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RESEARCH **Open Access**

Analysis of the Effect of Black Garlic (Allium sativum) Extract on Ovarian Follicular Atresia, Endometrial VEGF Expression, and Fallopian Tube Epithelial Cell Count in Rats (Rattus norvegicus) Exposed to Cigarette Smoke

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Abstract
Cigarette smoke exposure significantly impairs reproductive function in Rattus norvegicus. This study evaluated the protective effects of black garlic extract against such damage. Using a post-test-only control group design, 25 female Wistar rats were divided into five groups and exposed to cigarette smoke with or without varying doses of black garlic extract. After four weeks, results indicated that black garlic extract significantly increased Vascular Endothelial Growth Factor expression, enhanced fallopian tube secretory epithelial cell counts, and reduced ovarian follicular atresia in rats exposed to cigarette smoke. The group receiving 50 mg/kgBW of black garlic extract showed the most significant improvements. Statistical analysis, including One-way ANOVA, revealed significant differences between groups. Normality was assessed using the Shapiro-Wilk test, and homogeneity was confirmed with the Levene test. Significant decreases in ovarian follicular atresia ($p < 0.05$) and increases in Vascular Endothelial Growth Factor expression ($p < 0.05$) and secretory epithelial cell counts ($p < 0.05$) were observed in the 50 mg/kgBW treatment group compared to controls. In conclusion, black garlic extract offers dose-dependent protection against cigarette smoke-induced reproductive damage, with 50 mg/kgBW being the optimal dose. Further research should explore molecular mechanisms, long-term toxicity, and clinical applications in humans.

Keywords: Black Garlic Extract, Cigarette Smoke, Fallopian Tube Epithelial Cell, Follicular Atresia.

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1. INTRODUCTION

Cigarette smoke is known to have widespread negative impacts on human health, including increased risk of cardiovascular disease, lung cancer, and reproductive disorders. (Rahma et al., 2019; Widyanti et al., 2020), exposure to cigarette smoke raises the risk of coronary heart disease by 2.4 times in individuals over 45 years old and can reduce fertility by 30% in women who are frequently exposed (Ardiana, 2021). (GATS, 2021) shows an increase in the number of smokers in Indonesia, with 34.5% of the population, or around 70.2 million people, being smokers, most of whom are men.

More than 4800 harmful chemicals and free radicals can be found in cigarette smoke, including nicotine, carbon monoxide, and tar. Research shows that nicotine affects the expression of estrogen and progesterone receptors and vascular endothelial growth factor (VEGF), which play a role in cell survival" (Ardiana, 2021; Totonchi et al., 2016). Cigarette smoke exposure also induces abnormal proliferation of the inner lining of rat uterus and affects endometrial angiogenesis in human and rat endometrial cells (Budani et al., 2021; Lee et al., 2017). Cigarette smoke exposure impairs female fertility through specific mechanisms, including decreased VEGF expression and increased oxidative stress. VEGF is essential for maintaining endometrial thickness and embryo implantation and development (Hanum & Saleha, 2023; Kida et al., 2021).

Cigarette smoke contains various free radicals, such as nicotine and tar, that can cause oxidative stress by disrupting the balance between the body's antioxidant defense mechanisms and the production of reactive oxygen species (ROS) (Awaga et al., 2019). This disrupts the normal function of the hypothalamus-pituitary, ovaries, and endometrium, potentially disrupting the ovulation and implantation process (Susanti et al., 2020).

Oxidative stress, a condition where there is an imbalance between the production of free radicals and the body's ability to neutralize them, can damage DNA and cell membranes, and disrupt the normal function of reproductive cells. This imbalance leads to cell damage and impaired reproductive function, further highlighting the detrimental effects of cigarette smoke on female fertility.

VEGF is essential in the angiogenesis process required for healthy endometrial tissue's growth and development. Decreased VEGF expression due to cigarette smoke exposure can reduce endometrial thickness, which is essential for embryo implantation and normal embryo development. A thin and poorly developed endometrium can reduce the ability of the embryo to attach and survive, thus decreasing the chance of pregnancy.

In addition, oxidative stress caused by free radicals in cigarette smoke can cause damage to ovarian follicles and inhibit the production of reproductive hormones such as estrogen and progesterone. This damage can lead to follicular atresia, a condition where the ovarian follicles that should develop into mature eggs degenerate and die instead. This can significantly reduce the number of eggs available for fertilization, thereby reducing reproductive function and fertility in women.

The increase in the number of smokers globally has led to severe public health problems, including disorders of the female reproductive system. Cigarette smoke contains various harmful chemicals that can damage various organs, including the ovaries, endometrium, and fallopian tubes in women. Recent studies have shown that VEGF expression in the endometrium and the number of endometrial arterioles may decrease due to exposure to cigarette smoke. Chemicals in cigarettes, such as nicotine and free radicals, can induce oxidative stress, leading to cell damage and ovarian follicular atresia, disrupting normal ovarian function and reducing fertility.

Black garlic has been widely studied for its ability to improve health parameters, including overcoming free radical damage. Previous studies have shown various health benefits

of black garlic, such as anti-tumor, antioxidant, anti-allergic, hypocholesterolemic, and hepatoprotective activities (Zhang et al., 2015). The antioxidant properties of black garlic reportedly protect against free radical damage in the body (Chung, 2006). Aged garlic extract (AGE) produced from fresh garlic has also been shown to prevent oxidative damage by counteracting free radicals (Capasso, 2013). Research has also shown that black garlic produced at high temperatures and humidity has strong antioxidant properties (Lu et al., 2017). Black garlic is known to have more potent antioxidant activity than fresh garlic, with additional benefits such as anticancer, anti-allergic, hypocholesterolemic, and hepatoprotective activities (Yuan et al., 2016).

However, previous studies have some limitations. Firstly, many studies focus on general antioxidant properties without identifying the specific molecular mechanisms. Secondly, most studies were conducted in animal or cell models, thus requiring further confirmation through human clinical trials. In addition, variations in black garlic processing techniques, such as fermentation duration and temperature, affect its antioxidant activity (Herlina et al., 2019). However, there is yet to be a widely accepted standard for this process. Another study showed that fermentation with specific bacterial strains can increase antioxidant activity (Setiyoningrum et al., 2018), but this research has yet to be widely applied on a larger scale.

Although previous studies have highlighted the potential health benefits of black garlic, there is still a need for further research that explores specific mechanisms, confirms results through clinical trials, and establishes consistent processing standards to optimize its health benefits. It's important to note that while black garlic is generally considered safe for consumption, it may cause certain side effects such as bad breath, body odor, and heartburn in some individuals. The novelty of this study is that it focuses on evaluating the effects of black garlic on reproductive function affected by cigarette smoke exposure, taking into account the appropriate dosage for optimal protective effects. This makes natural treatment alternatives such as black garlic extract more attractive as they generally have fewer side effects, are safer, and are more affordable. Therefore, there is a growing need to look for safer and more affordable therapeutic alternatives, such as natural ingredients with antioxidant potential. One interesting natural ingredient is black garlic (Allium sativum), which is processed from garlic through fermentation, increasing its antioxidant content.

This research presents a novel approach to the negative impact of cigarette smoke exposure on the female reproductive system at the cellular and molecular levels, particularly oxidative stress damage. It addresses issues such as decreased expression of VEGF, vital for angiogenesis in the endometrium, increased follicular atresia indicating damage to the ovaries, and damage to fallopian tube epithelial cells, essential for ovum transportation. The study explores the potential of black garlic extract, a unique and unexplored therapeutic agent, to mitigate these adverse effects. It does so by harnessing the antioxidant properties of black garlic extract to reduce oxidative damage and improve reproductive function.

The study aims to comprehensively examine the therapeutic effects of black garlic extract on damage caused by cigarette smoke to the reproductive system of female rats. Specific objectives include evaluating the ability of black garlic extract to reduce ovarian follicular atresia, increase VEGF expression in the endometrium, and improve the number of secretory epithelial cells in fallopian tubes exposed to cigarette smoke. Through these objectives, the study seeks to provide insight into the potential of black garlic extract as an effective and safe therapeutic alternative to protect female reproductive function from the adverse effects of cigarette smoke.

This study highlights the increasing prevalence of smokers worldwide, particularly in Indonesia, where the number of female smokers and exposure to secondhand smoke has become a severe public health concern. The increased exposure in workplaces, homes, and restaurants necessitates addressing the gap in treatment and prevention of reproductive system damage due to cigarette smoke. By exploring the use of black garlic (Allium sativum) extract, which is easily accessible, potentially effective, and affordable, this study aims to offer a viable treatment alternative. Therefore, this research analyzed the effect of black garlic extract on ovarian follicular atresia, endometrial VEGF expression, and fallopian tube epithelial cell count in rats (Rattus norvegicus) exposed to cigarette smoke.

2. RESEARCH METHOD

This study used a true experimental method with a post-test-only control group design approach. This study was conducted at the Pharmacology Laboratory, Faculty of Medicine, Universitas Brawijaya, Indonesia, for 28 days. The subjects of this study were female Wistar rats (Rattus norvegicus), with an initial population of 25 animals divided into five experimental groups. The sampling technique, which was randomized to determine the experimental and control groups, was chosen for its ability to reduce bias and ensure a representative sample. Each group was exposed to cigarette smoke for five minutes per session, two sessions per day for 28 days. The following is the division of the groups and the duration of exposure:

1) CN (Negative Control): This group was not exposed to cigarette smoke and was not given black garlic extract.
2) CP (Positive Control): Exposed to cigarette smoke twice a day for five minutes per session for 28 days without black garlic extract.
3) P1 (Treatment 1): Exposed to cigarette smoke twice a day for five minutes per session and given a dose of 50 mg/kgBW black garlic extract for 28 days.
4) P2 (Treatment 2): Exposed to cigarette smoke twice a day for five minutes per session and given a dose of 100 mg/kgBW black garlic extract for 28 days.
5) P3 (Treatment 3): Exposed to cigarette smoke twice a day for five minutes per session and given a 200 mg/kgBW dose of black garlic extract for 28 days.

This duration of exposure was designed to mimic conditions of continuous exposure to cigarette smoke and to test the effectiveness of black garlic extract in reducing the adverse effects of such exposure. Data were collected through direct observation of biological changes in the rats after exposure to cigarette smoke and/or administration of black garlic extract. Each group was exposed to cigarette smoke for five minutes per session, two sessions per day for 28 days.. Specifically, this study measured the expression of VEGF in the endometrium, the number of arterioles in the endometrium, and the number of ovarian follicular atresia. Data were analyzed using parametric statistics and One-way ANOVA to determine significant differences between groups.

Data were presented in tables and graphs to facilitate interpretation of the results. Specifications of the tools used included an Olympus microscope for histological examination, a spectrophotometer for analysis of an image analyzer to calculate VEGF expression and number of arterioles. The materials used included black garlic extracts of specific concentrations prepared at the university's pharmacy laboratory, distilled water for control, and cigarette smoke generated from commercial cigarettes. The experimental conditions, including duration of exposure and dosage, were designed to minimize outside variables and improve the reliability of the results. The entire experimental procedure was approved by the Ethics Committee of the Faculty of Medicine, Brawijaya University, with ethical number 367/EC/KEPK-S2/11/2023.

3. RESULTS AND DISCUSSION

This study examined the effect of black garlic extract (Allium sativum) on ovarian follicular atresia, endometrial VEGF expression, and fallopian tube epithelial cell count in

Wistar female rats exposed to cigarette smoke. A total of 25 rats were divided into five groups, each of which was given different treatments: control with distilled water, exposure to cigarette smoke, and three groups that received black garlic extract at doses of 50, 100, and 200 mg/kgBW, and exposure to cigarette smoke.

Table 1. Group Distribution

Group	Cigarette Smoke (2 cigarettes /day for 28 days)	Black garlic Extract
CN	0	0
CP	√	0
P1	√	Dose of 50 mg/kgBW
P2	√	Dose of 100 mg/kgBW
P3	√	Dose of 200 mg/kgBW

Description: CN: Negative Control, CP: Positive Control, P1: Treatment 1, P2: Treatment 2, P3: Treatment 3.

Characteristics of the study subjects showed no significant difference in baseline weight between groups. Univariate analysis showed an overall decrease in body weight in all groups exposed to cigarette smoke, indicating a systemic effect of cigarette exposure.

After completion of the treatment period for four weeks (28 days), rats in the proestrus phase were vaginal swabbed and dissected. Bivariate analysis using the ANOVA test revealed significant differences in endometrial VEGF expression between the control group and the group receiving black garlic extract. In the group given a 50 mg/kg dose of black garlic extract, there was a significant decrease in an increase in VEGF expression compared to the group that was only exposed to cigarette smoke.

The decision criteria, namely p-value> from 0.05, then the data is usually distributed, and vice versa; if the p-value <0.05, then the data is not normally distributed. The Shapiro-Wilk test analysis obtained is shown in the table below:

Table 2. Data normality and homogeneity test results

Assumption Testing	Coefficient	p-value	Data distribution
Atresia Folikel			
Normality		0.765	Normal
Homogeneity		0.051	Homogen
VEGF Expression			
Normality	0.962	0.40	Normal
Homogeneity	1.133	0.36	Homogen
Fallopian Tube Secretory Epithelial Cell Count			
Normality	0.932	0.823	Normal
Homogeneity	2.400	0.087	Homogen

Description: If p-value <0.05 the data is not normally distributed and if p-value >0.05 the data is normally distributed.

The Effect of Black Garlic (Allium sativum) Extract on Ovarian Follicular Atresia in Rats (Rattus norvegicus) Exposed to Cigarette Smoke

The number of follicular atresias with the HE staining method was observed with an Olympus CX23 microscope with a magnification of 30x and 400x to reconfirm the follicles that experienced atresia. In the observation of Figure 1, it can be seen that the most follicular atresia occurs in group 3 (P3) exposed to cigarette smoke and BG extract 200 mg/kgBW. The least atresia was found in Group CN (Negative Control), which was not exposed to cigarette

smoke and only distilled water. In this group, the number of follicular atresia was the lowest compared to other groups exposed to cigarette smoke.

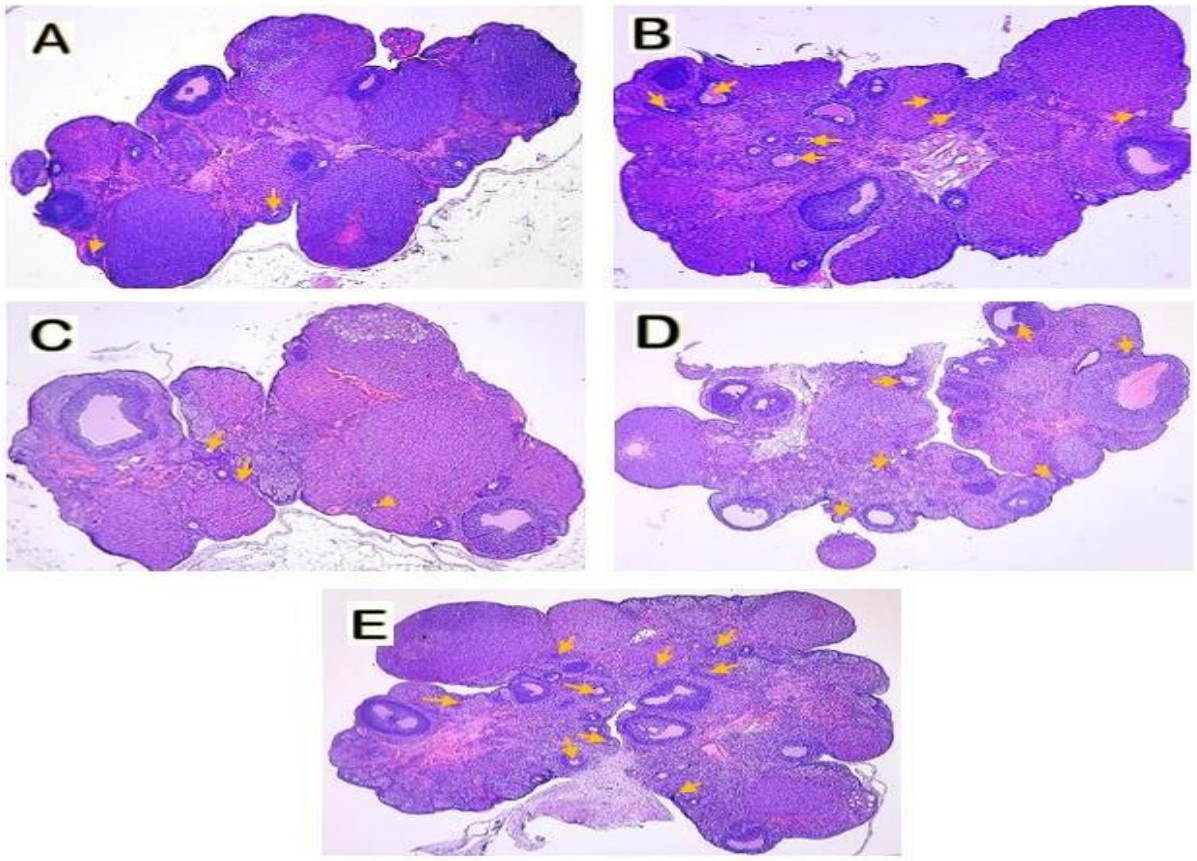
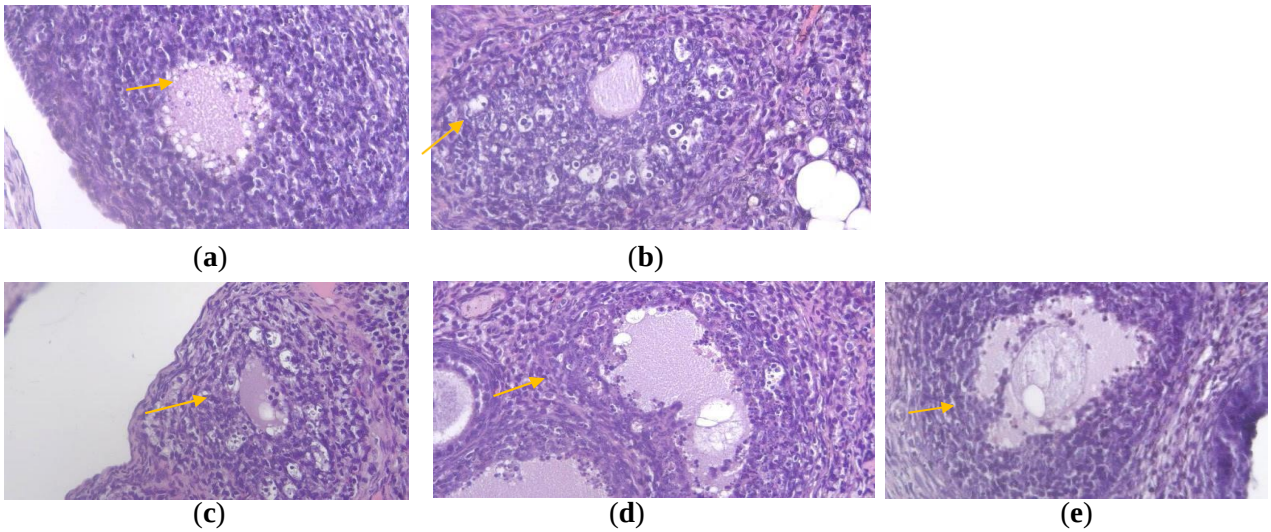


Figure 1. Follicular Histology

Observations in Figure 1 were made with an Olympus SZ51 microscope with 30x magnification. (a) observation on CN (without exposure to cigarette smoke); (b) observation on CP (exposed to cigarette smoke); (c) observation on P1 (cigarette smoke and BG 50 mg/kgBW); (d) observation on P2 (cigarette smoke and BG 100mg/kgBW); (e) P3 (cigarette smoke and 200 mg/kgBW BG extract). Follicular atresia appears more in P3.



(**a**) (**b**)

(**c**) (**d**) (**e**)

Figure 2. Follicular atresia

Observation of follicular atresia in Figure 2 with Olympus CX23 microscope with 400x magnification. (a) CN group, which was only given a distilled water sonde; (b) only exposed to cigarette smoke (CP); (c) P1 exposed to cigarette smoke and BG extract 50 mg/kgBW; (d) exposed to cigarette smoke and BG 100 mg/kgBW (P2); (e) exposed to cigarette smoke and BG 200 mg/kgBW (P3). Follicular atresia is characterized by apoptotic bodies and necrosis, as the yellow arrows indicate.

Table 3. T-test results of follicular atresia

Group	n	Mean±SD	p-value
CN	5	1.4 ± 1.67^{a}	
CP	5	7.2 ± 3.77^{b}	
P1	5	2.6 ± 1.34^{a}	0.000
P2	5	6.8 ± 0.84^{b}	
P3	5	8.2 ± 1.79^{b}	

Mean ± Standard Deviation indicates descriptive test results. p-value <0.05 indicates a significant difference.

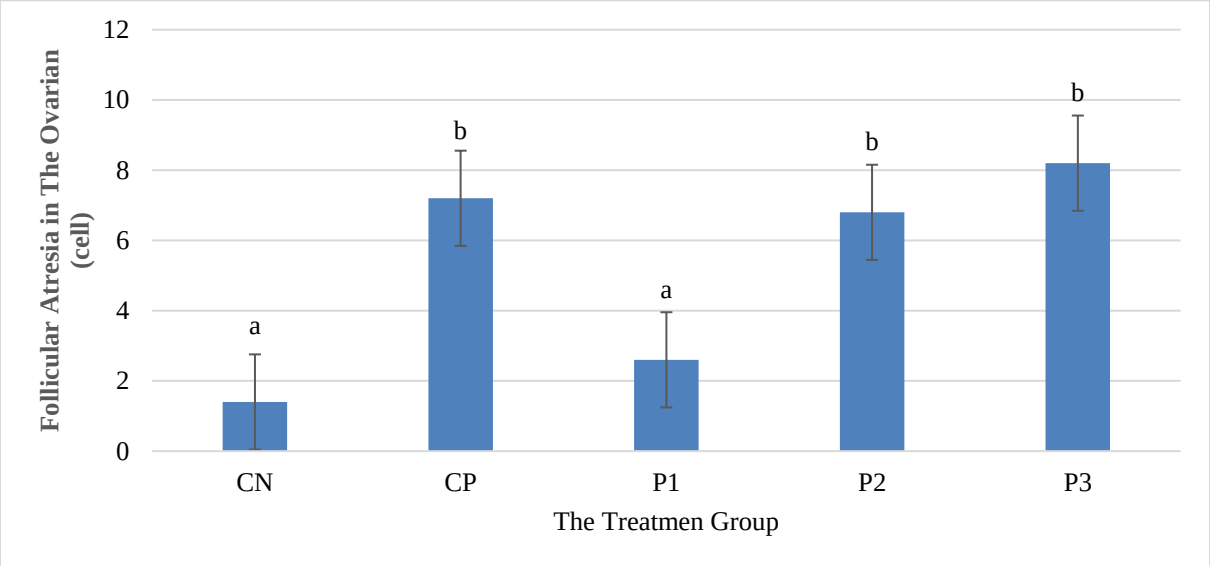


Figure 3. Follicular atresia in the ovarium on rat

Figure 3 shows a histogram of mean follicular atresia in female Rattus norvegicus that were not treated (CN) (mean 1.4 ± 1.67 standard deviation), exposed to cigarette smoke (CP) (mean 7.2 ± 3.77 standard deviation), and exposed to cigarette smoke and given BG at doses of 50 mg/kgBW (mean 2.6 ± 1.34 standard deviation), 100 mg/kgBW (mean 6.8 ± 0.84 standard deviation), and 200 mg/kgBW (mean 8.2 ± 1.79 standard deviation) (P1, P2, and P3). The mean follicular atresia appeared lower in group P1; administering BG at a dose of 50 mg/kgBW can reduce the mean follicular atresia in the ovaries. However, in groups P2 and P3, there was an increase in the mean follicular atresia, which was higher than in P1. BG administration with increasing doses can increase the average follicular atresia.

Table 4 shows that there is a significant relationship between BG dose and follicular atresia (p-value = 0.001 < α) in the cigarette smoke exposure and BG administration groups (50 mg/kgBW, 100 mg/kgBW, and 200 mg/kgBW). The test results of the correlation coefficient of dose to follicular atresia resulted in r = 0.791, which indicates a strong relationship or correlation. The correlation is positive, meaning that when the dose of BG is increased, there

will be an increase in follicular atresia in the group exposed to cigarette smoke. Vice versa, when the dose of BG is lowered, there will be a decrease in follicular atresia. Therefore, it can be concluded that there is a relationship between the dose of BG and follicular atresia in Rattus norvegicus rats exposed to subacute cigarette smoke, which refers to a level of smoke exposure that is less severe than chronic exposure but more severe than acute exposure.

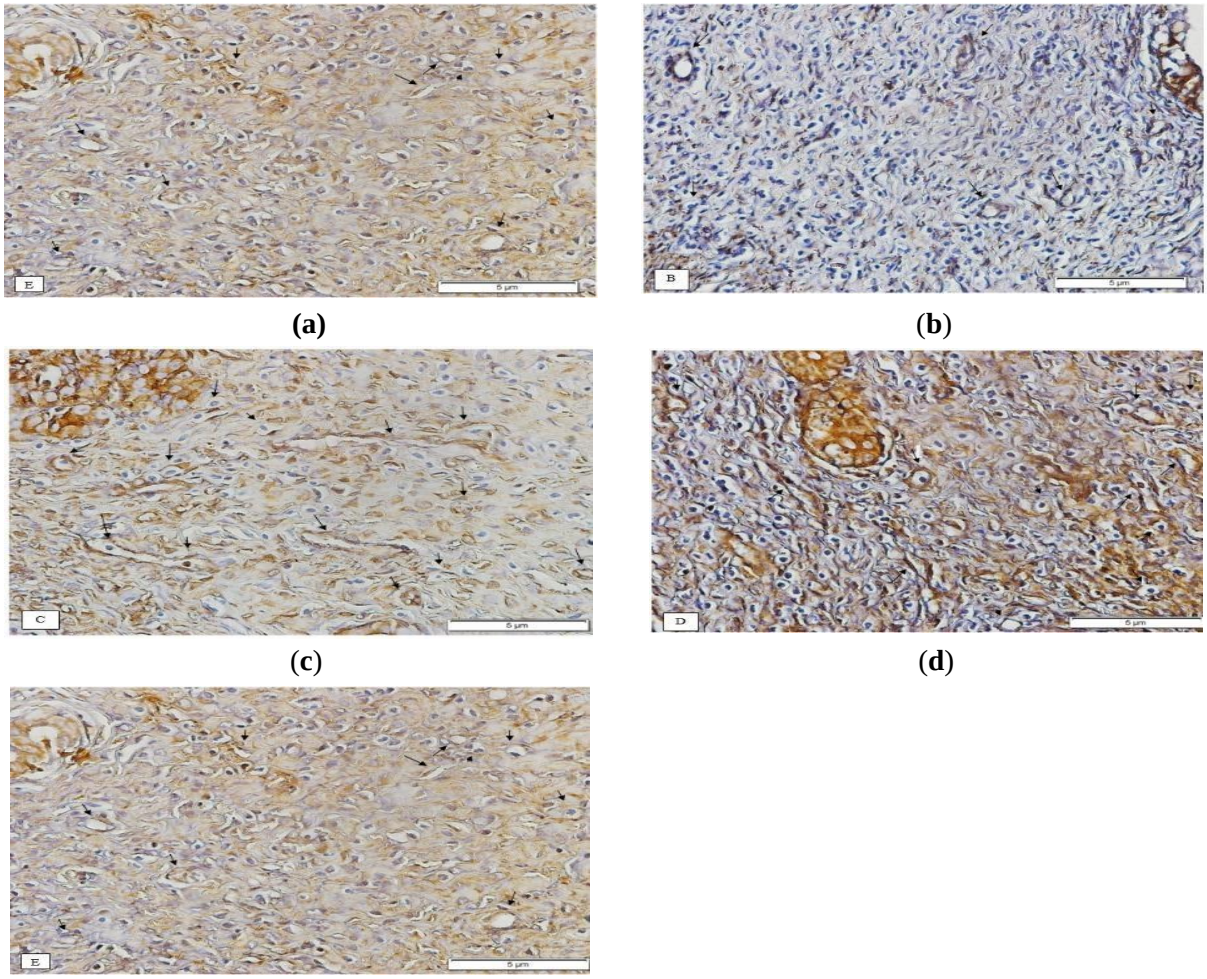
Table 4. Correlation Test Results with Pearson Correlation

Correlated variables	Correlation coefficient (r)	Meaning	*p-value*	Description
Dose with follicular atresia	0.791	Strong correlation	0.000	Significant

Description: The correlation coefficient column indicates a strong relationship, and the p-value column indicates a significant relationship between the two variables.

The Effect of Black Garlic (Allium sativum) Extract on Endometrial VEGF Expression in Rats (Rattus norvegicus) Exposed to Cigarette Smoke

Immunohistochemical images of VEGF expression in the uterus of rats (Rattus Norwegians) in the control group (negative and positive) and the treatment group of black garlic extract at a dose of 50, 100, 200 mg / kgBW in longitudinal sections and observed with an Olympus BX53 microscope with 400x magnification.



(**a**) (**b**)

(**c**) (**d**)

(**e**)

Figure 4. VEGF Expression

In Figure 4, the arrow shows positive VEGF expression, seen in the cytoplasm of endometrial blood vessels that are colored brown. (a) negative control group/no treatment; (b) positive control group/exposed to cigarette smoke; (c) treatment group 1 with cigarette smoke and black garlic 50 mg/kgBW; (d) treatment group with cigarette smoke and black garlic 100 mg/kgBW; (e) treatment group 3 with cigarette smoke and black garlic 200 mg/kgBW.

Table 5. Differential test results of VEGF expression

Group	n	Mean±SD	p-value
CN	5	32.95 ± 7.45[a]	
CP	5	5.87±1.08[a]	
P1	5	29.69 ± 15.39[b]	0.002
P2	5	25.18 ± 8.28[b]	
P3	5	24.04 ± 9.22[b]	

Different notations (a,b) give significant meaning to the mean ± sd obtained (p-value < α = 0.05), which means that there is a significant difference, and if the notation makes an unequal letter obtained (p-value > α = 0.05) then there is no significant difference in the HSD post hoc test.

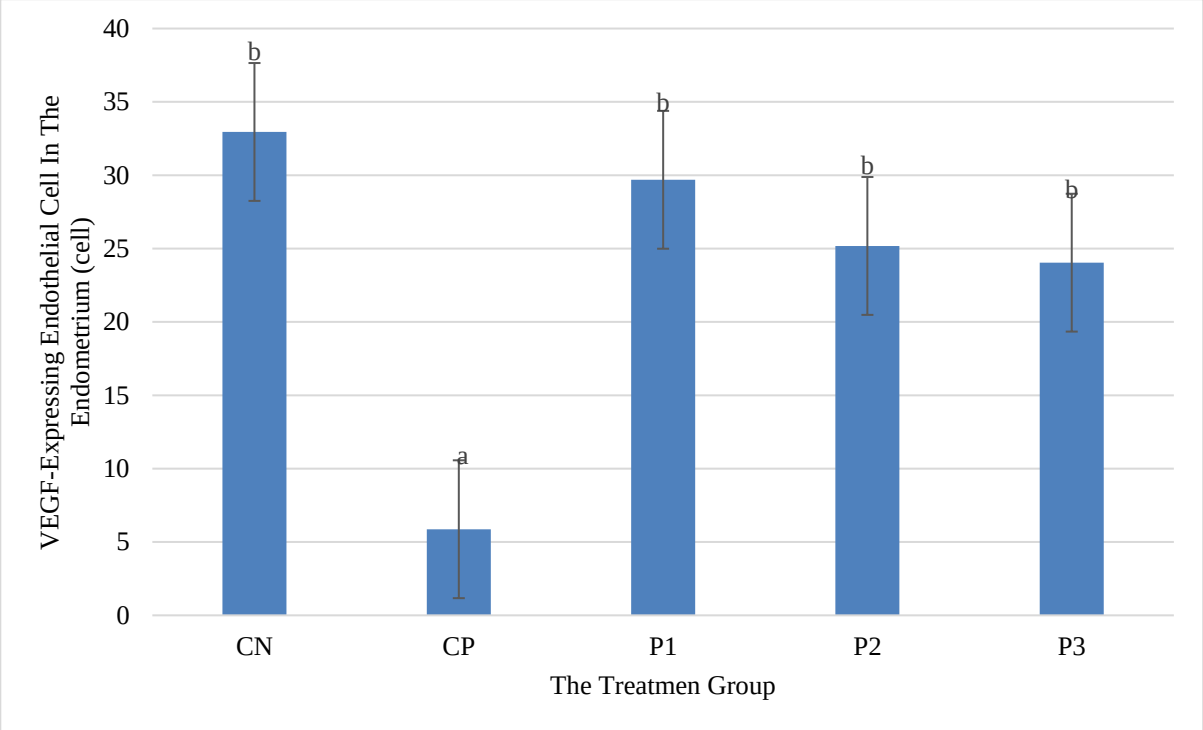
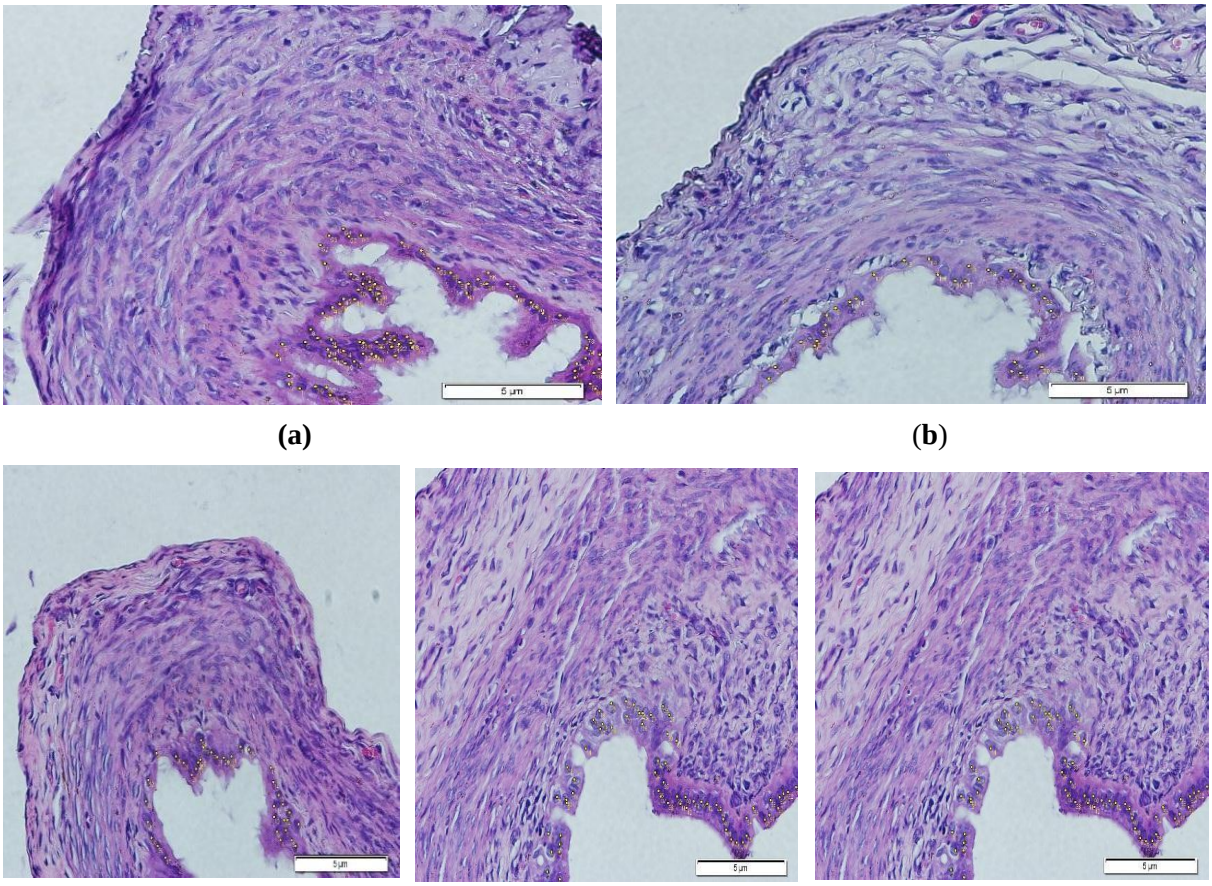


Figure 5. VEGF expressing endothelial cell in the endometrium in mice exposed to cigarette smoke

Figure 5 shows the mean expression of VEGF in the negative control group, positive control group (exposed to cigarette smoke two cigarettes/day), P1 (exposed to cigarette smoke + BG 50 mg/kg/BB), P2 (exposed to cigarette smoke + BG 100 mg/kg/BB), P3 (exposed to cigarette smoke + BG 200 mg/kg/BB). The mean ± sd obtained (p-value < α = 0.05) can mean that there is a significant difference, and if (p-value > α = 0.05), then there is no significant difference.

The Effect of Black Garlic (Allium sativum) Extract on Fallopian Tube Secretory Epithelial Cell Count in Rats (Rattus norvegicus) Exposed to Cigarette Smoke



(a) (**b**)

(**c**) (**d**) (**e**)

Figure 6. Histopathology of Fallopian tube secretory epithelial cell counts

In Figure 6, (a) negative control group CN; (b) positive control group CP cigarette smoke exposure; (c) treatment group 1 (cigarette smoke exposure and black garlic dose of 50mg/kg/BB); (d) treatment group 2 (cigarette smoke exposure and black garlic dose of 100mg/kg/BB); (e) treatment group 3 (cigarette smoke exposure and dose of 200 mg/kg/BB).

Table 6. Differential test results of VEGF expression

Group	n	Mean±SD	p-value
CN	5	51.240 ± 9.0856^{b}	0,001
CP	5	28.400 ± 3.3166^{a}	
P1	5	43.920 ± 8.1677^{b}	
P2	5	45.960 ± 4.0679^{b}	
P3	5	48.960 ± 10.8898^{b}	

In the mean ± SD if it contains different letters, there is a significant difference ($p < 0.05$) and if it contains the same letter, there is no significant difference ($p > 0.05$).

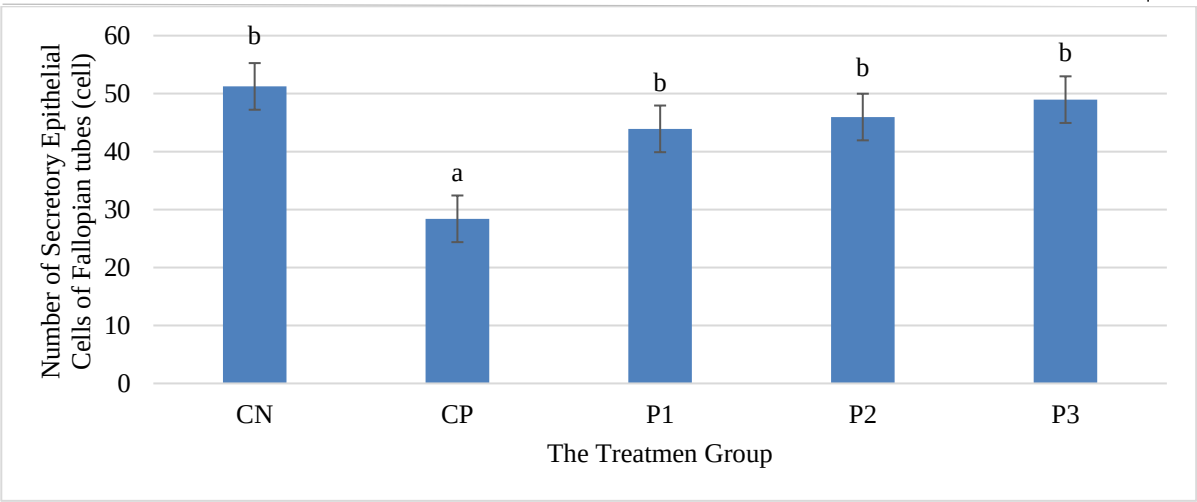


Figure 7. Effect of Black Garlic Extract on Increasing the Number of Secretory Epithelial Cells of Fallopian tubes of White Rats Exposed to Cigarette Smoke

Based on Figure 7, it is known that CN: without exposure to cigarette smoke and administration of black garlic extract; CP: exposed to cigarette smoke and without administration of black garlic extract; P1: exposed to cigarette smoke and given black garlic extract 50 mg/kgBW; P2: exposed to cigarette smoke and given black garlic extract 100 mg/kgBW; P3: exposed to cigarette smoke given black garlic extract 200 mg/kgBW.

Figure 7 above shows that the average number of secretory epithelial cells in the fallopian tubes of the positive control group has an average value of the number of secretory epithelial cells with a smaller value than the negative control group. This indicates that cigarette smoke exposure has an impact on reducing the number of fallopian tube secretory epithelial cells. The treatment group (P1, P2, and P3) had a mean value of the number of secretory epithelial cells with a value greater than the positive control group. This indicates that the administration of black garlic extract can increase the number of fallopian tube secretory epithelial cells.

Here are the calculations for the percentage change in follicular atresia for the doses of 100 mg/kgBW and 200 mg/kgBW compared to the group exposed to cigarette smoke only (CP):

1) Dose 50 mg/kgBW (P1):
   a) Mean follicular atresia in CP: 7.2
   b) Mean follicular atresia in P1: 2.6
   c) Percentage change: $\left(\frac{7.2-2.6}{7.2}\right) \times 100 \approx 63.89\%$
2) Dose 100 mg/kgBW (P2):
   a) Mean follicular atresia in CP: 7.2
   b) Mean follicular atresia in P2: 6.8
   c) Percentage change: $\left(\frac{7.2-6.8}{7.2}\right) \times 100 \approx 5.56\%$
3) Dose 200 mg/kgBW (P3):
   a) Mean follicular atresia in CP: 7.2
   b) Mean follicular atresia in P3: 8.2
   c) Percentage change: $\left(\frac{7.2-8.2}{7.2}\right) \times 100 \approx 13.89\%$

The results indicate that:

1) Administration of 50 mg/kgBW reduced follicular atresia by 63.89%.

2) Administration of 100 mg/kgBW reduced follicular atresia by 5.56%.
3) Administration of 200 mg/kgBW actually increased follicular atresia by 13.89%.

This shows that higher doses of black garlic extract do not provide better effects and may even increase damage to ovarian follicles.

DISCUSSION

The Effect of Black Garlic (Allium sativum) Extract on Ovarian Follicular Atresia in Rats (Rattus norvegicus) Exposed to Cigarette Smoke

The results indicate that cigarette smoke, as a source of oxidative stress, increases the number of follicles undergoing atresia, triggers granulosa cell apoptosis, and causes oxidative damage. Comparison of the mean follicular atresia between cigarette smoke-exposed and non-exposed groups showed a significant increase in exposed mice, which is in agreement with those who documented that nicotine exposure affects ovarian function by inducing follicular atresia through oxidative stress mechanisms (Li *et al.*, 2020).

Black garlic extract, known for its antioxidant properties, showed protective effects against this oxidative damage, especially at low doses (50 mg/kgBW). The group receiving a low extract dose (50 mg/kgBW) showed the best results compared to the higher doses (100 mg/kgBW and 200 mg/kgBW). The low dose (50 mg/kgBW) gave the best results compared to the higher dose. Administration of this extract decreased the number of follicular atresia, indicating that the black garlic extract ameliorated the free radical damage produced by cigarette smoke. These results support the findings (Chen *et al.*, 2021), which showed that antioxidants in black garlic can mitigate the toxic effects of free radicals on the reproductive system.

However, the study results also showed that increasing the dose of black garlic extract above 50 mg/kgBW did not continue the protective trend and instead may have caused prooxidative effects, as described who stated that high doses of certain compounds in black garlic can have toxic effects (Dutta *et al.*, 2021). Therefore, these findings demonstrate the importance of dosage in the therapeutic application of black garlic extracts, where an optimum point produces protective effects without causing additional damage.

This discussion contributes to a better understanding of the potential of interventions using natural materials in dealing with the negative impact of environmental exposures such as cigarette smoke on the reproductive system. More broadly, this study adds evidence to the corpus of literature regarding the use of antioxidants in preventing oxidative damage to ovarian function and supports the development of safer and more effective therapeutic strategies to maintain reproductive health in the presence of environmental risk factors.

The Effect of Black Garlic (Allium sativum) Extract on Endometrial VEGF Expression in Rats (Rattus norvegicus) Exposed to Cigarette Smoke

In this study, three doses of black garlic (Allium sativum) extract were given to female Wistar rats exposed to cigarette smoke to test its effect on Vascular Endothelial Growth Factor (VEGF) expression in the endometrium. The following is a description of each dose used:

1) Dose 50 mg/kgBW (Group P1): This group received 50 mg of black garlic extract per kilogram of rat body weight daily. Based on research, this dose showed the best results in increasing VEGF expression and the number of fallopian tube secretory epithelial cells, as well as reducing ovarian follicular atresia in rats exposed to cigarette smoke.
2) Dose 100 mg/kgBW (Group P2): This group received 100 mg of black garlic extract per kilogram of rat body weight daily. This dose was also influential in increasing VEGF expression and the number of fallopian tube secretory epithelial cells, but not as good as the 50 mg/kgBW dose in several parameters measured.

3) Dose 200 mg/kgBW (Group P3): This group received 200 mg of black garlic extract per kilogram of rat body weight daily. Although this dose increased VEGF expression and the number of fallopian tube secretory epithelial cells, the results were not as good as the lower dose. They may even lead to increased follicular atresia in some rats.

Exposure of female rats to cigarette smoke of two cigarettes per day was done to trigger a significant decrease in VEGF expression, which was further observed to be affected by the administration of black garlic extract at various doses.

Results showed that administration of black garlic extract significantly increased VEGF expression in mice exposed to cigarette smoke, with the highest increase occurring at the lowest dose (50 mg/kg/BB). This phenomenon can be interpreted through the antioxidant and anti-inflammatory activities of black garlic, which reduce oxidative stress and inflammation induced by cigarette smoke. This reduction in oxidative stress allows for more effective restoration of VEGF expression, which is essential for the maintenance and repair of endometrial tissue through angiogenesis.

Research (Jaleel et al., 2021) also showed a decrease in VEGF expression due to cigarette smoke exposure, strengthening the evidence that cigarette smoke hurts angiogenic factors. However, this study is unique in showing that black garlic extract can reverse this effect at specific doses. Research (You et al., 2019) supports these findings by showing that black garlic reduces molecular activities associated with inflammation and oxidative stress, which may explain its effect on VEGF.

The importance of this study lies in demonstrating the potential of black garlic extract in nutraceutical therapy to protect reproductive tissues from environmental stress, such as cigarette smoke (Wang et al., 2015). In science and technology, these results enrich the literature regarding using natural materials to develop safer and more effective treatment strategies against disorders induced by environmental factors.

Furthermore, this study emphasizes the importance of dosage in phytopharmaceutical use, as higher doses of black garlic extract did not increase VEGF as effectively as lower doses. This suggests the need for further research to explore the specific mechanisms involving black garlic and its interaction with cellular signaling pathways in the context of its effect on VEGF expression (Ahmed & Wang, 2021).

In the context of community development and public health, these findings provide insights for the development of therapies that can reduce the negative impact of cigarette smoke pollution, particularly for populations at high risk of impaired endometrial function. It also offers a natural alternative that may be more acceptable to individuals who avoid using synthetic drugs for health reasons or personal preference.

The Effect of Black Garlic (Allium sativum) Extract on Fallopian Tube Secretory Epithelial Cell Count in Rats (Rattus norvegicus) Exposed to Cigarette Smoke

Results showed that although there was no significant difference between groups exposed to cigarette smoke and those administered black garlic extract at different doses, there was an increase in the number of secretory epithelial cells. These findings provide insight into the protective potential of black garlic against cigarette smoke-induced damage in the fallopian tubes, which are crucial components in the reproductive system.

In the context of the effects of cigarette smoke, previous studies by (Bala et al., 2021) have shown that cigarette smoke causes impairment of reproductive organ function, including damage to ciliary and secretory epithelial cells (Wang et al., 2015). Cigarette smoke reduces the ability of the fallopian tubes to carry out ovum and sperm transportation functions

effectively, affects hormonal balance, and reduces the quality of the fallopian tube epithelium. These effects are due to oxidative stress induced by nicotine and other harmful components in cigarettes.

Black garlic extract, rich in antioxidants such as S-allyl cysteine (SAC), can potentially reduce these negative impacts. “SAC is known for its powerful antioxidant properties, which help neutralize free radicals and reduce oxidative stress, as described” (Jeong et al., 2016). In the context of this study, the antioxidant activity of SAC likely contributes to the improved condition of the fallopian tube secretory epithelium by reducing the damage caused by cigarette smoke. Antioxidant activity can reduce oxidative stress and improve follicular health, VEGF expression, and epithelial cell function through several essential mechanisms. First, antioxidants such as SAC (S-Allylcysteine) in black garlic extract can capture and neutralize free radicals generated by cigarette smoke. *Free radicals* are highly reactive molecules that can damage DNA, proteins, and lipids in cells, disrupting normal cell function and causing tissue damage. By neutralizing free radicals, antioxidants help protect cells from oxidative damage.

In addition, antioxidants reduce oxidative stress, which occurs when the production of free radicals exceeds the capacity of the body's antioxidant system to neutralize them. The antioxidants in black garlic extract help restore this balance by providing compounds that can interact with free radicals, reducing the amount of free radicals available to cause damage. By reducing oxidative stress, antioxidants also help increase the expression of VEGF (Vascular et al. Factor), a protein essential for angiogenesis, which is the formation of new blood vessels. Oxidative stress can reduce the expression of VEGF, which can disrupt the blood supply to endometrial tissue and affect tissue regeneration and function. Thus, antioxidants help maintain or increase VEGF expression, essential for endometrial tissue health and function.

Furthermore, antioxidants protect epithelial cells in the fallopian tubes, essential for ovum transport and the secretion of factors necessary for fertilization. Oxidative stress can damage these cells, impairing fallopian tube function. The antioxidants in black garlic extract help protect epithelial cells from oxidative damage, ensuring optimal function of the fallopian tubes. In addition, antioxidants reduce follicular atresia, a process in which ovarian follicles degenerate and die before they can mature into ova. Oxidative stress can accelerate this process, reducing the number of follicles available for ovulation. By reducing oxidative stress, antioxidants help protect follicles from damage, increasing the chances of follicles developing into mature ova. Overall, the antioxidant activity of black garlic extract contributes to protecting and repairing cells and tissues affected by cigarette smoke, thus improving overall reproductive health.

Research (Ahmed & Wang, 2021; Diputra et al., 2018) also supports these findings, suggesting that antioxidants in black garlic can effectively decrease the expression of enzymes that produce reactive oxygen species (ROS), repair tissue damage, and support normal cellular function. This is reinforced (Chen et al., 2021; Diputra et al., 2018; Tran et al., 2020), which found that black garlic can inhibit inflammation and histological changes in the context of other diseases.

This study adds evidence to the existing literature that black garlic extract could be a potential nutraceutical intervention to protect reproductive function from the adverse effects of cigarette smoke. This has significant implications for developing reproductive health protection strategies, especially in adverse environmental exposures such as cigarette smoke (Lu et al., 2017; Qiu et al., 2020). Furthermore, this study suggests that adequate doses of black garlic need to be further studied to optimize its benefits without crossing any possible toxicity limits (X. Zhang et al., 2016).

Integrating these findings into science and technology development could give consideration to using black garlic as part of supportive therapy for individuals exposed to

cigarette smoke (Tran et al., 2020), encouraging better health practices and preventive approaches in reproductive health management. Nevertheless, it's crucial to acknowledge the limitations of this study, which include the use of animal models (rats), a relatively small sample size, and a short exposure duration. These factors might restrict the applicability of the results to humans. Therefore, it's imperative to conduct further research in human studies, with larger sample sizes and longer exposure durations, to validate the potential therapeutic effects of black garlic on reproductive health.

4. CONCLUSION

This study demonstrates that black garlic extract (*Allium sativum*) has protective potential against cigarette smoke-induced damage in *Rattus norvegicus*. The extract significantly reduced ovarian follicular atresia increased VEGF expression in the endometrium, and improved the number of secretory epithelial cells in the fallopian tubes. These effects were most pronounced at a low dose (50 mg/kgBW), highlighting the importance of proper dosage. Higher doses (100 mg/kgBW and 200 mg/kgBW) were less effective and may have adverse effects.

Black garlic extract offers a promising therapeutic approach to mitigate the negative impact of cigarette smoke on reproductive health. Further research is needed to explore the mechanisms involved, optimize dosing strategies, and confirm the safety and efficacy of black garlic extract in clinical applications. Integrating black garlic extract into nutraceuticals could provide a preventive and complementary strategy for individuals at risk of exposure to cigarette smoke.

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