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THE EFFECT OF GIVING RED DRAGON FRUIT JUICE (HYLOCEREUS POLYRHIZUS) ON SPERMATOZOA CONCENTRATION IN WISTAR RATS EXPOSED TO CIGARETTE SMOKE

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ABSTRACT

Background : Cigarette smoke contains dangerous chemical components that can produce free radicals and cause oxidative stress, which can reduce sperm quality, including sperm concentration. Rat studies demonstrate that cigarette smoke exposure lowers sperm count by 13–17% and severely affects morphology and motility. Red dragon fruit (Hylocereus polyrhizus) contains vitamin C, vitamin E, beta carotene, lycopene, and flavonoids that function as antioxidants and can counteract the free radicals that enter the body. **Purpose** : To determine the effect of red dragon juice on the concentration of Wistar rat spermatozoa exposed to cigarette smoke. **Method**: This study used a post-test only control group design. The total sample was 35 male Wistar rats, divided into 5 groups. Healthy control group is the group without intervention. Negative control group was only exposed to cigarette smoke. Group P1 was exposed to cigarette smoke and given 2 grams/2.5 ml of red dragon fruit juice. Group P2 was exposed to cigarette smoke and given 4 grams/2.5 ml of red dragon fruit juice. Group P3 was exposed to cigarette smoke and given 8 grams/2,5 ml of red dragon fruit juice. Intervention is given for 28 days and on day 29 all rats are terminated and their spermatozoa concentration is checked. **Result** : The mean concentrations of spermatozoa were: Group K(-) = 42.83; Group K(+) = 31.17; Group P1 = 29.17; Group P2 = 63.33; Group P3 = 72.00. The One Way ANOVA test found a significant difference between the five treatment groups. The Post-Hoc test found significant differences in the K(-) group against K(+), P1, P2, P3. In the K(+) group toward P2 and P3 there was a significant difference. In the P1 to P2, P3 groups, there were significant differences. In the P2 to P3 groups, there was a significant difference. **Conclusion** : Giving red dragon fruit juice can affect the concentration of spermatozoa in Wistar rats exposed to cigarette smoke.

INTRODUCTION

According to the World Health Organization (WHO), infertility is the failure to get pregnant in a married couple who has had regular and active sexual intercourse for 12 months without using contraceptives.[1] There are 50–80 million couples in the world of childbearing age who experience infertility, while in Indonesia, around 10-15% of the population experiences infertility.[2]

Spermatogenesis is a complex process in undifferentiated primordial germinativum cells when spermatogonia with 46 diploid chromosome sets proliferate and then convert to specialized methyl spermatozoa with 23 haploid chromosome pairs. Spermatogenesis occurs in the seminiferous tubules which can occur during the sexually active perioddue to stimulation of anterior pituitary gonadotropic hormone.[3]

Cigarette smoke contains free radicals that suppress the decline of antioxidants and result in oxidative stress. Cigarette smoke is broken down into gaseous and particulate forms. Elements in the form of gas: carbon monoxide (CO), carbon dioxide (CO2), nitrogen dioxide (NO), hydrogen cyanide (HCN), and

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ammonia (NH3). Elements in particulate form in cigarette smoke, namely: nicotine, tar, and metal.[4]

Unhealthy lifestyles such as smoking can have harmful effects on health due to the entry of free radicals into the body which can cause an increase in ROS (Reactive Oxygen Species) resulting in DNA damage from spermatozoa, and can increase cell apoptosis.[5] Increased production of ROS (Reactive Oxygen Species) causes oxidative stress due to weakened defense from antioxidants.[6] Studies in rats exposed to smoke show decreased spermatogonia, spermatocytes and spermatids, along with thinner spermatogenic layers.[7]

The content of red dragon fruit such as vitamin C, beta-carotene, lycopene, vitamin E and flavonoids that function as antiproliferative antioxidants. Red dragon fruit contains high levels of vitamin C so it is able to ward off free radicals and can neutralize them before free radicals damage the body because Vitamin C can easily dissolve in water so it can neutralize free radicals.[8]

Flavonoids found in red dragon fruitfunction as antioxidants. Flavonoids can prevent oxidative stress, and flavonoids can inhibit lipid peroxidation, so free radicals cannot develop into new free radicals.[9] Betalains is pigments that responsible for the vibrant color of the fruit and also exhibit strong antioxidant activity. They play a role in neutralizing oxidative stress and may reduce inflammation.[10]

Until now, there has been no empirical study that reports the effect of giving red dragon fruit juice on spermatozoa concentration. With this study, the author wants to find outmore about the effect of giving red dragon fruit juice on spermatozoa concentration in male Wistar rats exposed to cigarette smoke.

METHODS

This study uses the form of experimental research with *post test only group design*. The samples for this study were male Wistar rats (*Rattus novergicus*) obtained from the Biology Laboratory of the Faculty of Mathematics and Natural Sciences (FMIPA), Semarang State University

The research sample was taken from the population randomly. The sample size uses the *Federer* formula, with the minimum number of samples in each group being 5 rats, so the total sample size is at least 25 rats. In this study, 7 Wistar rats were used in each group, so the total number needed was 35 rats.

Inclusion criteria are as follows:

- Male wistar rats
- 6-8 weeks old
- Body weight of 200-300 grams

Exclusion criteria are male Wistar rats with anatomical abnormalities and those that are not actively moving.

Drop out criteria are rats that died in the study.

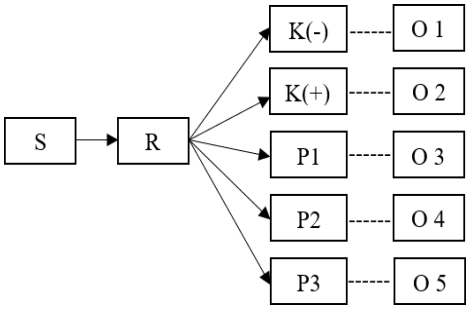

Figure 1: Schematic of research designDescription:
S = Sample
R = RandomizationK,
P = Treatment
O = Observation
K(-) = Negative control, mice that receiveda standard diet, without red dragon fruit juice and cigarette smoke exposure.
K(+) = Positive control, mice with astandard diet were exposed to 2 cigarettes per day of cigarette smoke, without the administration of red dragon fruit juice for 28 days.
P1 = Rats with a standard diet exposed to cigarette smoke, with the administration of 2 g/2.5 ml per day of red dragon fruit juice for 28 days.
P2 = Rats with a standard diet exposed to cigarette smoke, with the administration of 4 g/2.5 ml per day of red dragon fruit juice for 28 days.
P3 = Rats with a standard diet exposed to cigarette smoke, with the administration of 8 g/2.5 ml per day of red dragon fruit for 28 days.

Thirty-five male Wistar rats were randomly selected and met the inclusion criteria. Adapted for 7 days, then divided into 5 treatment groups, each consisting of 7 rats per group.

Red dragon fruit juice was made by homogenizing it with manual mashing, Then dividing the finished juice according to the respective doses of 2 grams per 2.5 ml, 4 grams per 2.5 ml, and 8 grams per 2.5 ml.

The treatment method is done by giving exposure to cigarette smoke, namely putting each group into a

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wooden box (30x25x15 cm) that has air vents at the top for the entry of cigarette smoke into the box and the box is connected to the channel of cigarette smoke originating from burning 2 cigarettes / day, then given red dragon fruit juice with a gastric sonde at the specified dose.

After 28 days, termination was carried out by cervical dislocation in rats that had been given chloroform cotton. Sperm sampling is done by cutting the vas deferens. Then the results of the semen coming out of the vas deferens are collected in a petri dish and dripped with NaCl and stirred so that it is homogeneous and examined. Examination of spermatozoa concentration can be done using a Hemocytometer.

DATA ANALYSIS

The data obtained from the five sample groups were processed using the SPSS computer program. Then, a normality test was conducted with the *Shapiro-Wilks* test because the number of samples was less than 50. Furthermore, the *Levene* testwas conducted to determine whether the groups had the same variance or not. Data distribution was normal, so parametric tests were carried out with the *One Way Anova* test and continued with *Post-Hoc LSD/Bonferroni*. Differences were considered significant if the p-value was <0.05 with a 95% confidence interval.

RESULTS

In this study results, the K(+) group was 31.17, K(-) 42.83, P1, P2 and P3 were 29.17, 63.33 and 72.00 respectively. These results show that the concentration of spermatozoa in K (-) is higher than in K (+). The P1 group produced a lower spermatozoa concentration than the K(-) and K(+) groups. The P2 group produced a higher increase in spermconcentration than the K(-) and K(+) groups. Group P3 produced a higherconcentration than P1, P2 and groups K(-)and K(+).

Table 1. Descriptive Analysis of Spermatozoa Concentration in each group

Group	*Average*	*Min*	*Max*
K-	42,83	35	53
K+	31,17	23	39
P1	29,17	25	34
P2	63,33	56	69
P3	72,00	59	80

Based on the table above, it can be seen that the average for the highest spermconcentration is in the 3rd treatment groupwhich is 72.00, while the lowest is in treatment group 1 which is 29.17.

Table 2. Results of ONE WAY Anova Test of Spermatozoa Concentration

Group	P-value
K-	
K+	
P1	< 0,001
P2	
P3	

One Way Anova test, p: significance value,meaningful if p < 0.05

From the *One Way Anova* test, the results obtained p = <0.001 (p<0.05) so it can be concluded that there is a significant difference between the five of each group. Furthermore, finding out the differences between treatment groups can be continuedby using the LSD *Post Hoc* test.

Table 3. *Post Hoc LSD* test results Spermatozoa Concentration

Group		p
I	**II**	
K-	K+	0,004*
	P1	0,001*
	P2	<0,001*
	P3	<0,001*
K+	P1	0,592
	P2	<0,001*
	P3	<0,001*
P1	P2	<0,001*
	P3	<0,001*
P2	P3	0,027*

From the results of the *Post Hoc* LSDtest, it was found that the K- group againstthe K +, P1, P2 and P3 groups had significant differences, the K + group against the P2 and P3 groups had significant differences, the P1 group against the P2 and P3 groups had significant differences, and the P2 group against the P3 group had significant differences.

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DISCUSSION

Cigarette smoke contains gaseous elements, namely carbon monoxide (CO), nitrogen oxides (NO), ammonia (NH3), hydrogen cyanide (HCN) and carbon dioxide (CO_2) while in particulate form there are tar, nicotine and metals. There are three main harmful elements in the content of cigarette smoke, namely carbon monoxide, nicotine and tar which can increase the production of free radicals.[4] The largest content in cigarettes is nicotine which is a dangerous insecticide substance, each cigarette contains 8-12 mg of nicotine.[11] In smokers, nicotine can accumulate in blood vessels and cause the narrowing of blood vessels.[12]

Free radicals in cigarette smoke are unstable so these compounds are highly reactive and can cause tissue damage. In one puff of a cigarette there are 1014 free radical molecules or ROS that can cause damage to sperm.[12] Some of the main ROS,such as superoxide anion, hydroxyl radical and hydrogen peroxide in semen plasma can cause infertility.[13]

Cigarettes contain chemicals that can disrupt spermatogenesis in seminiferous tubules and inhibit leydid cells which can inhibit the secretion of the hormone testosterone. Meanwhile, cigarette smoke contains carcinogens such as tar which can affect spermatozoa DNA and can reduce testosterone levels and increase cell apostosis.[14]

Increased production of free radicals will damage spermatozoa DNA, damage the membrane of spermatogenic cells, disrupt ion transport for proliferation and growth of spermatogenic cells, and can increase the occurrence of spermatozoa apoptosis.[15,16]

Cigarette smoke exposure induces lipid oxidation in unsaturated fatty acid chains. Lipids will oxidize and then undergo reactions that form free radical products, including: PUFA free radicals,peroxyl free radicals and superoxide free radicals. The increasing number of free radical products can cause the decomposition of unsaturated fatty acids into unstable lipid peroxides. Lipid peroxidation will cause damage to the structure and metabolic disorders of spermatozoa resulting in reduced spermatozoa concentration.[17] In this study in the same direction, namely in accordance with research conducted previously by Galih Bakti (2019) that exposure to cigarette smoke resulted in lower spermatozoa concentration, due to oxidative stress conditions caused by the excessive increase in free radicals, damaging the quality of spermatozoa.[15,16]

Red dragon fruit contains bioactive substances, namely antioxidants that can improve the quality of spermatozoa. Red dragon fruit is easily available because of its abundant production every year. The sweetness level of red dragon fruit reaches 13-15^0 brix. The redder the color of the reddragon fruit, the more betacarotene it contains. The percentage of water content in red dragon fruit reaches 90% which means red dragon fruit cannot be stored for too long, it can be stored for 7 to10 days at a temperature of 14 C.[18]

In this study, the P1 group that had been exposed to cigarette smoke andred dragon fruit juice at a dose of 2 grams / 2.5 ml daily produced a lower spermatozoa concentration than the K (-) and K (+) groups. This is because the red dragon fruitjuice given at a dose of 2 grams/2.5 ml has not been able to show a significant increasein spermatozoa concentration. The P2 group that had been exposed to cigarette smoke and red dragon fruit juice at a dose of 4 grams/2.5 ml every day resulted higher spermatozoa concentration compared to theK(-) and K(+) groups. The P3 group which has been exposed to cigarette smoke and red dragon fruit juice at a dose of 8 grams/2.5 ml daily showed a significant difference in spermatozoa concentration between P1, P2 and groups K(-) and K(+). This shows that an increase in the dose of red dragon fruit juiceresulted in a higher concentration of spermatozoa in Wistar rats exposed to cigarette smoke, which means that the administration of red dragon fruit juice at ahigher dose of 8 grams / 2.5 ml provides a higher concentration of spermatozoa compared to other groups.

Giving red dragon fruit juice at a dose of 8 gram/2.5 ml can result higher sperm concentration because it contains antioxidants such as Vitamin C, Vitamin E lycopene, beta-carotene and flavanoids.[19] Antioxidants in red dragon fruit juice are high in Vitamin C so they are able to ward off free radicals and can neutralize them before they damage the body.[19] Electron molecules from free radicals in the body that binds to antioxidant compounds can inhibit the oxidation process of metabolic cells. [20]

The limitations of this study are the absence of sperm concentration comparison before and after treatment and the inability to evaluate the amount of oxidative stress brought on by cigarette smoke exposure.

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CONCLUSIONS

Giving red dragon fruit juice can affect the increase in spermatozoa concentration in Wistar rats exposed to cigarette smoke. The highest concentrationof spermatozoa is in doses of 8 grams / 2.5 ml every day.

ADVICE

It is necessary to conduct further research on the effect of giving red dragon juice fruit on the concentration of Wistar ratspermatozoa with other substances and alsoto investigate MDA levels after beingexposed to cigarette smoke.

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