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Novel Therapeutic Potential of 'Ambon' Banana and Green Bean Sprout Extracts in Mitigating Cigarette Smoke-Induced Testicular Oxidative Stress and Sperm Quality Impairment

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Abstract: Cigarette smoke exposure generates free radicals, causing oxidative stress and adversely affecting reproductive organs, resulting in reduced sperm quality. The aim of this study was to investigate the therapeutic benefits of 'Ambon' banana and green bean sprout extracts, either individually or in combination, in mitigating testicular MDA levels and preserving spermatogenic cells in rats exposed to cigarette smoke. 35 Male rats, aged 3 months, were divided into five groups: negative control, positive control (cigarette smoke-exposed), P1 (cigarette smoke + 'Ambon' banana extract), P2 (cigarette smoke + green bean sprout extract), and P3 (cigarette smoke + 'Ambon' banana and green bean sprout extracts). Cigarette smoke exposure was administered at 4 sticks/day/group for 30 days, and extracts were orally administered using a sonde for the same duration. The study showed that 'Ambon' banana extract, green bean sprout extract, and their combination significantly reduced testicular MDA levels, increased the number of spermatogonia, spermatocytes, spermatids, testicular weight, diameter of seminiferous tubules, and the number of mouse Leydig cells in rats. This study highlights the potential therapeutic effects of 'Ambon' banana and green bean sprout extracts as protective agents against cigarette smoke-induced testicular oxidative stress and sperm quality impairment.

Keywords: Cigarette smoke; 'Ambon' banana extract; Sprout extract

Introduction

Infertility remains a significant global health concern, affecting millions of couples worldwide, including in Indonesia (Bennett, 2018; Damayanti et al., 2022; Gabrielsen & Tanrikut, 2016). Infertility is defined as the inability of a married couple of childbearing age, who have engaged in regular intercourse without contraception for 12 months, to conceive or have children (World Health Organization, 2021). Studies have estimated that approximately 8-12% of couples of childbearing age, representing 50 to 80 million couples, experience infertility globally.

Smoking has been identified as one of the factors that can negatively impact sperm quality and quantity. Cigarettes contain numerous toxic compounds, including tar, nicotine, nitrosamines, carbon monoxide, PAH compounds, and others, which are harmful to the body. The toxic content of cigarette smoke, particularly PAH, has been linked to testicular atrophy, impaired spermatogenesis, and damage to sperm morphology (Mendel et al., 2018; Soleimani et al., 2022; Zeng et al., 2022).

The detrimental effects of cigarette smoke on sperm quality are attributed to seminal oxidative stress induced by Reactive Oxygen Species (ROS). These ROS

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cause damage to sperm cells, disrupting spermatogenesis and resulting in abnormal sperm morphology. Moreover, the Poly Unsaturated Fatty Acids (PUFA) present in the sperm's plasma membrane are susceptible to damage by ROS, leading to lipid peroxidation and changes in membrane function. As a consequence, sperm metabolism, morphology, motility, and fertility are adversely affected (Sinenko et al., 2021).

To mitigate the harmful impact of excessive free radicals, consumption of antioxidants from external sources is essential. Natural ingredients, such as green bean sprouts (Phaseolus radiatus Linn) and Ambon banana (Musa paradisiaca var. sapientum (L.)), offer abundant antioxidants. Vitamin E in sprouts plays a vital role in reproductive health, as its deficiency can lead to degeneration of spermatogonia cells, which are the foundation of spermatogenesis. On the other hand, Ambon bananas are rich in nutrients, including potassium, magnesium, phosphorus, iron, calcium, vitamins C, E, B complex, B6, serotonin, flavonoids, and catechins, which act as potent antioxidants (Ruspita & Rahmi, 2022).

Given the potential benefits of Ambon banana and mung bean sprout extracts in countering oxidative stress and supporting reproductive health, this study aims to investigate the effects of these extracts, individually and in combination, on various parameters in rats exposed to cigarette smoke. These parameters include malondialdehyde (MDA) levels, the number of spermatogonia, spermatocytes, and spermatids, testicular weight, seminiferous tubule diameter, and Leydig cell count. By understanding the impact of these extracts on sperm quality in a smoking-induced oxidative stress environment, this research seeks to contribute to the advancement of infertility treatment and reproductive health enhancement.

Method

This research is an experimental laboratory study using a completely randomized design

Making Mung Bean *Sprout Extract.* The mung bean seeds are soaked in water overnight, then the soaked mung bean seeds are placed in a container that has holes and is covered with a cloth to keep moisture from rotting. Every day the beans are poured with water 4-5 times. After one day of germination, sprouts with a length of about one centimeter will be produced. The sprouts used are sprouts that are 48 hours (2 days) old, which is counted from the release of the radicle and plumule from the seed coat. Next, the extract is made.

In the manufacture of mung bean sprout extract, the sprouts were prepared and weighed as much as 13 kg and air-dried until dry. The dried sprouts were crushed with a blender and then sieved to obtain fine sprout powder. Then the sprout powder is soaked in 80% ethanol with a ratio of 1:4 (250 grams of sprout powder: 1 liter of 80% ethanol), for three days. The macerate is separated from the soaked sprout powder by filtering it. Ethanol evaporation was carried out using a rotary evaporator for 2 days and the result was mung bean sprout extract. The results obtained are ethanol extracts containing polar and non-polar compounds

Making Ambon Banana Extract

Peeled Ambon bananas, 12 kg of peeled Ambon bananas were cut into thin strips and then air-dried. After drying, grind it with a blender and sift it to get Ambon banana powder. Ambon banana powder soaked in 80% ethanol with a ratio of 1:4 (250 grams of Ambon banana powder: 1 liter of 80% ethanol) for 3 days. Maserate is separated by filtering. Evaporation of ethanol was carried out using a rotary evaporator for 2 days and the result was Ambon banana extract.

Experimental Animal Treatment

The population of this study were 35 male white rats (*Rattus norvegicus*) aged about 3 months, with a body weight of 140-150 grams, healthy condition, and no anatomical abnormalities obtained from the Biochemistry Laboratory of the Experimental Animal Unit, Faculty of Medicine, Airlangga University, Surabaya. Prior to treatment, male rats were acclimatized for 1 week. Mice were kept in cages, given feed in the form of pellets and drinking water ad libitum according to laboratory standards.

Treatment

Rats were randomly divided into five treatment groups namely the negative control group (K-) which was not exposed to cigarette smoke, the positive control (K+) which was exposed to cigarette smoke, and three treatment groups namely the group which was exposed to cigarette smoke and was given Ambon banana extract (P1) with dose of 0.5 g/Kg body weight for 30 days, the group exposed to cigarette smoke and given green bean sprout extract (P2) at a dose of 2 g/kg body weight for 30 days and the group exposed to cigarette smoke and given a combination extract, namely ambon banana extract mixed with green bean sprout extract (P3) with a ratio of 0.25 gr/KgBB of Ambon banana extract and 1gr/KgBB of mung bean sprout extract for 30 days.

Exposure to cigarette smoke is done in the following ways: Rats were exposed to cigarette smoke twice per day (07.00 in the morning and 16.00 in the evening) 2 cigarettes each for 30 days. Exposure to cigarette smoke was given by placing rats in a glass box 60 cm long, 45 cm wide and 20 cm high. This box has 2 holes on each side with a diameter of 1 cm. At the top of the box there are 2 holes which are used as a place for

the cigarette smoke hose to enter. Mice in the box were allowed to inhale cigarette smoke from burning 2 cigarettes for 4 minutes. On day 31, the rats were anesthetized with chloroform, then terminated and underwent surgery, the testes were isolated. The testicles are then weighed using an analytical balance to determine the weight of the testicles. Furthermore, the rat testicles that had been weighed were put in a container containing 10% formalin.

Measurement of testicular MDA levels

MDA measurements were carried out with thiobarbituric acid (TBA). Compounds 1,1,3,3-tetraethoxypropane were used in the preparation of the standard curve. The testes were crushed using a tissue grinder until dissolved, then centrifuged to take the supernatant. The supernatant obtained was added with 1 ml of TCA to separate it from the protein, then added 1 ml of 0.037% TBA. Then heated in a water bath at 80 ^{0}C for 15 minutes. Then chill for 1 hour, then centrifuge again at 3500 rpm for 15 minutes. Color absorption is read using a spectrophotometer at a wavelength of 532 nm. The MDA level assessment was calculated using the regression line equation from the standard curve (standard) of MDA solution.

Preparation of rat testis histology

The testes of the rats that had been fixed were made for testicular histology preparations using standard laboratory procedures, namely the paraffin method and Haematoxylin-Eosin (HE) staining. Observations were made on spermatogonium cells, spermatocytes and spermatids.

Procedure Observation of the diameter of the seminiferous tubules

Diameter of the seminiferous tubules measurement was carried out by measuring the longest and shortest distances from the seminiferous tubules which were round in shape or considered round by measuring using image j software, the number of seminiferous tubules measured was 5 tubules from each treatment group and then averaged. *Procedure for calculating the number of Leydig cells*. Calculation of the number of Leydig cells is done by counting the number of cells between three to four seminiferous tubules in 5 fields of view with 400x magnification.

Data analysis

Data on MDA levels were analyzed using regression. Differences in MDA levels in the treatment were carried out by the Brown-Forsythe test and Welch test and continued with the Games Howell test. Data on testicular weight, number of spermatogonia, spermatocytes, spermatids, leydig cells, diameter of the semiiferous tubules were analyzed using one way ANOVA and if the results were significant then the LSD test was continued. The analysis was carried out with the help of SPSS.

Result and Discussion

The results of the Brown-Forsythe test and the Welch test showed that the comparison of testicular MDA levels had a significant difference with p = 0.000. The results of the Games Howell test showed that the K-group compared to the K +, P1, P2, and P3 groups had a significant difference with p = 0.000. The K+ group compared to the P1, P2, and P3 groups also showed a significant difference with p = 0.000. Between groups P1 and P2 showed a significant difference p = 0.001. but did not show a significant difference compared to the P3 group, namely p = 0.719. Groups P2 and P3 showed significant differences with a value of p = 0.000.

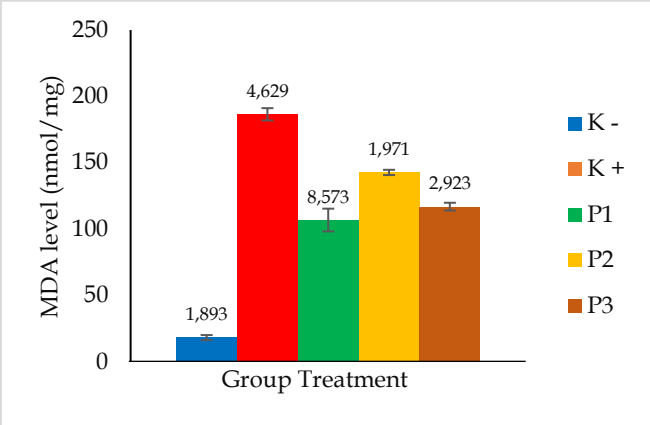


Figure 1. Graph of comparison of MDA levels (nmol/mg)

Comparison of MDA Levels in Group K- and K+

Giving exposure to cigarette smoke has the effect of increasing the number of free radicals in the body. The negative control group (K-) in this study was used as a parameter for the normal value of testicular MDA levels. Exposure to non-filter cigarette smoke in the morning and evening with a total of 4 cigarettes/day in the positive control group (K+) resulted in higher rat testicular MDA levels when compared to the negative control group (K-). This is supported by research by (Jaggi & Yadav, 2015) which stated that there was an increase in MDA levels in the testes of rats exposed to cigarette smoke compared to the group not exposed to cigarettes. This increase in levels indicates that exposure to cigarette smoke for 30 days can increase free radicals in the testicular organs.

Epidemiologically, it was found that smokers have higher concentrations of MDA in their blood. This is due to the high oxidative stress generated in smoking activities. The process of burning cigarettes will produce

ROS which are present in the gas phase and particulate phase of cigarette smoke. ROS present in the gas phase lasts only a short time and only affects the upper respiratory tract, whereas ROS present in the particulate phase, especially in the form of semiquinone radicals, will produce more secondary free radicals and induce more lipid peroxidation. This oxidative stress will increase lipid peroxidation products (Maestra et al., 2015). Endogenous antioxidants as superoxide dismutase and catalase were lower in smoker (Dai et al., 2015).

Effect of Administration of Ambon Banana Extract on MDA Levels in Rat Testes Exposed to Cigarette Smoke

In the P1 group, namely the treatment with Ambon banana extract, there was a significant decrease in testicular MDA levels. This is because the content of Ambon banana contains more antioxidant compounds in capturing free radicals caused by cigarette smoke compared to mung bean sprouts, one of which is catechin which is not owned by mung bean sprouts. Catechins play a role in protecting body cells from damage caused by free radicals by binding to free radicals thereby preventing inflammation and inflammation in body cells (Adethia & Sukarni, 2022; Kusudaryati & Prananingrum, 2022). These results were reinforced by research conducted by (Hu et al., 2018) who reported that the catechins contained in green tea extract were able to reduce MDA levels in rat testes.

The general structure of catechins has many –OH (phenol) groups which can bind to lipid radicals by removing the H+ group on the phenol group which will later bind to lipid radicals to form RH complexes, thereby inhibiting the initiation process. In other processes, peroxyl radicals can also bind to H+ groups to form ROOH (hydroperoxide) complexes which will later be broken down by metal chelating agents, thereby inhibiting the propagation process. The antioxidant radical compounds formed can also bind to hydroxyl radicals which are the forerunners of forming lipid radicals, so that free radical oxidation processes in the body can be completely prevented so that the body avoids the dangers of threatening free radicals (Asadi et al., 2017).

Ambon bananas also contain flavonoids which contribute to their antioxidant activity in vitro by binding (chelating) metal ions such as Fe and Cu. Flavonoids stop the initial stage of the reaction by releasing 1 hydrogen atom from the hydroxyl group which then binds to 1 free radical. Flavonoid radicals (FL-O*) can react again with the second free radical compound, forming a stable quinone structure. Flavonoid radicals (FL-O*) will undergo a termination reaction with free radicals (R*) to form stable and non-reactive flavonoid-radical compounds (FL-OR) (Pietta, 2000). In addition, Ambon bananas also contain vitamin C which has a strong ability to reduce and act as an antioxidant in various hydroxylation reactions (Adethia & Sukarni, 2022; Kusudaryati & Prananingrum, 2022). As an antioxidant, vitamin C works as an electron donor by transferring an electron to Cu metal compounds. Due to the ability of vitamin C as a free radical inhibitor, its role is very important in maintaining the integrity of cell membranes (Kusudaryati & Prananingrum, 2022)

Effect of Mung Bean Sprout Extract on MDA Levels in Rat Testes Exposed to Cigarette Smoke

In the P2 group, namely the treatment with mung bean sprout extract, there was a significant decrease in testicular MDA levels. Mung bean sprout extract contains various compounds including vitamin E, vitamin C, flavonoid compounds and phytosterol compounds which are antioxidants. Vitamin E is a fatty peroxide chain breaker in the membrane. Vitamin E controls fatty peroxides by donating hydrogen ions to the reaction, thus converting peroxyl radicals (results of lipid peroxidation) into less reactive tocopherol radicals, blocking the additional activity carried out by peroxides, thus breaking the chain reaction and limiting damage (Sharifi-rad et al., 2020).

When compared to the treatment group, the MDA level of the P2 group was higher than that of the P1 and P3 groups. This can be attributed to the mechanism of vitamin E in capturing free radicals. When vitamin E scavenges peroxyl radicals, it is con verted into vitamin E radical, which may be further oxidized into α-tocopheryl quinone or reduced by vitamin C or other reducing compounds to regenerate vitamin E. α-Tocopheryl quinone is a biomarker of the antioxidant action of vitamin E. Vitamin E deficiency may lead to various disorders such as infertility (Niki, 2015). Perhaps because the content of vitamin C is not too much in mung bean sprouts, namely 15 mg/100 g of mung bean sprouts so that the vitamin C contained in mung bean sprouts is not enough to reduce tocopherol radicals to non-radicals. So that vitamin E could not maximally reduce free radicals, and the impact on MDA levels in the P2 group was higher compared to the other treatment groups. Vitamin E is also a water-insoluble vitamin, so administration of mung bean sprout extract dissolved in distilled water cannot dissolve vitamin E and its absorption into the systemic circulation is also relatively small.

The Effect of Giving Ambon Banana and Mung Bean Sprouts Combined Extract on MDA Levels in Rat Testes Exposed to Cigarette Smoke

In the P3 treatment, namely the combination of Ambon banana extract and mung bean sprouts, there was a significant decrease in testicular MDA levels

compared to K+. In the P3 group, the two extracts worked together as antioxidants. Because the tocoferol radicals that are formed can be reduced with the help of vitamin C from Ambon bananas. After reacting with vitamin E radicals, vitamin C turns into monodehydroascorbate radicals. Then vitamin C undergoes reduction through 2 pathways, namely enzymatic and non-enzymatic. Through an enzymatic reaction, vitamin C is reduced by reduced glutathione (GSH), which is then catalyzed by glutathione peroxidase and selenium to become vitamin C again and oxidized glutathione (GSSG). Meanwhile, the non-enzymatic reaction works to reduce vitamin C through the reaction of two monodehydroascorbate molecules to form one molecule of ascorbate and dehydroascorbate, both of which are not radicals. GSSG is then converted to GSH again by the influence of the glutathione reductase enzyme. Based on the antioxidant action, consuming antioxidants will be better if given not in single form, but in combination (Yasin et al., 2015)

Differences in the Effect of Ambon Banana Extract, Green Bean Sprouts and Their Combination on Testicular MDA Levels

Based on the conducted research, it has been conclusively established that extracts from Ambon bananas, mung bean sprouts, and their combined application possess the capability to significantly decrease Malondialdehyde (MDA) levels within the testes of rats exposed to cigarette smoke. Notably, Ambon banana extract exhibited superior efficacy in reducing MDA levels, demonstrating an average reduction to 115.511 ± 8.57, surpassing the effectiveness of mung bean sprout extract (139.704 ± 1.97) and the combination (119.835 ± 2.92).

Table 1. Differences in the number of spermatogonia, spermatocytes and spermatids of rats not exposed to cigarette smoke (K-) and those exposed to cigarette smoke (K+).

Group	Mean ± SD		
	Spermatogonium	Spermatocytes	Spermatids
K-	73.71 ± 3.546[a]	70.71 ± 3.200[a]	83.71 ± 3.729[a]
K+	69.43 ± 4.791[b]	66.71 ± 4.309[b]	73.43 ± 5.062[b]

Note: Different letters on different lines indicate a significant difference (P<0.05) between groups

Table 2. Differences in the number of spermatogonia, spermatocytes and spermatids in the group exposed to cigarette smoke (K+), the group given Ambon banana extract (P1), mung bean sprouts (P2) and their combination (P3)

Group	Mean ± SD		
	Spermatogonium	Spermatocytes	Spermatids
K+	69.43 ± 4.791[a]	66.71 ± 4.309[a]	73.43 ± 5.062[a]
P1	76.43 ± 3.690[b]	75.57 ± 3.867[c]	88.57 ± 4.504[b]
P2	71.43 ± 3.409[ab]	69.14 ± 1.864[ab]	77.71 ± 5.155[a]
P3	75.43 ± 3.359[b]	74.00 ± 2.582[bc]	85.29 ± 4.855[b]

Note::Different letters on different lines indicate a significant difference (P<0.05) between groups

The data obtained was then analyzed and the results obtained were that there was a difference between the group not exposed to cigarette smoke (K-) and the group exposed to cigarette smoke (K+) in the number of spermatogonia (p = 0.043), spermatocytes (p = 0.030) and spermatids (p = 0.000). There was a difference between the group exposed to cigarette smoke (K+) and the group exposed to cigarette smoke and then given Ambon banana extract (P1) on the number of spermatogonia (p = 0.002), spermatocytes (p = 0.000), spermatids (p = 0.000). There was no difference between the group exposed to cigarette smoke (K+) and the group exposed to cigarette smoke and given mung bean sprout extract (P2) on the number of spermatogonia (p = 0.332), spermatocytes (p = 0.177), spermatids (p = 0.098). There was a difference between the group exposed to cigarette smoke (K+) and the group exposed to cigarette smoke and then given a combination of Ambon banana and mung bean sprout extract (P3) on the number of spermatogonia (p = 0.006), spermatocytes (p = 0.000) and spermatids (p = 0.000). These differences can be seen in Figure 2.

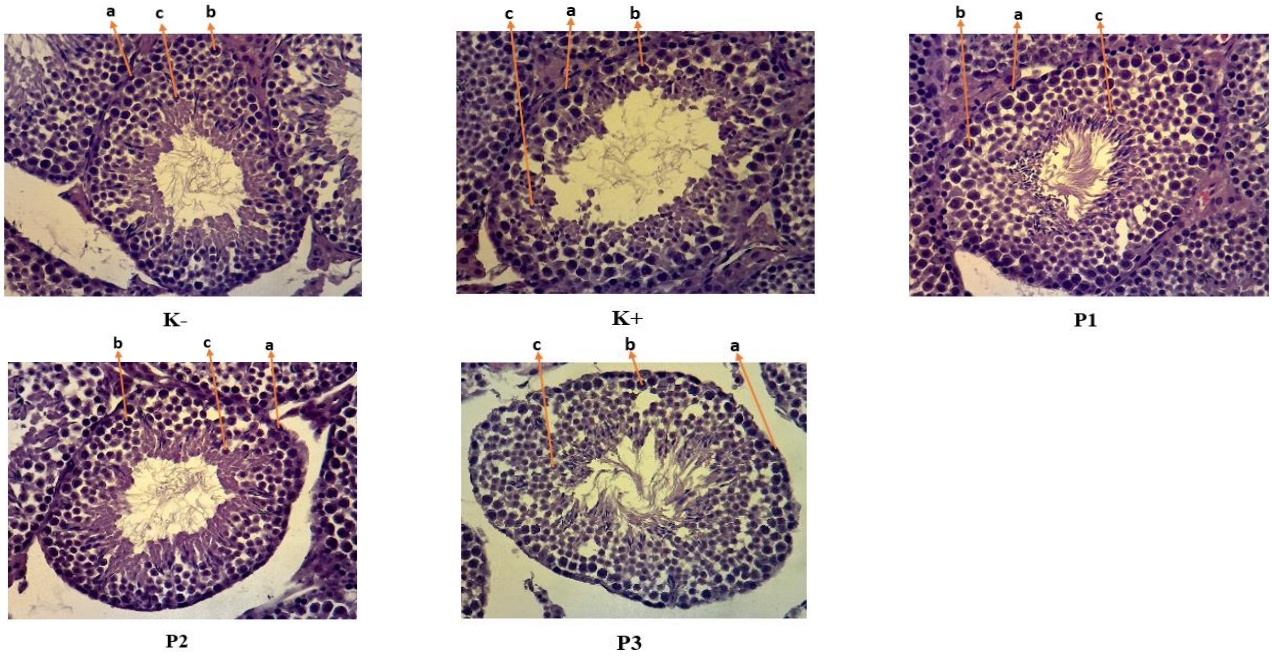


Figure 2. Testicular histology: K-: negative control, K+: positive control (cigarette smoke-exposed), P1: cigarette smoke + 'Ambon' banana extract, P2: cigarette smoke + green bean sprout extract, P3: cigarette smoke + 'Ambon' banana and green bean sprout extracts. Notes: a.) spermatogonia, b.) spermatocytes, c.) spermatids

In the K+ group, which was the group that was exposed to cigarette smoke, spermatogonia cells were seen lined up in the basal lamina, but with a loose arrangement of cells between the cells as well as a loose arrangement of spermatocytes and spermatids. Meanwhile, in the K- group, the group that was not exposed to cigarettes, the cells were denser. The tubular lumen in the K+ group appeared enlarged when compared to the K- group. In the P1 group, the inter-cell arrangement looked denser than the cell arrangement in the other groups and the diameter of the tubule lumen also appeared smaller when compared to the other groups. The P3 group has a tight inter-cell arrangement but the lumen diameter of the tubules is wider than the P1 group. Meanwhile, the lumen in the P2 group was wider compared to the P1 and P3 groups. The arrangement between cells is also tight.

Comparison of the number of spermatogonia cells, spermatocytes and spermatids of mice in the group not exposed to cigarette smoke (K-) and the group exposed to cigarette smoke (K+)

Table 1 shows that there are differences in the number of spermatogonia cells, spermatocytes and spermatids in the Positive Control (K+) group, namely the group exposed to cigarette smoke for 30 days in the morning and evening as many as 4 cigarettes/day, decreased compared to the group (K-) namely the group that was not exposed to cigarette smoke. This is supported by research conducted by (Ginting et al., 2019) which showed a significant decrease in the number of spermatid, primer spermatocytes, spermatogonium in the testes of mice treated with cigarette smoke Cigarettes contain many toxic compounds, including arsen, nicotine, benzena, carbon monoxide, nicotine, heavy metals (Kulaksiz et al., 2022). Cigarettes can cause testicular atrophy, inhibit spermatogenesis, and damage the morphology of spermatozoa, and result in apoptosis of spermatogonia. In addition, cigarettes also contain several other carcinogens and mutagens, namely polonium, benzo(a) pyrene, dimethylnitrosamine, naphthalane, CO2, H20x, NOx, SOx and CO, mercury, copper (Pb), and cadmium. According (Ramos-trevin et al., 2018) that lead showed impaired spermatogenesis and DNA damage to Sertoli cells.

Based on the above, there are many compounds contained in cigarettes that can reduce the number of spermatogonia cells, spermatids and rat spermatocytes so that there is a significant difference in numbers between the group exposed to cigarette smoke (K+) and the group not exposed to cigarette smoke (K-).

Comparison of the number of spermatogonium cells, spermatocytes and spermatids of mice in the group exposed to cigarette smoke (K+) and the group exposed to cigarette smoke and given Ambon banana extract (P1)

Administration of ambon banana extract to rats exposed to cigarette smoke (P1) was shown to significantly increase the number of spermatogonia, spermatocytes and spermatids when compared to the positive control group (K+), namely the group exposed only to cigarette smoke. Ambon bananas contain carbohydrates, minerals and vitamins such as vitamin C, B complex, B6, serotonin and vitamin E. In addition,

bananas also contain flavonoids and catechins. Catechins are a subclass of polyphenols which are chemical compounds contained in plants and are strong antioxidants. Catechins play a role in protecting body cells from damage caused by free radicals by binding to free radicals thereby preventing inflammation and inflammation.

Vitamin E plays a role in maintaining the motility or movement of spermatozoa. Vitamin E is able to inhibit lipid peroxidation and increase the activity of various antioxidants so that free radicals generated during the reduction of oxygen molecules and oxidative activity of enzymes can be bound (Niki, 2015) Deficiency of vitamin E causes degeneration of spermatogonia cells. Spermatogenesis begins with spermatogonia cell division so that if from the start the number of spermatogonia cells decreases, it can affect the development of subsequent spermatogenic cells (Saddein et al., 2019). Vitamin C is able to protect spermatozoa from damage caused by oxidative stress by neutralizing hydroxyl, superoxide and hydrogen peroxide radicals and preventing spermatozoa from experiencing agglutination (Agarwal et al., 2014). Meanwhile, the flavonoids in bananas play a role in binding metal ions such as Fe and Cu. Metal ions such as Fe and Cu can catalyze reactions which eventually produce free radicals (Sidhu & Zafar, 2018).

Comparison of the number of spermatogonia cells, spermatocytes and spermatids of mice in the group exposed to cigarette smoke (K+) and the group exposed to cigarette smoke and given mung bean sprout extract (P2)

The results of the study, administration of mung bean sprout extract increased the number of spermatogonium cells, spermatocytes and spermatids of rats exposed to cigarette smoke, although statistically there was no significant difference between the groups exposed to cigarette smoke and then given mung bean sprout extract (P2) and the control group (P2). K+), namely the group that was only exposed to cigarette smoke.

Vitamin E is able to inhibit lipid peroxidation and increase the activity of various antioxidants so that free radicals generated during the reduction of oxygen molecules and oxidative activity of enzymes can be bound. Vitamin E deficiency can result in a reduction in the number of spermatogenic cells in the seminiferous tubules (Agarwal et al., 2014). Vitamin E as an antioxidant works synergistically with the presence of other antioxidants in mung bean sprouts, for example vitamin C. Vitamin C is able to protect spermatozoa from damage caused by oxidative stress by neutralizing hydroxyl, superoxide and hydrogen peroxide radicals and preventing spermatozoa from experiencing agglutination (Agarwal et al., 2014) .

Comparison of the number of spermatogonium cells, spermatocytes and spermatids of mice in the group exposed to cigarette smoke (K+) and the group exposed to cigarette smoke and given a combination of Ambon banana extract and green bean sprouts (P3)

Based on the results of the study, the P3 group, namely the group that was given a combination of Ambon banana extract and mung bean sprout extract, showed a significant difference in number to the group exposed to cigarette smoke (K+). This happens because the two extracts work together as antioxidants that can neutralize ROS caused by cigarette smoke. Vitamins E and C work together synergistically by way of vitamin E donating hydrogen atoms to lipid radicals, so that vitamin E will be oxidized to become alpha tocopheroxyl radicals which can be regenerated into stable alpha tocopherols again through a reduction process with the hydroxyl groups of other antioxidants such as vitamin C (acidic acid). ascorbate). Reactive vitamin C will turn neutral with the help of NADPH through the enzyme NADH semidehydroascorbate reductase. In fact, the alpha tocopheroxyl radical can interact directly with lipid peroxy radicals, so that the alpha tocopheroxyl radical loses another hydrogen atom and becomes a stable and completely oxidized tocopheryl quinone (Harahap et al., 2017).

That the combination of vitamins C and E could increase the concentration, motility and morphology of rat spermatozoa exposed to smoke (Sutanto et al., 2017). Vitamin E deficiency can result in a reduction in the number of spermatogenic cells in the seminiferous tubules (Alam & Hoque, 2018). The results of this study indicate that Ambon banana extract, mung bean sprout extract and a combination of ambon banana extract and mung bean sprouts can increase spermatogonium cells, spermatids and spermatocytes of rats exposed to cigarette smoke. However, the best treatment was Ambon banana extract.

Table 3.Data on testicular weight, seminiferous tubule diameter, number of Leydig cells in positive control and negative control.

Group	Testicular weight (gr)	Mean± SD	
		Seminiferous tubule diameter (µm)	Number of leydig cells
K-	2.21 ± 0.03 [a]	65.47 ± 2.84 [a]	89.42 ± 1.71 [a]
K+	2.15 ± 0.04 [b]	60.17 ± 3.77 [b]	69.57 ± 1.51 [b]

Note: Different letters in different rows indicate significant differences (p=0.05) between groups

Table 4.Testicular weight data, seminiferous tubule diameter and Leydig cell count

Group	Mean ± SD		
	Testicular weight (g)	Seminiferous tubule diameter (µm)	Number of Leydig cells
K+	2.15 ± 0,04 [a]	60.17 ± 3,77 [a]	69.57 ± 1,51 [a]
P1	2.32 ± 0,02 [c]	66.99 ± 2,86 [b]	91.57 ± 1,81 [d]
P2	2.18 ± 0,03 [a]	61.66 ± 3,98 [a]	76.28 ± 1,11 [b]
P3	2.26 ± 0,05 [b]	64.65 ± 1,28 [ab]	86.85 ± 2,11 [c]

Note: Different letters in different rows indicate significant differences (p=0.05) between groups

In Table 3, it can be seen that there was a decrease in testicular weight, seminiferous tubule diameter and the number of Leydig cells in the K+ treatment (positive control) compared to the K- treatment (negative control) due to exposure to cigarette smoke. In table 4 above on the testicular weight parameter it can be seen that the P2 treatment did not differ significantly from K+. This shows that the dose of mung bean sprout extract is not sufficient to have the potential to significantly increase testicular weight exposed to cigarette smoke, such as P1 and P3 which show significant differences with K+.

In the diameter of the seminiferous tubules, it can be seen that the P2 and P3 treatments did not differ significantly with respect to K+. This shows that the dose of mung bean sprout extract and its combination has not had the potential to significantly increase the diameter of the seminiferous tubules exposed to cigarette smoke. and P1 which shows a significant difference with K+.

In the Leydig cell number parameter, it can be seen that in each treatment P1, P2 and P3 were significantly different from K+. This shows that doses of Ambon banana extract, mung bean sprout extract and their combination have the potential significantly to increase the number of Leydig cell exposed to cigarette smoke.

The following is an image of the diameter of the seminiferous tubules of K-, K+, P1, P2 and P3 mice with objective lens magnification (10x10):

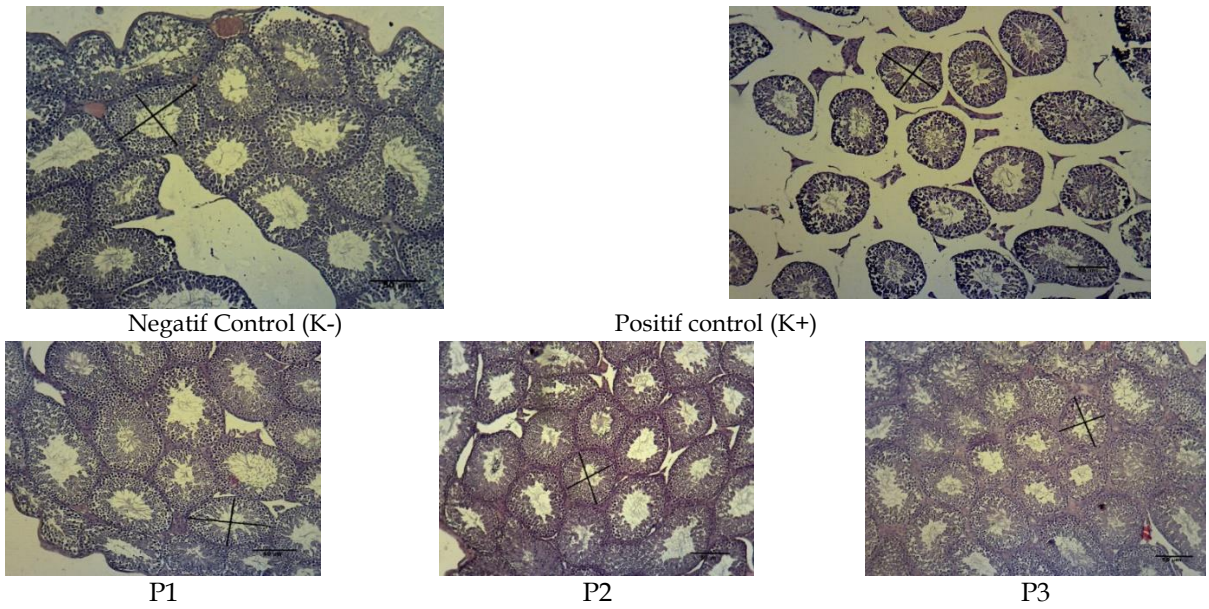
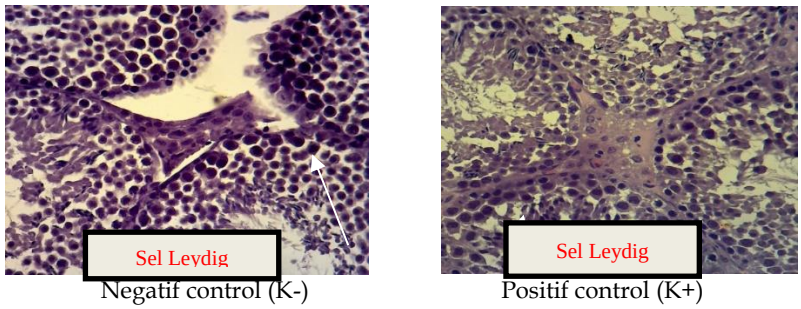


Figure 3. The diameter of the seminiferous tubules of K-, K+, P1, P2 and P3 mice with objective lens magnification (10x10)



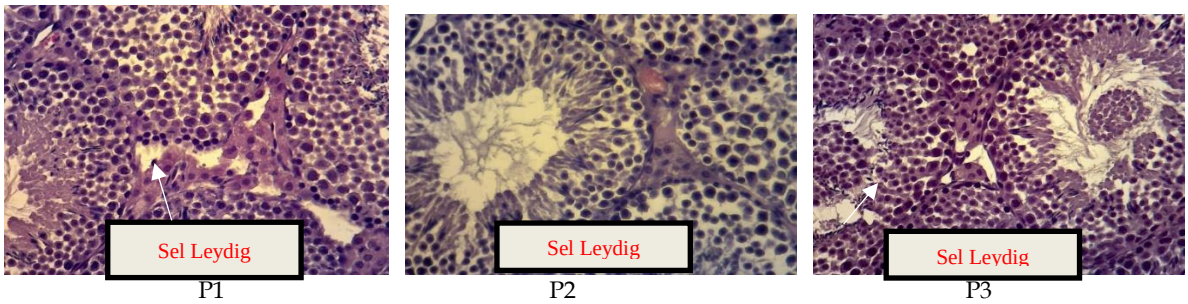


Figure 4. The number of Leydig cells in K-, K+, P1, P2 and P3 with an objective lens magnification (40x10) K-: negative control, K+: positive control (cigarette smoke-exposed), P1: cigarette smoke + 'Ambon' banana extract, P2: cigarette smoke + green bean sprout extract, P3: cigarette smoke + 'Ambon' banana and green bean sprout extracts

The results obtained in the previous table, it was found that smoking can reduce testicular performance in white rats. It can be seen from the positive control results in this study which included small testicular weights, seminiferous tubule diameter and the number of Leydig cells in white rats compared to control results. negative and other treatments caused by the ingredients contained in cigarettes. This is in line with (Batubara et al., 2013) said that exposure to 4 cigarettes had a negative effect on the quality of spermatozoa due to the presence of free radicals produced by cigarette smoke. Research conducted by (En et al., 2020) which was treated on experimental animals, it was found that exposure to cigarette smoke for 20 days had caused the diameter of the seminiferous tubules to decrease, so that the number of spermatozoa produced would be less than those that did not decrease. Disruption of spermatogenesis in the seminiferous tubules will result in reduced sperm quality, which will cause infertility.

Free radicals generated by cigarette smoke are unstable and have high reactivity. Cigarettes have a large amount of oxidants, so it is possible to damage the respiratory tract to the reproductive system. Tobacco smoke oxidants can deplete intracellular antioxidants in lung cells (in vivo) through a mechanism related to oxidant stress. It is estimated that each cigarette puff has a very large amount of oxidant ingredients, including aldehydes, epoxides, peroxides, and other free radicals that may be long enough to last and persist to cause tissue damage (Cirillo et al., 2019).. Other materials such as nitric oxide, peroxyl radicals and carbon-containing radicals are present in the gaseous phase. Also contains other radicals which are relatively stable in the tar phase. Examples of radicals in the tar phase include the semiquinone moieties produced from various quinones and hydroquinones. Mutagenic substances such as radioactive polonium, benzopyrene, methylbenzanthracene, methilnitrosamine, naphthalene and methnapthalene which are also inhaled by both active and passive smokers are absorbed into the vascular tissue in the lungs and the results are membrane plasma damage dan apoptosis cells (Horinouchi et al., 2016).

Mammalian sperm is rich in polyunsaturated fatty acids and is therefore highly susceptible to ROS attack. The ability of ROS to reduce sperm motility through peroxidation of sperm cell membranes induced by ROS causes a decrease in the flexibility and movement of the sperm tail. This sperm cell membrane lipid peroxidation can occur enzymatically and non-enzymatically. Enzymatically it involves the enzyme NADPH-cytochrome P450 reductase and reacts with the perferryl complex (ADP-FE3+O2.-). In addition to lipid peroxidation, direct damage to sperm mitochondria by ROS which causes a decrease in energy availability also causes a decrease in sperm motility(Korneyev, 2022).

ROS are also able to directly damage sperm DNA by attacking purine and pyrimidine bases. ROS can also initiate apoptosis in sperm, causing caspase enzymes to activate to degrade sperm DNA (Kowalczyk, 2022). Oxidative stress results in damage to the vascular endothelium and causes microangiopathy which can interfere with the delivery of nutrients through the blood vessels to the spermatozoa-forming tissues so that the stages of spermatogenesis in the testicular organs are imperfect. The testes in the reproductive process have two main functions, namely producing hormones and spermatozoa. The two functions are anatomically separate, namely the hormone testosterone is produced by the Leydig cells, while the spermatozoa cells are produced by the epithelial cells of the seminiferous tubules. Oxidative stress can also interfere with the hypothalamus-pituitary gonal axis so that hormone secretion becomes abnormal. If the cells and hormones in the testes are disrupted, the spermatogenesis stage will be disrupted, causing decreased spermatozoa production, and ultimately leading to infertility problems (Adelati et al., 2016).

The results of the treatment of mice exposed to cigarette smoke showed a significant increase in testicular weight, seminiferous tubule diameter and the number of Leydig cells in white rats. In the treatment of Ambon banana extract, mung bean sprout extract and a

combination of Ambon banana extract and mung bean sprouts, compared to positive control. The order of increasing testicular weight, seminiferous tubule diameter and the highest number of Leydig cells occurred in the treatment of ambon banana extract, then combination of ambon banana extract and mung bean sprout extract and then mung bean sprout extract. The increase in each treatment could occur because the Ambon banana and sprouts contained several antioxidant compounds, namely flavanoids, catechins and vitamin C in the Ambon banana extract and phytosterols and vitamin E in the green bean sprout extract. Flavonoids contribute to their antioxidant activity in vitro by binding (chelating) metal ions such as Fe and Cu. These metal ions such as Cu and Fe, can catalyze reactions that eventually produce free radicals (Cherrak et al., 2016).

Flavonoids stop the initial stage of the reaction by releasing 1 hydrogen atom from the hydroxyl group which then binds to 1 free radical. Meanwhile, vitamin C as an antioxidant works as an electron donor by transferring one electron to the Cu (Kuprum) metal compound. In addition, vitamin C can also donate electrons in intracellular and extracellular biochemical reactions. Vitamin C can directly react with superoxide anions, hydroxyl radicals, singlet oxygen and lipid peroxide. As a reducing agent, ascorbic acid will donate an electron to form semidehydroascorbate which is not reactive and then undergoes a disproportionation reaction to form dehydroascorbate which is unstable. Dehydroascorbate will be degraded to form oxalic acid and threonic acid. Due to the ability of vitamin C as a free radical inhibitor, its role is very important in maintaining the integrity of cell membranes, prevent sprm chromatine and apoptosis (Mangoli et al., 2018). This is in line with research by (Yuniarifa et al., 2020) that vitamin C can maintain the number of leydig cells in mice exposed to cigarette smoke. And the catechins contained in the Ambon banana extract have many –OH (phenol) groups which can bind to lipid radicals by removing the H+ group on the phenol group which will later bind to lipid radicals to form RH complexes, so that they can inhibit the initiation process. In other processes, peroxyl radicals can also bind to H+ groups to form ROOH (hydroperoxide) complexes which will later be broken down by metal chelating agents, thereby inhibiting the propagation process. The antioxidant radical compounds formed can also bind to hydroxyl radicals which are the forerunners of forming lipid radicals, so that free radical oxidation processes in the body can be completely prevented so that the body avoids the dangers of threatening free radicals. When the body is damage exogenously or endogenously, the oxidation capacity of the body is enhanced, producing excessive free radicals and releasing a large number of reactive oxygen species (ROS) (Liang et al., 2021).

Phytosterols are plant sterol compounds. This compound is actually contained in many vegetable oils associated with hypocholesterolemic properties. Phytosterols and their components (β-sitosterol, stigmasterol, and campesterol) can fight lipid peroxidation which can be caused by an increase in low density lipoprotein (LDL) (Alves, 2020). Phytosterols chemically act as an antioxidant, free radical scavenger, and physically as a membrane stabilizer. While Vitamin E is a fatty peroxide chain breaker on the membrane. Vitamin E controls fatty peroxides by donating hydrogen ions to the reaction, thereby converting peroxyl radicals (the result of lipid peroxidation) into less reactive tocopherol radicals, blocking additional activity carried out by peroxides, thereby breaking the chain reaction and limiting damage. The most vitamin E in nature is in the form of α-tocopherol. This compound has been known as an antioxidant capable of maintaining the integrity of cell membranes. This compound is also reported to work as an oxygen free radical scavenger, lipid peroxidation, and singlet oxygen (Eslamian et al., 2020). As an antioxidant, vitamin E functions as a hydrogen ion donor which is able to convert peroxyl radicals into less reactive tocopherol radicals, so they are unable to damage fatty acid chains (Doostabadi & Asgharzadeh, 2021). The content of vitamin E is considered as the highest antioxidant content in mung bean sprouts when viewed from the antioxidant effect that can be caused (Asrullah et al., 2019). This is in line with research conducted by (Wardhani et al., 2014) that vitamin E doses of 11 and 20 mg/day can have an effect on reducing the number of Leydig cells that undergo necrosis in mice due to oxidative stress due to exposure to tetrachlorodibenzo-p-dioxin (TCDD).

Conclusion

This study concluded that Ambon banana extract, mung bean sprout extract and a combination of both extracts act as protective agents against testicular oxidative stress and protect sperm quality due to exposure to cigarette smoke.

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Author Contributions

Conceptualization, Methodology, writing—original draft preparation: S, A, N, F. Software, Validation, formal analysis, investigation, Resources, data curation: S. A. N, F. writing—

review and editing: S. All authors have read and agreed to the published version of the manuscript

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