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RESEARCH ARTICLE

THE PROTECTIVE EFFECT OF *RHIZOPHORA APICULATA* BARK EXTRACT AGAINST TESTICULAR DAMAGE INDUCED BY CIGARETTE SMOKE IN MALE RATS

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ABSTRACT

Background: The mangrove bark extract (*Rhizophora apiculata*) is known to have the ability to inhibit the formation of free radicals, act as antioxidants, and anti-inflammatory.

Objective: This study was attempted to investigate the potency of *Rhizophora apiculata* bark extracts as an antioxidant to protect rat testes from the damage due to cigarette smoke exposure.

Methods: An experimental study using a posttest-only control group design was employed. Samples consisted of 25 male rats divided into 5 groups, namely K (-) not treated, K (+) exposed to cigarette smoke without the administration of mangrove bark extract, groups P1, P2, and P3 were exposed to cigarette smoke and each group received a dose of *Rhizophora apiculata* bark extracts every day for 30 days. Furthermore, P1 obtained 28.275 mg/KgBW, P2 was about 56.55 mg/kgBW, and P3 got 113.10 mg/kgBW.

Results: Analysis using One Way ANOVA showed that there were significant effects of administration of extracts on the average number of primary spermatocytes and the thickness of the seminiferous tubules in the rats that have been exposed to cigarette smoke when compared to controls. The dose of extract that has the best effect was 113.10 mg/kgBW.

Conclusion: *Rhizophora apiculata* bark extract is indicated to have a protective effect that can prevent damage in rats testes exposed to cigarette smoke.

Keywords: Antioxidants, Cigarettes, Mangrove, *Rhizophora apiculata* extract, Testes

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INTRODUCTION

Free radicals are molecules that lose an electron from their free electron pair or are the result of monolithic separation of a covalent bond. As a result of monolithic breakdown, a molecule will split into free radicals that have unpaired electrons. Free radicals can be formed in the human body from the influence of the environment and the body's metabolism. Besides, the body's metabolisms that can produce free radicals are cellular respiration and inflammation. Many human diseases, including accelerated aging, cancer, inflammation, cardiovascular and neurodegenerative disease, and infertility are linked to excessive amounts of free radicals. Therefore, exogenous antioxidants are necessary to help the natural antioxidant in our body to defend our health.[1]

In line with free radicals, infertility is one disease which is caused by it. It affects the population globally up to 15%. Male infertility is the cause in about 20% of cases but may contribute to 40% of infertile couples. Reactive oxygen species (ROS) is well known as a potential contributor to male infertility. Oxidative stress leading to defective sperm function was demonstrated in early studies illustrating the toxic effect of endogenously generated hydrogen peroxide (H_2O_2) on sperm metabolism and motility. The current literature reports that ROS may be a contributing factor in 30–80% of male infertility.[2] In the case of infertility, antioxidants take an important role to solve it.

Furthermore, antioxidants are an important component of the body's defense against oxidative stress. Some antioxidants can be made by the body and some are obtained from food. However, synthetic antioxidants might be unsafe. Therefore, more attention is being given to search for natural antioxidants from plants to prevent oxidative damage.[3]

Mangroves are potential plants for natural antioxidant. They are identified as a big group of plants which consist of many kinds of species that resist salt in the sea. They vegetate along subtropical and tropical coastline and they have good resistance to an extreme condition because they have already adapted to their environment.[4] Indonesia is rich in mangrove forests. But Mangrove plants have not been used optimally by the Indonesian. These plants are usually only left alone or even considered nuisance plants. Whereas there are many mangrove's benefits for ecosystems and other advantages that have not been revealed yet.

Associated with mangroves, *Rhizophora apiculata* (*R. apiculata*) is a part of it that produce a plentiful of bioactive components. This plant might be a potential source of natural antioxidants which is very useful for the community. Also, a big number of it can commonly be found in Indonesia. There are ethnopharmacological studies of *R. apiculata*. They were traditionally used by some Indonesian people to treat stomach aches and heartburn by drinking boiled water from their leaves, flowers, and fruits.[5] Moreover, traditional healer in India used it to treat diarrhea, amoebiasis, vomiting, and nausea.[6] It was reported that the bark extract of *R. apiculata* showed antioxidant activities. The main active ingredients that act as antioxidants and free radical scavenger in *R. apiculata* extract are tannin[4] and pyroligneous acid[7]. The highest content of antioxidant property of *R. apiculata* was located in the bark.[8] The tannin contained in *R. apiculata* is stronger than

standard antioxidant in the case of scavenging activity related to hydrogen peroxide.[9] This study was conducted to investigate the potency of *R. apiculata* bark extracts as an antioxidant to protect rat testes from the damage due to exposure to cigarette smoke.

MATERIAL AND METHODS

This study was an experimental using a posttest-only control group design. It was undertaken in January - February 2019 in University of Lampung. Ethical Clearance was approved by Ethical Commission of Medical Faculty in Universitas Lampung (5291/UN26.18/PP.05.02.00/2018).

The population was male white rats (Rattus norvegicus) Sprague Dawley strain aged 2.5-3 months obtained from the Palembang Veterinary Center. Samples were 25 animals chosen randomly and then divided into 5 groups based on Federer's formula. The sampling criteria consisted of inclusion criteria, namely 1) White rats (Rattus norvegicus) male Sprague Dawley strain, 2) Healthy (mice with hair that was not dull, moved actively, normal consumption of feed, there was no abnormal exudate from the eye, mouth, anus and genital), 3) Age 2.5-3 months (adult), 4) Weight 200-250 grams, 5) Normal activity and behavior. Exclusion criteria were a weight loss of more than 10% after the adaptation period and the rat died during the study.

Rhizophora apiculata bark was extracted by using the maceration method. The bark was obtained from Pasir Sakti sub-district, East Lampung shoreline. The barks were cleaned and subsequently sundried. Next, 1600 grams of mangrove bark were cleaned and cut into small pieces, and put into machine-grinded until the bark turned into powder. After that, the bark powder was sank in 2.6 liters of 95% ethanol solvent and blended homogenously for 24 hours. The mixture with 95% ethanol solvent was filtered with filter paper to obtain the filtrate. The obtained filtrate was evaporated by rotatory evaporator 50^0C until all the ethanol has evaporated.

The study started with the acclimation of rats for 7 days. Next, researchers separated them into 5 groups randomly. The first group was a negative control (healthy group), named group K (-) which was only given standard food and drink. The next 4 groups were exposed to 24 cigarettes smoke every day for 30 days. The positive control group K (+) was only given exposure to cigarette smoke. Treatment group 1 (P1) was given exposure to cigarette smoke and mangrove extract at a dose of 28.275 mg/kgBW. Treatment group 2 (P2) was given exposure to cigarette smoke and mangrove extract at a dose of 56.55 mg/kgBW. Treatment group 3 (P3) was given exposure to cigarette smoke and mangrove extract at a dose of 113.10 mg/kgBW. This intervention spent for 30 days. On 31st day, the rats were terminated. Their testicular organs were sampled for histopathological preparations. The data was obtained from histopathological observations under a microscope with 200x magnification in five fields of view. Researchers calculated the number of primary spermatocytes and measured the thickness of the seminiferous tubules.

The data was tested for statistical analysis using SPSS statistical analysis software using the Shapiro-Wilk normality test and followed by the One Way ANOVA test.

RESULTS

Based on this study, it indicated that there was a remarkable difference in the number of primary spermatocytes from each group. Two groups had a low number of primary spermatocytes namely K+ and P1 group. While the group that had the highest number of primary spermatocytes was the healthy control group. Exposure to cigarette smoke caused a low number of primary spermatocytes (p=0.000) (Figure 1). The addition of *R. apiculata* extract was observed to influence the average number of primary spermatocytes in the P2 group (p=0.006) and P3 group (p=0.031) (Table 1).

Table 1. The average number of primary spermatocyte in 5 visual fields (cell)

Sample	Groups				
	K(-)	K(+)	P1	P2	P3
1	33.25	22.5	21	29.5	31.25
2	39	26.75	27.5	26.5	26.25
3	37.5	28.25	22.5	31.75	33
4	34.75	29	24.5	32	33.75
5	32.25	30.25	25	30.5	32.5
Average	35.35	27.35	24.10	30.05	31.35
±SD	±2.84	±2.99	±2.49	±2.22	±2.99

Researchers observed that exposure to cigarette smoke caused the narrowing of the seminiferous tubules (Figure 2). There was a seminiferous tubules thickness decrease in a group of rats exposed to cigarette smoke alone compared to the control (p=0.026). Administration of extracts with multilevel doses in the group of rats that were smoke exposed maintained the thickness of the seminiferous tubules due to exposure to cigarette smoke. In fact, in the P3 group, the thickness of the subsequent tubules was not different from the healthy group (p=1.000) (Table 2).

Table 2. The average measurement of tubular diameter of the seminifer (μm)

Sample	Groups				
	K(-)	K(+)	P1	P2	P3
1	293.82	270.18	271.30	267.94	277.55
2	332.69	264.68	253.96	302.52	293.07
3	327.59	268.19	273.54	269.27	312.69
4	284.88	261.23	272.1	347.59	337.79
5	282.45	272.96	298.33	254.15	314.11
Average	304.28	267.45	273.85	288.69	307.04
±SD	±24.04	±4.60	±15.85	±37.17	±22.87

DISCUSSION

In this study, the number of primary spermatocytes and the diameter of seminiferous tubules in rats exposed to cigarette smoke was significantly lower compared to the control group. This was consistent with Ahmadnia's studies concluding that cigarette smoke caused impaired testicular histology, reduced diameter of seminiferous tubules, and decreased index of the Sertoli cells in rats.[10] Also, a big number of it commonly can be found in Indonesia. There are ethnopharmacological studies of *R. apiculata*. They were traditionally used by some Indonesian people to treat stomach aches and heartburn by drinking boiled water from their leaves, flowers, and fruits.[5] Moreover, traditional healer in India used it to treat diarrhea, amoebiasis, vomiting, and nausea.[6] Cigarette smoke is a common source of reactive oxygen species (ROS), which is proven to be associated with decreased male fertility and poor semen quality.[17] Spermatogenesis is affected by cigarette smoke because it contains toxic components that stimulates testes histology damage. It is caused by oxidative stress triggered by cigarette smoke.[10] Moreover, early exposure to cigarette smoke affects adult sperm quality in mice.[18]

Related to oxidative testicular damage, it can be caused by excess ROS. They are active byproducts of aerobic metabolism. They are created by our body continuously in the process of cell metabolism via mitochondria. But, they are neutralized by endogenous antioxidant systems. If oxidant-antioxidant balancing can be maintained well, our body can be always in healthy state. On the other hand, this balancing can be disrupted by increasing number of ROS production and it takes a long time, oxidative stress will occur. Spermatogenesis normally produces ROS by process of circulating leucocyte and the capacitation. Even though, an increasing number of external free radicals will incline the number of ROS production in the epididymis and testis, stimulate to a decline in sperm motility and viability. Related to this case, male infertility is increased. Moreover, if a patient is in condition of pro-oxidative for example smoking.[19]

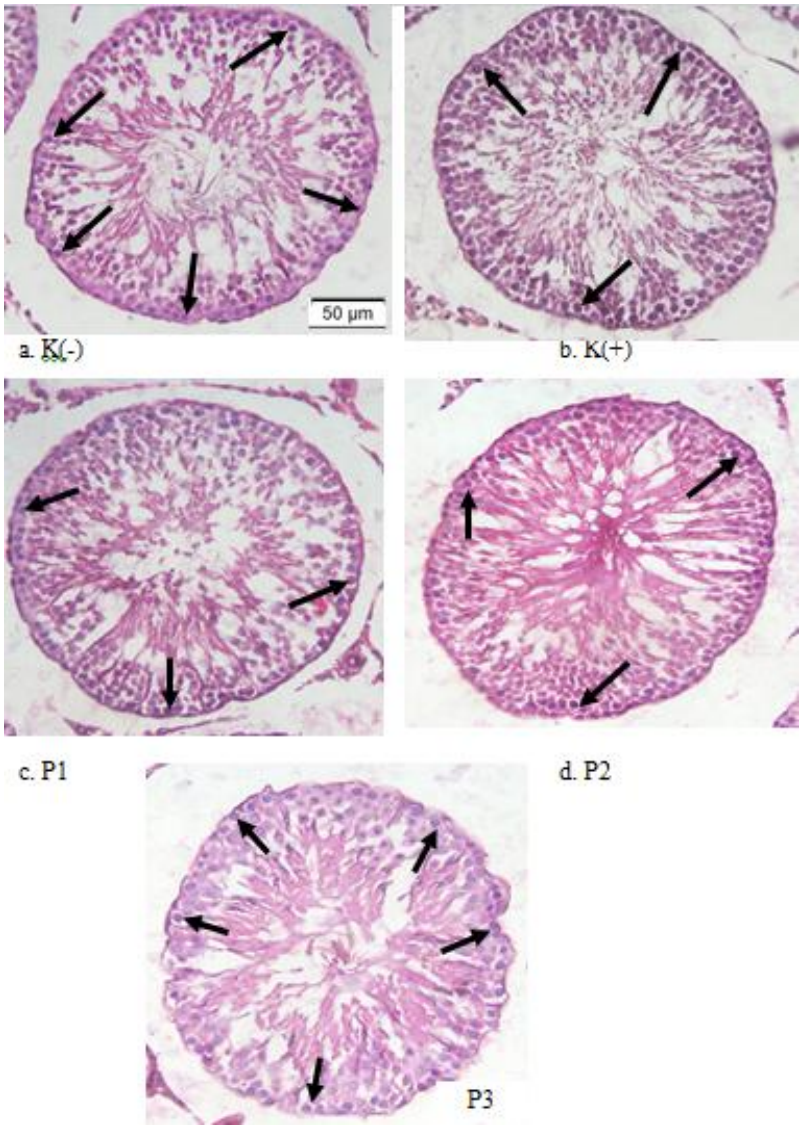

Figure 1. Histopathological picture of rat's seminiferous tubules of 200x magnification. Arrows indicate primary spermatocytes. (Note : K(-): Healthy control ; K(+): cigarette smoke only ; P1: cigarette smoke + 28.275 mg/kgBW R. apiculta bark extract ; P2: cigarette smoke + 56.55 mg/kgBW R. apiculta bark extract ; P: cigarette smoke + 113.10 mg/ kgBW R. apiculta bark extract.

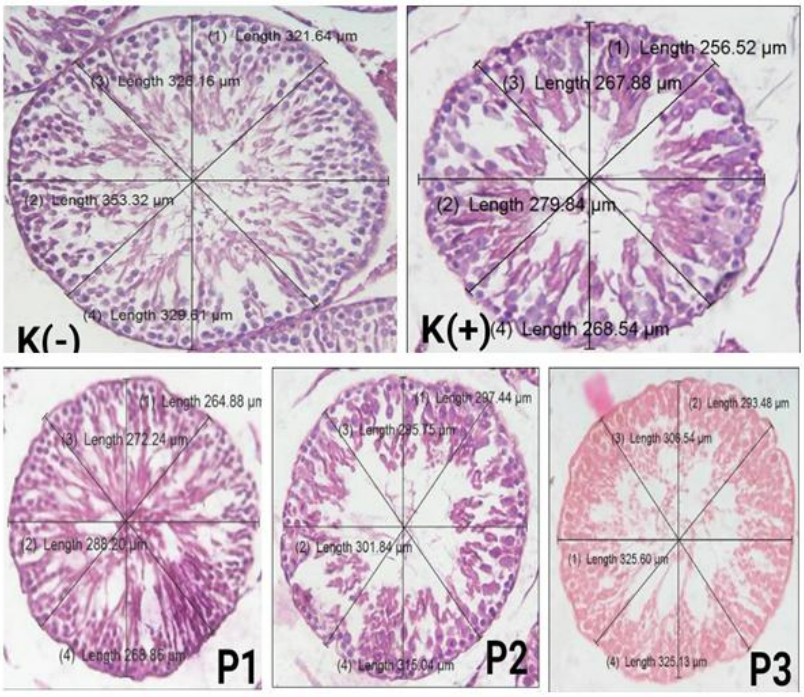

Figure 2. Histopathological picture of 200x magnification of the rat's seminiferous tubule thickness. (Note : K(-): Healthy control ; K(+): cigarette smoke only ; P: cigarette smoke + 28.275 mg/kgBW extract ; P2 : cigarette smoke + 56.55 mg/kgBW extract ; P3: cigarette smoke + 113.10 mg/ kgBW extract.

Furthermore, an antioxidant can scavenge ROS to protect against the effect of excess free radical to enhance sperm function. The sperm is nourished and maintained by seminal fluid which has abundants antioxidants. They are classified into two groups; non-enzymatic and enzymatic antioxidant system. The non-enzymatic system is constructed of variant components that are eaten via food or as supplements. On the other side, the enzymatic system consists of catalase, superoxide dismutase, and glutathione peroxidase. They are found in the seminal plasma or sperm cell naturally and they are undertaken from the prostate originally. Increasing number of ROS can cause oxidative stress that can damage both nuclear and mitochondrial DNA.[20] This case is not suitable for spermatozoa because this content is very low of enzymatic antioxidants for maintaining the sperm from a high number of ROS rates.[21] So, it is very important to involve antioxidant regimens to decrease the oxidative stress burden.[2][17]

Antioxidants give benefits to male fertility. Also, free radical can be scavenged by them, they also can neutralize radical chain reaction that triggers to oxidative stress. To solve infertile patient's oxidative stress, doctors prescribe antioxidants that are relatively cheap and easy to get.[21] People can find antioxidant in plants naturally. The human redox defense system can be protected by it.[3] In that case, one plant that can be developed as a natural antioxidant source is *R. apiculata*.[4]

This study found that there was a significantly higher number of primary spermatocytes in the groups of rats exposed to cigarette smoke that got

R. apiculata extract compared to the control group. This case also occurred in the rats' seminiferous tubule diameter length. It was indicated that this extract had a protective effect on testicular exposure to cigarette smoke. The protection mechanism of this extract against rat testicular damage due to exposure to cigarette smoke are antioxidant and anti-inflammatory.[22,23]

In the case of antioxidants properties, *R. apiculata* bark extract contains tannin[4], and the pyroligneous acid[7]. The *R. apiculata* tanin indicated a higher number of antioxidant activity compared than standard antioxidant.[4] Vijayavel et. al, showed that *R. apiculata* bark extract protects rats from naphthalene stress. They investigated that the lipid peroxidation was decreased, but on the other hand, the activity of mitochondrial enzymes was increased and glutathione was also enhanced to near control rates. These findings ensure there was preservative contribution through their free radical scavenging properties by *R. apiculata.*[24] Not just in the stem, methanol extracts of *Rhizophora* spp. fruit consistently showed antioxidants activity.[25]

In the case of anti-inflammatory, our prior research performed that the administration of mangrove bark extract could protect pancreatic damage of rats uncovered to cigarette smoke.[26] In other research, it indicated the anti-inflammatory effect of this extract can prevent thickening of the coronary arteries of rats exposed to cigarette smoke.[27] In line with a study of Prabhu et.al, which discussed the anti-inflammatory impact of the *R. apiculata* extract.[28] Moreover, their other research indicated that *R. apiculata* could avoid mouse from colitis that is useful as a natural agent for Inflammatory Bowel Disease therapy.[23]

CONCLUSION

The findings of this study confirm that there was protective effect of the ethanol extract *R. apiculata* bark against testicular damage induced by cigarette smoke in rats. The best dose of these extract which had an effect in anticipating adverse effects of cigarette smoke was 113.10 mg / KgBW

The future direction of this study is to test further toxic dose of *R. apiculata* bark extract. It is also important to explore parameters of antioxidant activities such as superoxide dismutase (SOD) and malondialdehyde (MDA). As well as it is involved as parameter of anti-inflammatory activity for instance tumor necrosis factor-α (TNF-α).

Conflict of Interest

The authors declare that there is no conflict of interest degrading the publication of this paper.

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