Identification of Active Compounds of Kepok Banana Peel and the Effect on Testosterone Concentration in Male Rats with High-Fat Diet

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Abstract. Our body needs an adequate supply of antioxidants to maintain physiological functions. Antioxidant compounds are found in natural plants, including inside of kepok bananas peel. This research was conducted to determine the potential of kepok banana peels to maintain testosterone concentration in male rats (*Rattus norvegicus*) with a high-fat diet. This study used 20 male rats 2.5-3 months old, which grouped into 5 groups: normal group given standard feed (K1), negative control group given high-fat diet (K2), positive control group given high-fat diet and simvastatin (K3), high-fat diet group and banana peel extract administered at 100 mg/kg BW (K4), and high-fat diet group and banana peel extract administered at 200 mg/ kg BW (K5). The blood serum was collected after 60 days of the treatment. The blood serum testosterone levels were measured by enzyme-linked immunosorbent assay (ELISA) method at a wavelength of 450 nm. The major findings of this study were that there is a significant difference between testosterone concentrations by the K4 group compared to K2 and K5 groups (P<0,05). The result suggests that the dose 100 mg/kg BW of banana peel extract has the potential to maintain the testosterone concentration in male rats with a high-fat diet.

Keywords: kepok banana peel, active components, high-fat diet, testosterone.

1 Introduction

The development of derived herbal medicines as alternative medicines has been intensively studied in recent years. Herbal medicines contain antioxidants such as vitamin E, vitamin C, phenol and flavonoids [1]. These bioactive compounds can be found in plants one of which is in the banana peel. Someya et al. [2] reported that banana peels have higher antioxidant activity than the banana flesh. The antioxidants in banana peel compounds are pectin, tannins, saponins, and flavonoids [3]. The results of Edenta et al. [4] research proved that the effect of banana peel extract in decreasing low-density lipoproteins (LDL) and triglycerides concentration was comparable to Atorvastatin which commonly used for cholesterol-lowering drug and hyperlipidemia. This proves the banana peels are the potential to inhibit the LDL increase that can reduce the possibility of hyperlipidemia caused by a high-fat diet.

Excessive high-fat diets can interfere with cholesterol homeostasis and then disrupts the normal functioning of the reproductive organs (Pushpendra and Jain, 2015). The interfere should occur for active cholesterol transport system disorders that cause lipid peroxidation in the Leydig cell membrane [5]. According to Rachmadi [6] and Al-Damegh [7], Leydig cells play an important role in producing the testosterone hormone which provides sexual stimulation (libido) in male animals. In addition, testosterone also plays an important role in spermatogenesis, especially in the spermiation stage [8-10]. Disruption of the spermatogenesis process might cause infertility in male animals [11].

In recent years, Many alternative medicines have been developed from herbal plants that can inhibit the negative effects of a high-fat diet. However, the effectiveness of the active compounds of kepok banana peel extract on testosterone concentration in male rats with a high-fat diet is still absent.

2 Materials and Methods

This study used 7 kg kepok banana peel and 20 adult male rats (Rattus norvegicus) aged 2.5-3 months and body weight ranged from 120-160 grams. The samples used in this study were blood plasma. Permit for use of experimental animals has been approved by the

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2.1 Animal Treatments

This research used experimental designed with a completely randomized design consists of five groups. Each of the groups consisted of four replications, coded K1, K2, K3, K4, and K5. Before treatment, all groups were adapted for two weeks. After the adaptation phase, K1 (normal control) is given standard feed, K2 (negative control) is given high-fat feed, K3 (positive control) is given high-fat feed and simvastatin, K4 is given high-fat feed and banana peel extract is 100 mg/kg BW, and K5 were given high-fat feed and banana peel extract as much as 200 mg/kg BW. The high-fat feed is modified from Heriansyah [12], it was 5% wheat flour, 2% waste cooking oil, 1,5% yolk and 5% beef fat. High-fat feeds are given ad-libitum, while *carboxyl methylcellulose* (CMC), simvastatin and kepok banana peel extract are given by oesophageal tube feeding method. Standard fed was given as much as 7 gr/day.

2.2 Preparation of Kepok Banana Peel Extract

The kepok Banana sent to the Botanical Laboratory of Biology-LIPI Research Center for identification and determination. The procedure of kepok banana peel extraction based on the modification research by Pratama *et al*. [13]. The kepok banana peel which used in this study was the raw green. 7 kg of kepok banana peel were cleaned and cut into small pieces (± 0.5 cm) and then dried on a tray by aerated in the room for 2 weeks. Dry banana peels were mashed by blender to form a powder. Banana peels powder weighed 100 g and macerated using 70% ethanol for 72 hours and then filtered. This maceration process is repeated until maserat produced. The obtained extract ware concentrated with a vacuum rotary evaporator at 60^{o}C until obtained a thick extract. The effective dose of kepok banana peel extract in mice refers to Onansanwo's research [14], which were 100 mg/kg body weight and 200 mg/kg body weight given orally with a volume of 1 ml.

2.3 Testosterone Measurement

Measurement of testosterone concentrations was conducted using a commercial testosterone enzyme-linked immunsorbent oassay (ELISA) kit (Cat. No. EIA-1559, DRG, Instrument Diagnostic GmbH, Germany) as described by Gholib et al. [27]. This assay has been used and validated for other animals [27,40,41]. Plasm samples were diluted using aquabidestilates in a ratio of 1: 4. Before the analysis is carried out, standard solutions prepared in concentrations of 0.2 ng/ml to 16 ng/ml and controls (QCs). Samples, standard solutions, and QCs were filled into ELISA microplate wells as much as 25 µL, then added 200 µL of conjugate enzymes to each well except blank wells, then covered with cling film and homogenized by slowly shaking for 10 seconds. After that, microplate incubated for 60 minutes at room temperature. After incubating, microplate was washed with 300 µL washing solution each wells 4 times. After that, 200 µL substrate solution filled into each well and then covered with cling film and incubated for 15 minutes at room temperature. The enzymatic reaction was stopped by adding 100 µL stop solution (5 M H_2SO_4) into each well and absorbance was read using an ELISA reader at 450 nm of wavelength. Concentration of testosterone was calculated using the MPM6 program.

2.4 Data analysis

The testosterone concentration data from each treatment group were analyzed using analysis of variance (ANOVA) and Post hoc test using a Duncan's test.

3 Results and Discussion

3.1 Results of Kepok Banana Peel Extract Compounds

Based on the results of the identification and determination of banana peel samples at the Botanical Laboratory of Biology-LIPI Research Center, it was found that the type of banana peel is *Musa acuminate x Musa balbasiana* (ABB).

Based on phytochemical tested of the presence of the active compounds in kepok banana peel extract as much as 25,82 gr obtained results in Table 1.

Table 1. Active compounds of banana peel.

Type of compounds	Results
Alkaloid	+
Flavonoid	+
Tannin	+
Saponin	+
Steroid/Triterpenoid	+

Note: (+) = positive representation of compounds.

Conducted phytochemical examination aimed to detect the presence of active compounds in natural materials. Saponin test on banana peel extract was positive if foam formed with a lot of intensity for 10 minutes after the solution stirred and allowed to stand for 10 minutes. Saponin is a bioactive compound that can inhibit exogenous cholesterol biosynthesis [15]. Saponin compounds work by binding cholesterol with bile acids so that they can reduce levels of low-density lipoprotein (LDL) fat in the blood [16].

The samples of banana peel extract showed positive for alkaloids. Alkaloid testing is positive if the Mayer test formed white or yellow deposits, Bouchardat test formed brown deposits, and Dragendorf test formed orange-yellow deposits. Samples tested positive for tannins when blue or green are formed in the reagent solution. While samples that contain positive flavonoids characterized by a yellow, red or brown color change that is very striking when tested using H_2SO_4 reagents. Flavonoids contained in kepok banana peels belong to the largest group of phenol compounds consisting of fifteen carbon atoms with two benzene rings [17]. According to Bigoniya and

Singh [18], the mechanism of flavonoids to protect our body from free radicals is by preventing lipid peroxidation. As an antioxidant, flavonoids act by reducing LDL in the body [19].

Banana peel extract also contains active compounds of steroids which evidenced by changes in blue, purple or green in reagents and contain triterpenoids due to red or brown discoloration. Steroids are a group of antioxidant that has antifungal effects and commonly used as raw materials for the biosynthesis of corticosteroid drugs. While triterpenoids are derivatives of 11 β-amyirine which easily crystallized through acetylation and can be used to reduce levels of LDL [20, 21].

Based on the results of phytochemical tests (Table 1), kapok banana peel extract contains five types of active compounds. This proves that banana peel extract is a potential candidate for antioxidants and natural herbal medicines. Several studies have shown that from all parts of the banana plant, banana peels have the highest antioxidant activity. The antioxidant activity on banana peels reaches 94.25% at a concentration of 125 μg/ml compared to the portion of bananas flesh which only 70% at a concentration of 50 mg/ml [22-24]. This antioxidant activity that causes banana peels can reduce LDL [25, 26].

3.2 Testosterone concentrations

The results of the serum testosterone concentrations in male rats in each treatment group for 60 days were presented in Table 2.

Table 2. The statistical analysis results of testosterone concentration in ng/mL by the Duncan test.

Treatment group	testosterone concentration (Mean ± SE)
K1	$3{,}8443 \pm 2{,}84^{ab}$
K2	$1{,}2783 \pm 0{,}67^{bc}$
K3	$1{,}8780 \pm 1{,}54^{abc}$
K4	$4{,}3248 \pm 1{,}61^{a}$
K5	$0{,}5045 \pm 0{,}14^{c}$

Note: Different superscript in each treatment group show a significant difference ($p<0.05$)

Based on the results of the statistical analysis of the five male rat treatments showed a decrease in the concentration of testosterone hormone in the K2 treatment group compared with the K1 treatment group although this decrease did not show any significant difference ($P> 0.05$). Testosterone was seen to be slightly increased in the K3 treatment group compared to the K1 and K2 treatment groups.

Decrease of the testosterone hormone concentration in the K2 treatment group (high-fat feeding) was thought to be due to the body's inability to reduce the negative effects of high-fat feeding. According to Bashandi [28], long-term a high-fat diet can cause hyperlipidemia followed by increasing production of reactive oxygen species (ROS) and lipid peroxidation. The high-fat concentration in bloodstream positively correlated with the onset of various disorders of reproductive function such as inhibition of testosterone and luteinizing hormone (LH) secretion, degeneration of Leydig cell, and spermatogenesis disorders. According to Khatimah [29], the inhibition of gonadotropin-releasing hormone (GnRH) inducing Leydig cells in rat closely related with modification in three pathways, (1) loss of hormone receptors (downregulation hormone), (2) decreased cyclic adenosine monophosphate (cAMP) response and (3) decrease in androgen production capacity.

According to Darbandi et al. [30], many factors can influence the increase in ROS in the reproductive system. If the ROS increases continuously, it will cause activation of the hypothalamic-pituitary-adrenal axis (HPA) to release corticosterone (in animals) or cortisol (in humans) to respond to the oxidative stress. This stress hormone stimulates the hypothalamic-pituitary-gonadal axis (HPG) to reduce LH secretion in the pituitary gland. The low concentration of LH causes a decrease in the stimulation of Leydig cells to produce enough testosterone.

Leydig cells play an important role in testosterone hormone regulation. According to Widhiantara et al. [31], Rats fed with high-fat feed in the long period will experience a decrease in the number of Leydig cells caused by hyperlipidemia. Hyperlipidemia can trigger to increase ROS compounds, disruption of the hypothalamus-pituitary axis, thereby decreasing LH secretion and disrupting the stimulation of Leydig cells to produce testosterone [28, 32].

The K4 treatment group showed the highest testosterone increase compared to the K1 and K3 treatment groups but did not show any significant difference ($P> 0.05$). The K4 treatment showed a marked difference in the concentration of testosterone hormone increase compared to the K2 treatment ($P <0.05$). This increase caused by the inhibiting negative process of high-fat feed by banana peel extract. The high-fat feed can induce oxidative stress, disrupt in membranes permeability, impaired function of sodium pumps in cell membranes and followed by the formation of ROS. According to Werdhasari [33], antioxidants needed by the body to neutralize the effects of ROS and prevent damage caused by it. Antioxidant compounds contained in banana peels are reported to have biological and pharmacological effects which can reduce the negative effects of free radicals [34, 35].

A decrease in testosterone concentration was shown in the K5 treatment group ($P <0.05$). This is probably due to the excessive breaking of the saturated fat chain that disrupts the function of the LH formation thereby reducing the formation process of the testosterone hormone. According to Brinkmann [36], LH increases the activity of enzymes that will convert cholesterol into the testosterone hormone. Decrease of LH secretion from the anterior pituitary causes degeneration of Leydig cells which results in low production of the testosterone hormone. The results of this study are in line with the statement of Bast and Haenen [37], that antioxidants derived from natural contents also have a limit does consumption.

The previous study has proven that the decrease in testosterone affects libido decreasing, disruption of

spermatogenesis and seminiferous tubule diameter. If the concentration of the testosterone hormone is too high or too low (below the normal threshold) will result in negative feedback to the hypothalamus which further interferes with spermatogenesis. Conversely, if the testosterone hormone concentration is normal, it will stimulate the testes to process the spermatogenesis [38, 39]**.**

4 Conclusion

Kepok banana peel extract with a dose of 100 mg/kg body weight given for 60 days has the potential to increase the concentration of white rat testosterone. While means giving kepok banana peel extract with a dose of 200 mg/kg bodyweight for 60 days can interfere with the formation of testosterone in white rats.

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