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EFFECT OF GIVING PINEAPPLE JUICE (Ananas comosus) ON THE VIABILITY OF WISTAR RAT SPERMATOZOA THOSE EXPOSED TO CIGARETTE SMOKE

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ABSTRACT

Background: Infertility affects 15% of couples worldwide, with 40-50% of cases attributed to unhealthy lifestyle factors in men, such as smoking, alcohol consumption, drug use, obesity, poor dietary habits, coffee intake, and psychological stress. Smoking has a negative impact on reproductive health and reduces the chances of having children. Smoking leads to oxidative stress, lowers antioxidant levels in the testes, thereby disrupting spermatozoa formation. The antioxidant content in pineapple is expected to prevent and repair damage caused by oxidative stress. **Objective:** To investigate the effects of pineapple juice administration on sperm viability in Wistar rats exposed to cigarette smoke. **Methods:** An experimental study was conducted with a post-test only group design involving 25 male Wistar rats divided into 5 groups: K (no treatment), K+ (exposure to cigarette smoke only), T1 (exposure to cigarette smoke and 1 ml of pineapple juice per day), T2 (exposure to cigarette smoke and 2.5 ml of pineapple juice per day), and T3 (exposure to cigarette smoke and 4 ml of pineapple juice per day). All groups received treatment for 28 days. **Results:** The mean percentage of spermatozoa viability in Wistar rats was K = 90.00; K(+) = 76.80; T1 = 76.80; T2 = 89.20; T3 = 93.40. Significant differences were observed between groups K and K+ and T1, with respective p-values of 0.005. Significant differences were also found between groups K+ and T2 (p = 0.008) and T3 (p = 0.001), while no significant difference was observed in group T1 (p = 1.000). Furthermore, there were significant differences between groups T1 and T2 (p = 0.008) and T3 (p = 0.001). Finally, no significant difference was found between groups T2 and T3 (p = 0.331). **Conclusion and Recommendations:** Pineapple juice can prevent and inhibit spermatozoa damage caused by free radicals from cigarette smoke. Further research should measure oxidative stress, provide exposure to each rat, and use varying doses for optimal effectiveness.

INTRODUCTION

Infertility is a global problem in 15% or around 48.9 million couples in the world.[1]Around 40-50% of all cases are caused by male factors and as many as 90% of them are related to less than optimal spermatozoa numbers and parameters. This is caused by at least one of the conditions of low spermatozoa concentration, poor spermatozoa motility, abnormal morphology, or poor viability, namely less than 58% of viable spermatozoa.[1–3]

A bad lifestyle, such as smoking, drinking alcohol, using illegal drugs or narcotics, being obese, being over 35 years old, having a poor diet, drinking coffee, and experiencing psychological stress, can all reduce the quality of spermatozoa.[4]

A smoker has significantly higher spermatozoa

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abnormalities compared to a non-smoker.[5,6]Substances contained in cigarettes such as carbon monoxide, tar and nicotine have a profound effect formation and increase in the amount of Reactive Oxygen Species (ROS) as well as reducing antioxidants in the body.[7]

Antioxidants are substances that can inhibit or stop the oxidation of other substances. Antioxidants, which prevent the body's overreactivity to free radicals, can reduce oxidative stress.[8]Antioxidants can come from endogenous (in the body) and exogenous (outside the body). The body needs exogenous antioxidants to help significantly inhibit free radical reactions.[9]

Pineapple is a type of fruit that has antioxidant levels and is produced abundantly in Indonesia.[10,11] Pineapples are also liked by many people, both local and foreign. Pineapples contain flavonoids and phenolic acids, both of which are types of antioxidants which play a role in inhibiting damage to body cells from the effects of free radicals which can trigger chronic diseases.[10,12,13]Research by Leko BJ et al (2020) proves the ability of pineapple juice to fight free radicals originating from aluminum.

Based on the description above, there has been no research investigating the impact of giving pineapple juice on the viability of rat spermatozoa exposed to free radicals from conventional cigarette smoke. Therefore, researchers want to get more detailed information about how pineapple juice can affect sperm viability in mice exposed to conventional cigarette smoke.

METHODS

This research will be carried out in July – August 2023 at the Biology Laboratory, FMIPA, Semarang State University.The research carried out was experimental research with a post test only control group design using experimental animals as research objects, namely male Wistar rats (Rattus norvegicus L.).

*Ethical clearance*this research hasobtained from the Health Research Ethics Commission (KEPK) Faculty of Medicine, Diponegoro University, Semarang. In this research, sample selection was carried out using a simple random sampling method. There were 5 treatment groups, each containing 5 mice. Treatment in this study was carried out for 28 days with the following conditions:

a. The control group (K) received standard feed without giving pineapple juice and exposure to cigarette smoke.
b. The positive control group (K+) received standard feed with exposure to cigarette smoke without giving pineapple juice.
c. Treatment group 1 (T1) received standard feed, exposure to cigarette smoke, and 1 ml pineapple juice per day. Pineapple juice is given 2 times a day with a dose of 0.5 ml per dose.
d. Treatment group 2 (T2) received standard feed, exposure to cigarette smoke, and 2.5 ml pineapple juice per day. Pineapple juice is given 2 times a day with a dose of 1.25 ml per dose.
e. Treatment group 3 (T3) received standard feed, exposure to cigarette smoke, and 4 ml pineapple juice per day. Pineapple juice is given 2 times a day with a dose of 2 ml per dose.

Termination of Wistar rats was carried out *after* 28 days of treatment, this aimed to remove the vas deferens *and* take sperm samples. The samples were collected, smeared, dried, fixed, and stained by eosin. The sperm are then examined using a microscope, grouped according to live and dead cells, and the number of spermatozoa is counted in percentage for every 100 spermatozoa.

Data from five sample groups will be analyzed *using* the Shapiro-Wilk test to assess normality, the One Way ANOVA test for normal distribution, and the Post Hoc test for analysis of differences. The Kruskal-Wallis and Mann-Whitney tests are used if the data distribution is not normal.

RESULT

Based on table 1, it can be seen that the highest average percentage of spermatozoa viability was in the T3 group (93.40% ± 1.52), namely the group that received treatment given exposure to cigarette smoke and pineapple juice 4ml/day, while the lowest average was in K+ group. (76.80% ± 8.56), namely the group that received the treatment, was exposed to cigarette smoke only and T1 (76.80% ± 10.90),

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namely the group that received the treatment, was exposed to cigarette smoke and pineapple juice 1ml/day.

Table 1. Descriptive Mean and Standard Deviation of Percentage of Spermatozoa Viability

Group	Rat Number	Viability (%)	Average viability of each group (%) ± SD
K	1	90	
	2	95	
	3	93	90.00 ± 4.12
	4	85	
	5	87	
K+	1	85	
	2	75	
	3	79	76.80 ± 8.56
	4	63	
	5	82	
T1	1	60	
	2	76	
	3	75	76.80 ± 10.90
	4	86	
	5	87	
T2	1	89	
	2	85	
	3	93	89.20 ± 3.35
	4	92	
	5	87	
T3	1	94	
	2	95	
	3	93	93.40 ± 1.52
	4	94	
	5	91	

Based on table 2, data on spermatozoa viability (%) for all groups in this study shows that the data is normally distributed, so to find out whether there are differences between groups, a different test is then carried out using the One Way Anova test.

Table 2. Normality Test Results (Shapiro Wilk Test)

Group	Mean ± SD	Median (min–max)	p
K	90.00 ± 4.12	90 (85 – 95)	0.832*
K+	76.80 ± 8.56	79 (63 – 85)	0.460*
T1	76.80 ±10.90	76 (60 – 87)	0.376*
T2	89.20 ±3.35	89 (85 – 93)	0.737*
T3	93.40 ±1.52	94 (91 – 95)	0.492*

Based on table 3, the difference between spermatozoa viability data (%) for all groups produces a p value of 0.001 and a lavender value of 0.078, because the p value is <0.05 it can be concluded that there is a significant difference with homogeneous data variants. To determine the differences between treatment groups, the test was continued using the Post Hoc LSD test.

Table 3. One Way Anova Test Results Spermatozoa Viability (%)

Group	Mean ± SD	p	Lavene
K	90.00 ± 4.12	0.001*	0.078**
K+	76.80 ± 8.56		
T1	76.80 ± 10.90		
T2	89.20 ± 3.35		
T3	93.40 ± 1.52		

Notes: * Significant (p < 0.05); ** Homogeneous (p > 0.05)

Based on table 4, between group K and groups K+ and T1 there is a significant difference, between group K+ and groups T2 and T3 there is a significant difference, and between group T1 and groups T2 and T3 there is a significant difference.

Table 4. LSD Post Hoc Difference Test Results of Spermatozoa Viability (%)

Group		p
I	II	
K	K+	0.005*
	T1	0.005*
	T2	0.851
	T3	0.430
K+	T1	1,000
	T2	0.008*
	T3	0.001*
T1	T2	0.008*
	T3	0.001*
T2	T3	0.331

Information: *Significant (p < 0.05)

DISCUSSION

This study showed a significant difference in the average percentage of spermatozoa viability in the group treated with exposure to cigarette smoke alone (76.80%) and the control group (90.00%). Zhanhui, et al. (2020) stated in their research that there was a difference in the average percentage of spermatozoa viability of smokers and non-smokers, namely 59% and 65%.[5]Previous studies also confirmed that one of the causes of spermatozoa death (necrozoopermia) is exposure to toxic substances, for example tobacco.[14–16]

The most dangerous component of cigarette smoke is cotinine, a metabolite of nicotine.17Khae-

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Hawn Kim et al. (2005), revealed that the nicotine content in cigarettes will reduce the percentage of spermatozoa viability and induce apoptosis through DNA fragmentation and changing chromatin density.[18]Apart from that, nicotine also causes degeneration of the seminiferous tubules (Nesseim, 2011). Kuladip Jana (2010) also revealed that nicotine interferes with the spermatogenesis process by reducing plasma concentrations and the hormone testestron in the testicles. Apart from that, LH and FSH concentrations will also decrease due to inhibited release of gonadotropins in the pituitary gland.[19]

This study shows data from a group of Wistar rats that were exposed to cigarette smoke by administering 1ml/day of pineapple juice (T1); 2.5ml/day (T2); and 4ml/day (T3) respectively produced an average percentage of spermatozoa viability of 76.80 ± 10.90%; 89.20 ± 3.35%; and 93.40 ± 1.52%. The T3 group had a higher percentage of spermatozoa viability compared to the T1 and T2 groups. This research also showed that there was quite a large difference between the positive control group and the two treatment groups who were exposed to cigarette smoke and pineapple juice, namely 2.5 ml and 4 ml per day respectively. However, the pineapple juice dose of 1ml/day was not significantly different from the positive control group. The T1 group and the T2 group and the T1 group and the T3 group had significant differences. Groups T2 and T3 did not differ from each other. This shows that at certain concentrations, pineapple juice can protect spermatozoa from damage or cell death.

Compounds in pineapple that are rich in antioxidants are vitamin C, flavonoids and phenolic acids. One type of flavonoid, namely anthocyanin, has the ability to reduce lipid peroxidation and malondialdehyde (MDA) by repairing damaged tissue.[20]Pineapples can be served in various preparations, including pineapple juice. According to research by Junian (2020), vitamin C can inhibit oxidative stress caused by excessive physical activity.[21]Administration of vitamin C can significantly reduce serum MDA and inhibit lipid peroxidation.[21]

This study has limitations, including no comparison of viability before and after treatment, no measurement of oxidative stress due to exposure to cigarette smoke, and potential bias due to uneven distribution of exposure to male Wistar rat spermatozoa and pineapple processing.

CONCLUSION

The viability of rat spermatozoa is 90% in conditions without exposure to cigarette smoke. Spermatozoa viability was significantly lower with exposure to cigarette smoke. Pineapple juice at doses of 2.5ml and 4ml significantly improved and prevented damage to the viability of Wistar rat spermatozoa exposed to cigarette smoke, while the 1ml dose had no significant effect.

Future research is recommended to measure oxidative stress due to exposure to cigarette smoke in mice to avoid bias and use varying doses to determine the best effective dose range for increasing spermatozoa viability.

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