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Original Article

The Effect of Dragon Fruit Juice on the Serum Testosterone Level, Sperm Count, and Morphology in Wistar Albino Rats Exposed to Cigarette Smoke

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Cigarette smoke contains three primary toxins: carbon monoxide (CO), nicotine, and tar, which can lead to an increase in Reactive Oxygen Species (ROS) and a decrease in antioxidant reserves. The depletion of antioxidant reserves can damage testicular cells, inhibiting the division of germinal testicular cells. Consequently, testosterone production is also inhibited, adversely affecting the quality of spermatogenesis. Red dragon fruit juice may serve as a potential antioxidant that mitigates ROS, thereby preventing damage to testicular cells. This research employed a "post-test only control group design." Twenty-five laboratory rats were divided into groups: KP (positive control), KN (negative control), P1, P2, and P3 (exposed to cigarette smoke for 15 minutes and subsequently administered dragon fruit juice at doses of 1.44 cc, 2.88 cc, and 4.32 cc, respectively) for 24 days. On the 32nd day, serum testosterone concentration was determined using ELISA. Additionally, sperm count and morphology were assessed using a digital microscope. Data were analyzed using one-way ANOVA, with a significance level set at $p<0.05$.The results of this research are summarized as follows: the average serum testosterone concentrations were KN 2.29 ng/ml, KP 2.65 ng/ml, P1 2.35 ng/ml, P2 2.86 ng/ml, and P3 2.39 ng/ml. The sperm counts were as follows: KN 399 million/ml, KP 269 million/ml, P1 312 million/ml, P2 357 million/ml, and P3 362 million/ml. The morphology of spermatozoa was recorded as follows: KN 72.01%, P1 81.09%, P2 82.33%, and P3 87.70%. Statistical analysis using one-way ANOVA indicated that the concentration of serum testosterone was not significantly different ($p=0.109$), while significant differences were observed in sperm count ($p=0.005$) and sperm morphology ($p=0.005$).Based on the statistical analysis, red dragon fruit juice did not significantly affect serum testosterone hormone concentration. However, it did positively influence the quality of spermatozoa in rats exposed to cigarette smoke, resulting in increases in both sperm count and morphology.

Keywords: Cigarette Smoke, Spermatozoa, Testosterone, Dragon Fruit

Indonesia is a developing country where the majority of the population consumes cigarettes (1). According to the World Health Organization (WHO) in 2008, Indonesia ranked third in the world for the largest number of active smokers, following China and India (2). Recent research published by Cleveland's Clinical Urology News indicates that an increased number of abnormal free radicals can damage cells (3).

Data from Riskesdas (2013) show that smok-ing habits among Indonesians aged over 15 years increased from 34.2% in 2007 to 34.7% in 2010, and further rose to 36.3% in 2013. Among these smokers, 64.9% were male, 2.1% were female, and 1.4% were children aged 10 to 14 (4).

Cigarette smoke contains approximately 4,000 compounds, including nicotine, tar, 3,4-benzopyrene, carbon monoxide (CO), carbon dioxide (CO2), nitrogen oxides (NOx), ammonia (NH3), sulfur (S), as well as over 200 other toxins

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and 40 carcinogenic chemicals (5). These compounds can interact with body cells to form free radicals or Reactive Oxygen Species (ROS), leading to oxidative stress (6). Oxidative stress can result in lipid peroxidation (7).

Lipid peroxidation is a chain oxidation reaction that produces malondialdehyde (MDA) (8). The concentration of MDA in the blood can serve as an indicator of free radicals present in the body (9). Antioxidants are compounds that capture free radicals and prevent oxidative reactions caused by ROS (10). The body has a natural defense mechanism against free radicals through the formation of endogenous antioxidants (11).

When the body's antioxidants are unable to neutralize free radicals, cellular damage occurs (12). Most natural antioxidants in plants contain phenolic compounds distributed throughout the plant, including in wood, seeds, leaves, fruits, roots, and flowers (13). Phenolic compounds, such as flavonoids, have the ability to convert and reduce free radicals. One such plant that contains these beneficial compounds is dragon fruit (14).

Materials and methods

This research employed a "post-test only control group design." The cultivation of test animals and exposure to cigarette smoke were conducted in the Physiology Laboratory of the Faculty of Pharmacy at Andalas University, Padang. The study population consisted of male Wistar albino rats (Rattus norvegicus) obtained from the aforementioned laboratory. The inclusion criteria for the samples were male white rats aged 2 months, with a body weight ranging from 200 to 250 grams.

A total of twenty-five male rats were assigned to different exposure groups: KP (positive control), KN (negative control), P1, P2, and P3. The rats in the P1, P2, and P3 groups were exposed to cigarette smoke for 15 minutes and then administered dragon fruit juice at doses of 1.44 cc, 2.88 cc, and 4.32 cc, respectively, once daily for 24 consecutive days. The positive control group consisted of rats exposed to cigarette smoke without any dragon fruit juice, while the negative control group included rats that were not exposed to cigarette smoke. Testosterone levels were monitored using the enzyme-linked immunosorbent assay (ELISA) method, and the quality of spermatozoa (both quantity and morphology) was assessed using a digital microscope.

A normality test was conducted on the obtained data, followed by analysis using one-way ANOVA with a confidence level of 95% ($p<0.05$ indicating significance).

Results

Data on testosterone hormone levels were analyzed using one-way ANOVA followed by Bonferroni post hoc testing (Table 1).

The table indicates that there is no significant difference in hormone concentration between the control group (K) and the exposed group (P). The results reveal that all groups have a p-value greater than 0.05.

Based on the one-way ANOVA test, the obtained p-value was 0.005 ($p<0.05$), indicating a significant difference between the positive control group and the exposed groups receiving dragon fruit juice (Table 2). An increase in the average spermatozoa count was observed starting from the first exposed group (P1).

The Post Hoc Bonferroni test demonstrates significant differences in averages between each group, particularly between KN and KP, as well as between KN and P1 (Table 3).

Based on According to the one-way ANOVA method, the p-value obtained from this experiment was 0.034 ($p<0.05$), indicating a significant difference between the positive control group and the exposed groups receiving dragon fruit juice (Table 4). The highest average spermatozoa morphology was observed in the third exposed group (P3).

To further examine the differences between the control and exposed groups, refer to the results of

the multiple comparisons (post hoc Bonferroni test) in Table 5.

Table 5 shows the Post Hoc Test Bonferroni for the significant difference of averages of each and every research group, in which there is, with a highly significant difference noted between KN and KP.

Table 1. Average concentration of testosterone hormones (ng/ml) for the control and exposed groups after administration of red dragon fruit juice

Group	n	Hormone concentration (ng/ml) Average ± SD	p value
KN	5	2,29 ± 0,30	
KP	5	2,65 ± 0,16	
P1	5	2,35 ± 0,44	0,109
P2	5	2,86 ± 0,50	
P3	5	2,39 ± 0,30	

Table 2. Average amount of spermatozoa (million/ml) for the control and exposed groups after administration of red dragon fruit juice

Group	n	Spermatozoa (million/ml) Average ± SD	p value
KN	5	399,20 ± 23,95	
KP	5	296,60 ± 43,59	
P1	5	312,80 ± 56,25	0,005
P2	5	357,40 ± 34,84	
P3	5	362,20 ± 37,28	

Table 3. Results of post hoc Bonferroni test on the degree of significance for the amount of spermatozoa in each research group

K	KN	KP	P1	P2	P3
KN	-	0,007*	0,31*	1,000	1,000
KP	0,007*	-	1,000	0,281	0,189
P1	0,031*	1,000	-	0,978	0,687
P2	1,000	0,281	0,978	-	1,000
P3	1,000	0,189	0,687	1,000	-

Table 4. Average morphology of spermatozoa (%) for the control and exposed groups after administration of red dragon fruit juice

Group	n	Morphology of Spermatozoa X ± SD	p value
KN	5	87,84 ± 8,07	
KP	5	72,01 ± 8,95	
P1	5	81,09 ± 10,50	0,030
P2	5	82,33 ± 3,47	
P3	5	87,70 ± 6,75	

Table 5. Presents the results of the Post Hoc Bonferroni test, highlighting significant differences in averages among each research group

K	KN	KP	P1	P2	P3
KN	-	0,049*	1,000	1,000	1,000
KP	0,049*	-	0,844	0,522	0,522
P1	1,000	0,844	-	1,000	1,000
P2	1,000	0,522	1,000	-	1,000
P3	1,000	0,522	1,000	1,000	-

Discussion

The effect of red dragon fruit juice on the concentration of serum testosterone hormones in cigarette smoke-exposed Wistar albino rats (Rattus norvegicus)

Results from this experiment indicated a decrease in the average testosterone concentration in the negative control group (KN) compared to the positive control group (KP). Additionally, a decrease in average testosterone concentration was observed in the third exposed group (P3) relative to the first (P1) and second (P2) exposed groups. These findings suggest that hormone levels are unstable due to exposure to cigarette smoke, leading to a reduction in serum testosterone concentration. Statistically, the one-way ANOVA method revealed a non-significant difference, with a p-value of 0.109 ($p > 0.05$). Based on this statistical analysis, we conclude that the presence of red dragon fruit juice does not significantly affect the concentration of serum testosterone hormones in Wistar albino rats exposed to cigarette smoke. According to the Post Hoc Bonferroni test, no significant differences were found among the experimental groups, as all p-values were greater than 0.05.

As shown in Table 1, there is a slight increase in the average testosterone concentration in the second exposed group (P2) compared to the first (P1) and third (P3) exposed groups. This suggests that 2.88 cc (133 grams) of red dragon fruit juice may have the ability to mitigate free radicals generated by cigarette smoke and to increase serum testosterone concentration. However, increasing the dose of red dragon fruit juice to 4.32 cc (200 grams) did not yield any notable effect on testosterone concentration, as indicated by the results for the third exposed group (P3) in Table 1.

The hypothalamus synthesizes a decapeptide known as gonadotropin-releasing hormone (GnRH), which subsequently binds to gonadotropins and stimulates the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (to a relatively small degree) into the general circulation. LH is carried to Leydig cells, where it binds to specific membrane receptors. This binding activates adenyl cyclase, leading to the formation of cyclic adenosine monophosphate (cAMP) and initiating the secretion of testosterone hormones. Conversely, the concentration of testosterone inhibits LH release from the anterior pituitary gland through a direct effect on the pituitary or an inhibitory effect on the hypothalamus, a phenomenon commonly referred to as hypothalamic negative feedback (15).

Cigarette smoke exposure is known to induce oxidative stress, deplete antioxidants, and degrade spermatozoa quality, including concentration, motility, and morphology (16). Depletion of antioxidant activity can result in increased reactive oxygen species (ROS) concentration, thereby causing oxidative stress. Oxidative stress is a leading cause of damage to Leydig cells, which play a crucial role in testosterone secretion with the aid of LH. Damage to Leydig cells contributes to decreased testosterone concentration (17). In addition to smoking habits, testosterone concentration can be influenced by other factors such as age, nutrition, and certain diseases.

The observation that the intake of up to 4.32 cc

of dragon fruit juice does not improve serum testosterone concentration in cigarette-exposed rats may be attributed to the possibility that the substance does not affect hormone metabolism directly. Another potential explanation is that the administered dose may not be sufficient to enhance serum testosterone hormone levels effectively.

The effect of red dragon fruit juice on the amount of spermatozoa in cigarette smoke-exposed Wistar albino rats (Rattus norvegicus)

The results of the one-way ANOVA indicate a significant effect of dragon fruit juice intake on the amount of spermatozoa in rats (Rattus norvegicus), with a p-value of 0.005. This suggests that red dragon fruit juice positively influences the quantity of spermatozoa in Wistar albino rats. As shown in Table 2, the amount of spermatozoa increases proportionally with the dosage of dragon fruit juice (from P1 to P3).

As mentioned in the previous section, exposure to cigarette smoke can lead to oxidative stress due to an imbalance between reactive oxygen species (ROS) and antioxidants. During oxidative stress, the concentration of ROS or free radicals increases, which is also indicated by elevated levels of malondialdehyde (MDA). Koeman (1987) stated that an excessive amount of free radicals in the liver causes lipid peroxidation, leading to inhibited energy production from mitochondria (18). This inhibition of energy production subsequently affects protein synthesis as well.

Lipid peroxidation damages germinal cells, which are precursors to spermatozoa. It also adversely affects Sertoli cells in the seminiferous tubules, which provide nutrition to spermatogenic cells and produce androgen-binding protein (ABP) to enhance testosterone levels. Additionally, Leydig cells in the interstitial tissue of the seminiferous tubules are also damaged by lipid peroxidation. When Leydig cells are compromised, the synthesis of testosterone, which is essential for spermatogenesis, is inhibited (19).

The process of spermatogenesis is regulated by the hypothalamus, which secretes gonadotropin-releasing hormone (GnRH) to stimulate the anterior pituitary gland to release follicle-stimulating hormone (FSH) and luteinizing hormone (LH). In the testes, these hormones have distinct functions: FSH triggers spermatogenesis in Sertoli cells and promotes the production of ABP, while LH stimulates Leydig cells to produce testosterone, which is necessary for spermatogenesis. ABP binds to testosterone and transports it from the seminiferous tubules to the receptors on germinal cells, facilitating subsequent stages of spermatogenesis (20).

Red dragon fruit is rich in antioxidants with antiproliferative properties. Key components of red dragon fruit include vitamins C and E, beta-carotene, lycopene, and flavonoids (10). Vitamin C, in particular, is abundant in red dragon fruit and serves as an effective antioxidant, neutralizing free radicals before they can cause cellular damage. Its water-soluble nature allows it to easily access all body cells, aiding in the neutralization of free radicals (21). Furthermore, vitamin C is crucial for spermatozoa formation.

According to Linder (2006), vitamin E acts as a fertility enhancer, normalizing the epithelial tissues of the seminiferous tubules (22). A deficiency in vitamin E can lead to degeneration of these tubules, inhibiting the spermatogenesis process and sperm cell production. As an antioxidant, vitamin E in dragon fruit helps protect spermatozoa DNA from damage (23).

This research aligns with previous findings by Nasriyah (2017), which demonstrated that rats given red dragon fruit juice induced with alloxan exhibited increased spermatozoa production in both quantity and rate compared to rats that did not receive red dragon fruit juice.

In this study, it was found that the amount of spermatozoa in the negative control group (KN), which was not exposed to cigarette smoke, was greater than that in the positive control group (KP), which was exposed to cigarette smoke, as shown in

Table 2. This indicates that cigarette smoke contains ROS that decrease the average amount of spermatozoa in rats. As previously stated, ROS can damage DNA, proteins, and lipids, leading to accelerated apoptosis of germinal cells and a subsequent decrease in spermatozoa quantity, contributing to male infertility (24). In groups where cigarette-exposed rats were administered dragon fruit juice (P1, P2, P3), a proportional increase in the average spermatozoa count was observed compared to the positive control group (KP). Thus, the intake of red dragon fruit juice appears to enhance the average amount of spermatozoa in cigarette-exposed Wistar albino rats.

The effect of red dragon fruit juice on the morphology of spermatozoa in cigarette smoke-exposed Wistar albino rats (Rattus norvegicus)

Similar to the previous results from the one-way ANOVA analysis, a significant effect was observed between the intake of dragon fruit juice and the morphology of spermatozoa in rats (Rattus norvegicus), with a p-value of 0.030. This indicates that the intake of red dragon fruit juice improves the morphology of spermatozoa in Wistar albino rats. As shown in Table 4, the average morphology of spermatozoa improves proportionally with the dosage of dragon fruit juice intake (from P1 to P3).

According to the results presented in Table 4, the morphology of normal spermatozoa in the positive control group (KP) is lower than that in the negative control group (KN), demonstrating that cigarette smoke negatively impacts spermatozoa morphology. The morphology of the exposed groups (P1, P2, and P3) increases proportionally compared to the positive control group (KP), further supporting the conclusion that the intake of red dragon fruit juice enhances the average morphology of spermatozoa in cigarette smoke-exposed rats.

Cigarette smoke exposure is known to inhibit the spermatogenesis process by generating free radicals and reactive oxygen species (ROS). These free radicals and ROS damage the overall structure of mature spermatozoa, thereby weakening their morphology. The data in Table 4 indicates that the cigarette smoke-exposed control group (KP) has a lower average morphology value compared to the unexposed control group (KN). This evidence reinforces the notion that cigarette smoke adversely affects spermatozoa morphology in Wistar albino rats.

The presence of free radicals in the body can lead to various diseases, including those affecting the reproductive system. Under normal conditions, there is a balance between ROS formation and antioxidant activation in male reproductive organs, with only a small amount of ROS needed to regulate the formation of normal spermatozoa (25). However, exposure to cigarette smoke or other pollutants increases ROS production, leading to an imbalance in the peroxidation system. Oxidative stress in spermatozoa occurs due to the abundance of polyunsaturated fatty acids (PUFA) in the plasma membrane, while the cytoplasm contains only limited amounts of antioxidant enzymes.

When oxidative stress levels are high, there is a need for a neutralizing agent in the form of additional antioxidants. One viable option for an additional antioxidant is found in red dragon fruit. Antioxidants are compounds that can prevent the formation of ROS (26). Endogenous antioxidants or anti-free radical mechanisms are present in the body, typically in the form of superoxide dismutase (SOD), glutathione peroxidase, catalase, and non-enzymatic antioxidants. The presence of these antioxidants can inhibit the formation of free radicals by acting as hydrogen atom donors, thereby converting free radicals into their stable forms (27).

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Conflict of interest

The authors declared no conflict of interest.

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