal sperm morphology (Rompis et al., 2018).

The study shows that there were differences in the results of each group. In the comparison data, each group was found to have increased or decreased abnormalities in spermatozoa. Observation of morphology of spermatozoa, found the normal spermatozoa, spermatozoa with no
head, spermatozoa with no tails, spermatozoa with tails that are too short, spermatozoa with bent neck, and spermatozoa with bent tails (Figure 3).

Figure 1. The average body weight of rats. Group 1 (G1)= HFD; Group 2 (G2)= normal diet; Group 3 (G3)= HFD; Group 4 (G4)= HFD; Group 5 (G5)= HFD. The data was analyzed with statistical difference analysis.

Figure 2. Graphic of average abnormal sperm morphology. Group 1 (G1)= HFD only; Group 2 (G2)= RPE with a dosage of 30 mg/kg BW; Group 3 (G3)= HFD + RPE with a dosage of 15 mg/kg BW; Group 4 (G4)= HFD + RPE with a dosage of 30 mg/kg BW; Group 5 (G5)= HFD + RPE with a dosage of 60 mg/kg BW (data analyzed with statistical difference analysis).

Figure 3. The picture of abnormal spermatozoa morphology in Wistar rats with obesity, are normal spermatozoa (a), spermatozoa with no tails (b), spermatozoa with bent neck (c), spermatozoa with tails that are too short (d), spermatozoa with bent tails (e), and spermatozoa without head (f) (all the picture was taken in experimental research in obese rats using a microscope magnification of 400x).
DISCUSSION

Obesity is currently a global health problem caused by an unhealthy lifestyle aside from that people do not regulate their diet, it can also be caused by a lack of physical activity. People who are obese have more adipose tissue. This adipose tissue can produce adipokines, which are independent factors in the formation of systemic Reactive Oxygen Species (ROS). In obese people, oxidative stress can also be formed due to an imbalance of endogenous peroxides and antioxidants in the body, so it accumulates in the body (Susantiningsih, 2015). ROS that formed in the body will stimulate the formation of pro-inflammatory cytokines, which produce Tumor Necrosis Factor alpha (TNF-α) and Interleukin-6 (IL-6), due to the formation of these 2 cytokines, the body will assume the low-grade inflammation is developing (Rahmawati, 2014; Susantiningsih & Mustofa, 2018).

Obesity is associated with alteration in spermatozoa parameters. Spermatozoa are susceptible to Reactive Oxygen Species (ROS) because their membranes are formed from Poly Unsaturated Fatty Acids (PUFA) and have single electrons that are easily bound to ROS, the increase in ROS can lead to gonadal cell degeneration. Therefore, the process of spermatogenesis can be disrupted. Damaged sertoli cells will interfere with the spermiogenesis process, while damaged Leydig cells will disrupt the synthetic testosterone hormone and affect the spermatogenesis process (Sharma et al., 2013). Obesity can lead to hormonal disorders. Obese men will have aromatase enzymes in their bodies, these enzymes can convert testosterone to estrogen, so the body will suffer from hyperestrogenemia & hypotestosteronemia, both of these disorders will provide negative feedback to the hypothalamus and pituitary to reduce GnRH, FSH, and LH secretion which causes hormonal imbalance that interferes with the process of spermatogenesis (Hammoud et al., 2008).

Obese people are also associated with a decrease in Sex Hormone Binding Globulin (SHBG), this is due to an increase in insulin in the blood for a long time which can lead into insulin resistance. They will suffer from type 2 diabetes (DM type 2). Someone who suffers from type 2 diabetes will decrease the synthesis of SHBG in the liver and affect the disruption of spermatogenesis. Obese men have more fat distribution in the upper thigh and scrotal area, this can cause an increase temperature in the scrotal area which can lead to disruption of the spermatogenesis process (Du Plessis et al., 2010). A Study by Rompis comparing the treatment group (diet-induced obesity, rats were given 22 g of food every day for 50 days) with the control group (given a normal diet of 20 g of food every day for 50 days) shows that the control group had a lower number of abnormal sperm morphology by 28.4%, compared to the treatment group (diet induced obesity), shows the results of having a higher number of abnormal sperm morphology by 44.8%. According to previous research, it can be concluded that obese men have higher sperm morphology abnormalities (Rompis et al., 2018). The experimental research shows the increase of abnormal sperm morphology in obese rats caused by adipose tissue because as the adipose tissue increase, the amount of leptin in the body increases so it can be damage the body, especially for spermatogenesis process.

RPE has polyphenols, which act as antioxidants. Rambutan peel has a higher antioxidant compared with other parts of rambutan fruit, because it contains phenolic compounds such as geraniin, corilagin, and ellagic acid compounds. Geraniin compounds are effective in counteracting free radicals (Thitilertdecha et al., 2008; 2010; Thitilertdecha & Rakariyatham, 2011). Tjandra revealed that RPE contains compounds such as steroids, phenols and flavonoids which are antioxidants (Tjandra et al., 2011). Statistical results from these studies with a significance value of P <0.05 showed that there was a significant difference in the group of rats induced by an HFD and given RPE. In the treatment group, the results of the group induced by a HFD and RPE at a dose of 15mg/kg BW had the least amount of abnormal morphology by 40.83% compared to other treatment groups. This explains that RPE is proven as an antioxidant. The antioxidants contained in RPE in the treatment group aimed to protect spermatozoa from exposure to free radicals due to an HFD.

The group with HFD and RPE with a dosage of 30mg/kg BW and 60mg/kg BW had more abnormal spermatozoa morphology, 43.67%, and 42.50%, this might be caused by the higher
extract used it can be toxic to the body and form oxidative stress that can interfere the process of spermatogenesis. This relevant to a study by Mahmudah, this study proves the results that polyphenol compounds in rambutan fruit extract can form covalent bonds with liver glutathione and make these compounds have fewer toxic characteristics. At an extract dose of 60 mg/kg BW, liver glutathione is unable to form covalent bonds, thus making these compounds toxic to the body and can form oxidative stress (Mahmudah et al., 2018). In a study conducted by a group of rats that only induced HFD, it had the highest number of abnormal morphologies. This proves that the group of rats induced by an HFD will lead to obesity, form ROS inside the body and cause hormonal imbalance in rats and disrupt the process of spermatogenesis. A group that was only induced by an HFD, the number of abnormal spermatozoa morphology was 50.33%. The group that was not induced by an HFD, only given RPE at a dosage of 30mg/kg BW had the least an abnormal morphology, which is 33.67%, this explains that RPE has an antioxidant effect that could help the body to produce more endogenous antioxidants, so it can be stabilize free radicals in the body, and does not affect spermatogenesis.

A study by Sipahutar et al. (2020) revealed that RPE can increase the total sperm count. In this study, using RPE with the best dose of 15mg/kg BW had a sperm count of 17,021.66. This is related to flavonoid compounds as antioxidants found in RPE. Flavonoids act as electron donors for the H⁺ group and reduce reactive peroxidative chains to produce more stable oxidant radicals. This research concluded that the best efficacy for increasing sperm count was RPE at a dose 15 mg/kg BW. This shows different results from other high doses 30 mg/kg BW and 60 mg/kg BW (Sipahutar et al., 2020). This proves the HFD group with an extract dosage of 15 mg/kg BW had the lowest abnormal sperm morphology. In a previous study, the application of RPE at a dose of 30 mg/kg BW tends to reduce body weight, leptin levels in visceral fat, and nutrient intake in obese rats.

A research by Lestari clarified that RPE can weight gain, especially in rats that were given extract at a dose of 30 mg/kg BW could reduce the weight gain of rats by 25%. Aside from offering a benefit as a powerful antioxidant, this proves that rambutan fruit extract also has benefits as an anti-obesity agent (Lestari et al., 2014). A study by Hammoud in 2008 revealed that a person who initially obese and experienced hormonal disorders due to obesity, if the person's weight returned to normal or norm weight, can cause hormonal responses that return to normal, for example, increased SHBG and testosterone levels (Hammoud et al., 2008). Rahmawati 2014 also stated that decreasing body fat can improve oxidation markers and increase antioxidant activity, which had previously been damaged by obesity (Rahmawati, 2014).

CONCLUSION AND SUGGESTIONS

The conclusion of this study shows that obesity can increase abnormal sperm morphology in Wistar rats. Obesity causes increase in Reactive Oxygen Species (ROS) and an imbalance hormone regulation, affect the process of spermatogenesis. RPE can prevent toxic effects on spermatogenesis due to obesity because it has antioxidant compounds. RPE significantly reduced the average abnormal sperm morphology in obese rats with an effective dose of 15 mg/kg BW. In the future, it is necessary to develop phytochemical test which is developed from this rambutan peel extract for recovering sperm morphology.

ACKNOWLEDGEMENTS

We gratitude to the Therapeutics and Pharmacology Laboratory, Faculty of Medicine, University of Padjadjaran, Bandung, West Java; the School of Life Sciences and Technology, Institut Teknologi Bandung (SITH-ITB); and the Bogor Province Research on Spice and Medicinal Crops (BALITRO) for providing experimental animals of this research.

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