Comparison of Histology of The Kidneys of Rats Exposed To Cigarette Smoke After Administration of Ethanol Extract Methanol and N-Hexane Rhizophora apiculata Bark

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Abstract. Rhizophora apiculata is a plant species that yields medicinal raw materials. This research compares the protective effects of ethanol, methanol, and n-hexane extracts of Rhizophora apiculata mangrove bark on the kidneys of rats exposed to cigarette smoke. The study used a post-test-only control group design on 30 rats divided into six groups for 30 days. All groups were exposed to 24 cigarette smokes/day except for the Control group (C); the negative control group (NC) only received exposure to cigarette smoke. The positive control group (PC) was given vitamin C 9 mg/kgBW/day, and the E, M, and N groups were each given ethanol, methanol, and n-hexane extracts of 56.55 mg/kgBW/day. On the 31st day terminated preparations and scoring of renal histology were carried out. The data were analyzed using the Shapiro-Wilk normality test, then the Kruskal-Wallis non-parametric test, and finally the Mann-Whitney post-hoc test. The average kidney damage score for C, NC, PC, E, M, and N was 0.24, 3.48, 2.6, 2.4, 2.44, and 2.4, respectively. There was a significant difference ($p<0.05$) between PC, E, M, and N versus NC. The PC, E, M, and N damage scores showed no significant difference ($p > 0.05$). Ethanol, methanol, and n-hexane extracts of Rhizophora apiculata mangrove bark at a dose of 56.55 mg/kgBW/day protect the kidneys from cigarette smoke exposure. The three extracts have the same effect as vitamin C at 9 mg/kgBW/day.

Keywords: Cigarette Smoke, Rhizophora apiculata, renal histology

Introduction

Cigarettes are paper-based tobacco products that are burned and contain a variety of substances that are extremely dangerous for both active and passive smokers.Passive smokers face the same risks as active smokers because they inhale cigarette smoke from active smokers. The smoke inhaled by smokers contains 25% harmful substances, while the byproducts of cigarette smoke contain 75% harmful substances, so passive smokers face greater health risks [1]. Cigarette smoke is a harmful particle; cigarette smoke has more than 4700 toxic chemicals, including tar, nicotine, carbon monoxide, and other particles. There are two phases to smoking: the gas phase and the tar phase, both of

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which contain a large number of free radicals. The majority of free radicals found in cigarettes are in the gas phase, which contains organic free radicals such as reactive oxygen species (ROS), superoxide anions, hydroxyl radicals, peroxyl radicals, and others [2].

Active smokers in Indonesia have the highest number in Southeast Asia, with the number of male smokers at 67% (57.6 million) and the number of female smokers at 2.7% (2.3 million). In 2011, rural areas had a higher prevalence of smoking (37.7%) than urban areas (31.9%) [3]. One of the leading cigarette toxins is nicotine; nicotine can increase adrenaline, which makes the heart pound faster and work harder, heart frequency increases, and heart contractions increase so that it causes blood pressure to increase; the second is tar, tar is smoke condensate, the total residue produced when cigarettes are burned after deducting nicotine and water, both of which are carcinogenic. Carbon monoxide is a compound in the form of a gas, colourless, odourless, flammable, and used to manufacture various organic and inorganic compounds. The carbon monoxide gas contained in cigarettes will not cause poisoning in smokers, but the adverse effects will occur slowly in the airways [4]. Exposure to 24 cigarette smoke per day by channelings cigarette smoke through a hose with the help of an air pump has succeeded in causing damage to the kidneys of rats; this is marked by the discovery of Bowman's spatial oedema, oedema in the epithelium of the tubules, inflammatory cell litter, congestion in the histopathological picture of white rats [5]. Concerning the study, the researcher used 24 cigarettes per day.

Indonesia has 4.5 million hectares of forest, accounting for 25% of the world's mangrove forests. It contains 45 mangrove plant species. One of the plants from the mangrove forest ecosystem in Indonesia is Rhizophora apiculata [6]. It has been reported that the highest concentration of antioxidants is found in the extract of the stem part of Rhizophora apiculata, which contains antioxidants such as alkaloids, flavonoids, triterpenoids and saponins [7].

Antioxidants are compounds that can neutralize free radicals and thus inhibit their oxidation reaction. There are two types of antioxidants: endogenous and exogenous. Endogenous antioxidants and glutathione peroxidase are synthesized from within the body; Exogenous antioxidants are antioxidants obtained outside of the body, such as vitamin C and vitamin E in food and beverages [8]. In this study, the researcher used an exogenous antioxidant, namely vitamin C, as a control group with a 9 mg/KgBW dose. Researchers used vitamin C because vitamin C is a standard antioxidant and is a water-soluble compound, so it is easy to secrete, while vitamin E is fat-soluble, so it is difficult for the body to excrete.

Given the large amount of harmful content in cigarettes to organs of the body, including the kidneys, previous studies have found that the antioxidant activity of ethanol extracts is better than the polar and nonpolar fractions that use methanol and n-hexane solvents. Methanol solvents have polar properties, and n-hexane solvents have nonpolar properties. Research has never been conducted on the two extracts, namely methanol and n-hexane extracts from mangrove bark. As a result, it is necessary to conduct a study that compares the renal histopathology of white rats (Rattus norvegicus) of the Sprague Dawley strain exposed to cigarette smoke after administration of ethanol, methanol, and n-hexane extracts from mangrove bark (Rhizophora apiculata).

Method

This study has a proper experimental design, including a post-test-only control group design. Data was collected once at the end of the study, after the subjects had received treatment, and then compared between the control and treatment groups.

This study's experimental animals were maintained and treated in the animal house at the Faculty of Medicine, University of Lampung. Mangrove bark extract is produced at the Botanical Laboratory of the Faculty of Mathematics and Natural Sciences. The kidney preparations will be performed at the Laboratory of Histology and Anatomical Pathology, Faculty of Medicine, University of Lampung.

Thirty male white rats were selected according to the inclusion and exclusion criteria. Acclimatization was carried out for 7 days, and randomization was done so that each group had five rats. In the control group (C), rats was only be given a standard diet, in the negative control group (NC), rats were exposed to 24 cigarette smoke for 30 days; in the positive control group (PC), rats were exposed to 24 cigarette smoke for 30 days and given vitamin C 9 mg/kg BW, in treatment group 1 (E) rats were exposed to 24 cigarette smoke for 30 days and given ethanol extract of Rhizopora apiculata stem bark at a dose of 56.55 mg/kgBW, In treatment group 2 (M), the rats were exposed to 24 cigarette smoke for 30 days and given methanol extract of Rhizopora apiculata stem bark at a dose of 56.55 mg/kg BW. In treatment group 3 (N), rats were exposed to 24 cigarette smoke for 30 days and given n-hexane extract of Rhizopora apiculata stem bark at a dose of 56.55 mg/kgBW. The rats are kept in cages covered with woven wire, placed indoors with sufficient light and ventilation, and not exposed to direct sunlight. The cages are cleaned at least three times a week so that the rats avoid feces that can cause infections.

On day 31, the rats were terminated, and their kidneys were collected for renal histology preparation. Histopathological preparation examination is examined under a light microscope with 100x and 400x magnification. Researchers and expert supervisors made observations. Histological kidney damage can be seen in the renal tubules and glomeruli. The scale of cell damage is calculated semiquantitatively in the entire field of view. Damage to the glomerulus and renal tubules is calculated with a score of 0-3. the score was 0 if there was no histological damage, 1 if inflammatory cell infiltration was found, 2 if Bowman's spatial oedema was found, and three if necrosis was present.

Data analysis was carried out using a non-parametric statistical test. The Health Research Ethics Commission, Faculty of Medicine, University of Lampung has approved ethical approval for this study.

Result

Renal histology was performed using a light microscope with magnifications of 100 and 400 times. The following is a picture of the rats kidney histology examination results (fig.1).

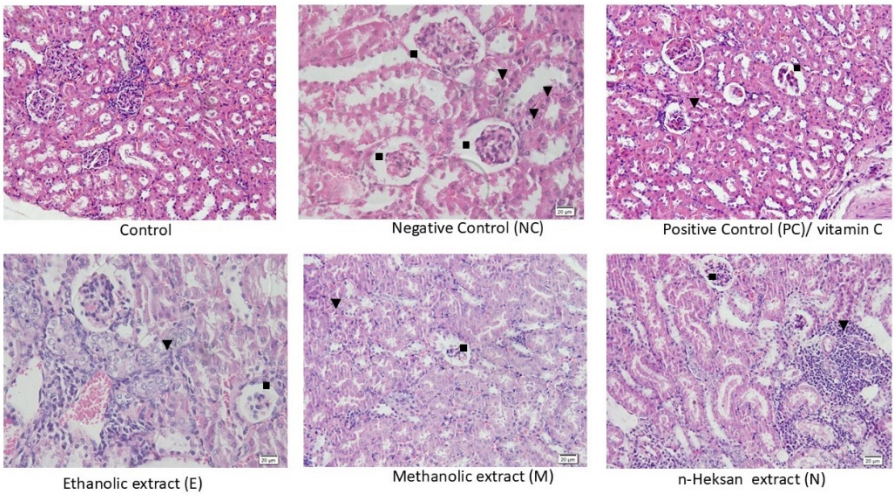

Fig. 1. The histological image of the rat kidneys was examined with 400 magnifications. The black triangle symbol indicates infiltration of inflammatory cells, while a black box indicates Bowman's Spatium oedema. The negative control group appears to have sustained the most severe kidney damage. Kidney damage was less severe in both the positive control group and the group that received mangrove extract.

After calculating and summarizing the tubule and glomerulus damage scores at five fields of view in each preparation, the average amount of kidney damage in the six treatment groups differed. The average kidney damage is shown in Table 1.

Table 1. Histological renal damages score

No	Group	Subject	Glomerulus score	Tubular score	Summary	Averages
1	C	A	0.4	0	0.4	
2		B	0	0.4	0.4	
3		C	0.2	0	0.2	0.24
4		D	0.2	0	0.2	
5		E	0	0	0	
6	NC	A	2	1.6	3.6	
7		B	1.8	1.8	3.6	
8		C	2.4	1.4	3.8	3.48
9		D	1.6	2	3.6	
10		E	1.4	1.4	2.8	
11	PC	A	1.4	1.2	2.6	
12		B	1.2	1.4	2.6	
13		C	1.4	1.2	2.8	2.6
14		D	1.2	1.6	2.8	
15		E	1.2	1.2	2.4	
16	E	A	1.2	1	2.2	

17		B	1.2	1.2	2.4	
18		C	1	1.4	2.4	2.4
19		D	1.2	1.4	2.6	
20		E	0.8	1.6	2.4	
21	M	A	1.4	1	2.4	
22		B	1.6	1.2	2.8	
23		C	1.2	1.2	2.4	2.44
24		D	1.4	1.2	2.6	
25		E	1.2	0.8	2	
26	N	A	1	1.2	2.2	
27		B	1.6	0.8	2.4	
28		C	1.8	0.6	2.4	2.4
29		D	1.4	1.2	2.6	
30		E	1.6	0.8	2.4	

The Shapiro-Wilk normality test indicated that the negative control group (NC) had a p-value of 0.018, which is less than 0.05. The normality test revealed that the data was not distributed normally, so the analysis was continued with the Kruskal-Wallis non-parametric test.

The Kruskal-Wallis test resulted in a p-value of 0.000, indicating a significant difference between more than two groups. A post-hoc analysis revealed that there was no significant difference between the treatment groups E (0.095), M (0.421), and N (0.095) and the positive control group (PC) ($P>0.05$). There was a significant difference ($P<0.05$) in the histological picture of the kidneys of rats exposed to cigarette smoke after administration of ethanol, methanol, and n-hexane extracts, supporting the hypothesis. The NC group had a higher damage score with an average of 3.48 than the average damage score of the E (2.4), M (2.44), and N (2.4) groups, which means that the E, M and N groups had a lower damage rate.

Discussion

Based on microscopic glomerulus and renal tubular scoring, the highest average result was the NC group of 3.48. The renal histology from the NC group showed tubule necrosis, inflammatory cell infiltration, Bowman's spatial oedema, and swelling of tubule epithelial cells. The high damage score in the NC group was because the NC group was only exposed to 24 cigarette smoke/day for 30 days without the administration of mangrove bark extract. Exposure to cigarette smoke contains ROS, which will cause an oxidative stress reaction and play a role in kidney damage [5].

The Normal Group (C) is the group of Rats with the lowest damage score of 0.24. The kidney histology generally still looks normal. This is because the C group is only given food and drink every day without any treatment for 30 days.

Groups E, M, N, and PC had damage scores that were not much different, and the average damage of each treatment group was 2.4, 2.44, 2.4, 2.6 when compared to the average damage from NC, which was 3.48; it can be interpreted that there is a protective effect of ethanol, methanol and n-hexane extracts. This protective effect cannot be separated from, vitamin C's antioxidant content and mangrove bark's antioxidant content such as saponins, steroids, alkaloids, flavonoids and terpenoids [9]. The results showed

no significant difference (p-value > 0.05) between the E, M, and N groups. This means that ethanol, methanol and n-hexane extract of mangrove bark oil at a dose of 56.55 mg/kgBW/day have an equally protective effect on the kidneys.

The PC group, namely the group of rats exposed to cigarette smoke and vitamin C, had a better damage score of 2.6 when compared to the damage score of the NC group of 3.48; this happened because vitamin C is a powerful antioxidant. Vitamin C, as a natural antioxidant, will donate electrons and free radical-reducing agents. Vitamin C prevents other compounds from oxidizing by donating electrons. Vitamin C can be an antioxidant for lipids, proteins, and DNA, usually the main targets of free radicals that take electrons because they are large molecules [10].

Although the group of rats given extracts of ethanol, methanol, n-hexane, and vitamin C had a protective effect against kidney damage exposed to cigarette smoke, this was no better than the C group, which was not exposed to cigarette smoke at all.

Conclusion

Ethanol, methanol, and n-hexane extracts of mangrove bark (Rhizophora apiculata) have a protective effect against kidney damage in rats exposed to cigarette smoke. These three extracts have a protective effect just as good as vitamin C.

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References

1. Soleimani, F., Dobaradaran, S., De-la-Torre, G. E., Schmidt, T. C., & Saeedi, R.: Content of toxic components of cigarette, cigarette smoke vs cigarette butts: A comprehensive systematic review. *Science of the Total Environment*, *813*, 152667 (2022).
2. Marques, P., Piqueras, L., & Sanz, M. J.: An updated overview of e-cigarette impact on human health. *Respiratory research 22*(1), 151 (2021).
3. Holipah, H., Sulistomo, H. W., & Maharani, A.: Tobacco smoking and risk of all-cause mortality in Indonesia. *PloS one 15*(12), e0242558 (2020).
4. Lei, T., Li, M., Zhu, Z., Yang, J., Hu, Y., & Hua, L.: Comprehensive evaluation of serum cotinine on human health: novel evidence for the systemic toxicity of tobacco smoke in the US general population. *Science of The Total Environment*, *892*, 164443 (2023).
5. Mustofa, S., & Dewi, S. N.: Rhizophora apiculata Bark Ethanolic Extracts Prevent Kidney Damage Caused by Cigarette Smoke in Male Rats. *Sriwijaya Journal of Medicine 6*(1), 17-23 (2023).
6. Rahman, Lokollo, F. F., Manuputty, G. D., Hukubun, R. D., Krisye, & Wardiatno, Y.: A review on the biodiversity and conservation of mangrove ecosystems in Indonesia. *Biodiversity and Conservation 33*(3), 875-903 (2024).
7. Mustofa, S., Adjeng, A. N. T., Kurniawaty, E., Ramadhita, L., & Tamara, T.: Influence of Rhizophora apiculata barks extract on Cholesterol, Triglyceride, LDL, and HDL Levels of

Rattus norvegicus (Sprague Dawley) fed high-cholesterol diet. *Research Journal of Pharmacy and Technology 17*(1), 396-400 (2024).
8. Kaźmierczak-Barańska, J., Boguszewska, K., Adamus-Grabicka, A., & Karwowski, B. T.: Two faces of vitamin C—antioxidative and pro-oxidative agent. *Nutrients 12*(5), 1501 (2020).
9. Ramya, R., Kamoona, S., Hatta, F. A. M., Sulaiman, W. S. H. W., Latiff, N. H. M., & Othman, R.: A Study on an Active Functional Group and Antimicrobial Properties From Rhizophora apiculata Extracts Used in Traditional Malay as Medicine. *Malaysian Applied Biology 52*(4), 153-160 (2023)..
10. Jodh, R., Tawar, M., Mude, G., Fasate, A., Sutane, R., & Patanray, P.: An Updated Review on Vitamin C-An Excellent Drug Having a Great Scavenging Property. *Asian Journal of Pharmaceutical Research 13*(1), 25-30 (2023).