

THE EFFECT OF GUAVA FRUIT (*PSIDIUM GUAJAVA L.*) EXTRACT TO THE AMOUNT OF LEYDIG CELLS OF MALE RATS (*RATTUS NORVEGICUS*) EXPOSED TO CIGARETTE SMOKE

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ABSTRACT

The purpose of this research is to prove the effect of guava fruit extract to the amount of Leydig cells testes in rats exposed to cigarette smoke. This experimental research using 25 rats which criteria 2-3 month old, male and average weight of 150-200 g. Group of negative control (K-) was given CMC Na 0.5% 2 mL without exposed by cigarettes smoke, group of P0 was exposed with cigarette smoke and given CMC Na 0.5% 2 mL, treatment groups P1, P2 and P3 treated with guava fruit extract at dose of 3.78 mg/200g BB, 7.56 mg/200g BB, 11.34 mg/200g BB and exposed to cigarette smoke. Exposure cigarette smoke had been given three pieces cigarettes per group each day. All Treatment and exposure cigarette smoke was given about 45 days. The result showed that guava fruit extract could improve the amount of Leydig cells exposed to cigarette smoke in the highest dose 11.34 mg/200g BB. Based on statistical data analysis using Analysis of Variance (ANOVA) it showed a different significant ($p=0.000$) with mean \pm SD as follows: K(-) $6.20d \pm 0.32$; K(+) $2.00a \pm 0.14$; P1 $3.88b \pm 0.70$; P2 $4.32b \pm 0.42$; P3 $5.32c \pm 0.39$, further analysis using Duncan Multiple Range Test (DMRT). The conclusion of this research showed that guava fruit extract could improved the Leydig cells count on testes in male rats that exposed to cigarettes smoke.

KEY WORDS: Guava Fruit, Extract, Leydig Cells

INTRODUCTION

Smoking is an unhealthy lifestyle that is still often found and affects the health of the body, even causing death. According to the World Health Organization, by 2020 it is estimated that 10 million smokers in the world will die each year (Putra, 2016). Smoked cigarette smoke caused 6 million people to suffer from some deadly diseases, while more than 600 thousand people died due to exposure to cigarette smoke. In 2008, the number of cigarette consumers in Indonesia was ranked third in the world after China and India (WHO, 2013).

Cigarettes contain harmful compounds that can endanger health. Every cigarette that is burned produces about 4000 kinds of chemicals. Components of these chemicals after identification, found compounds that can endanger health include nicotine, tar, carbon monoxide (CO), nitrosamines, nitrogen oxides (NO), polynuclear aromatic

hydrocarbons (PAHs) compounds (Tirtosastro and Murdiyati, 2017).

Cigarette chemicals will enter the lungs quickly and then spread throughout the body through blood vessels. Chemicals in cigarette smoke can cause oxidative stress by increasing the formation of Reactive Oxygen Species (ROS) and can affect health problems that occur in the reproductive system (Intania, 2006).

Leydig cells are cells that have an important function in male reproduction as a producer of the hormone testosterone that functions for the process of spermatogenesis in the seminiferous tubules (Soenardirahardjo *et al.*, 2006). Damage to Leydig cells can interfere with the process of steroidogenesis and cause an imbalance of testosterone hormone synthesis so that it can interfere with the process of spermatogenesis, if it lasts a long time will cause infertility (Creasy, 2001). Infertility can be prevented by consuming high antioxidants that are found in

fruits. Natural ingredients are generally safer for consumption and have a relatively higher effectiveness and can improve body health. Antioxidants are substances that are needed by the body to prevent cell damage caused by free radicals (Xu *et al.*, 2019).

Guava fruit is known to contain high antioxidants because it contains vitamin C, arotenoids (beta-carotene, lycopene, and beta-cryptoxanthin) and polyphenols are high in antioxidants so they can increase endurance (Barbalho *et al.*, 2012). According to Paniandy *et al.*, (2000) in guava fruit there are chemicals that can affect antioxidant activities, such as flavonoid compounds, saponins, oleanolic acid, guaijavarin and quercetin.

Vitamin C is an antioxidant that has high polarity properties because it contains a lot of hydroxyl groups so that it dissolves easily in water; this gives the advantage of being easily changed by the body. Vitamin C has a high effectiveness to protect body molecules such as protein, fat, carbohydrates and DNA by free radicals and oxygen reactivation that can be produced during normal metabolism which can eliminate free radicals (He and Venant, 2004).

Based on these descriptions can be the basis of research to find out effect of guava fruit extract (*Psidium guajava* L.) on the number of Leydig testis cells of white rats (*Rattus norvegicus*) exposed to cigarette smoke.

MATERIALS AND METHODS

Research Tools and Materials

This study used 25 male rats (*Rattus norvegicus*) as many as 200 g of weight, red salt cellar clove cigarettes. Material for making guava fruit extract (*Psidium guajava* L.): guava fruit powder, sterile aquadest, ethanol 96%, CMC Na 0.5%, chicken feed in the form of pellets for rat feed, drinking water and wood dust for mouse cage pads. The tools used in this study include a smoking box measuring 30 x 20 x 12 cm³ which has 2 connecting holes in the front, which are connected by hose and syringe.

Research Method

- 25 male rats were divided into 5 groups. In the control group negative (C-) was not given exposure to cigarette smoke and only CMC Na 0.5% 2 mL/head. On the 8th day the treatment was started with 3 cigarettes daily exposure in groups (C +), P1, P2 and P3 then the P0 group was only given CMC Na 0.5%, guava fruit extract was given with 3 different

doses for P1, P2 and P3 is 3.78 mg/200g BB, 7.58 mg/200g BB, 11.34 mg/200g BB per day. Exposure to cigarette smoke and administration of guava fruit extracts was carried out for 45 days and on the 46th day all the mice were sacrificed and their testicular organs taken and histopathological preparations made to count the number of Leydig cells.

Leydig Cell Number Calculation

Calculation of the number of Leydig cells through haematoxylin-eosin (HE) staining with light microscope observations that have been calibrated with 400x magnification to count the number of Leydig cells from five tubules in one preparation. By means of each testicular histopathological preparation observed in five different testicular interstitial tissue fields that start from left to right, then down from left back then right then photographed using OptiLab®. The results of the number of Leydig cells in each field were then added up on average using the help of the Image Raster Program. Data analysis using statistical tests one way Analysis of Variance (ANOVA). Followed by Duncan's Multiple Range Test (DMRT) if there was any significant different. All data were analyzed using the Statistical Product for Service Solutions (SPSS) program version 20.0.

RESULTS AND DISCUSSION

The results were obtained based on the treatment of testicular preparations in rats exposed to cigarette smoke. The cross section of the testicular preparations for each treatment is shown in Image 1.

Based on the results of research on the number of Leydig cells in rat testes showed an increase in the number of Leydig cells after being given guava extract given to mice. The highest increase in the number of Leydig cells was in the P3 group with an extract dose of 11.34 mg/kg/day and the lowest was in the positive control group exposed to cigarette smoke. There was an increase in the number of Leydig cells in groups P1, P2 and P3. The results of the analysis using *Analysis of variance* (ANOVA) show the value of $p = 0.00$ ($p > 0.05$), meaning that there are differences evident in at least two treatments. This shows that there is a significant effect of guava extract on the number of Leydig cells ($p < 0.05$).

From the histopathology result it can be revealed that K(+) group has highest number of necrosis cells of Leydig cell compared to the other groups. P3

group has the best effect of treatment because it revealed the highest amount of normal Leydig cells that similar to K(-) group. The histopathological changes among all treatment groups are shown in Figure 1.

The results of the study showed that the highest number of Leydig cells was treatment 3 with a dose of 11.34 mg and the lowest number of Leydig cells in positive controls exposed to cigarette smoke. Decrease in the number of Leydig cells is thought to occur due to cell damage due to cigarette smoke content which has the potential to increase free radicals in the form of Reactive Oxygen Species (ROS) in the body such as Superoxide (O_2^-), Hydroxyl (OH) and Peroxyl (H_2O_2) radicals. Free

radicals in excessive amounts can cause oxidative stress, if it lasts a long time will cause lipid peroxidation of cell membranes so that it damages spermatogenic cell membranes, disrupts ion transport, suppresses the process of steroidogenesis and increases cell death to necrosis so that the number of Leydig cells becomes smaller (Aina, 2005; Karim, 2011). Increased oxidative stress will interfere with sexual function due to decreased testosterone secretion due to decreased number and function of Leydig cells.

Guava fruit extract contains several active compounds, namely vitamin C and quercetin, which is one of the active compounds that has a dominant role in counteracting free radicals. Physicochemical properties are antioxidants that can inhibit COMT (*Catechol-O-Methyl Transferase*), an enzyme that reduces the breakdown of epinephrine and inhibits heat shock proteins that can cause apoptosis in cells (Jusuf, 2010).

Quercetin and vitamin C compounds function against ROS caused by cigarette smoke by activating the enzymes *Superoxide Dismutase* (SOD) and *Catalase* (CAT), so that it can result in affecting the activity of SOD and CAT. SOD activity decreases due to its reaction with O_2^- to form H_2O_2 and O_2 . The effects of quercetin and vitamin C can reduce excessive ROS production (Paunoviæ *et al.*, 2016). The combination of quercetin and vitamin C compounds can protect the phospholipid layer with

Table 1. The average yield and standard deviation of the number of Leydig cells in each treatment

Treatment	Mean \pm SD of Leydig Cells Number
K (-)	6.20d \pm 0.32
K(+)	2.00a \pm 0.14
P1	3.88b \pm 0.70
P2	4.32b \pm 0.42
P3	5.32c \pm 0.39

Note: K(-) : solvent CMC Na 0.5%; K(+):solvent CMC Na 0,5%; P1: guava extract (*Psidium guajava* L.) dose 3.78 mg/200gBW; P2: guava extract (*Psidium guajava* L.) dose 7.56 mg/200g BW; P3: guava extract (*Psidium guajava* L.) dose 11.34 mg/200gBW.

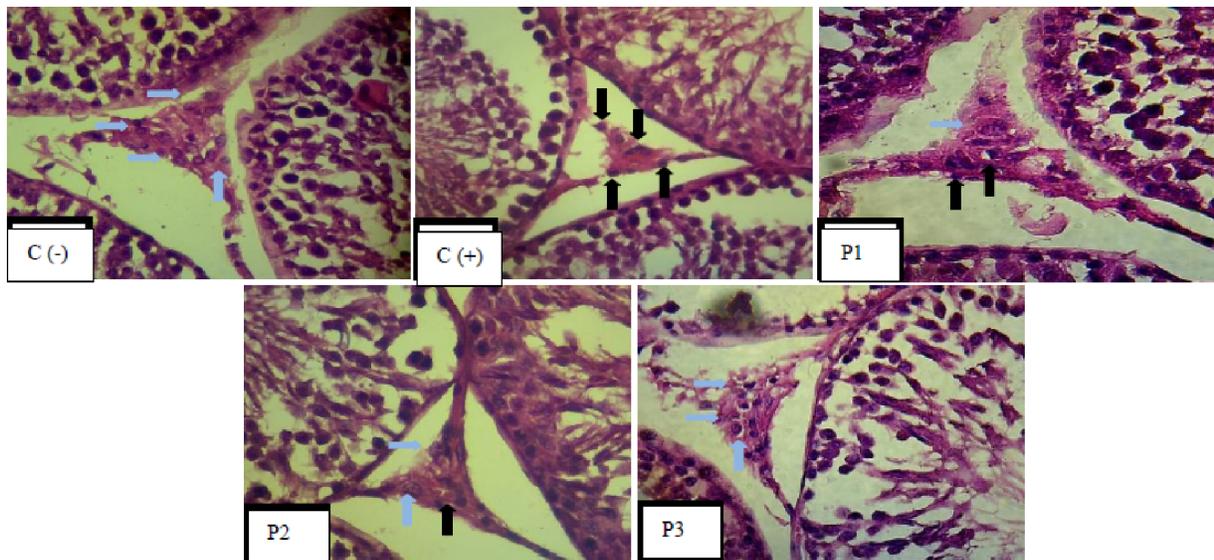


Fig. 1. Histopathology changes in albino rats (*Rattus norvegicus*) negative control group (C-), positive control (C +), P1 group, P2 group and P3 group in 400x magnification, with H.E staining. Noted that blue arrows indicate normal Leydig cells and black arrows indicate Leydig cells undergo necrosis.

non-covalent bonds; this way can increase the regeneration of endogenous antioxidants (Fabre *et al.*, 2015). One mechanism of action of the quercetin antioxidant is by scavenging the free radicals such as SOD produced by xanthine compounds (Dok-Go *et al.*, 2003)

Flavonoids have the ability as an antioxidant that can inhibit oxidative stress against the danger of free radicals and can enhance the process of spermatogenesis. The effect of Flavonoides can also increase regeneration in a way detract free radicals, provide competitive substrates for unsaturated lipids in membranes and or accelerate the repair mechanism of damaged cell membranes (Sarma and Babu, 2011).

Research result shows that guava fruit extract has a significant effect on increasing the number of white rat Leydig cells exposed to cigarette smoke. The higher dose of extract given showed a high improvement, with increasing numbers of Leydig cells.

CONCLUSION

Based on the results of research that has been done guava fruit extract (*Psidium guajava* L.) can maintain the number of Leydig testes cells of male white rats (*Rattus norvegicus*) exposed to cigarette smoke.

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