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The Effect of Red Dragon Fruit Juice (*Hylocereus Polyrhizus*) on Liver Histopathological Images in Wistar Rats Exposed to Cigarette Smoke

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ABSTRACT: Cigarette smoke contains toxic components, carbon monoxide, hydrogen cyanide, carbon dioxide, and tar, which can trigger the formation of ROS (Reactive Oxygen Species) and reduce antioxidant reserves, which impact oxidative stress and are known to cause damage to liver cell membranes. Red dragon fruit (*Hylocereus polyrhizus*) is a fruit that is rich in antioxidants that inhibit ROS and prevent damage to liver cells by cigarette smoke. This study aims to assess the effect of administering red dragon fruit juice on liver microscopic images in Wistar rats exposed to cigarette smoke. It was an experimental study with a post-test-only control group design. Thirty-five male Wistar rats (*Rattus novergicus*) were randomized into five groups. Four groups were exposed to cigarette smoke (K-, P1, P2, and P3), and the fifth group was used as a control (K). All the P-groups were given red dragon fruit juice 2 g/2.5 ml, 4 g/2.5 ml, and 8 g/2.5 ml, respectively, for twenty-eight days. On the 29th day, the livers of Wistar rats were taken for preparation and microscopic observation. The research data were analyzed using the Kruskall-Wallis and Mann-Whitney post hoc tests to determine group differences. The average liver cell damage in K, K-, P1, P2, and P3 were 1.23, 2.51, 1.57, 1.51, and 1.43, respectively. There was a significant difference in damage between the K- group and the P1 ($p = 0.002$), P2 ($p = 0.004$), and P3 ($p = 0.002$) groups. The administration of red dragon fruit juice can protect the liver cells of rats exposed to cigarette smoke.

KEYWORDS: Red Dragon Fruit Juice; Liver; Microscopic; Cigarette smoke

I. INTRODUCTION

Smoking is a lifestyle of many people which is harmful to health. About 70% of smokers come from developing countries, one of which is Indonesia.[1] The Basic Health Research (Riskesdas) stated in 2018 that the prevalence of smokers aged ≥10 years in Indonesia had reached 28.9%.[2] One of the smoking effects that smokers can experience is liver damage.[3,4] Apart from harming active smokers, the content of chemicals contained in cigarettes is also a threat to passive smokers.[2]

Free radicals are an atom or a group of atoms that have one or more unpaired electrons.[5] Cigarette smoke contains about $10^{14} – 10^{15}$ free radicals in one puff of cigarette smoke.[5] Free radicals contained in cigarette smoke, such as carbon monoxide (CO), carbon dioxide (CO_2), nitrogen monoxide (NO), hydrogen cyanide (HCN), nitrosamines, and formaldehyde will be inhaled through the respiratory tract to the lungs and then transported by the blood to the heart and will be circulated to the liver.[6,7] These compounds interact with cells, which then will form free radicals. The liver acts as a filter for toxic compounds that enter the body, but if the amount of free radicals is excessive, it will cause liver cell damage.[4]

Antioxidants can stop the formation of free radicals by giving one of their Hydrogen ions to the radical, thereby creating a more stable free radical.[8] Naturally, a body has a protective mechanism against free radicals, which is played by endogenous antioxidants. However, if the amount of free radicals in the body is excessive, the endogenous antioxidants cannot neutralize them, resulting in oxidative stress.[9] Cells that are exposed to oxidative stress will experience cell membrane damage.[10] This causes the body to need exogenous antioxidants to increase endogenous antioxidant defences in counteracting against free radicals.[7,11]

Red dragon fruit (*Hylocereus polyrhizus*) is a nutritious fruit that acts as a natural antioxidant, and it has been proven effective in many previous studies.[12–14] Gita *et al*. demonstrated that red dragon fruit juice significantly affects the quality of spermatozoa

of the *Rattus norvegicus* Wistar albino strain exposed to cigarette smoke.[9] Nurliyana et al. proved that 1.0 mg/mL of red dragon fruit flesh could hamper free radicals by 27.45 ± 5.03%.[13] Rebecca *et al*. demonstrated that there is a total phenolic content of 86.13 ± 17.02 mg in 0.50 g dry extract of *H. polyrhizus.*[12]

This presentation shows the importance of further research regarding the antioxidant effects of red dragon fruit on the liver function of Wistar rats exposed to cigarette smoke. Therefore, the authors are interested in researching the effect of giving red dragon fruit juice on the histopathological appearance of the liver of Wistar rats exposed to cigarette smoke.

II. METHOD

This experimental research uses a post-test-only control group design. The samples of this study were 35 Wistar rats, selected using the simple random sampling method based on the inclusion and exclusion criteria. The experimental animals chosen for this study were male Wistar rats aged 2-3 months, weighing 150-250 grams, in good health condition without any anatomical abnormalities, and actively moving. Experimental animals were declared drop-out if they died during the research period.

The red dragon fruit, washed and peeled, is cut into small pieces. The pieces were then crushed using a mortar tool and filtered using gauze to separate the flesh and seeds of the fruit. Fruit flesh filtered will be taken and weighed according to the treatment dose: 2 g, 4 g, and 8 g. Each dissolved in 2.5 ml of aquades.[14]

After an adaptation period of 7 days, 35 Wistar rats were divided into five groups. The healthy control group (K) was given standard food and beverages for 28 days. The negative control group (K-) was given standard food and beverages, with exposure to cigarette smoke equal to two cigarettes per day for 28 days. Treatment groups P1, P2, and P3 were given standard food and beverages, with exposure to cigarette smoke equal to 2 cigarettes per day, and each group was given red dragon fruit juice 2 g/2.5 ml, 4 g/2.5ml, and 8 g/2.5ml each day, respectively, in 28 days.

On the 29th day, termination was carried out by administering chloroform anaesthesia and doing cervical dislocation, and then the liver organs of Wistar rats were harvested for preparation. Preparations were observed under a 400x magnification microscope at five different areas of view. In each area of view, 20 cells were randomly counted, and the score for each cell was assessed using Manja Roenigk's scoring by dividing the degree of liver cell damage as follows: Score 1 (normal), score 2 (parenchymatous degeneration), score 3 (hydropic degeneration), score 4 (necrosis). Then, the average score was calculated from five areas of view to be analyzed.

The results of the research were then processed using the SPSS program. The first data will be tested using the Shapiro-Wilk normality test, followed by the Kruskal Wallis non-parametric test and the post hoc Mann-Whitney test.

III. RESULTS

On the tenth day, one rat from the P3 group dropped out because it died during the research period. Microscopic images of the data from each group are displayed in Figure 1. The results of the research were analyzed using the Shapiro-Wilk normality test in Table 1.

Table 1. Normality test for liver cell damage

Groups	Means ± SD	*P**
K	1.23 ± 0.2	0.249
K-	2.51 ± 0.5	0.041
P1	1.57 ± 0.4	0.117
P2	1.51 ± 0.5	0.014
P3	1.43 ± 0.4	0.566

*Shapiro-Wilk test. Significant if p>0.05.

Based on the normality test results, it can be inferred that the data were not normally distributed (p <0.05), so it was continued with the non-parametric Kruskal-Wallis test.

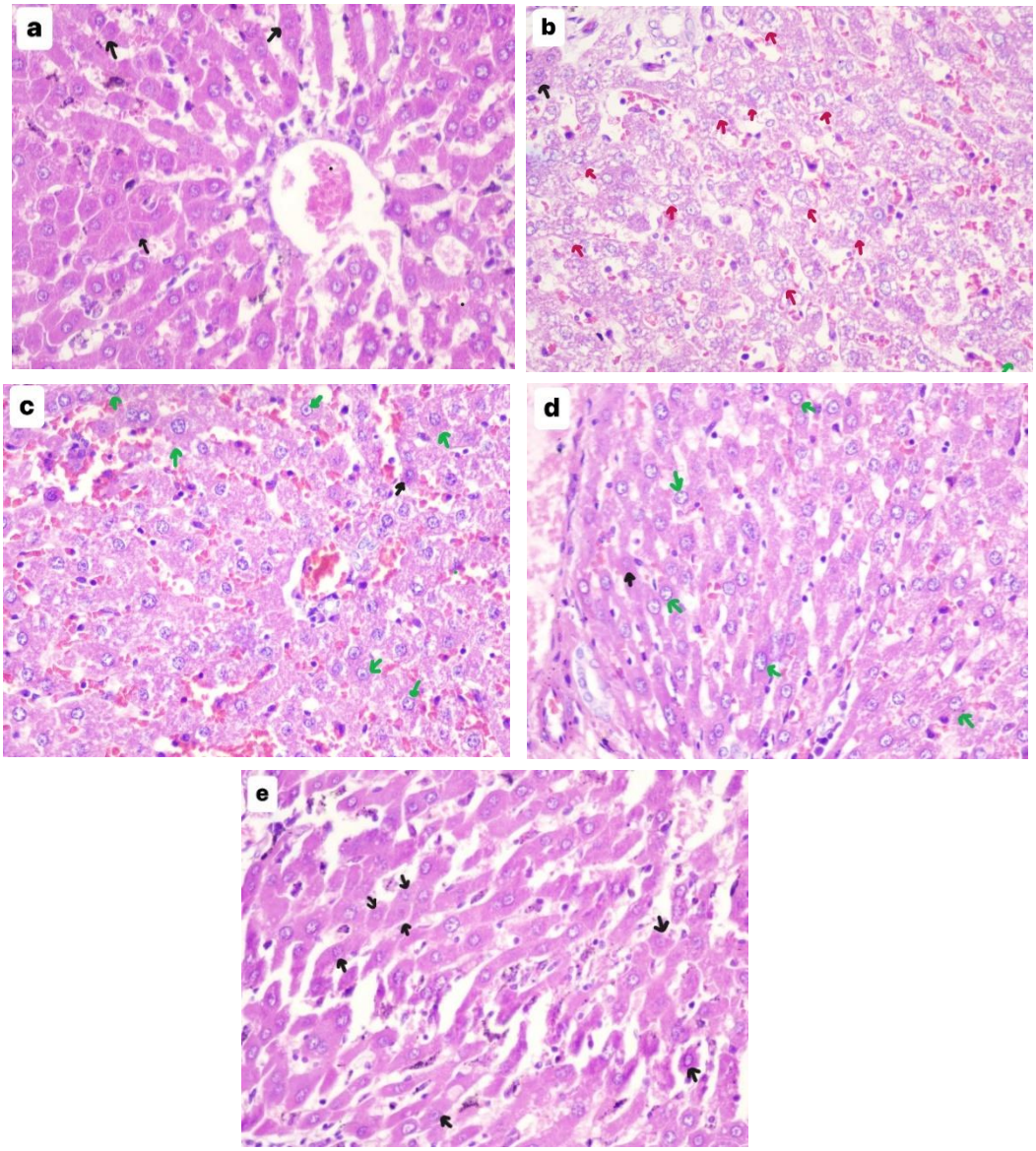

Figure 1. Microscopic view of the liver cells of Wistar rats (magnification 400x). (a) Healthy rat hepatocyte cells (K) scored 1, with the appearance of normal hepatocytes (black arrow). (b) Hepatocyte cells of negative control rats (K-) scored 3, with the appearance of parenchymatous degeneration of cells (red arrow). (c) Hepatocyte cells of treated rats 1 (P1) scored 2, with the appearance of a cell experiencing hydropic degeneration (green arrow). (d) Hepatocyte cells of treated rats 2 (P2) scored 2, with the appearance of a cell experiencing hydropic degeneration (green arrow). (e) Hepatocyte cells of treated rats 3 (P3) scored 1, with the appearance of normal hepatocytes (black arrow).

Table 2. Hypothesis test for liver cell damage

Groups	Means ± SD	*P**
K	1.23 ± 0.2	0.003
K-	2.51 ± 0.5	
P1	1.57 ± 0.4	
P2	1.51 ± 0.5	
P3	1.43 ± 0.4	

*Kruskal-Wallis test. Significant if $p<0.05$.

Based on the Kruskal-Wallis hypothesis test, it can be inferred that there is a significant difference in damage between the five groups of experimental animals, so it can be continued with a post hoc Mann-Whitney test to determine the differences between each group.

Table 3. Differences test results

Groups	K	K-	P1	P2	P3
K	-	0,001*	0,165	0,456	0,366
K-		-	0,002*	0,004*	0,002*
P1			-	0,946	0,557
P2				-	0,882
P3					-

Based on the Mann-Whitney differences test results, damage was significantly different between the K group and the K- group, the K- group and the P1 group, the K- group and the P2 group, the K- group and the P3 group.

IV. DISCUSSION

Microscopic differences in the livers of Wistar rats between the K- and K group.

There was a significant difference in damage between the K- and K groups. This difference indicates a significant increase in liver cell damage due to exposure to cigarette smoke, two cigarettes per day. Based on Table 2, the highest average liver cell damage was found in the K- group, so the level of damage to K- liver cells was more severe than K group.

Cigarette smoke contains toxic chemical compounds, including tar, carbon monoxide (CO), hydrogen cyanide (HCN), benzo-a-pyrine (BaP), Tobacco Specific Nitrosamine (TSNA), and nitrogen monoxide (NO) or gas components that will interact with cells in the body to form free radicals.[15–17] Free radicals are atoms with unpaired electrons, so they attract electrons from other molecules to achieve stability and form new free radicals, creating a chain reaction and damaging the rat's liver cells.[5,6] This effect was proven in the microscopic picture of the K- liver. The scores 2 and 3 were obtained, indicating the presence of parenchymatous and hydropic degeneration.

The results of this research are in line with the research of Ulilalbab *et al*., which proved that exposure to cigarette smoke that is equal to two cigarettes per day for 28 days can cause significant liver histological damage.[4]

Parenchymatous degeneration is a reversible degeneration with the mildest degree. Parenchymatous degeneration is characterized by swelling and granules in the cytoplasm due to the shift of extracellular water into the cell. However, the cytoplasm swelling is still not up to twice normal. This damage only occurs in a small part of the cell structure and can cause cell oxidation to be disrupted. The water elimination process is also disrupted, causing water to heap up in the cell. The water will create small to large vacuoles in the cytoplasm, which could push the nucleus to the edge. This feature of injury is known as vacuolar degeneration or hydropic degeneration.[8]

Hydropic degeneration is a reversible degeneration characterized by cytoplasm swelling up to 2x normal, which forms a vacuole image filled with water and does not contain fat. It is often called ballooning degeneration (swelling cells), with wide clear cavities and irregular cytoplasm. Hydropic degeneration has a higher degree of severity than parenchymatous degeneration.[8]

Microscopic differences in the livers of Wistar rats between the K- and the treatment group.

There were significant differences in damage between the K- group and the P1, P2, and P3 groups. These differences indicate significant protection for the liver cells in the treatment group caused by giving them red dragon fruit juice.

Red dragon fruit contains phenolic compounds, flavonoids, tannins, catechins, betanins, polyphenols, and vitamin C, which could reduce and stop the formation of free radicals.[12,13,18] This aligns with previous research, which was proven through the 2,2-diphenyl-1-picrlhydrazyl (DPPH) test of red dragon fruit juice. It showed that red dragon fruit juice has antioxidant activity that can prevent high amounts of free radicals.[12,13] A study conducted by Gita *et al*. stated that red dragon fruit is a source of antioxidants that contain bioactive flavonoid compounds and can counteract free radicals caused by exposure to cigarette smoke.[9]

Components in cigarette smoke enter the body through the respiratory tract to the lungs, are transported by the blood to the heart, and then circulated to the liver. These components interact with cells to form free radicals. Free radicals are an atom or a group of atoms that have one or more unpaired electrons.[5–7] If the number of free radicals is excessive and endogenous antioxidants cannot neutralize them, it will trigger oxidative stress.[4,9] Cells exposed to oxidative stress will experience cell membrane damage.[10]

Red dragon fruit plays a role as an antioxidant that can reduce and stop the formation of free radicals.12–14,18 Antioxidants in red dragon fruit will donate/give one of its hydrogen ions to radicals so that antioxidants can stop further radical reactions.[8]

Based on Table 2, the average damage to K-liver cells was more severe than the treatment groups because groups P1, P2, and P3 had protection in the form of antioxidants contained in red dragon fruit juice, which played a role in stopping the formation of free radicals so that liver cell damage did not occur.

Microscopic differences in the livers of Wistar rats between the K group and the treatment group.

There were no significant differences in damage between the K group and the three treatment groups. Based on Table 5, the average damage to liver cells P1, P2, and P3 was slightly different from the K group. This slight difference happens because the P1, P2, and P3 groups are protected by antioxidants, which can stop the formation of free radicals. However, the protection is not optimal, so the P1, P2, and P3 groups still experience mild damage and have a liver picture that is almost the same as the K group, but there is still damage.

In addition, there were insignificant differences in damage between the P1, P2, and P3 groups. The best reduction in liver cell damage occurred in the P3 group compared to the P1 and P2 groups. This means there is no significant difference between the three doses, so using the smallest or the largest dose will not have such a significant effect. In other words, there are insignificant differences between treatments.

V. CONCLUSIONS

In this study, it can be concluded that the administration of red dragon fruit juice affects the microscopic picture of the liver by protecting the liver cells of Wistar rats exposed to cigarette smoke.

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