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The Potential of Binahong Leaf (*Anredera cordifolia*) Ethanol Extract on Male Fertility in Mice Exposed to E-Cigarette Smoke

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ABSTRACT

Binahong leaf is known for its strong ability to prevent oxidation due to its active flavonoid compounds. Exposure to electronic cigarette smoke can increase reactive oxygen species (ROS), as a contributing factor to infertility. This study aims to identify the potential of Binahong leaf (*Anredera cordifolia*) ethanol extract on testosterone levels and spermatogenic cells in adult mice (*Mus musculus*) exposed to electronic cigarette smoke. A post-test control group design was used, with 28 male Balb/c mice allocated to four groups. The control group received 0.3% CMC, while the three treatment groups were administered Binahong leaf ethanol at doses of 50, 100, and 200 mg/kg body weight, in a volume of 0.1 ml orally each evening for 35 days. All mice were exposed to an electronic cigarette containing 6 mg of nicotine for 20 minutes. On day 36, blood samples were obtained to measure testosterone levels via ELISA. Testicular tissue was observed using Hematoxylin-Eosin staining. A marked elevation in testosterone concentrations and spermatogenic cell count was observed in the group treated with 50 mg/kg BW of Binahong leaf ethanol extract in contrast to the control group ($p = 0.014$ and 0.008, respectively). Higher doses reduced testosterone/spermatogenesis compared to the optimal dose, potentially implying a toxic or inhibitory effect. Binahong leaf ethanol extract administered at 50 mg/kg BW is effective in increasing testosterone levels and spermatogenic cells in adult mice (*Mus musculus*) exposed to electronic cigarette smoke.

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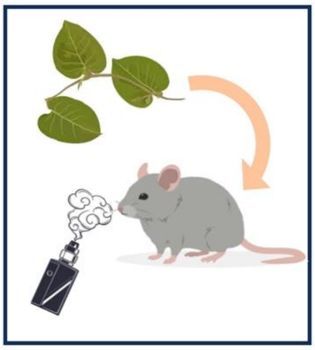
Key Messages:

- The oxidative stress contained in electronic cigarette smoke can disrupt spermatogenesis, leading to a decrease in testosterone levels, which ultimately results in infertility.
- The ethanol extract of Binahong leaf (*Anredera cordifolia*) has the ability to improve the fertility of rats exposed to electronic cigarette smoke.
- The ethanol extract of Binahong leaf (*Anredera cordifolia*) exhibits strong antioxidant activity that may be associated with spermatogenic cell counts and testosterone levels.

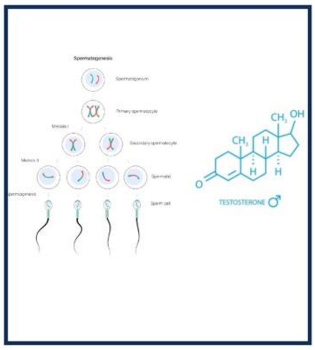
GRAPHICAL ABSTRACT

The Potential of Binahong Leaf (*Anredera cordifolia*) Ethanol Extract on Male Fertility in Mice Exposed to E-Cigarette Smoke

E-cigarette smoke leads to decreased testosterone levels, suppress spermatogenesis, and impair sperm morphology



Binahong leaf (*Anredera cordifolia*) ethanol extract is believed to increase testosterone levels and spermatogenic cells in adult mice (Mus musculus) exposed to e-cigarette smoke.



Binahong leaf ethanol extract is effective in increasing testosterone levels and spermatogenic cells in adult mice (*Mus musculus*) exposed to e-cigarette smoke.

INTRODUCTION

Smoking is a lifestyle habit adopted by a portion of the population. Currently, 1 in 3 individuals over the age of 15 is a smoker. It is estimated that around 1.2 billion people worldwide are smokers, with approximately 800 millions of them residing in developing countries. According to statistics from World Health Organization (WHO), Indonesia ranked third in the world in terms of the number of smokers, following China and India, with 34.7% of the total population or approximately 82 million people being smokers (1). The use of electronic cigarettes has become increasingly popular in Indonesia in line with advancements in the technology used, including device size, design, colour, battery life, and other features. Electronic cigarettes are nicotine delivery systems that utilize heat to vaporize a mixture of propylene glycol, flavourings, nicotine, and glycerol (2).

Nicotine present in electronic cigarette smoke has the potential to stimulate the adrenal medulla to secrete catecholamines, which may subsequently impact the central nervous system and thereby disrupt the regulatory feedback mechanism involving the testes, anterior pituitary gland, and hypothalamus. As a result, testosterone synthesis and spermatogenesis may also be impaired (3). Nicotine exposure leads to decreased testosterone levels by inhibiting the function of Leydig cells, which are responsible for producing testosterone. Moreover, several studies have demonstrated that polynuclear aromatic hydrocarbons (PAHs) present in cigarette smoke can induce testicular atrophy, suppress spermatogenesis, and impair sperm morphology. The formation of reactive oxygen species (ROS) increases with exposure to cigarette smoke. These reactive oxygen species are regarded as key mediators of infertility. Subtances like antioxidant protect cells from oxidative stress triggered by ROS (4,5).

Studies have estimated that infertility affects 15% of couples globally. Based on the total world population, this equates to approximately 48.5 million couples experiencing infertility. In Indonesia, the prevalence of infertile couples in 2013 was reported to be between 15–25% of all couples. Males are estimated to account for 20–30% of infertility cases and account for approximately 50% of cases when combined with female or unexplained factors (6,7). In relation to infertility, systematic review studies have concluded that exposure to electronic cigarette smoke can impact the male reproductive system and potentially lead to infertility. This can be demonstrated through microscopic to molecular-level sperm analysis. Spermatogenesis plays a crucial role in male fertility and ultimately holds a vital function in the continuation of human life (8). Spermatogenesis occurs in the seminiferous tubules under the regulation of testosterone and Follicle Stimulating Hormone (FSH), which involve the hypothalamic-pituitary-gonadal

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axis. FSH directly affects the Sertoli cells within the seminiferous tubules, while Luteinizing Hormone (LH) influences spermatogenesis indirectly through testosterone produced by Leydig cells, which are situated within the testicular interstitial tissue. Testosterone is essential for the proliferation and differentiation of germ cells during the process of spermatogenesis (9).

Currently, a variety of herbal treatments with diverse therapeutic properties have been developed. Indonesia's tropical forests harbor approximately 30,000 plant species, approximately 9,600 of these species are recognized for their medicinal properties, with around 200 utilized as raw materials in traditional medicine industries. One such plant with notable medicinal potential is Binahong (*Anredera cordifolia*), which has been recognized for various healing properties (10). The use of Binahong leaves (*Anredera cordifolia*) as a medicinal plant has been previously studied, demonstrating both empirical and scientific benefits. Binahong leaves contain functional flavonoid constituents. Flavonoids act as antioxidants by inhibiting the formation of free radicals, preventing lipid peroxidation, and modifying cell membrane structures (11).

Numerous studies have indicated that antioxidants have the capacity to protect sperm DNA from oxidative stress-related cellular damage, enhance the stability of the blood-brain-testis barrier, and improve both sperm quality and male fertility. In addition, Binahong leaves (*Anredera cordifolia*) are believed to increase spermatozoa motility due to their rich antioxidant content, which can regulate gene expression, chromatin remodeling, and the expression of inflammatory genes induced by cigarette smoke. This suggests that the antioxidant compounds in Binahong leaves (*Anredera cordifolia*) are considered effective in improving spermatogenesis (12). There have been few studies specifically analysing the effectiveness of ethanol extract of Binahong leaves (*Anredera cordifolia*) in enhancing spermatogenesis in species exposed to electronic cigarette smoke. Although previous study have demonstrated the protective effects of antioxidants on male reproductive parameters, most existing research has primarily focused on conventional cigarette smoke exposure rather than electronic cigarette smoke. In addition, the available study on a related topic mainly evaluated reproductive outcomes without assessing the spermatogenic cell profiles. Thus, there remains a knowledge gap regarding the mechanistic relationship between ethanol extract of Binahong leaves and spermatogenic cell.

Despite its known potential, the optimal dosage of Binahong leaves requires further study. Previous preclinical studies, also using Binahong leaf extract, assessed the extract's effectiveness at doses of 50-200 mg/kgBW as an anti-anemia agent in rat models, demonstrating measurable pharmacological activity without overt toxicity (13). Another study also showed that acute toxicity evaluation of Binahong leaf ethanol extract in rats showed no mortality at doses up to 2400 mg/kgBW, indicating a wide safety margin well above the experimental doses selected in this study. This study also found no dead rats in any of the groups, indicating that no LD50 was identified (14). This can be considered in assessing the optimal dosage for fertility. Therefore, the goal of this research is to evaluate the potential of the ethanol extract of Binahong leaves (Anredera cordifolia) at doses of 50-200 mg/kgBW to improve male fertility, including testosterone levels and spermatogenic cells, in adult mice (*Mus musculus*) exposed to electronic cigarette smoke.

METHODS

Research Design

This study employed an analytical experimental approach, using a quasi-experimental design with post-test control groups, to evaluate the effect of Binahong leaves (Anredera cordifolia) ethanol extract on improving male fertility in adult mice (*Mus musculus*) exposed to e-cigarette smoke. The research was carried out at the Integrated Biomedical Laboratory Unit (UPT Laboratorium Biomedik Terpadu), Faculty of Medicine, Udayana University, located on Jl. PB. Sudirman, Denpasar, Bali.

Research Subjects

The experimental subjects used in this study were male mice (*Mus musculus*), Balb/c strain, aged 2–3 months, weighing 20–30 grams, with no anatomical abnormalities, exhibited normal health and activity throughout a 7-day acclimatization period. The exclusion criteria encompassed mice that refused

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to eat, while dropout criteria included death during the study. The sample size, based on Federer's formula, was 28 mice, divided into 4 groups of 7 mice each.

Materials and Instruments

The primary materials used in this study were Binahong leaves (*Anredera cordifolia*), e-liquid containing 6 mg/mL nicotine, 96% ethanol, and 0.3% carboxymethyl cellulose (CMC). Histological and biochemical analyses required 10% formalin buffer, Hematoxylin–Eosin stain, a testosterone ELISA kit (BT LAB, Bioassay Technology Laboratory, size 96T, Cat. No. EA0023Ra), phosphate-buffered saline (PBS) LabotiQ No. Cat: L-1252.0500, ethanol series (70–100%), xylol, and paraffin. The instruments utilized included an analytical balance RADWAG AS 220.R2 PLUS, porcelain mortar and blender, Whatman filter paper, and rotary evaporator (68°C) BUCHI rotavapor R-200. Laboratory analysis was conducted using a centrifuge Sorvall Biofuge Prior R. Centrifuge, microtome from Leica number 820, water bath from BIOMERIEUX (Washer 470), microscope (Olympus CX31) with OptiLab camera, and an ELISA microplate reader StatFax 4200.

Extract Preparation

A total of 250 grams of *Binahong* leaves (*Anredera cordifolia*) were dried in the shade and then ground in a porcelain mortar and blender until a fine powder was obtained. The powdered leaves were then macerated in 96% ethanol with occasional stirring. Filtration was conducted using Whatman filter paper number 42 to obtain the initial filtrate. The residue was re-macerated and subsequently filtered to obtain the second filtrate. Both filtrates were then combined, and the solvent was concentrated using a rotary evaporator under reduced pressure at 68°C for 1 hour. An ethanol-based extract derived from Binahong leaves was prepared at doses of 50, 100, and 200 mg/kg BW, administered orally in a volume of 0.1 ml via oral gavage once daily in the evening for 35 days.

Research Protocol

A total of 28 male *Mus musculus* mice of the Balb/c strain were randomly assigned to four groups. This study did not include a normal control group without exposure and treatment because the primary objective was to evaluate the potential protective effect of binahong leaf ethanol extract on male fertility under e-cigarette smoke exposure. Therefore, all experimental groups were uniformly exposed to e-cigarette smoke to ensure internal validity and to allow direct comparison of the effects of different extract doses. The first group served as the Control group (K), which received a placebo (0.3% CMC). The second group, Treatment 1 (P1), received *Binahong* leaf ethanol extract administered at a dose of 50 mg/kg BW. The third group, Treatment 2 (P2), was given 100 mg/kg BW, and the fourth group, Treatment 3 (P3), was given 200 mg/kg BW. All treatments were given orally at a dose of 0.1 ml each evening for 35 days. Prior to the administration of treatments, all groups were exposed to electronic cigarette smoke containing 6 mg/ml nicotine for 20 minutes daily. The electronic cigarette was administered as one puff for 1 minute every 5 minutes. Thus, over a 20 minutes exposure period, the sample received a total of four puffs in a chamber measuring 80 cm in length, 20 cm in width, and 25 cm in height. On day 36, blood samples were obtained from the medial canthus to assess testosterone hormone levels. The mice were then euthanized for testis collection. The testes were fixed in 10% formalin buffer for a duration of 24 hours, sectioned using a microtome at a thickness of 3–5 microns, and stained with Hematoxylin-Eosin (HE). Histological evaluation was conducted using an Olympus CX31 microscope equipped with an OptiLab camera at 400x magnification to assess spermatogenesis by counting the number of spermatogenic cells, including spermatogonia A (SG), primary spermatocytes (SP), round spermatids (SP1), and elongated spermatids (SP16). Testosterone levels were measured using a competitive ELISA assay, and the optical density (OD) of the resulting color was recorded as numerical data.

Data Analysis

Data analysis was performed utilizing the SPSS software package version 27.0. When the data

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exhibited a normal distribution, differences between groups were analyzed using analysis of variance (ANOVA). Nevertheless, in instances where the data did not conform to a normal distribution, the Kruskal-Wallis test was employed. Statistical significance was determined at a p-value threshold of less than 0.05.

CODE OF HEALTH ETHICS

The study received ethical approval from the Research Ethics Committee of the Faculty of Medicine, Udayana University, with approval number 2098/UN 14.2.2.VII.14/LT/2022.

RESULTS

Based on the antioxidant level testing using the DPPH method, the ethanol extract derived from Binahong leaves (*Anredera cordifolia*) showed an inhibition concentration (IC50) value of 91.20 μg/mL, a total flavonoid content of 366.1 mg quercetin equivalent (QE)/100 g extract, and a total phenolic content of 438.6 mg gallic acid equivalent (GAE)/100 g extract. The results of the antioxidant content analysis of *Binahong* leaves (*Anredera cordifolia*) are presented in Table 1.

Table 1. Antioxidant Content Test Results of *Binahong* Leaves (*Anredera cordifolia*) Ethanol extract

Parameter	Value
IC50%	91.20 μg/mL
Flavonoid total	366.1 mg QE/100 g extract
Fenol	438.6 mg GAE/100 g extract

The testosterone levels measured in the samples from each group showed varied results. The control group exhibited a testosterone level of 19.15 ± 2.77 mmol/L. Group P1 (50 mg/kg BW extract) had the highest testosterone level at 22.87 ± 4.30 mmol/L. A decrease in testosterone level was observed in group P2 (100 mg/kg BW extract) with 18.35 ± 4.42 mmol/L, while group P3 (200 mg/kg BW extract) showed a further decline with a testosterone level of 16.08 ± 0.93 mmol/L. This study also evaluated the presence of significant differences in testosterone levels among the groups. The analysis revealed a significant difference (p=0.014) in the mean testosterone level between the control group and group with Binahong leaf ethanol extract, 50 mg/kg BW. The mean testosterone levels in male mice (*Mus musculus*) for each group are presented in **Figure 1**.

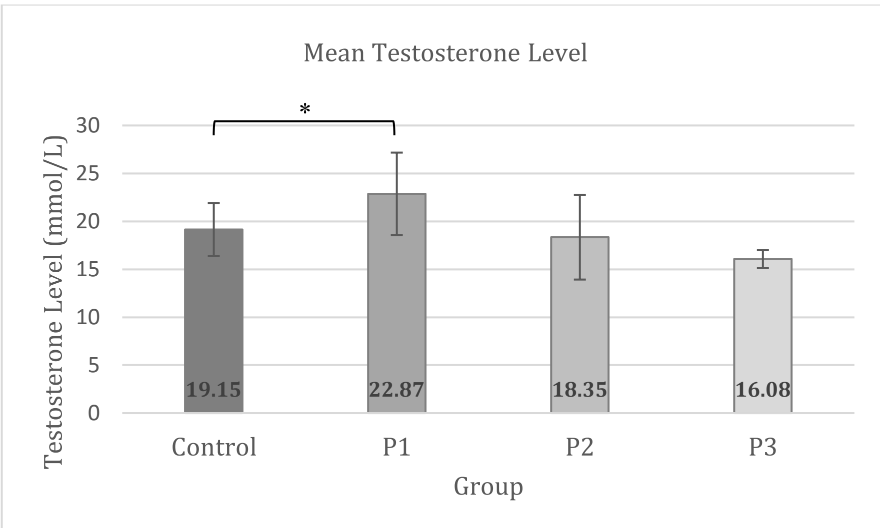

Figure 1. Mean Testosterone Level in Each Group. The data presented are the mean ± SD; *p = 0.014

The results of spermatogenesis with spermatogenic cells observed in each group are shown below. Based on the mean number of spermatogenic cells found, group P1 had the highest average count compared to the other groups, with 83.72 ± 2.57 cells for spermatogonium A (SG), 67.48 ± 5.21 cells for

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primary spermatocytes (SP), 74.24 ± 4.20 for spermatid 1 (SP1), and 78.95 ± 1.99 cells for spermatid 16 (SP16). The lowest number of spermatogonium A was found in group P3, with 45.57 ± 3.60 cells, while the lowest counts for primary spermatocytes, spermatid 1, and spermatid 16 were observed in the control group, with values of 34.76 ± 2.44 cells, 35.14 ± 6.74 cells, and 35.90 ± 4.66 cells, respectively. This study also assessed the presence of significant differences in the number of spermatogenic cells among the groups. The analysis revealed a p-value of 0.008, showing a statistically significant difference between the control group and group with Binahong leaf ethanol extract, 50 mg/kg BW. The distribution of spermatogenic cell counts can be seen in Figure 2.

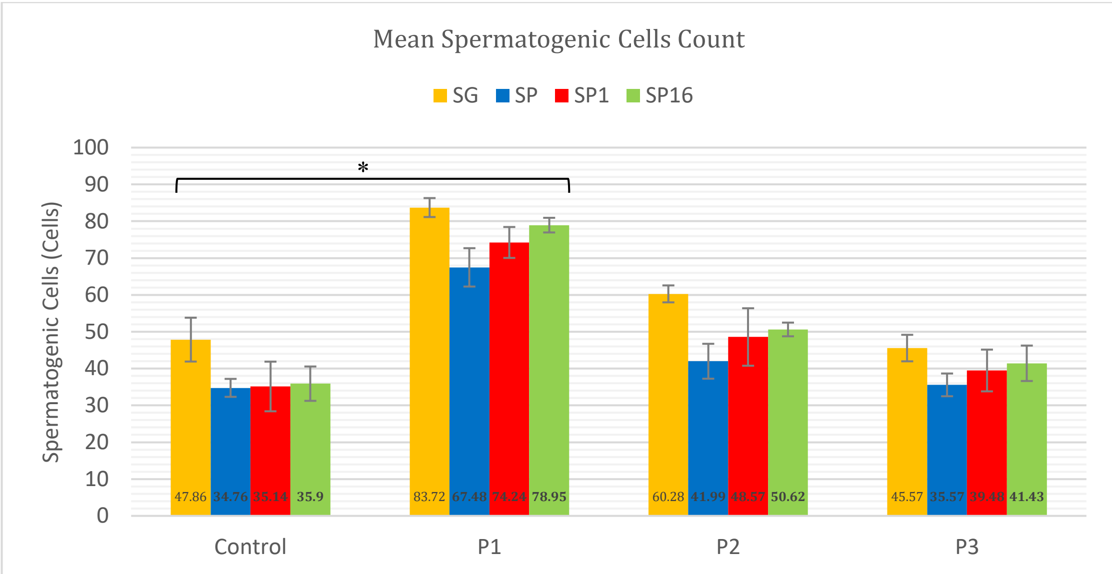
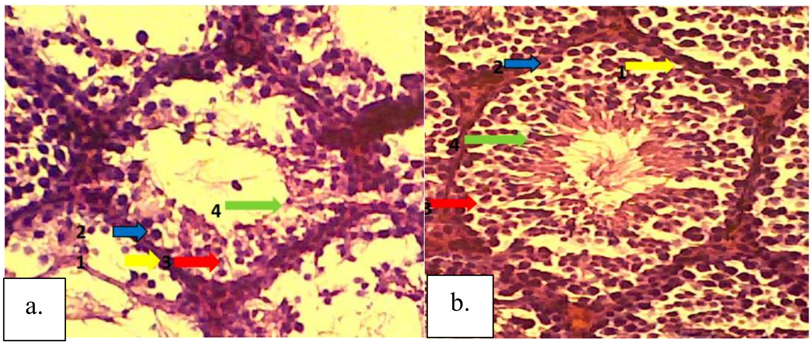


Figure 2. Spermatogenic Cells Count in Each Treatment Group. The data presented are the mean ± SD; *p = 0.008

This study also examined the histological appearance of the testes in male *Mus musculus* mice across the treatment groups. Each tissue section showed a relatively dense arrangement of spermatogenic cells (several of these are indicated by blue arrows); however, the highest number of spermatogenic cells was observed in group P1, which was administered the ethanol-based extract from *Binahong* leaves given at a dose of 50 mg/kg BW. In contrast, groups P2 and P3, treated with ethanol extracts at 100 and 200 mg/kg BW respectively, exhibited a sparser distribution of spermatogenic cells compared to group P1. The histological images of the testes in male mice from each treatment group are presented in **Figure 3.**



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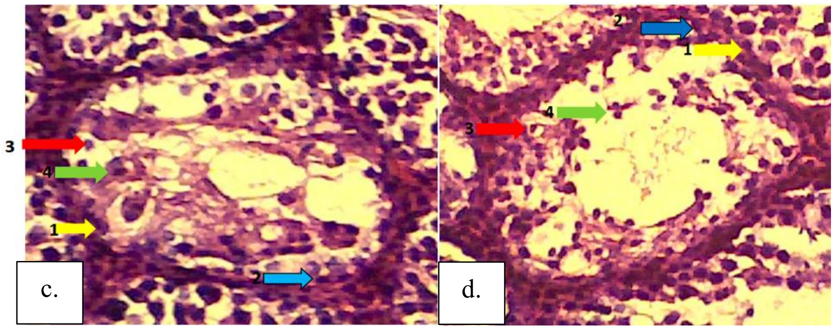

Figure 3. Histological images of testis sections from male *Mus musculus* mice in each treatment group. (a) Control group given placebo (0.3% CMC), (b) group treated with ethanol-based extract from Binahong leaves given at a dose of 50 mg/kg BW, (c) group at a dose of 100 mg/kg BW, (d) group at a dose of 200 mg/kg BW. Yellow arrows indicate spermatogonia, blue arrows indicate primary spermatocytes (SP), red arrows indicate primary spermatocytes (SP1), and green arrows indicate spermatids (SP16).

DISCUSSION

Cigarettes are highly effective at dispersing toxic chemicals. It has been established that smoking contributes to about 25 distinct diseases across different human organ systems (15,16). Exposure to electronic cigarette smoke has been extensively reported to have various adverse effects on male reproduction, particularly disrupting the process of spermatogenesis. Multiple mechanisms and factors contribute to how smoking affects sperm quality and function, mainly through oxidative stress, hormonal alterations, and toxic effects on testicular cells. The primary mechanism causing impaired spermatogenesis due to smoke exposure is the generation of ROS. Smoking introduces various toxins into the body that increase oxidative stress on sperm. Studies have demonstrated that harmful components such as polycyclic aromatic hydrocarbons and heavy metals exacerbate sperm quality deterioration through oxidative stress (17). The oxidative environment also contributes to reduced sperm count, motility, and abnormal morphology. This oxidative effect is also reported to disrupt the overall balance of sperm production in the seminiferous tubules of the testes. Biologically, smoke exposure elevates apoptotic levels, particularly caspase-3, which plays a role in germinal cells of the testes (18).

Another significant impact is the disruption of hormonal regulation, especially testosterone. Nicotine in electronic cigarettes impairs testosterone production and sperm count by altering epigenetic modulation of the promoter region of the Steroidogenic Acute Regulatory protein (StAR), responsible for mediating cholesterol transport to the mitochondria for testosterone biosynthesis in Leydig cells. Leydig cells, situated in the interstitial tissue surrounding the seminiferous tubules, play a vital role in testosterone synthesis and the regulation of secondary sexual characteristics. Nicotine ultimately reduces testicular androgenic enzyme activity, plasma testosterone concentration, and testicular tissue levels. This condition also alters plasma gonadotropin concentrations and testicular antioxidant levels, leading to disrupted spermatogenesis and reduced male fertility (19). Additional hormonal disturbances include reduced levels of LH and FSH, which reduce spermatogenic activity. The cellular impact of electronic cigarette smoke on testicular tissue is substantial, as smoke toxins cause direct damage to sperm DNA, resulting in chromatin alterations and increased DNA fragmentation. Smoke-induced apoptosis extends to spermatogonial stem cells, which are vital for maintaining spermatogenesis (20).

The consumption of ethanol extract derived from Binahong leaves (*Anredera cordifolia*) has garnered significant attention due to its various potentials in enhancing spermatogenesis through hormonal modulation. Several studies have explored the phytochemical contents of Binahong leaves and their biological effects, demonstrating that the extract's composition is effective in influencing reproductive health. Binahong leaves are rich in secondary metabolites, including flavonoids, tannins, and saponins, which are well-established for their potent antioxidant effects. These compounds are believed to benefit spermatogenesis through multiple mechanisms, including hormonal modulation (11). Polyphenols found in Binahong leaves can increase testosterone levels. Polyphenols suppress the activity of the enzyme

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5-α reductase, which catalyzes the conversion of testosterone into dihydrotestosterone, thereby elevating testosterone concentrations. Polyphenols, particularly flavonoids, also competitively bind to the enzyme aromatase, reducing its enzymatic expression and inhibiting the conversion of testosterone to estrogen, consequently increasing testosterone levels. The flavonoid content has been reported to enhance testosterone production by directly acting on Leydig cells in the testes (11,12).

An increase in testosterone levels is critically important, as testosterone is the principal androgen required for spermatogenesis. Elevated testosterone is associated with improved sperm production and motility (21). This study is further supported by other research documenting that administration of Binahong leaf extract elevates testosterone levels in animal models (11). Recent studies also indicate that oral supplementation with Binahong leaf extract can influence endocrine function, optimally promoting hormone levels essential for spermatogenesis, particularly testosterone (22). Moreover, ethanol extract of Binahong has been observed to exhibit protective effects on reproductive organs. The antioxidant properties of natural compounds, such as those found in Binahong leaves, can reduce oxidative stress, which is known to impair testicular function in testosterone production and negatively affect sperm quality. This protection is vital since oxidative stress compromises sperm DNA integrity and disrupts the hormonal balance necessary for effective spermatogenesis (11).

This study found that male mice (*Mus musculus*) administered with 50 mg/kg BW of ethanol extract from Binahong leaves exhibited significantly higher testosterone levels and spermatogenic cell counts compared to the control group. At optimal concentrations, compounds such as flavonoids, saponins, and alkaloids can stimulate Leydig cells in the testes to enhance testosterone synthesis. Conversely, excessive or suboptimal doses may lead to adverse effects. These findings align with previous research indicating that certain lower doses of phytoestrogens can optimize testosterone levels by improving the androgen-estrogen ratio, thereby positively impacting male spermatogenesis (23). The study further demonstrated that the antioxidant properties of the ethanol extract of Binahong leaves (*Anredera cordifolia*) are most pronounced at a dosage of 50 mg/kg BW. Elevated oxidative stress is known to reduce testosterone production and the number of spermatogenic cells. Lower doses appear to sufficiently harness the antioxidant effects without overburdening cellular mechanisms that might otherwise suppress testosterone synthesis due to excessive ROS production (24,25).

Furthermore, the ethanol-based extract from Binahong leaves administered at a dose of 50 mg/kg BW showed potential in increasing spermatogenic cell proliferation. Active components in the extract effectively promote the proliferation of Sertoli cells, which are essential for supporting spermatogenesis. Appropriate dosing is essential to achieve optimal results, as higher doses may inhibit Sertoli cell proliferation due to the disruption of hormonal feedback mechanisms, thereby impairing testosterone biosynthesis and function (26).

However, this study also showed that administration of Binahong leaf extract (*Anredera cordifolia*) at doses of 100 mg/kg and 200 mg/kg BW led to a reduction in testosterone levels and spermatogenic cell counts. Several studies support these findings. Although a dose of 50 mg/kg BW increased testosterone levels and spermatogenic cells, the flavonoid and saponin content in Binahong leaf extract is associated with estrogenic effects or acts as phytoestrogens in the body. Higher doses lead to elevated phytoestrogen levels, which can negatively impact testosterone synthesis and function by downregulating androgen receptor expression and disrupting the feedback mechanism of the hypothalamic-pituitary-gonadal axis (27). This finding is further supported by Hanafiah et al., who reported that while the antioxidant properties of Binahong leaf extract are generally beneficial, excessive reduction of reactive oxygen species may inhibit steroidogenesis, potentially causing a decrease in testosterone levels (28). The optimal dose observed in this study (50 mg/kg BW in mice) does not directly correspond to an equivalent human dose. Based on body surface area–based conversion, this dose corresponds to an approximate human equivalent dose (HED) of 4 mg/kg BW, while the higher doses of 100 and 200 mg/kg BW correspond to substantially higher HED values (29).

Additional experimental animal studies by Trisnawati et al. have highlighted the dose-dependent effects of increased Binahong leaf extract on testosterone levels. These effects are linked to alterations in lipid metabolism and hormonal profiles following dose escalation. Their study demonstrated significant

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hormonal and lipid profile changes, indicating a dose-dependent modulation of hormone levels with increasing Binahong extract doses (30). Supporting this, other research indicates that the saponin content in Binahong leaf extract can interfere with lipid metabolism crucial for steroidogenesis. Saponins are known to significantly reduce lipid profiles, which are essential for cholesterol production. Consequently, higher doses of Binahong leaf extract may suppress testosterone synthesis in Leydig cells by limiting cholesterol availability (31).

There are several limitations to this study. Animal model research requires cautious interpretation as results cannot be directly extrapolated to humans. This study was conducted over a short duration, thus not assessing the long-term effects of ethanol extract obtained from Binahong leaves on spermatogenesis. This study did not include a healthy control group (no exposure to e-cigarette smoke and no treatment), which limits the ability to determine whether the administration of Binahong leaf ethanol extract is capable of restoring male fertility parameters to normal baseline levels or merely provides a relative improvement compared to the smoke-induced damaged condition. The absence of a healthy control therefore represents a significant limitation in interpreting the full restorative potential of the extract. Furthermore, this study did not evaluate the potential side effects or toxicity of the ethanol extract obtained from Binahong leaves.

CONCLUSION

This study showed that ethanol-based extract from Binahong leaves (*Anredera cordifolia*) demonstrates the potential to enhance testosterone levels and spermatogenic cell counts at an optimal dose of 50 mg/kg BW, as evidenced by quantitative analyses and histopathological examination of testicular tissue in adult *Mus musculus*. These effects were more pronounced than in the control group and in the experimental groups receiving doses of 100 mg/kg and 200 mg/kg BW. Further research is warranted to investigate the long-term effects and the safety profile, including potential toxicity, of ethanol-based extract from Binahong leaves in mice exposed to e-cigarette smoke. Additionally, future studies should include a healthy control group to better elucidate the restorative potential of Binahong leaf ethanol extract on male fertility.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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