PURPLE ROSELLA (*Hibiscus sabdariffa Linn.*) PETAL EXTRACT PREVENTS HEPATOCYTE DEGENERATION IN WISTAR RATS EXPOSED TO CIGARETTE SMOKE

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ABSTRAK

Saat ini, jumlah perokok di Indonesia masih tinggi. Asap rokok mengandung radikal bebas. Penelitian ini bertujuan untuk menganalisis pengaruh ekstrak kelopak rosella ungu terhadap pencegahan degenerasi hepatosit tikus Wistar yang dipapar asap rokok. Total sampel 20 tikus jantan dan dibagi menjadi empat kelompok: kontrol negatif, kontrol positif, perlakuan ekstrak kelopak rosella ungu dosis 270 mg/kg BB, dan ekstrak kelopak rosella ungu dosis 540 mg/kg BB. Kontrol negatif hanya diberi pakan standar. Kontrol positif diberi pakan standar dan dipapar 2 rokok per hari. Kelompok perlakuan diberi pakan standar dan ekstrak rosella di pagi hari dan setelah itu dipapar 2 batang rokok sampai rokok tersebut habis dan dilakukan setiap hari. Penelitian ini dilakukan selama 28 hari. Di akhir penelitian, dibuat sediaan histopatologi hepar dengan pewarnaan hematoxilin eosin untuk dilihat degenerasi hepatosit. Pemberian ekstrak kelopak rosella ungu dosis 540 mg/kg BW dan dosis 270 mg/kg BB secara bermakna (p < 0,05) dapat mencegah degenerasi hepatosit. Ekstrak kelopak rosella ungu dapat mencegah degenerasi hepatosit pada tikus Wistar yang dipapar asap rokok. ***(FMI 2018;54:96-101)***

Kata kunci*: Antioksidan; radikal bebas; degenerasi hepatosit; rosella ungu*

ABSTRACT

Currently, the number of smokers in Indonesia is still high. Cigarette smoke contains free radicals. This study aimed to analyze the effects of purple rosella petal extract on the prevention of hepatocyte degeneration in Wistar rats exposed to cigarette smoke. Twenty male rats were divided into four groups: negative control, positive control, treatment group receiving 270 mg/kg BW purple rosella petal extract, and treatment group receiving 540 mg/kg BW purple rosella calyx extract. Negative control was only given with standard feed. Positive control was given with standard feed and exposed to 2 cigarettes a day. Treatment groups were fed with standard feed and administered with rosella extract in the morning then exposed to 2 cigarettes every day. This study was conducted for 28 days. At the end of the study, hepatocyte degeneration was observed in liver histopathology stained with hematoxilin eosin. The administration of purple rosella petal extracts in the doses of 540 mg/kgBW and 270 mg/kgBW significantly (p<0.05) prevented hepatocyte degeneration. Purple rosella petal extract is able to prevent hepatocyte degeneration in Wistar rats exposed to cigarette smoke. ***(FMI 2018;54:96-101)***

Keywords*: Antioxidant; free radical; hepatocyte degeneration; purple rosella*

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INTRODUCTION

Smoking is familiar to Indonesians. Smoking behavior among the population of >15 years of age in Indonesia in 2007 was found in as much as 34.2%. There was an increase of the prevalence of smoking behavior in the population as shown from the results of RISKESDAS 2013 which was 36.3% (Kemenkes 2015). Smoking habits in society is difficult to stop. Nicotine in cigarettes can cause addiction and disorders of the heart and lungs (Voges 2000). However, eliminating smoking habit is not easy. Cigarette smoke contains free radicals that harm the body, thus an innovation is necessary to develop product that can minimize negative impacts caused by cigarettes.

Chemical components contained in cigarette smoke are in the form of gas and particles (Tirtosastro & Murdiyati 2010), some of which are radical. Although most free radicals are short-lived, the radicals of nitric oxide and quinones can reach the lungs (Subandi 1999). From the lungs, free radicals are carried by the bloodstream to the heart and are circulated throughout the body, including to the liver (Guyton & Hall 1997). This leads to complex biochemical changes that can ultimately lead to liver cell damage (Soini & Lehto 1998).

Exposure to cigarette smoke can cause hepatic cell necrosis (Muliartha et al 2009). The liver is one of the most vulnerable organs since it is a filter of toxic substances that enter the body. In addition, the liver has a double circulatory system (Vollmar & Menger 2009), so the accumulation of toxic materials in the liver is higher. Free radicals produced by toxic substances can cause hepatocyte damage (Jawi et al 2006). In addition to causing hepatic damage, cigarette smoke may damage the alveolar lung (Marwan et al 2006), leading to a reduction in the number of spermatogonium cells in rat testis (Wistar strain *Rattus norvegicus*) (Kurnia et al 2011), leading to increased levels of malondialdehyde (MDA) (Nasution et al 2016, Kurnia et al 2011, Ambrose & Barua 2004, Jain et al 2009). MDA is a biomarker that indicates oxidative stress has occurred.

Ironically, many people are unaware of the negative effects of smoking, even though the dangers are listed in the cigarette packs saying that smoking can cause cancer, heart attacks, impotence, pregnancy and fetal disorders. The impact is related to the presence of free radical content in cigarette smoke, such as NO, CO, NOx, H2-O2, aldehydes, trace elements and nitroso compounds (Valvanidis & Haralambous 2001). Free radicals can cause oxidative stress which is the results of degenerative diseases. The source of free radicals can be from motor vehicle fumes, waste cooking oil, and cigarette smoke (Park et al 1998).

Normally, free radicals are present in the body (endogenous free radicals). The body naturally also has antioxidants as inhibitors that act to inhibit oxidation by reacting with free radicals to form relatively free radicals. However, if the free radicals are excessive, then the natural antioxidants are not able to cope. In such circumstances the body needs antioxidant supply. Antioxidants are all compounds that can reduce the negative effects of oxidants, especially in the inhibition and cessation of oxidative damage to a target molecule (Simanjuntak & Sudaryati 1998). Source of antioxidants from outside the body can be found, for example, on rosella petals (Esa et al 2010, Bolade & Ojo 2009, Hirunpanich et al 2005). Rosella extract at doses of 150 mg and 300 mg can improve the oxidative stress state, improve endothelial function and decrease the risk of atherosclerosis as characterized by increased SOD activity, decreased amount of F2-isoprostan, ADMA and Foam cell in endothelial tissue of rats receiving an atherogenic diet (Yusmiati et al 2012).

One of the alternatives we proposed in this study was the use of rosella petal extract in water solvent. This is because the rosella extract in the water solvent can be applied as functional food addition. Water solvents are suitable for extracting anthocyanins that have the same polarity as water. In addition, water solvents are chosen because they are safer and easier to apply on a household scale. The extract was taken from rosella petals because it had the highest total antioxidant activity as high as 54.1% compared to rosella seeds, leaves, or rods (Esa et al 2010). Rosella contains many anthocyanins in which 1 gram of rosella contains 56.5 mg delphinidin-3-O-sambubioside and 20.8 mg cyanidin-3-O-sambubioside (Alarco-Alanso et al 2012). Antioxidants derived from anthocyanin extract of rosella potentially overcome oxidative damage in rat liver induced by CCl4 (Ajiboye et al 2011, Adetutu & Owoade 2013).

The antioxidants contained in purple rosella effervescent have been shown to be effective in preventing hepatic necrosis in Wistar rats (Maulana et al 2014) and counteracting free radicals from waste cooking oil (Ulilalbab et al 2012). Further studies are needed whether antioxidant rosella extract can counteract free radicals from exposure to cigarette smoke. So far, the antioxidant effect on purple rosella petal extract in its ability to counteract free radicals from cigarette smoke is not known. Studies on the benefits of rosella petal extract through testing on experimental animals exposed to cigarette smoke by observing indicators of oxidative stress in the form of hepatocyte degeneration needs to be done. The purpose of this study was to determine the effect of purple rosella petal extract on hepatocyte degeneration of Wistar rats exposed to cigarette smoke.

MATERIALS AND METHODS

The instruments used in this study were spatula, beaker glass, whatman 42 filter paper, hot plate magnetic stirrer, measuring cylinder, dark bottle, digital scales, vacuum evaporator, mouse cage, drinking bottle for experimental animals, smoking pump, vortex, spectrophotometer, binocular microscope with 400x magnification, OptiLab microscope camera, and hematoxylin eosin. Experimental animals used were Wistar rats (*Rattus norvegicus*), standard feed, mineral water, clove cigarettes, and purple rosella.

Research design

This was a laboratory experimental study. The in vivo stage of this study was true experimental laboratory with post test only control group design, while for the treatment, we used complete randomized design (CRD). The samples consisted of 20 male rats of *Rattus norvegicus* of Wistar strain aged 3-4 months weighing 180-200 g with a healthy condition. Samples were chosen by random sampling to be divided into one group of negative control (normal), one positive control

group, and two treatment groups. Each group consisted of 5 rats. Negative control (normal) group received standard feed without rosella extract and no exposure to cigarette smoke. Positive control group received standard feed with 1 ml p.o water (orally), then exposured to 2 cigarette smoke. Treatment 1 group received standard feed and 270 mg/kg BW p.o 1 ml purple rosella extract, then exposured to the smoke of 2 cigarettes. Treatment 2 group received standard feed, 540 mg/kg BW p.o 1 ml purple rosella extract, then exposured to the smoke of 2 cigarettes.

The experimental animal feed used was POKPHAND CP 591 with composition of ≤ 13.0% water, 18.0-20.0% protein, ≥ 3.0% fat, ≤ 6,0% fiber, ≤ 7.0% ash, ≥ 0.9% calcium, and ≥ 0.6% phosphor. The ingredients used in feed formulations included corn, bran, fish meal, soybean meal, coconut meal, meat and bone meal, wheat fraction, peanut cake, flour, canola, vitamin, calcium, phosphate and trace minerals.

The cigarettes used in this study contained 2.1 mg nicotine and 34 mg tar. The dose of smoke exposure was 2 cigarettes/rat/day in the afternoon. Cigarette smoke exposure was done after the administration of rosella extract p.o in the treatment groups. Exposure to cigarette smoke and extract administration were carried out for 28 days. The study was conducted at In Vivo Laboratory, Faculty of Medicine, Wijaya Kusuma University, Pharmacology Laboratory, Brawijaya University, Anatomic Pathology Laboratory, Faculty of Medicine, Universitas Airlangga, and Anatomic Pathology Laboratory, GDC, Dr. Soetomo Hospital, Surabaya.

Each treatment group consisted of five rats. In the early stage, normality analysis with Shapiro-Wilk test and homogeneity test with Levene Statistic were performed. If the data were not homogeneous, non-parametric test was performed using Kruskal Wallis test. Whereas, to observe the differences between each group, Mann-Whitney test was used. Mean hepatocyte degeneration was defined as the mean of total degenerated hepatic cells in three observations using kraticule in each treatment group. Each observation examined the number of degenerated cells from 100 cells by using microscope in 400x magnification.

RESULTS

Antioxidant test was done by DPPH method (α, α diphenyl picryl hydrazil) with absorbance of λ=517 nm. The analysis results of antioxidant activity (DPPH test) of rosella extract are presented in Table 1.

Table 1. Antioxidant activity (DPPH test) of of rosella extract

Samples	% Inhibition
BHT (butylated hydroxytoluene)	91.620
540 mg/kg BW	83.888
270 mg/kg BW	59.858

The highest antioxidant activity (DPPH test) was BHT (butylated hydroxytoluene) as control (91.620%), followed by 540 mg/kg BW purple rosella extract (83.888%) and 270 mg/kg BW purple rosella extract (59.858%).

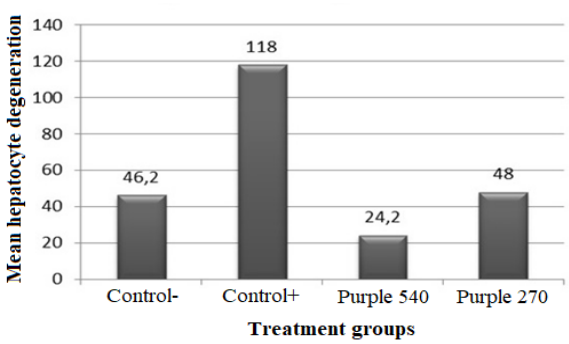

Fig. 1. Mean hepatocyte degeneration

The mean hepatocyte degeneration in the group given with 540 mg/kg BW purple rosella extract was 24.2. Whereas, mean hepatocyte degeneration in group receiving 270 mg/kg BW purple rosella extract dose ie 48. Mean hepatocyte degeneration in positive control group was 118, while mean hepatocyte degeneration in negative control group was 46.2. The highest degree of hepatocyte degeneration was found in positive control group, whereas the lowest was in group receiving purple rosella petal extract dose of 540 mg/kg BW.

Table 2 shows that the number of hepatocyte degeneration for the group receiving purple rosella extract dose of 270 mg/kg BW did not differ significantly from that of the normal group. The hepatocyte count in positive control group was significantly different from all groups, ie purple rosella extract dose of 540 mg/kg BW, purple rosella extract dose 270 mg/kg BW, and negative control. Whereas, the negative control group was not significantly different from the group receiving purple rosella extract dose of 270 mg/kg BW. Negative control group was significantly different from the group receiving purple rosella extract dose of 540 mg/kg BW and positive control.

Table 2. Mean hepatocyte degenerations in different groups

Groups	Mean hepatocyte degeneration
540 mg/kg BW purple rosella extract	24.2 (a) ± 3.493
270 mg/kg BW purple rosella extract	48.0 (b) ± 7.165
Positive control	118.0 (c) ± 58.189
Negative control (normal)	46.2 (b) ± 6.017

Note: The averages accompanied by the same letter states no significant difference (Mann Whitney test)

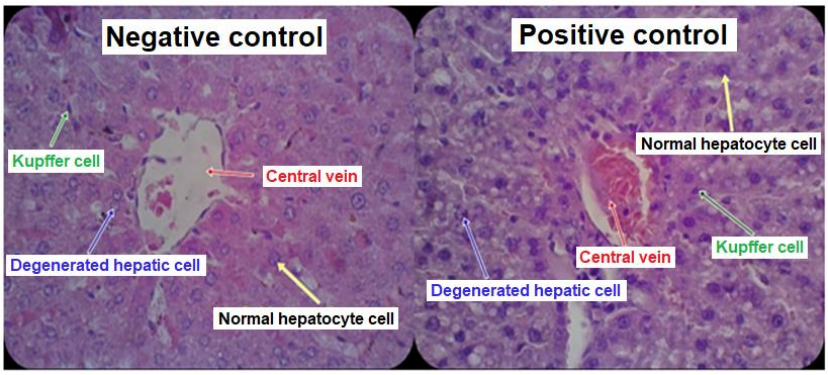

Fig. 2. Hepatic histological section of rats (*Rattus norvegicus*) with HE staining in negative control group (left) and positive control group (right) in a 400x magnification microscope.

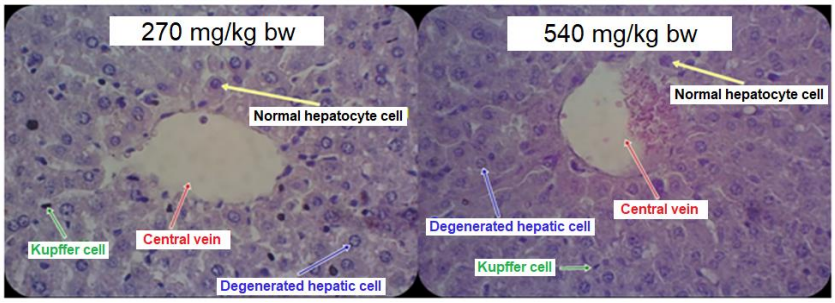

Fig. 3. Hepatic histological section of rats (*Rattus norvegicus*) with HE treatment in treatment groups receiving purple rosella extract dose of 270 mg/kg BW (left) and 540 mg/kg BW (right) in 400x magnification microscope. Red arrows indicate central vein, yellow arrow shows normal hepatocyte cells, green arrow indicates kupffer cell, blue arrow indicates cell liver degeneration.

DISCUSSION

DPPH test results revealed that the highest antioxidant activity of purple rosella petal extract was in BHT (butylated hydroxytoluene) as control, followed by purple rosella petal extract doses of 540 mg/kg BW and 270 mg/kg BW. The higher the dose indicates the higher the value of its antioxidant activity. The main character of the antioxidant compounds is its ability to capture and stabilize free radicals (Prakash 2001). This study found that the group of purple rosella extract dose of 540 mg/kg BW had the lowest hepatocyte degeneration. This was because the group received the extract p.o with the highest antioxidant activity (83.888% inhibition) compared to the other treatment groups. Conversely, positive control group had the highest number of degenerated hepatic cells because the experimental animals in the group were exposed to cigarette smoke alone without the intake of antioxidants. The heavily necrotic liver cells were present in the positive control treatment group (Muliartha et al 2009).

The group receiving purple rosella extract in a dose of 540 mg/kg BW differed significantly from the other groups. This indicates that giving purple rosella extract dose of 540 mg/kg BW provides a remarkable effect on the prevention and reduction of oxidative stress caused by cigarette smoke. In studies on rosella activity as

antihepatotoxic in streptozotocin-induced rats as indicator of free radical, rosella has been found to have an effect of repairing tissue damage (Adeyemi et al 2014). The group receiving purple rosella extract in a dose of 270 mg/kg BW did not differ significantly with the negative group, so it can be concluded that the purple rosella extract group of 270 mg/kg BW was able to match the normal condition despite being treated with exposure to cigarette smoke. This indicates that the purple rosella dose of 270 mg/kg BW is able to prevent hepatocyte degeneration of Wistar rats exposed to cigarette smoke. This is confirmed by some studies suggesting that rosella extract is able to decrease serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) serum activity as biomarkers of hepatic damage (Famurewa et al 2015, Usoh et al 2012).

Groups of rats exposed to tobacco smoke showed higher hepatocyte degeneration rates than the treatment group or negative control group. This is because cigarette smoke contains tar-free radicals or gas components (Muliartha et al 2009). Free radicals from cigarette smoke enter the lungs through the airways, then carried by the bloodstream to the heart and circulated throughout the body, including to the liver (Guyton & Hall 1997). Oxidants and free radicals present in cigarettes have the potential to trigger lipid peroxidation of cell lipid membranes (Frei et al 1991). ROS (Reactive Oxygen Species) in cigarette smoke triggers the destruction of endogenous antioxidants (enzymatic vitamins and antioxidants), thereby reducing the role of antioxidants in cellular defense (Cross et al 1999).

CONCLUSION

Purple rosella petal extract is able to prevent hepatocyte degeneration in Wistar rats exposed to cigarette smoke. In this study, treatment group receiving the extract of 540 mg/kg BW was able to optimally prevent hepatic cell degeneration compared to the group receiving 270 mg/kg BW. However, negative control group, which did not receive any extract, also showed hepatocyte degeneration because free radicals are also naturally produced as byproduct of metabolic activity in the body.

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