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Submission date: 21-Nov-2022 09:11AM (UTC+0700)
Submission ID: 1959712675
File name: jpnr-2022-04-155_1118-1123.pdf (303.62K)
Word count: 4800
Character count: 25604

Original Article

THE EFFECT OF GIVING PLANTAIN PEEL EXTRACT (Musa paradisiaca) ON MALONDIALDEHYDE LEVELS IN WISTAR RATS EXPOSED TO FILTER CIGARETTE SMOKE

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Abstract

Background: Free radicals from filter cigarettes can contribute to the body's oxidative stress. Malondialdehyde is a substance that the body produces in response to oxidative stress. Antioxidants found in plantain peel extract can be used to counteract oxidative damage.

Aim: This study sought to determine whether Wistar rats exposed to filtered cigarette smoke could have their MDA levels reduced by a plantain peel extract.

Methods: On Wistar rats, this study used an actual experimental design with a post-test-only control group. Twenty-eight rats from the research sample were split into 4 separate groups. K+ received exposure to cigarette smoke, P1 received exposure to cigarette smoke and extracted at a dose of 200mg/kg BW, and P2 received exposure to cigarette smoke and extracted at a dose of 400mg/kg BW. K- serves as the negative control. K+ received exposure to cigarette smoke. The retroorbital plexus vein was used to draw blood from the dead rats afterward. MDA levels in the plasma were measured using the TBARS technique.

Results: The average output of MDA levels in the K1, K2, P1, and P2 groups was 1.695 ppm, 2.430 ppm, 1.791 ppm, and 3.115 ppm, respectively. The K- and K+ groups showed a significant difference in the Mann-Whitney test (p=0.037), as did the K+ and P1 group (p=0.010), K+ and P2 group (p=0.025), and P1 and P2 group (p=0.004).

Conclusion: MDA levels in Wistar rats exposed to filter cigarette smoke could be decreased by administering plantain peel extract.

Keywords: Malondialdehyde, filter cigarettes, plantain peel.

Introduction

Tobacco use is widely recognized as a risk factor for early morbidity and mortality. However, the relative mortality risk and the adverse effects of smoking differ between nations. As one of the world's major markets for tobacco use, Indonesia is severely impacted by diseases linked to tobacco use (Holipah et al., 2020).

Indonesian clove cigarettes, also known as kretek, are a blend of tobacco and cloves rolled into a cigarette and sprayed with oils, plant extracts, and food flavorings in an unknown quantity and composition, which varies by brand and is usually kept hidden(Picanço et al., 2022).

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Quick Response Code:

Website: www.pnrjournal.com

DOI: 10.47750/pnr.2022.13.04.155

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How to cite this article: Satria Damarjati, Lusiana Batubara, Puspita Kusuma Dewi, Amallia Nuggetsiana Setyawati, THE EFFECT OF GIVING PLANTAIN PEEL EXTRACT (Musa paradisiaca) ON MALONDIALDEHYDE LEVELS IN WISTAR RATS EXPOSED TO FILTER CIGARETTE SMOKE, J PHARM NEGATIVE RESULTS 2022;13: 1118-1123.

Based on the 2018 Basic Health Research, 33.8% of Indonesia's population aged 15 years and over are active smokers (Balitbangkes Kemenkes, 2019). Cancer and immune-mediated inflammatory illnesses are two conditions for which smoking is a significant risk factor. Reactive oxygen and nitrogen species (ROS and RNS), among other compounds, are present in tobacco smoke and be capable of causing harm to lipids, proteins, and nucleic acids, which are cellular and sub-cellular targets (Caliri et al., 2021). Aldehydes, epoxides, peroxides, and other free radicals are among the numerous oxidants in high concentrations in each cigarette puff (Caliri et al., 2021; Thimmulappa et al., 2020). Cigarette smoke can indirectly create more significant amounts of oxidants in the body by activating anti-inflammatory cells to produce free radicals (Emma et al., 2022). Due to their reactive nature, Reactive Oxygen Species (ROS) can arise due to an abundance of free radicals in the body and harm tissue (Venditti & Di Meo, 2020).

Malondialdehyde (MDA) is a dialdehyde molecule produced as a by-product of the body's processes for enzymatic and non-enzymatic lipid peroxidation. Because MDA readily reacts with thiobarbituric acid, it is a biomarker for the peroxidation of omega-3 and omega-6 fatty acids (Fuloria et al., 2020). The Thiobarbituric Acid (TBA) test can determine MDA levels. Because there are free radicals in the body, the appearance of MDA suggests an oxidation process in cell membranes. An increase in ROS from cigarette smoke exposure causes increased fat peroxidation in smokers (De Leon & Borges, 2020).

Antioxidant-rich substances can reduce the harm caused by free radical oxidation. Southeast Asia is where the monocot plant, known as the banana, first appeared. Each year, Indonesia produces more than 6 million tons of bananas. The plantain is a variety of bananas that is quite popular in Indonesia (Ningsih, 2019). Most of the time, individuals consume the fruit and discard the skin of the banana fruit without realizing its many health benefits. One of them is the presence of antioxidants in the skin, such as flavonoids and phenolic compounds. The plantain peel extract's flavonoids have the potential to serve as antioxidants to lower MDA levels that increase as a result of exposure to free radicals, which in this study was focused on filtered cigarette smoke (Adetuyi et al., 2022). This finding is evident from the reason given above. The findings of this study should shed light on the possibility of using plantain peels to lower MDA levels.

METHODS

Sample

This study's sample consisted of Wistar (Ratus norvegicus) strain rats who met the following inclusion, exclusion, and dropout criteria Wistar rat (Rattus novergicus) 6-8 weeks old Active movement. 200-300 g, without anatomical flaws.

Exposure to Filter Cigarette Smoke

A smoking chamber was used to house animal samples. The smoking chamber has two holes: one for the outlet of filtered cigarette smoke exposure and the other for inserting lit filter cigarettes. Blowing cigarette filters is accomplished by using a smoking pump until the cigarette is extinguished. A cigarette smoke filter is administered twice daily, at 09.00 and 15.00, in the amount of two sticks, one at a time.

Extraction of Plantain Peel

The maceration process produced plantain peel extract. The maceration method is used without heating to ensure that the compounds in the plantain peel are adequately extracted and do not decompose. The tools and materials prepared were knives, an oven, scales, a maceration vessel, plantain, cotton, water, and 96% ethanol. The procedure was carried out based on previous research. The following is the procedure for extracting plantain peels; Peel the plantain to separate the skin from the fruit, plantain peel washed with water until clean, and plantain peel cut into small pieces. The plantain skin was dried in an oven at 50°C until dry. The dried plantain peels were sorted and weighed for the extraction process. The plantain peel was placed in a maceration vessel, and 96% ethanol solvent was added until the entire sample was completely submerged. The plantain peel and 96% ethanol solution were evenly stirred, and the maceration vessel was tightly closed to produce maceration. Recurrent stirring is done while the maceration process is kept at room temperature in a dark area. Filtration of the macerate with a cotton swab results in a filtrate. A thick plantain peel extract was made by evaporating the filtrate.

Dosage of Plantain peel extract

Another study stated that the most significant decrease in MDA levels occurred with the administration of 200 mg/kg BW/day kepok banana peel extract. 45 The dose from the prior study was used, and a second dose was added by multiplying the initial dose by two. The second dose is 400 mg/kg BW daily a result.

Animal Treatment

At our animal laboratory, all rats were housed in cages. Throughout the study, Wistar rats were fed and given water. The adaptation treatment lasted seven days and consisted of ad libitum traditional food and drink. The Wistar rats were randomly divided into four groups on the eighth day following the adaptation treatment, namely:

Rats in Group K- received standard feed and water. For 28 days, rats in Group K+ were given standard feed and water and were exposed to filtered cigarette smoke. Group K1: After 28 days of exposure to filtered cigarette smoke, rats were given standard feed and a sonde of plantain peel extract at 200mg/kg BW/day. Group K2: After 28 days of exposure to filter cigarette smoke, rats were given standard feed treatment and 400mg/kg BW/day of plantain peel extract. The

treatment period for the animals lasted 28 days.

Analytical Statistics

The information obtained is primary data. The information is entered into a computer and analyzed using the Statistical Product and Service Solutions (SPSS) for Windows software.

The Shapiro-Wilk homogeneity test was used to determine the data distribution's normality. Data is classified as generally distributed at a 95% confidence interval if the p-value is 0.05. If the data is normally distributed, the one-way Anova test is used to determine whether there is a general difference in rats' average blood MDA levels across all groups. Then conduct the Post Hoc Test to determine the various groups. A p-value of 0.05 indicates that the data is not generally distributed at a 95% confidence level. If the data is not normally distributed, the Kruskal-Wallis test can be used to compare the average blood MDA levels of rats across groups. The Mann-Whitney test is then used to determine the various groups.

Results

This study used a sample of 28 Wistar rats with a minimum age of 6 weeks before adaptation and a body weight of 200-300 grams. Each group had one rat drop out during the treatment, so six samples were collected. The MDA levels in blood from groups K-, K+, P1, and P2 were then determined using the TBARS method. The Shapiro-Wilk test was used to determine data normality and distribution.

Table 1: Average and Normality Test of MDA Levels (ppm)

Group	$\bar{x} \pm SD$	Normality test (p-value)
K-	1,695 ± 0,78	0,113*
K+	2,430 ± 0,31	0,248*
P1	1,791 ± 0,22	0,282*
P2	3,115 ± 0,44	0,960*

*Data are normally distributed (p>0.05).

Note Group K-: received standard feed and water. Group K+: exposed to filtered cigarette smoke. Group K1: exposed to filtered cigarette smoke and plantain peel extract at a 200mg/kg BW/day dose. Group K2: exposed to filtered cigarette smoke and plantain peel extract at a 200mg/kg BW/day dose.

In the Shapiro-Wilk normality test, the data distribution was normal (p>0.05) in all groups, so the mean was used as the measure of data concentration, and the standard deviation was used as the size of the spread. Deviation.

The findings of this study did not meet the statistical test requirements—one-way ANOVA. Unpaired numerical data and more than two groups, data with a normal distribution and homogeneous data variants are all required for the one-way ANOVA test. The data variance was not homogeneous in the Levene test, with p = 0.007 (p 0.05).

As a result, the Kruskal-Wallis test was used to determine the significant differences between each group.

Table 2: Kruskal-Wallis Analysis

	Plasma MDA Levels (ppm)
Kruskal-Wallis H	15,820
df	3
p-value	0,001

Description: *significant (p<0.05)

Note Group K-: received standard feed and water. Group K+: exposed to filtered cigarette smoke. Group K1: exposed to filtered cigarette smoke and plantain peel extract at a 200mg/kg BW/day dose. Group K2: exposed to filtered cigarette smoke and plantain peel extract at a 200mg/kg BW/day dose.

The table shows the results of the Kruskal-Wallis test with a p-value of 0.001 (p 0.05), indicating significant differences between each group. To ascertain the differences between each group, the Mann-Whitney non-parametric test was used.

Table 3: Pairwise Mann-Whitney Test Group Comparison

Group-Group	p-value
(K-)-(K+)	0,037*
(K+)-(P1)	0,010*
(K+)-(P2)	0,025*
(P1)-(P2)	0,004*

Description: *significant (p<0.05)

Note Group K-: received standard feed and water. Group K+: exposed to filtered cigarette smoke. Group K1: exposed to filtered cigarette smoke and plantain peel extract at a 200mg/kg BW/day dose. Group K2: exposed to filtered cigarette smoke and plantain peel extract at a 200mg/kg BW/day dose.

According to the Mann-Whitney test results table above, there was a significant difference between the K- group (negative control) and the K+ group (positive control) with a p value = 0.037 (p<0.05), the K+ group and the P1 group (extract dose 200mg/kgBW) with a p value = 0.010 (p<0.05), group K+ and group P2 (extract dose 400mg/kgBW) with a p value = 0.025 (p<0.05) and between group P1 and group P2 with a value of p= 0.004(p<0.05).

According to the Mann-Whitney test results, the results of this study accepted the study's hypothesis, namely that there was an effect of giving plantain peel extract on the malondialdehyde levels of Wistar rats exposed to filter cigarette smoke. The findings supported the hypothesis that administering plantain peel extract at a dose of 200mg/kg BW could reduce MDA levels in Wistar rats exposed to filtered cigarette Kosmoke. However, the results of this study rejected the hypothesis that the administration of plantain peel extract at a dose of 400mg/kg BW could reduce MDA levels in Wistar rats exposed to filtered cigarette smoke.

Discussion

MDA level measurements discovered that rats in the K- group had the lowest mean MDA levels because they did not receive any treatment other than standard feed. The mean of the K- group (1.695 ppm) was lower than that of the K+ group (2.430 ppm). However, based on the findings of the research data analysis, the difference between the two groups was statistically significant (p>0.05). According to epidemiological research, fruits, vegetables, and less processed staple foods provide the best defense against the emergence of oxidative stress-induced diseases (Igwe et al., 2021). Both banana peel and pulp contain antioxidant activity, though the amount varies depending on the variety. Because of the presence of potassium, a single banana meal can significantly reduce plasma oxidative stress in a healthy human (Qamar & Shaikh, 2018).

Bananas are renowned for being the superior reservoir of potassium, which helps maintain muscle functioning and prevent muscle spasms. In particular, the fruits include vitamin A and vitamin B6, which, when ingested, will benefit., C and D enhance immunity and ensure appropriate metabolic functions (Kumar et al., 2012). Not only are the fruits of Musa spp. Edible and therapeutic, other parts of the plant, including the blooms, peels, roots, and seeds, also possess these qualities (Kumar et al., 2012; Padam et al., 2014).

The nutritional content of plantain peel varies. According to previous research, plantain peel contains a variety of energy and mineral sources. Carbohydrates (59mg/g), fiber (31.7mg/g), fat (1.7mg/g), and protein (0.9mg/g) are the energy sources in question. Numerous banana peels can be used for a variety of purposes. There are 19.2mg/g calcium, 24.3mg/g sodium, 78.1mg/g potassium, 76.2mg/g manganese, 0.61mg/g iron, 0.21mg/g rubidium, bromine is 0.04mg/g, strontium is 0.03mg/g, zirconium is 0.02mg/g, and niobium is 0.02mg/g minerals in banana peels(Pyar & Peh, 2018). Banana peels can be utilized in a variety of ways. Due to their capacity to donate hydrogen atoms to free radicals, phenolic compounds are bananas' main antioxidants (Afzal et al., 2022).

The average MDA levels of rats in the P1 group (1.791 ppm) were lower than those in the K+ group (2.430 ppm), while those in the P2 group (3.115 ppm) were higher than those in the K+ group (2.430 ppm) and P1 group (1.791 ppm) (1.791 ppm). According to the findings of statistical analysis of research data, the difference in MDA levels between the K+ and P1 groups was statistically significant (p>0.05), and the difference in MDA levels between the P2 group and the K+ and P1 groups was statistically significant (p0.05).

Antioxidants are free radical-neutralizing chemicals that can be gained through dieting(Showell et al., 2014). Compared to synthetic antioxidants, phenolic substances are more potent and safer (Zeb, 2020).

The antioxidant effect of flavonoids can be attributed to the uptake of free radicals by hydrogen proton donors from the flavonoid hydroxyl group. The antioxidant effect of flavonoids is strongly influenced by substituting hydroxy groups in the ortho and para positions to the OH and OR groups (Lopes et al., 2020).

Cellular ROS are neutralized or eliminated by the antioxidant defense system. GSH is mainly present in its reduced form, which functions as an important intracellular antioxidant. In addition to halting DNA production and mending damaged DNA pieces, it helps to protect cells from oxidative damage, harmful substances, and radiation. Malondialdehyde is one of these by-products and a widely recognized sign of oxidative stress (Denk et al., 2022). The flavonoids extracted from Musa paradisiaca in rats were found to stimulate the activities of superoxide dismutase (SOD) and catalase, which may be to blame for the decreased levels of peroxidation products like hydroperoxides (Galani, 2019). Consuming flavonoids have a favorable influence on oxidative, glucose, and lipid metabolisms (Neri-Numa et al., 2020).The phenolic components in banana peel range from 0.90 to 3.0 g/100 g in dry weight(Singh et al., 2016). Flavonoids inhibit the action of the Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase and xanthine oxidase, as well as binding to metals (Cu2+ and Fe2+), which have a preventive effect on redox reactions that can generate free radicals. In vivo, flavonoids can be present sufficiently high quantities to exhibit pharmacological activity at receptors, enzymes, and transcription factors (Azzi, 2022). Flavonoids can preserve redox tone alterations and enhance oxidative stress markers (Oteiza et al., 2021).

Our result is in line with research on the results of administering rat exercise-induced plantain peel extract. In this experiment, rats' MDA levels were considerably lowered by administering banana peel extract following high and low-intensity exercise (Kinanti et al., 2021). In addition, research on diabetic rabbits with hyperlipidemia demonstrates that kepok banana peel extract can lower MDA levels. This study found that giving banana peel extract to rabbits resulted in considerably decreased MDA levels (Samiasih et al., 2019).

In high fat fed/low dose streptozotocin-induced diabetic rats,

diets supplemented with boiled unripe plantain (20%-40%) prevented lipid peroxidation compared to acarbose administration. When unripe plantains are boiled, they can provide the necessary natural therapeutic measures to be considered a potential economic means of managing diabetes in underdeveloped countries (Ajiboye & Shodehinde, 2022).

In this study, rats exposed to cigarette smoke and given a dose of 200 mg/kg BW plantain peel extract had significantly lower MDA levels than rats exposed to cigarette smoke alone. However, rats exposed to cigarette smoke and given plantain peel extract at a dose of 400mg/kg BW had significantly higher MDA levels than rats exposed to cigarette smoke and given plantain peel extract at a dose of 200mg/kg BW and rats exposed to cigarette smoke only. This result is because plantain peel extract at 200mg/kg BW does not exceed the maximum dose in Wistar rats, allowing the administration of plantain peel extract to reduce MDA levels. In contrast, plantain peel extract at 400mg/kg BW exceeds the maximum dose, causing a toxic effect on the body. MDA levels in rats rise as a result of thisWhen there is an imbalance between antioxidant and oxidant compounds in the body, or when antioxidant levels are high while oxidant levels are low, the body will form oxidant compounds to balance the levels, resulting in an increase in peroxidation and an increase in MDA levels. According to another study, the ethanol extract Musa paradisiaca L. contains secondary metabolites and can potentially be a weak antioxidant. Several restrictions apply to this study, including determining antioxidant active ingredient concentrations. This study did not include any histopathological analysis (Nurmazela et al., 2022).

Conclusion

Plantain peel extract was found to lower MDA levels in Wistar rats exposed to filter cigarette smoke. The plausible mechanism is that the presence of phenolic compounds in banana peel reduces lipid peroxidation

activity.

Ethical clearance

KEPK has granted ethical clearance with the number 05/EC/H/FK-UNDIP/1/2022.

Acknowledgment

There was no financial support for this study. We want to express our appreciation for the technical assistance provided during this study course.

Contribution of Author

Formal analysis, ANS; investigation, PKD, and ANS; data curation, ANS; writing—original draft preparation, ANS; writing—review and editing, ANS; and funding acquisition, ABS. Conceptualization, SD, ANS, and PKD; methodology, PKD, and ANS validation, PKD, LB, and ANS; formal analysis, ANS; and investigation, PKD, and ANS. All authors have read and approved the final text.

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