



The Effectiveness of Binahong (*Anredera cordifolia* (Ten.) Steenis) Extract in Promoting Fertility in Male Wistar Rats after Exposure to Cigarette Smoke

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Abstract

BACKGROUND: Cigarette smoke has a variety of dangerous chemicals and free radicals that can potentially cause infertility. One of the plants that are reported to have medicinal properties and have active compounds as antioxidants is Binahong (*Anredera cordifolia* (Ten.) Steenis).

AIM: This study aims to investigate the effectiveness of *A. cordifolia* leaf extract (ACLE) in increasing the fertility of male Wistar rats after exposure to cigarette smoke.

METHODS: Twenty-four adult male rats were divided into six groups (age 8–10 weeks, weight 200–250 g): Group 1 (normal control) received aquades (1 ml/day) without being given cigarette smoke, Group 2 received exposure to smoke without being given ACLE, Group 3 received cigarette smoke exposure + 25 mg/kg ACLE, Group 4 received cigarette smoke exposure + 50 mg/kg ACLE, Group 5 received cigarette smoke exposure + 75 mg/kg ACLE, and Group 6 received exposure to cigarette smoke + 100 mg/kg of ACLE. To produce smoke from cigarettes and expose rats to the smoke, a Smoke chamber is used. Rats in the treatment group were exposed to cigarette smoke for 2 weeks (40–60 s daily for 6 days each week). The evaluation of male rat fertility was carried out by measuring body weight, genital weight (testis and epididymis), and spermatozoa spermogram (velocity, motility, morphology, and total number).

RESULTS: The least significance different test results showed no significant difference in the rate of weight gain between treatments, standard control, and negative control groups. The average testicular weight of rats exposed to cigarette smoke for 14 days was significantly different from normal controls and doses of 50, 75, and 100 mg/kg body weight. However, at a dose of 25 mg/kg of body weight had not shown a significant increase compared to control. The average spermatozoa velocity reached 12.43 mm²/s and 13.36 mm²/s. The spermatozoa velocity increased significantly at a dose of 100 mg/kg body weight.

CONCLUSIONS: ACLE at a dose of 100 mg/kg body weight was effective in increasing the fertility of male Wistar rats exposed to cigarette smoke.

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Introduction

Cigarette smoke is a source of free radicals. Free radicals are generated due to incomplete combustion [1]. Cigarette exposure can result in cardiomyopathy and an immune reaction in the liver [2]. Epidemiological studies reveal that exposure to Second Hand Smoke leads to an increased risk of lung cancer by 20–30%, heart disease by 25–30%, stroke by up to 82%, and an increased risk of other non-fatal respiratory diseases [3]. Cigarettes can also affect the decrease in serum antioxidants, which triggers oxidative stress, DNA damage, and cell apoptosis [4]. As an air pollutant, unfiltered tobacco smoke contains 3 times more nicotine. Apart from nicotine, tobacco smoke contains more than 4000 types of constituents, including tar, carbonate monoxide, polycyclic aromatic hydrocarbons, heavy metals, and other pyrolysis derivatives [5], [6].

Several reproduction studies report that smoking can cause decreased semen quality, reproductive system dysfunction, hormone disturbances, spermatogenesis,

sperm maturation, and spermatozoa function [5], [7], [8]. The mechanism of decreased sperm function under oxidative stress has been shown to involve excessive reactive oxygen species (ROS). To counteract the effects of ROS, spermatozoa and seminal plasma have many antioxidant systems. Spermatozoa have a low number of cellular ROS defense systems consisting of catalase, superoxide dismutase, glutathione peroxidase, and Vitamin E. Similarly, seminal plasma has an antioxidant buffer capacity [9]. However, other studies have also revealed that infertile men have a low seminal plasma non-enzymatic antioxidant capacity, so the likelihood of being exposed to ROS is very high [10].

Several studies have reported that antioxidants and Vitamins A, B, C, and E in food can protect sperm DNA from free radicals and increase blood-barrier-testis stability and can improve sperm quality and improve fertility sperm quality rates in men [11], [12]. Many plant-derived substances, called phytonutrients, or phytochemicals, are becoming recognized for their antioxidant activity. About 3000 flavonoids from phenol derivatives found in plants

are known [13]. It is known that one of the plants that have active compounds is binahong (*Anredera cordifolia* (Ten.).

The binahong plant (*A. cordifolia*) is a plant from the Basellaceae family. This plant is known as Madeira vine or Mignonette vine, in Indonesia, it is known as binahong. This plant can grow in tropical, subtropical, and Mediterranean areas. This plant is often used by the community as a traditional medicine for various diseases. This plant contains flavonoids, alkaloids, triterpenoids, saponins, and essential oils and Vitamin C. Binahong leaves also contain phytoestrogens, which work as estrogens that can affect the production and breakdown of the hormone estrogen by the body. Therefore, nutritional factors in reproductive and fertility care are critical.

This study was conducted to assess the ability of binahong leaf extract (*A. cordifolia* (Ten.)) to increase fertility parameters of male Wistar rats after exposure to cigarette smoke.

Methods

Experimental animals

The experimental animals used were 24 male Wistar rats preserved in the Biology Laboratory of Faculty of Teacher Training and Education (FKIP), Tadulako University. The maintenance room was illuminated for 12 h (06.00–18.00). The experimental rats fed with domestic chicken feed granules produced by PT Charoen Pokphand Indonesia (protein value 14–16) and given *ad libitum* drinking water. The rats used were aged 3 months old, with an average initial body weight of 150–200 g. The experimental animals were divided into six groups, each group consisting of four rats (replication).

Research design

The research was conducted in November 2018 at the Biology Education Laboratory, Faculty of Teacher Training and Education (FKIP), Tadulako University. The research design used a laboratory experiment with a completely randomized design, six treatments, and four replications. The treatment groups were detailed as follows:

- Group 1 = Standard control (without smoke exposure and extracts treatment)
- Group 2 = Negative control, exposed to cigarette smoke without extract.
- Group 3 = Exposed by cigarette smoke and had extract at a dose of 25 mg/kg body weight
- Group 4 = Exposed by cigarette smoke and had extract at a dose of 50 mg/kg body weight
- Group 5 = Exposed by cigarette smoke and had extract at a dose of 75 mg/kg body weight
- Group 6 = Exposed by cigarette smoke and had extract at a dose of 100 mg/kg body weight

Research procedure

Binahong leaf extract preparation

Binahong leaf extract was prepared initially by 10 days air-dried of 250 g of binahong leaves. The dried leaves then ground and made into simplicia. The simplicia was immersed in 500 ml of methanol for 48–72 h and filtered using a Buechner funnel and concentrated using a rotary evaporator.

Exposure to cigarette smoke and intervention of binahong leaf extract

Smoke exposure to Wistar rats was conducted in a smoking chamber, a vacuum desiccator equipped with an open tube [14]. The cigarette was positioned at one end. The tube was connected to the vacuum with a stopcock placed at the top. Rats were placed in the smoking chamber, and cigarettes were lit. Puffs of smoke were drawn into the room using an air straw for 20 s. The rats were exposed to the accumulated smoke for 40 s. After that, the room was ventilated for 1 min with fresh air. Exposure of cigarette smoke to experimental animals was carried out for 14 days. On day 15, the experimental animals had binahong leaf extract for about 48 days, about 0.5 ml extract/day for each.

Assessment of Wistar male rats fertility profile

The fertility profile of rats after being exposed to cigarette smoke and given binahong leaf extract was assessed by measuring the response: (1) Body weight, (2) genital weight (testes, epididymis), and (3) spermogram. Measurements of the observed spermogram response included velocity, motility, morphology, and number of spermatozoa.

Data analysis

Statistical analysis of the data was performed using Univariate analysis/analysis of variance (ANOVA). F value or $p < 0.05$ was considered significant. Ethical committee approval with number: 9681A/UN28.1.31/PT/2020 by Animal Ethics Commission for Animal Research, Faculty of Animal Husbandry and Fisheries, Tadulako University, June 20, 2020

Results

The response of body weight and weight gain rate of male Wistar rats

The observation results on the effectiveness of binahong extract on the response to body weight and the rate of weight gain of rats after being exposed to cigarette smoke are presented in Table 1.

Table 1: The body weight and weight gain rate of male Wistar rats exposed to cigarette smoke and given binahong leaf extract

Intervention groups	Average body weight (g)		Average weight gain rate (g/day)
	Before (day 0)	Average Weight Gain Rate (g/day)	
Group 1 (control normal)	151.05	216.97	1.37 ^a
Group 2 (control negative)	150.12	211.87	1.28 ^a
Group 3	151.47	213.00	1.28 ^a
Group 4	152.12	213.35	1.27 ^a
Group 5	150.12	215.55	1.36 ^a
Group 6	152.20	215.42	1.31 ^a

LSD test results at the 95% confidence level (p<0.05). The same letter in one column between treatments shows no significant difference. LSD: Least significance different.

Based on the results of the least significance different (LSD) test, it was found that there was no significant difference in the weight gain rate of rats between treatments and with standard control and negative control.

Weight of Wistar male rats reproductive organs

The ANOVA showed that binahong leaf extract had a significant effect on rats' testicular weight, but had no significant effect on epididymal weight. The average weight of the reproductive organs based on the LSD test is shown in Table 2.

In Table 2, the average testicular weight of rats exposed to cigarette smoke for 14 days was significantly different from standard control treatment or with doses of 50, 75, and 100 mg/kg body weight, but at a dose of 25 mg/kg body weight (Group 4) has not shown a significant increase when compared to control (Group 1).

Male Wistar rat spermatozoa spermiogram

The ANOVA showed that the binahong leaf extract had a significant effect on the spermatozoa spermiogram of rats. The data from the calculation results and the LSD test for each spermiogram response are shown in Table 3.

From Table 3, it is clear that the negative control (Group 2) and the treatment dose of 25 mg/kg body weight, spermatozoa velocity until day 48 had not

seen a significant increase when compared with normal controls. The average velocity reached 12.43 mm²/s and 13.36 mm²/s. The spermatozoa velocity increased significantly at Group 6 (dosage 100 mg/kg body weight).

The percentage of motile sperm in an experimental animal exposed to cigarette smoke for 14 days without extract (Group 1) decreased significantly. The mean decrease in motility when compared with normal controls was 26%. The percentage of motile sperm between doses of 25, 50, and 75 mg/kg body weight showed an increase with increasing doses given. However, only a dose of 100 mg/kg body weight showed a significant difference (Table 3).

Observations on sperm morphology indicated that sperm morphology abnormalities caused by exposure to cigarette smoke were mainly in the form of separate sperm with a head and tail or a broken tail. The percentage of broken sperm between negative controls with doses of 25, 50, 75, and 100 mg/kg body weight showed a significant difference. However, with binahong leaf extract administration, the tendency to improve sperm morphology was seen, especially at the dose of 100 mg/kg body weight (Table 3).

The mean sperm count of rats exposed to cigarette smoke for 14 days decreased compared to normal controls (Group 1). Likewise, at doses of 25, 50, and 75 mg/kg body weight, there was a decrease in the number of spermatozoa. Although the binahong leaf extract was given for 48 days, the sperm count statistically did not show a significant increase when compared to normal controls. The increase in sperm occurred significantly at a dose of 100 mg/kg body weight (Table 3).

Discussion

White rat (*Rattus norvegicus*) fertility profile

The body weight measurement showed that cigarette smoke-exposed to male rats did not cause

Table 2: Weight of male Wistar rats' reproductive organs exposed to cigarette smoke for 14 days and intervened with binahong leaf extract

Reproductive organs	Duration of treatment (Day)	Average organ weight (mg/100 g body weight)					
		Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Testicles	48	305.75 ^a	291.00 ^b	297.50 ^b	303.00 ^a	301.25 ^a	303.50 ^a
Epididymis	48	122.00 ^a	114.50 ^b	118.75 ^b	117.75 ^b	118.50 ^b	119.25 ^b

LSD test results at the 95% confidence level (p<0.05). The same letter in a row shows no significant difference. LSD: Least significance different.

Table 3: Spermatozoa spermiogram after exposure to cigarette smoke for 14 days and given Binahong leaf extract for 48 days

Dosage (mg/kg BW)	Spermatozoa velocity (0.2 mm ² /XS) (X ± SD)	Motility (%) (X ± SD)	Σ sperm morphology abnormalities (X ± SD)	Sperm count (106/ml) (X ± SD)
Group 1 (control normal)	39.07 ^a ± 1.37	82 ^a ± 4.95	10 ^a ± 2.22	4.05 ^a ± 0.37
Group 2 (control negative)	12.43 ^b ± 1.92	56 ^b ± 7.18	34 ^b ± 2.75	3.07 ^b ± 0.61
Group 3	13.36 ^b ± 1.35	59 ^b ± 3.64	30 ^b ± 2.22	2.87 ^b ± 0.46
Group 4	16.33 ^c ± 1.33	61 ^b ± 3.00	28 ^b ± 2.22	3.45 ^b ± 0.39
Group 5	26.67 ^c ± 1.35	67 ^b ± 2.12	22 ^b ± 2.22	3.65 ^b ± 0.13
Group 6	38.47 ^d ± 3.17	80 ^b ± 1.41	17 ^b ± 2.94	3.95 ^{ab} ± 0.37

The result of the LSD test at the 95% confidence level (P<0.05). The letters a, b, c, d, e, f is used as codes to describe the significant difference or not. The same letter in a column between treatments shows no significant difference. LSD: Least significance different.

weight loss, as did the treatment given binahong leaf extract. It indicates that the rats exposed to cigarette smoke for 14 days and given binahong leaf extract for 48 days did not interfere with the rats' eating activities so that their body weight did not decrease. The lowest average weight gain rate was 1.27 g/day, and the highest was 1.37 g/day. Based on the weight gain rate data, it can be assumed that cigarette smoke and binahong leaf extract do not interfere with the transit time of food in the intestine and the absorption of food substances to increase the rats' weight. It also shows that giving cigarette smoke for 14 days has not caused a toxic effect on experimental animals (negative control). Likewise, binahong leaf extract given for 48 days at doses of 25, 50, 75, and 100 mg/kg body weight has not been able to cause toxic effects in mice. An active substance's effectiveness is strongly influenced by the administration's dose and duration [15]. Symptoms of toxicity can be caused by the accumulation of active substances in the animal's body due to the low elimination rate.

The results of measuring the reproductive organs' weight showed that the treated rats' testicular weight exposed to cigarette smoke decreased significantly. In treatment 1 (rats exposed and given binahong leaf extract at a dose of 25 mg/kg body weight) did not show any increase in testicular weight. However, in Group 4, Group 5, and Group 6 treatments, there was a significant increase in testicular weight. The decrease in testicular weight in rats exposed to cigarette smoke was probably due to decreased spermatids 19 and spermatids 7, thus causing the testes to be slightly smaller. However, this still needs further research. The epididymis reproductive organs' weight did not significantly affect weight reduction compared to controls or between treatments. If there is a decrease in epididymal weight, this indicates a disturbance in the epididymis' development and function. It is caused by decreased testosterone levels produced by Leydig cells, causing changes in the epididymal duct. However, it is not yet certain whether cigarette smoke affects the epididymis' histological structure, still needs to be further proven.

White rat (*R. norvegicus*) spermatozoa spermiogram

The results showed that cigarette smoke caused a significant decrease in sperm quality. The decrease in spermatozoa function (speed, motility, morphology, and quantity) is due to cigarette smoke containing nicotine and carcinogenic substances [16]. The findings in this study are supported by research conducted by Aryanpur *et al.* that cigarette smoke can directly or indirectly cause interference with spermatogenesis. The main content possessed by cigarettes is nicotine [17]. The decrease in spermatozoa's speed exposed to cigarette smoke in the negative control was due to cigarette smoke containing nicotine and carcinogenic substances. Apart from nicotine, tobacco smoke contains more than 4000

types of constituents, including tar, carbonate monoxide, polycyclic aromatic hydrocarbons, heavy metals, and other pyrolysis derivatives [18]. Cigarette smoke is a source of free radicals [19]. Free radicals are generated due to incomplete combustion [1]. The mechanism of decreased sperm function under oxidative stress has been shown to involve excessive ROS [20]. Various cigarette smoke components can cross the blood-testis barrier, causing significant changes in sperm quality, such as decreased sperm concentration, morphology, and motility of spermatozoa [21]. Although not very clear, proposed mechanisms for cigarette smoke effects on reproductive function include increased oxidative stress and DNA damage. Smoking is the primary exogenous source of ROS capable of inducing lipid peroxidation, DNA damage, and apoptosis of sperm cells [22]. Other studies have also shown that smoking can reduce spermatogenesis, and the number of mature sperm cells produced and reduce fertile quality and capacity [23].

Giving binahong leaf extract can increase the speed of spermatozoa, this is because binahong leaves contain various antioxidant agents, such as natural polyphenol products containing endogenous antioxidants, can capture free radicals and oxidants, and regulate redox gene expression and glutathione biosynthesis genes, chromatin remodeling, and inflammatory gene expression. Spermatozoa motility in rats exposed to cigarette smoke decreased significantly with an average motility percentage of 56%. The increase in spermatozoa motility occurred after administering binahong leaf extract at a dose of 100 mg/kg body weight. The administration of binahong leaf extract at a dose of 25 mg/kg body weight to 75 mg/kg body weight has not significantly increased spermatozoa motility. The decrease in spermatozoa motility is thought to be caused by cigarette smoke, affecting the epididymis structure, namely, the degeneration of the epididymis's epithelial ductal head cauda epididymis. Epithelial degeneration of the epididymis disrupts epididymal function, namely, reabsorption and secretion. It is suspected that a change in the fluid content in the lumen of the epididymis, which ultimately disrupts the spermatozoa spermogram. Epididymis provides a specific chemical environment for spermatozoa maturation. One of the epididymal lumen fluid characteristics is several particular proteins that can interact with spermatozoa membrane proteins. Along the epididymal duct, there is a process of addition or reduction of epididymal fluid protein.

Abnormal spermatozoa have head and tail defects [24]. Besides, the possibility of abnormal spermatozoa occurs when spermatozoa are released from the Sertoli cells in the seminiferous tubules [25]. Another possibility is that the spermatozoa's morphological abnormality is caused by disruption of the spermatozoa membrane's permeability [22]. It is suspected that cigarette smoke can cause cell shrinkage to interfere with integrating proteins and lipid bilayers on the membrane. Thus, the

membrane permeability decreases; thus, sodium and potassium transport involving Na⁺, K⁺, and ATPase are disrupted. It will cause fluid to enter the cell in large enough numbers to experience edema. In the observation, several spermatozoa with large heads (macrocephalus) were found, which is thought to be due to the influx of large amounts of fluid. Na⁺, K⁺, and ATPase are integral membrane proteins that function to maintain and maintain a balance of sodium and potassium transportation into cells. The administration of binahong leaf extract at a dose of 100 mg/kg body weight can increase normal spermatozoa in rat epididymis cauda. This increase is thought to be related to the active compounds found in binahong leaves which can cause an increase in the number of normal spermatozoa forms. A decrease in spermatozoa occurred in mice exposed to cigarette smoke and not given binahong leaf extract. In mice given binahong leaf extract, there was an increase in the number of white rats' spermatozoa. The increase in number starts at a dose of 50, 75, and 100 of body weight. The increasing concentration (number) of spermatozoa in cauda epididymis is thought to be binahong leaves with moderate to high doses can increase the secretion of proteins, carbohydrates, lipids, and other specific components required for the physiological maturation process of spermatozoa in the epididymis. As it is known, there are many active sites of androgens on the head of the epididymis, which stimulate the synthesis of various peptides. These peptides will be secreted into the epididymis lumen, and there will be incorporated into the surface layer of spermatozoa. However, this still needs further investigation.

Conclusions

A. cordifolia leaf extract at a dose of 100 mg/kg body weight was effective in increasing the fertility of male Wistar rats exposed to cigarette smoke.

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