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The Number of Leydig Cells in High-Fat Diet-Fed Rats After Administration of Kepok Banana Peel Extract

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Abstract

The impact of free radicals on testicular Leydig cells can result in the disruption of male reproductive health. The present study aimed to evaluate the effects of a high-fat diet on Leydig cells and the potential of kepok banana peel extract as a medication to mitigate these effects. In total of 20 adult male Wistar rats were assigned to five groups and treated with standard feed (P1), high-fat feed (P2), high-fat feed with simvastatin (P3), and high-fat feed with banana peel extract at doses of 100 and 200 mg/kg BW (P4 and P5). After 60 days of treatment, the number of Leydig cells was determined using HE staining methods. Data were analyzed using an One Way ANOVA test. The results showed that the administration of 100 mg/kg BW of kepok banana peel extract (P4) was able to maintain the number of Leydig cells and counteract the negative effects of a high-fat diet. These findings suggest that kepok banana peel extract may have the potential as a herbal medicine for supporting male reproductive health.

Keywords: Kepok banana peel extract, Leydig cells, high-fat diet-fed, free radicals

Background

Herbal plants are natural products that are easy to obtain, inexpensive, and still being thoroughly studied for their potential as herbal medicines development of pharmaceuticals derived from herbal plants, including the banana plant (Imam *et al*., 2011; Kirtida *et al*., 2013). Consuming bananas into food ingredients such as chips, sale, fried foods, or consumed directly will produce waste in the form of banana peels. Banana peels have only ever been utilized in the past as animal feed or as junk (Berry Satria and Ahda, 2009). However, another promising finding on the banana peels confirms the existence of fiber, pectin, and phytosterol from the unripe pulp and peel (Emaga *et al*., 2008; Oliveira *et al*., 2008). In addition, another study has shown that banana peel extract can be used as an alternate component in pharmaceuticals (Andini, 2014). This has a strong correlation to the existence of secondary metabolites in banana peels that have high antioxidant activity (Pane, 2013).

The active compounds showing antioxidant activity in banana peel extract are of scientific interest. Previous studies have shown that banana peels' antioxidant activity was higher than other parts of the plant. Banana part antioxidant activity is only about 70% at a concentration of 50 mg/ml, compared to 94.25% for banana peel antioxidant activity at a concentration of 125 g/ml (Andini, 2014). This antioxidant activity effectively protects body cells from

the risks of oxidative stress, such as superoxide anions, hydroxyl, peroxyl, and alcoholic radicals (Alanko *et al*., 1999).

Numerous antioxidant compounds, including flavonoids and phenols, are present in the Kepok banana peel (*Musa acuminata*) (Baskar *et al*., 2011). This compound, which belongs to the category of anti-oxidation chain breakers, is an antioxidant that will manage and lessen lipid peroxidation in the body. Lipid peroxidation is a form of oxidative stress that can cause chain reactions that damage tissue organs (Priyanto, 2007; Gemayangsura, 2015). At the level of the testes, oxidative stress can disrupt Leydig cells capacity to produce steroids and the germinal epithelium's ability to differentiate the spermatozoa (Naughton *et al*., 2001). This condition can be triggered as a result of consuming a high-fat diet-fed. According to Zulkifli *et al*. (2020), a high-fat diet-fed contributes to oxidative stress that harms membrane permeability, disturbs sodium pump function in cell membranes, and leads to the production of ROS.

There is no research has revealed the role of kepok banana peel extract on the number of Leydig cells in rats with high-fat diet-fed. Therefore, this study was conducted to obtain information on the number of Leydig cells in high-fat diet-fed rats after administration of kepok banana peel extract.

Materials and method

This descriptive study used 20 adults male wistar rats (*Rattus norvegicus*), high-fat diet-fed, and kepok banana peel extract. Rat testicles were the source of the study's sample to inspect Leydig cell numbers by histological staining. Before starting the research investigation, the Veterinary Ethics Committee of the Universitas Syiah Kuala, Faculty of Veterinary Medicine provided ethical approval.

Treatment

For two weeks, every rat was acclimated to the new environment. The standard diet was administered to all test animals during the adaption phase. Before receiving treatment, male rats' specific age, weight, size, and clinical diagnosis were recorded. After two weeks, the rats were randomly assigned to one of five treatment groups: P1 (normal control) received only standard feed and CMC; P2 (negative control) received high-fat feed and CMC; P3 (positive control) received high-fat feed and simvastatin; P4 (high-fat feed and 100 mg/kg BW banana peel extract); and P5 (high-fat feed and 200 mg/kg BW banana peel extract). In high-fat feed, ingredients including wheat flour, used cooking oil, egg yolk, and beef fat are present. Simvastatin, CMC, and kepok banana peel extract were provided orally, and 7 g of standard and high-fat meals were given ad libitum each day.

Extraction of the kepok banana peel

The kepok banana peel used in this study was raw and green peel. The Pratama *et al*. (2018) modification was applied in this study's extraction process. Kepok banana peels weighing 7 kg were washed, sliced into small pieces (0.5 cm), and dried at room temperature on a tray for two weeks. The dried banana peels are blended to create banana peel powder. After being macerated in 70% ethanol for 72 hours, the banana peel powder was filtered. Until the liquid extract is obtained, this maceration process is repeated. A vacuum rotary evaporator was used to concentrate the extract at a temperature of 60 ^{o}C until a thick extract was produced. Onansanwo (2013) determined that the effective dose of kepok banana peel extract for rats is 100 mg/kg BW and 200 mg/kg BW when given orally.

The process of the Leydig cells examination

Leydig cell histology tissue was examined under a light microscope at 200 µm. The Topview program was installed on a computer, which was used to perform the examination. Five separate seminiferous tubules from each treatment sample were used in this examination.

Histological processing

The testicular tissue was removed from the rat's scrotum at the end of the

treatment. After being cleaned with 0.9% physiological NaCl solution, the testicles remained the next 24 hours submerged in 10% neutral buffered formalin (NBF). After being properly processed, the fixed tissues were moved from 10% NBF solution to 70% alcohol solution, embedded in paraffin, blocked, sectioned, and stained with the standard hematoxylin and eosin (HE) method (Kmiec, 2016). Examining the testicle stained using HE methods allowed researchers to quantify the presence of Leydig cells.

Data analysis

The statistical analyses for this study were carried out using the SPSS Statistics software package. In this study, any indicators of differences were discovered using one-way analysis of variance (ANOVA) and Duncan's test, with p 0.05 being regarded as statistically significant.

Results and Discussion

Leydig cells are polyhedral-shaped cells that surround the seminiferous tubules' blood channels (Wrobel and Bergmann, 2006), as shown in Figure 1. Free radicals or reactive oxygen species (ROS) can harm this cell (Colón, 2007). The synthesis of the hormone testosterone will decrease as the number of Leydig cells declines (Chen and Zirkin, 1999). According to some literature, the concentration of the hormone testosterone has a significant impact on how Leydig cells develop. The hormone testosterone is involved in preserving the morphology of immature sperm cells at a developmental stage and promoting them to become mature cells (Misro *et al*., 1993; Mendis-Handagama *et al*., 1998). Table 1 displays the findings of the examination of the Leydig cell count.

The average number of Leydig cells revealed that each treatment group differed. The K4 treatment group displayed the highest average number of Leydig cells compared to the other treatment groups. The P2 treatment group revealed the lowest amount of Leydig cells. ANOVA statistically analyzed the number of Leydig cells, and the results showed no significant differences between the P1, P2, and P3 treatment groups (P>0.05), as well as between the P3 and P4 treatment groups (P>0, 05). Compared to the P1, P2, and K5 treatment groups, the P4 treatment group showed a significant difference in the number of Leydig cells (P<0.05). This indicates that 100 mg/kg BW of kepok banana peel extract can enhance the quantity and reduce the impact of exposure to high-fat diets.

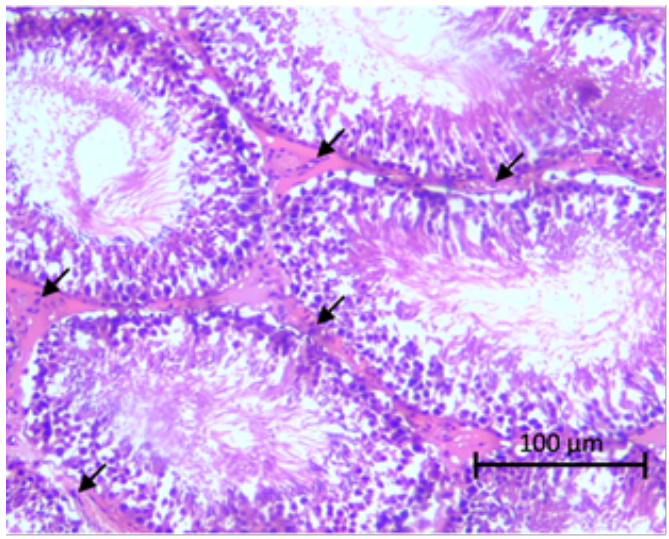

Figure 1. Testicular tissue in rat testes shown in histological. The Leydig cell distribution is shown by the black arrow.

Table 1. the average number of Leydig cells in each of the five treatment groups of white rats, as determined by Duncan's test.

Groups	Number of Leydig cells (Mean ± SD)
P1	16.60 ± 2.98^{bc}
P2	12.95 ± 4.71^{bc}
P3	17.55 ± 2.26^{ab}
P4	22.40 ± 4.14^{a}
P5	11.65 ± 2.16^{c}

Leydig cells are the main actors to control the testosterone hormone. Long-term high-fat diet-fed to rats results in hyperlipidemia, which reduces the number of Leydig cells (Widhiantara *et al*., 2018). Increased levels of ROS, disruption of the hypothalamus-pituitary axis, decreased LH secretion, and interference with the activation of Leydig cells to produce testosterone are all possible effects of hyperlipidemia (Bashandy, 2007; Kartiko and Siswanto, 2018).

Kepok bananas impact on Leydig cells

Previous researchers have thoroughly investigated the antioxidant properties of kepok banana peel. Kepok banana is a free radical scavenger with a very high antioxidant content (Singhal and Ratra, 2013). Several mechanisms related to the antioxidant activity of kepok banana peels as a free radical scavenger include chelation of transition metal ions, inhibition of free radicals produced by cells, and regeneration of α-tocopherol from α-tocopheroxyl radicals (Gemayangsura, 2015). However, the procedure to optimize the number of Leydig cells and protect them from free radicals is still unclear.

In the in vitro and in vivo case studies performed by Bae *et al*. (2017), natural herbal components as antioxidants can protect Leydig cells from harm caused by oxidative stress responses and boost the survivability of Leydig cells. Additionally, in terms of observations on testicular function, serum testosterone levels, oxidative stress, and cell apoptosis, these results were attained in studies with androgen-deprived rats.

A recent study by Zulkifli *et al*. (2020) showed that rats on a high-fat diet might have their testosterone levels normalized by administering banana peel extract at the correct dose. The study's findings also showed that excessively administering kepok banana extract could decrease the number of Leydig cells (shown by the P5 treatment group). These findings are in line with the results of Bast and Haenen (2002), which found that low testosterone production promotes Leydig cell degeneration due to decreased anterior pituitary secretion of the luteinizing hormone.

Conclusion and recommendation

According to the study's findings, maintaining the number of Leydig cells can help counteract the harmful effects of high-fat diets. This can be done by giving kepok banana peel extract at a dose of 100 mg/kg BW. Since the clinical implications of this activity are currently unknown, further research is needed before kepok banana peel extract can be for counteracting the harmful effects of high-fat meals on the body.

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