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The Effect of Using Absorbent Carbon on Air Exposed to Air Fresheners on The Histology of The Cornea of The White Rat (*Rattus Novergicus*)

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Abstract

Air freshener contain hazardous chemical such as formaldehyde. Absorbent carbon is used as an adsorbent to reduce air pollution. The goal of this research is to know the use of absorbent carbon to cornea histology on *Rattus novergicus* that was induced with air freshener. This research was an experimental study with a post-test only control group. The subjects were 28 one-month old male *Rattus novergicus* Wistar strains divide into 4 groups: Control (K), air freshener (P1), absorbent carbon (P2) and air freshener plus absorbent carbon (P3).The treatment was performed 8 hours/day for 35 days. Data of anterior epithelial thickness were analyzed by *One Way Anova* test. Data of the overall thickness of the corneal layer and keratocyte number were analyzed by *Kruskal Wallis* test. The overall thickness of the corneal layer and anterior epithelial thickness showed that there were not significant difference between subject groups (p>0,05). Anterior epithelial thickness showed that there were not significant difference between subject groups (p>0,05). Keratocyte number showed significant difference between subject groups (p<0,05), *Mann Whitney* test for Keratocyte number showed results P1>P3>K>P2 Absorbent carbon gives postitive effect to reduce cornea damage on *Rattus novergicus* that induced by air freshener.

Keywords: Cornea, Absorbent carbon, Air Freshener, *Rattus novergicus*, Keratocyte

INTRODUCTION

Air pollution is increasingly alarming. The sources of air pollution are growing more numerous around humans, which, ironically, are often not recognized by people as contributing to air pollution. Air fresheners are one such source of air pollution that is not commonly recognized by the public. People enjoy the freshness and fragrance provided by air fresheners.

Over the past few decades, research has been conducted on various types of air fresheners, revealing that they contain Volatile Organic Compounds (VOCs). VOCs are hydrocarbon compounds that easily evaporate and have high vapor pressure. One type of VOC found in air fresheners is formaldehyde. Research conducted on rabbits has shown that direct exposure to formaldehyde can cause damage to the corneal surface of the eyes. Low-concentration exposure to formaldehyde does not significantly damage the cornea; however, prolonged exposure leads to morphological changes in cells and abnormalities in tear production.

Given the negative impacts and the presence of hazardous chemicals in air fresheners, efforts are needed to reduce these negative effects on organs, particularly the cornea. One way

to achieve this is by using granular absorbent carbon. In the chemical industry, granular absorbent carbon is used to purify liquids and gases. Granular absorbent carbon can also be used to reduce pollutants originating from VOCs. This study aims to determine the effect of using granular absorbent carbon on the histological structure of the cornea in *Rattus novergicus* induced by air fresheners.

RESEARCH METHODS

This study initially used 28 male white rats (*Rattus novergicus*) of the Wistar strain as research samples. However, during the observation process, 4 specimens were found to be damaged and could not be observed, so the total number of samples observed was 24. The rats were divided into four groups: the control group (K), the gel air freshener group (P1), the granular absorbent carbon group (P2), and the granular absorbent carbon combined with air freshener group (P3). Each group consisted of 6 rats. The exposure duration was eight hours per day, with a total exposure time of 35 days.

Every day, the four groups of rats were given standard feed, starting with an initial portion of about 250 grams, which was increased as the rats aged. The rats were also provided with mineral water for drinking. The bedding was replaced every two days, coinciding with the weighing of the rats.

The rats were dissected on the 36th day of exposure. Before dissection, the rats were placed in a sealed jar containing chloroform until they lost consciousness, and then dissection was performed. The dissection was done to retrieve the organ under study, the eyes (cornea), to prepare histological specimens using the paraffin block method and Hematoxylin-Eosin (HE) staining. The specimens were observed in 5 fields of view with a magnification of 40x10 to measure the thickness of the anterior layer, the total thickness of the cornea, and the number of keratocytes.

The independent variables in this study were the exposure to the gel air freshener and the granular absorbent carbon. The dependent variables were the total corneal thickness, the thickness of the anterior epithelium, and the number of keratocytes.

The equipment used in this study included 4 rat cages, 4 treatment cages, glassware, treatment tools, large and small jars, minor surgical instruments, 4 drinking bottles, Optilab software, and a microscope. The materials used in the study included orange-scented gel air fresheners containing 0.62 ppm formaldehyde, two types of granular absorbent carbon, rat feed, Aqua drinking water, and rice husks for bedding.

Maintenance and treatment were carried out in the Biomedical Laboratory of the Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta, for 35 days, including a 7-day acclimatization period. The preparation of corneal histology specimens was done at the Laboratory of Anatomic Pathology, Faculty of Medicine, Universitas Gadjah Mada (UGM). Observation and evaluation of the specimens were conducted at the Histology Laboratory, Universitas Muhammadiyah Yogyakarta.

The data obtained were subjected to a normality test using the Shapiro-Wilk test. Anterior epithelial thickness data were analyzed with One-Way ANOVA. Data on total corneal thickness and the number of keratocytes were analyzed using the Kruskal-Wallis test, followed by the Mann-Whitney test.

RESULTS AND DISCUSSION

a. Overall Corneal Thickness

The normality test for the overall corneal thickness data distribution using the Shapiro-Wilk Test showed the following results: Control group (K) had p=0.969, the group exposed to the air freshener (P1) had p=0.062, the group exposed to absorbent carbon (P2) had p=0.998, and the group exposed to both absorbent carbon and the air freshener (P3) had p=0.529. The

Test of Homogeneity of Variances resulted in a variance of 0.011 ($p<0.05$), indicating that the data were not homogeneous. Therefore, non-parametric testing was performed, as the data did not meet the requirements for normal distribution and homogeneity. The non-parametric test used was the Kruskal-Wallis test. The results of the Kruskal-Wallis test showed a p-value of 0.198 ($p>0.05$), indicating that there was no significant difference among the data from the four groups.

Table 1. The average overall corneal thickness

Treatment Group	Average (µm) (x ± SD)
Control (K)	683,12 ± 57,44
Air Freshener (P1)	817,28 ±153,56
Absorbent carbon (P2)	753,05 ± 79,40
Carbon + Air Freshener (P3)	770,33 ± 65,30

It can be seen in Table 1 that, although the Kruskal-Wallis test results show no significant difference between the four groups compared, the average results indicate that the overall corneal thickness in the air freshener group is the highest. Meanwhile, the corneal thickness in the absorbent carbon group is close to that of the control group.

b. Anterior Epithelial Thickness

The Shapiro-Wilk test results for the anterior epithelial thickness variable showed the following: the control group (K) had $p=0.461$, the air freshener group (P1) had $p=0.913$, the carbon group (P2) had $p=0.526$, and the carbon plus air freshener group (P3) had $p=0.708$. Data from all four groups met the requirement of $p>0.05$, indicating that the data distribution is normal. The Test of Homogeneity of Variances yielded a result of $p=0.156$ ($p>0.05$), indicating that the data are homogeneous. The One-Way ANOVA test resulted in $p=0.979$, which means it did not meet the criteria of $p<0.05$, suggesting that there are no significant differences among the four groups.

Table 2. Average Anterior Epithelial Thickness

Treatment Group	Rata-rata (µm) (x ± SD)
Control (K)	157,38 ± 22,40
Air Freshener (P1)	160,08 ± 11,59
Absorbent carbon (P2)	155,72 ± 28,95
Carbon + Air Freshener (P3)	159,97 ± 15,52

As seen in Table 2, although the Kruskal-Wallis test results show no significant differences among the four groups compared, the average results indicate that the anterior epithelial thickness is greatest in the air freshener group. In contrast, the anterior epithelial thickness in the absorbent carbon group is the thinnest.

c. Number of Keratocytes

Table 3. Average Number of Keratocytes

Treatment Group	Rata-rata (µm) (x ± SD)
Control (K)	$17,83 \pm 1,50^{a}$
Air Freshener (P1)	$25,80 \pm 3,34^{c}$

Absorbent carbon (P2)	$17,47 \pm 3,07^{ab}$
Carbon + Air Freshener (P3)	$20,90 \pm 0,43^{b}$

Note: Different letters indicate significant differences in the Kruskal-Wallis test with Post Hoc Mann-Whitney Test at a 95% significance level.

The normality test results using the Shapiro-Wilk test for the keratocyte count variable are as follows: the control group (K) had p=0.956, the air freshener group (P1) had p=0.531, the carbon group (P2) had p=0.525, and the carbon plus air freshener group (P3) had p=0.964. Data from all four groups met the criterion of p>0.05, indicating that the data distribution is normal. However, the Test of Homogeneity of Variances showed a result of p=0.037 (p<0.05), meaning that the data across the four groups are not homogeneous. Therefore, non-parametric testing using the Kruskal-Wallis test was conducted. The Kruskal-Wallis test results showed p=0.001 (p<0.05), indicating significant differences among the four groups.

The presence or absence of differences among groups K, P1, P2, and P3 was analyzed using the Post Hoc Mann-Whitney test.

The Post Hoc Mann-Whitney test results showed the following:

- For Group K compared to Group P1, the Mean Rank difference resulted in a p-value of 0.004 (p<0.05), indicating a significant difference between Group K and Group P1.
- For Group K compared to Group P2, the p-value was 0.688 (p>0.05), indicating no significant difference between Group K and Group P2.
- For Group K compared to Group P3, the p-value was 0.005 (p<0.05), indicating a significant difference between Group K and Group P3.
- For Group P1 compared to Group P2, the p-value was 0.006 (p<0.05), indicating a significant difference between Group P1 and Group P2.
- For Group P1 compared to Group P3, the p-value was 0.005 (p<0.05), indicating a significant difference between Group P1 and Group P3.
- For Group P2 compared to Group P3, the p-value was 0.054 (p>0.05), indicating no significant difference between Group P2 and Group P3.

Microscopic observation results representing each treatment group can be seen in the following images:

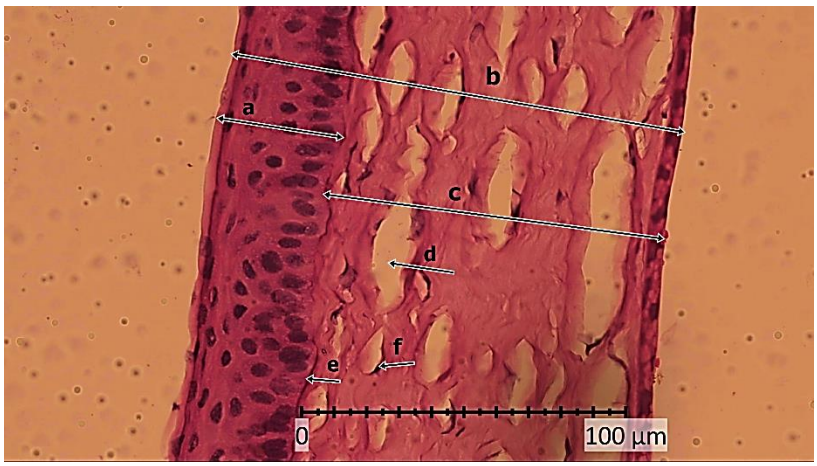

Figure 4. Histological Image of the Cornea of *Rattus novergicus* Control Group (HE, 400x)
Description: (a) Anterior epithelium; (b) Overall corneal thickness; (c) Stroma
(d) Vacuolization; (e) Bowman's membrane; (f) Keratocytes

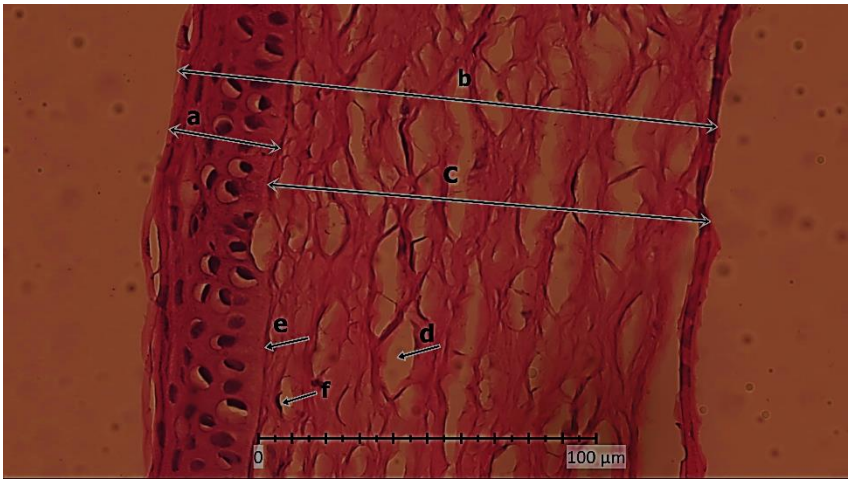

Figure 5. Histological Image of the Cornea of *Rattus novergicus* Exposed to Gel Air Freshener for 8 Hours/Day for 35 Days (HE, 400x)
Description: (a) Anterior epithelium; (b) Overall corneal thickness; (c) Stroma
(d) Vacuolization; (e) Bowman's membrane; (f) Keratocytes

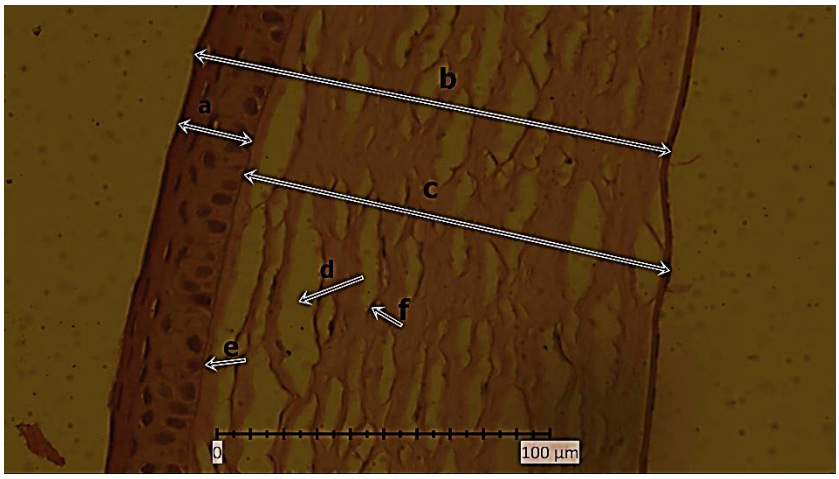

Figure 6. Histological Image of the Cornea of *Rattus novergicus* Exposed to Granular Absorbent carbon for 8 Hours/Day for 35 Days (HE, 400x)
Description: (a) Anterior epithelium; (b) Overall corneal thickness; (c) Stroma
(d) Vacuolization; (e) Bowman's membrane; (f) Keratocytes

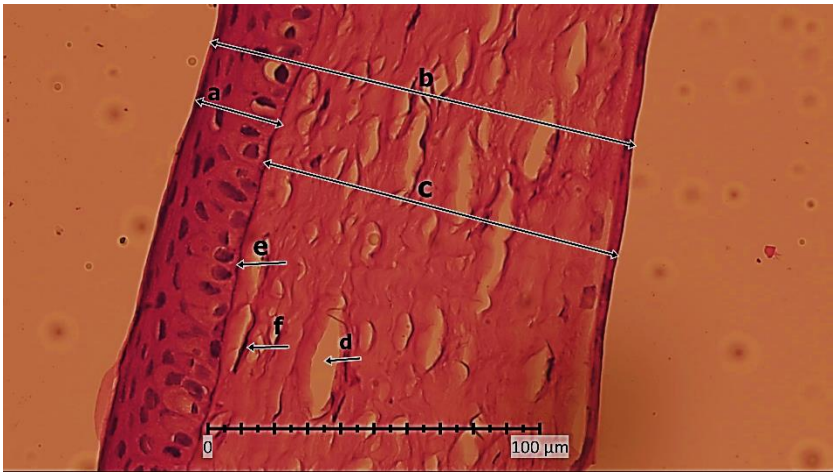

Figure 7. Histological Image of the Cornea of *Rattus novergicus* Exposed to Granular Absorbent carbon and Gel Air Freshener for 8 Hours/Day for 35 Days (HE, 400x)
Description: (a) Anterior epithelium; (b) Overall corneal thickness; (c) Stroma
(d) Vacuolization; (e) Bowman's membrane; (f) Keratocytes.

a. Overall Corneal Thickness

The research results for this variable are not significant; however, between the control group (K) and the gel air freshener group (P1), the overall corneal thickness is greater in group P1. Group P1 used an air freshener with a formaldehyde concentration of 0.62 ppm. Maurer (2001) conducted a study to investigate the effects of exposure to acetone, cyclohexanol, parafluoroaniline, and formaldehyde in rats. The study found that formaldehyde exposure caused the most severe corneal damage, leading to corneal swelling and significant changes in corneal thickness before and after treatment (Maurer et al., 2001). Besides formaldehyde, air fresheners also contain other chemicals, such as toluene and xylene, which were not measured in this study but could affect corneal thickness by causing inflammation (5).

The overall corneal thickness is an accumulation of the anterior epithelial layer, stroma, and endothelium. The stroma is the largest layer in the cornea, accounting for about 90% of the total corneal thickness. It consists of an extracellular matrix rich in collagen, which is a key factor influencing corneal thickness, along with keratocytes and the endothelial cell layer. The extracellular matrix of the stroma primarily consists of type I collagen, with some type V collagen and four proteoglycans. The core proteins of proteoglycans and type V collagen play a role in regulating collagen fibril growth. Collagen synthesis in the stroma affects the corneal wound healing process. In addition to type I and type V collagen, type XII collagen also plays a role in maintaining stromal shape and fibril formation [(6)].

The stromal response to injury is an extension of the initial healing response by the anterior epithelium. This response leads to stromal swelling and the invasion of inflammatory cells, which can occur even if the injury does not directly affect the stroma [(7)].

Comparing the average thickness of group K with groups P2, P1, and P3, it is observed that the average thickness in group P2 is close to that of group K. Absorbent carbon is a material used to filter harmful chemical compounds contaminating water and air [(8)]. Granular absorbent carbon can absorb various pollutants, including radioactive pollutants like radon. Pari (2004), who studied the effectiveness of using absorbent carbon to absorb formaldehyde from plywood, also showed that absorbent carbon could reduce formaldehyde emissions and lower free formaldehyde levels in the materials tested [(9)]. Based on this explanation, it can be understood why the overall corneal thickness in group P2 is closer to that of group K, due to the function of absorbent carbon, especially granular absorbent carbon, in filtering harmful chemical pollutants. In this study, the known pollutant level was formaldehyde at 0.62 ppm. Granular absorbent carbon filters the air inside the treatment cages, thus reducing the exposure of the male rats' eyes to pollutants, which are the subjects of the research.

b. Anterior Epithelial Thickness

The anterior epithelium, being the outermost layer of the cornea, is the most vulnerable to injury and is the first to be affected by such injuries. When an injury occurs to the cornea, the epithelium is the most rapid and effective layer in the healing process to restore its structure and function to normal[(10)].

Corneal epithelial wound healing can be divided into four specific stages. The first stage is called the latent phase because there is no cell movement or change in cell number. During this stage, there is an increase in metabolic activity and restructuring of cell structures in preparation for the next stage. The second stage is migration, characterized by the movement of cells around the wound area to cover it. The third stage is proliferation, where cells begin to divide, restore epithelial structure, and intercellular connections. The final stage is the return of the cell substrate in the epithelium. Often, subsequent phases occur before the previous phase

is complete, but the sequence of stages remains the same. Thus, during epithelial healing, there are four overlapping phases to restore the epithelium's structure and function (10).

The time required from epithelial injury to corneal response is rapid. The response begins within the first hour after injury. Following epithelial injury, cytokines are released from the injured epithelium and basal membrane, including Interleukin-1 (IL-1) and Tumor Necrosis Factor alpha-1 (TNF α-1), Bone Morphogenic Proteins (BMP) 2 and 4, Epidermal Growth Factor (EGF), and Platelet-Derived Growth Factor (PDGF). The time needed from cytokine release to the onset of early epithelial healing is 12-48 hours. Growth factors like EGF and PDGF, as well as other cytokines, assist in this healing process. Epithelial cells will replicate and cover the epithelial wound (7).

Data analysis of anterior epithelial thickness shows no significant difference between the control group and the treated exposure groups. As explained earlier, this may be because the epithelium can repair itself quickly.

The results of this study are consistent with research by Nastiti (2015), which investigated the effects of exposure to spray and gel air fresheners. Nastiti (2015) noted that the lack of significant difference in anterior epithelial thickness was due to the ongoing wound healing process, which causes the epithelial thickness to return to normal [(11)].

The epithelial wound healing process described may be why there is no significant difference between the control and treatment groups. The exposure in this study lasted for 35 days, allowing the epithelial wound healing process to progress, resulting in no significant difference in epithelial thickness. Observations show that the absorbent carbon exposure group had better results than the control group. The gel air freshener exposure group had the thickest epithelial layer among all groups.

The gel air freshener used in this study has a formaldehyde concentration of 0.62 ppm. The Occupational Safety and Health Administration (OSHA) recommends that exposure to formaldehyde should not exceed 0.75 ppm for 8 hours (12). This may also influence the study's results as the formaldehyde concentration used was lower than the maximum limit. Additionally, exposure time and method may also affect the results.

The absorbent carbon used in this study was granular. Granular absorbent carbon has a broad range, capable of neutralizing various air and water contaminants. Akpa (2014) found that benzene adsorption decreased when the particle size of absorbent carbon increased, and increased adsorption occurred with additional doses of adsorbent [(13)]. The adsorption process of granular absorbent carbon filtering contaminants occurs within minutes. Since this study lasted 35 days, the better average anterior epithelial thickness in the absorbent carbon group compared to the control group may be due to the granular absorbent carbon functioning as an air filter from contaminants.

c. Number of Keratocytes

The Mann-Whitney test results for the number of keratocytes showed a significant difference between the Control (K) group and the gel air freshener exposure group (P1). This finding is consistent with the study by Nastiti (2012), which reported that exposure to gel and spray air fresheners adversely affected the histological appearance of the corneas of male *Rattus novergicus*. The study also revealed that the number of keratocytes was higher in rats exposed to gel air fresheners compared to those exposed to spray air fresheners [(11)]. There is a similarity in the significant results, where the gel air freshener exposure group had more keratocytes compared to other groups. This is related to the particle size of gel air fresheners, which is very small, about 0.1 µm. The tiny size facilitates these particles in penetrating the corneal epithelial layer, leading to a reaction from dormant keratocytes, making them active, proliferating, and involved in repairing the damaged tissue caused by the chemical particles from the gel air freshener [(14)] [(11)] [(15)].

Besides particle size, the compounds contained in air fresheners contribute to corneal damage. Air fresheners are known to contain substances such as benzyl alcohol, phthalates, acetone, benzyl benzoate, butane, terpenes, limonene, benzyl salicylate, toluene, and formaldehyde. These substances or their derivatives formed during reactions between the air freshener and the environment can have harmful health effects on humans [16]. The gel air freshener used in this study contained formaldehyde at a concentration of 0.62 ppm. The presence of formaldehyde contributes to corneal damage and an increase in keratocyte numbers. Exposure to materials containing formaldehyde at concentrations of 0.05-2.0 ppm can cause eye irritation [17].

Corneal stromal cells or keratocytes are located between collagen lamellae and are responsible for secreting extracellular matrix (ECM) components, which are essential for maintaining the cornea's normal structure and function [18]. Keratocytes have a dendritic morphology. These cells produce lumican and keratocan, two types of keratan sulfate proteoglycans that help maintain the transparency and shape of the stroma. Although keratocytes are generally quiescent, they respond quickly to injury. Keratocytes typically change from a dendritic to a fibroblastic form during the wound healing process [19] [20]. Keratocyte function begins when the corneal epithelium is injured or irritated. Following epithelial injury, cytokines are released from the injured epithelium and basal membrane, with IL-1 playing a significant role. If the epithelial barrier is damaged, IL-1 can reach the stroma and bind to its receptors on keratocytes. This binding modulates keratocyte apoptosis. After keratocyte apoptosis, proliferation and migration of keratocytes begin within 12 to 24 hours. The healing process and return to normalcy can take several months to years to resolve the injury and restore keratocytes to their inactive form [7]. Thus, the higher number of keratocytes in the gel air freshener group indicates that the healing process due to irritation is still ongoing.

A significant difference was also found in the Mann-Whitney test between the P1 and P2 groups. In comparison with the P1 group, the P2 group also showed no significant difference compared to the K group. The average number of keratocytes in the P2 group was found to be the lowest. The effect of granular absorbent carbon on the number of keratocytes does not directly impact the target organ but works by affecting the surrounding air.

Absorbent carbon is known as an adsorbent that efficiently removes pollutants from soil, air, and water. Other adsorbents include silica gel, active alumina (moisture-absorbing), zeolites, molecular sieves, and synthetic resins. However, absorbent carbon is more efficient at reducing or even eliminating pollutants. Another study by Wasewar (2007) investigated the effectiveness of granular absorbent carbon for adsorbing benzaldehyde. The results showed that granular absorbent carbon effectively removed benzaldehyde from solutions. The study also explained that the effectiveness of granular absorbent carbon in adsorbing pollutants depends on the amount of pollutant to be adsorbed and the availability of the granular carbon as an adsorbent [21].

Absorbent carbon comes in various forms, including granular, powder, extruded, and pellet. Granular absorbent carbon was chosen for this study due to its larger particle size and smaller external surface area, which enhances diffusion and adsorption of air and better filters various types of pollutants, especially air pollutants (22).

When comparing the gel air freshener + granular absorbent carbon group (P3) with the K, P1, and P2 groups, no significant differences were found. Granular absorbent carbon, with particle sizes ranging from 0.2-5 mm, is capable of absorbing various pollutants. Gel air fresheners have the smallest particle size, down to 0.1 µm. Since the exact particle size of the absorbent carbon used was unknown, it is possible that the absorbent carbon absorbed pollutants from the air freshener but did not adsorb some of these pollutants. As mentioned earlier, adsorption on absorbent carbon depends on the amount of pollutant to be adsorbed and the quantity of granular absorbent carbon used. If there is an imbalance, where the pollutants

to be adsorbed exceed the capacity of the absorbent carbon, adsorption will take longer and some pollutants may not be adsorbed. Based on these explanations, granular absorbent carbon can reduce the effects of exposure to the gel air freshener pollutant with a formaldehyde concentration of 0.62 ppm, based on the number of keratocytes in this study.

CONCLUSION

The use of granular absorbent carbon influences the reduction of corneal damage as observed through the measurement of overall corneal thickness, anterior epithelial thickness, and the number of keratocytes in white rats (*Rattus novergicus*) induced by air fresheners.

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