Comparison of conventional and electronic cigarette on formaldehyde in blood and alveolus histopathology in vivo

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ABSTRACT

Background: An e-cigarette is a cigarette that does not contain tobacco. The most toxic compounds in conventional cigarette smoke and e-cigarette vapors are carbonyls, such as formaldehyde which induce lung tissue damage. This study aims to compare the effect of exposure and the difference in duration of exposure to conventional cigarettes and electronic cigarettes on blood formaldehyde levels and alveoli histopathological features in Sprague dawley rats.

Method: This research is an experimental study with 30 white Sprague dawley rats, male, aged 10-12 weeks, and with body weights of 150-250 grams. Rats will be necropsied after intervention for two weeks and four weeks. Blood samples will be examined for formaldehyde levels, and lung tissue will be assessed for alveolar damage. The results were analyzed by one-way ANOVA statistical for blood formaldehyde levels and Mann Whitney for the degree of histopathological damage to the alveoli.

Result: There was no significant difference in blood formaldehyde levels between groups (p>0.05). There was an increasing trend in the intervention group compared to the control and in the fourth-week group compared to the second-week group. There was a significant difference in the degree of alveolar damage in the intervention group compared to the control (p<0.05). There was no significant difference between exposure for 2 and 4 weeks (p>0.05). Between rats given conventional cigarettes and e-cigarettes, there was no significant difference in the degree of alveolar damage at weeks 2 and 4 (p>0.05).

Conclusion: Exposure to conventional cigarette smoke or electronic cigarette vapor has a significant effect on alveoli histopathological features.

Keywords: conventional cigarettes, electronic cigarettes, blood formaldehyde levels, alveolus histopathological damage.

INTRODUCTION

An electronic cigarette or vape is a device in the form of a cigarette, cigar, or pen that does not contain tobacco.1 Initially, electronic cigarettes were considered a means of stopping tobacco consumption, but the present supporting data is inadequate to state this.2 Over the recent year, news about the potential health risks and benefits of using e-cigarettes are increasingly rampant.3 However, this is a controversial topic due to conflicting data.4 Public Health England recommends e-cigarettes as an alleged alternative, 95% safer than conventional cigarettes.5 Data from the European Respiratory Society task force in 2019 stated that the long-term effects of vaping are unknown, no evidence states e-cigarettes are safer than conventional cigarettes, and based on current knowledge, the negative impact on health cannot be ruled out.7 Conflicting pieces of information cause the public to be unaware of the harmful effects e-cigarettes have on health.1 The evidence is that since 2014 sales of electronic cigarettes in the world have continued to increase rapidly, from around US$2,500 to US$10,000 in early 2018.8 Given that several professional bodies recommend e-cigarettes to be clinically prescribed as an alternative to conventional cigarettes, it is crucially important to know the difference between the two.9

The most toxic compounds in conventional cigarette smoke are carbonyls such as formaldehyde which are associated with oncogenesis, entering the International Agency for Research on Cancer (IARC).10,11 Carbonyl content also supports the occurrence of respiratory diseases by inducing lung tissue damage. The primary carbonyl source in conventional cigarettes comes from sugars naturally present in tobacco. Sugar is additionally included by factories as a flavoring and flavoring for cigarettes.11,12 Another alternative, electronic cigarettes, was equally found to produce carbonyl.13 The main constituents (about 80-97%) of e-cigarette liquids are glycerol and propylene glycol, which become aerosols when the user inhales air through an electronic cigarette. Carbonyl in e-cigarettes is obtained from the pyrolysis and oxidation of glycerol and propylene glycol caused by the vaporization of e-cigarettes during
use. At the same time, flavorings can also be an additional carbonyl source. Several previous studies have identified the presence of aldehydes, especially formaldehyde, in e-cigarette vapors and conventional cigarette smoke. Electronic cigarettes are also considered to produce fewer carbonyl emissions than conventional cigarettes, although there are still conflicting data between existing studies. Research on the effects of electronic and conventional cigarettes has also been carried out and stated that there was no difference in histopathology of the alveoli between the two groups.

In electronic and conventional cigarettes, no studies have examined the levels of formaldehyde in the blood. The histopathological studies of the alveoli that have been carried out did not compare the duration of exposure. They also did not use the cigarette brand that was most widely used by the public, so it does not reflect the influence of conventional cigarette smoke and electronic cigarette vapor. This study aimed to compare the effect of exposure and the difference in duration of exposure to conventional cigarette smoke and electronic cigarette vapors on blood formaldehyde levels and histopathological features of the alveoli in Sprague dawley rats.

MATERIAL AND METHODS

Research design
This study represents an experimental study, using male Sprague dawley rats aged 10-12 weeks and weighing 150-250 grams.

Study subjects
The research sample was a white rat (Rattus norvegicus) of the Sprague dawley strain that met the inclusion criteria, namely male, aged 10-12 weeks, weighed 150-250 grams, had never been a research subject, male, aged 10-12 weeks, weighed 150-250 grams, had never been a research subject, norvegicus

The exclusion criteria for the research sample were rats that died during the study, did not want to eat or drink, and had other diseases. Sampling in this study is to take samples by random sampling. The rat sample size was determined by calculating the number of samples exerting the degree of freedom ANOVA formula. The research subjects were divided into four intervention groups and one control group, with six rats in each group. Mice were allowed to acclimatize one-week post-purchase for seven days to prevent stress-induced disease before intervention. Provision of standard food and drinks will also continue until the day of the intervention. Rats will be weighed weekly to ensure the mice consume enough food and drink. The experimental animals were assigned to the following groups: control (C), conventional cigarette for two weeks (CC2), electronic cigarette for two weeks (EC2), conventional cigarette for four weeks (CC4), and electronic cigarette for four weeks (EC4).

Intervention
The intervention was carried out by inserting conventional cigarette smoke or electronic cigarette vapor into a specially built smoking chamber containing one experimental rat. Each smoking chamber used in this study has been assembled and connected to a 20-cc syringe through a fumigation device. First, the smoke from burning cigarettes will be channeled into a 3-way infusion, and the smoke will then enter and fill the 20-cc syringe because there is a partition that blocks the smoke from entering the smoking cage. Once the syringe is fully charged, the smoke will be pushed out and flow into the smoking chamber. After the intervention, the rats were returned to the rearing cage in a separate room. Each rat in the intervention groups was given up to 12 syringes of 20 cc of smoke each day, which the combined amount of nicotine being around 0.34 mg.

Parameter measurements
A cardiac puncture approach was used to obtain blood samples, preceded by the injection of 70 mg ketamine and 8 mg xylazine mixture per 100 milligrams of body weight. The reason for using this method is that the rats will be terminated after taking blood. The blood samples will be checked for formaldehyde levels using the Picoprobe® Formaldehyde Fluorometric Assay kit.

Lung tissue was also prepared with Hematoxylin and Eosin staining. The histopathological damage will be assessed using a modified score of the degree of histopathological damage to the alveoli. This score was modified to improve the specifics of the assessment carried out. Each preparation will be viewed in 5 fields at the corner and center with 100x magnification by Yulvian Sani, DVM., Ph.D. from IPB University without blinding. Three parameters are used: alveolar edema, destruction of the alveolar septum, and inflammatory cell infiltration. Here are the categories: 1) No specific damage with a score of 0. 2) Very light damage (≤ 20% of 5 fields of view) in the measurement parameters with a score of 1. 3) Light damage (21-40% of 5 fields of view) on measurement parameters with a score of 2. 4) Moderate damage (41-60% of 5 fields of view) on measurement parameters with a score of 3. 5) Heavy damage (61-80% of 5 fields of view) in the measurement parameters with a score of 4. 6) Very heavy damage (>80% of 5 fields of view) on measurement parameters with a score of 5.

Statistical analysis
The collected data were tested for normality of distribution and variance. Normally distributed data of identical variance were analyzed using one-way ANOVA. Posthoc Bonferroni showed significant differences between intervention groups (p<0.05). Non-normally distributed Kruskal-Wallis tested data of differing variances. Mann-Whitney followed up significant differences between intervention groups (p<0.05).

RESULTS

Post-Intervention in Blood Formaldehyde
Mean formaldehyde levels are shown in Table 1. There is an increasing trend in the intervention group compared to the control group. An increasing trend occurred in the fourth-week group compared to the second-week group, both in the conventional and electronic cigarette groups. The collected data were suitable for parametric tests, and one-way ANOVA testing found no significant difference in formaldehyde levels between intervention groups (p=0.248).

Post-Intervention Alveolus Histopathological Images
The degree of histopathological damage to the alveoli in each study group is
demonstrated in Table 2. There was an increasing trend in the intervention group compared to the control group. The collected data were unsuitable for parametric tests and were therefore analyzed using non-parametric tests. Kruskal Wallis testing obtained significantly different degree of alveolus histopathological damage between intervention groups (p=0.003).

Furthermore, using the Mann-Whitney test, which is shown in Table 3, significant differences were found between C vs. CC2 (p=0.002); C vs. EC2 (p=0.002); CC vs CC4 (p=0.002); C vs. EC4 (p=0.002). No significant differences were found between CC2 vs EC2 (p=0.268); CC2 vs CC4 (p=0.268); C vs EC4 (p=0.434); EC2 vs CC4 (p=0.1); EC2 vs EC4 (p=0.665); CC4 vs EC4 (p=0.665).

**DISCUSSION**

In this study, there was no significant difference in blood formaldehyde levels between the control group and the conventional cigarette smoke group or the electronic cigarette vapor group (p>0.05). Based on the data obtained, it can be concluded that there is no difference in exposure to conventional cigarette smoke and exposure to electronic cigarette vapors on changes in blood formaldehyde levels in Sprague dawley rats. Although there were no significant differences in statistical tests, there was an increasing trend in the intervention group compared to the control group. An increasing trend also occurred in the fourth-week group compared to the second-week group, both in the conventional and electronic cigarette groups.

Based on the extant literature, the increase in formaldehyde levels in the blood can be explained by the following two mechanisms. First, the cumulative buildup of formaldehyde that can come from conventional cigarettes or electronic cigarettes can downregulate ALDH2 gene expression, causing a decrease in the production of the ALDH2 enzyme that plays a role in formaldehyde metabolism. Second, the cumulative buildup of formaldehyde can also cause liver damage, resulting in decreased liver anabolic functions such as mRNA synthesis and protein synthesis, which ends with decreased ALDH2 enzyme production in the liver.18,19

In this study, the liver, as the organ that plays the most role in xenobiotic metabolism, may not have experienced adequate damage, so the level of formaldehyde in the blood has not demonstrated a significant difference. More prolonged exposure durations may increase the cumulative buildup of formaldehyde, which induces liver damage and reduce the production of the ALDH2 enzyme causing an increase in formaldehyde levels in the blood.18,19

The cumulative buildup of formaldehyde is not only affected by the duration of exposure but also by the level of formaldehyde exposure. In this study, the researchers adjusted the dose from humans to mice and the number of cigarettes given to the smoking cage based on the average respiratory rate and tidal volume so that the mice inhaled the predetermined dose.20 However, the results obtained were not meaningful and accompanied by an increasing trend, which implies that increasing the number of cigarettes used increases the probability of obtaining a meaningful result.

In addition, it is perceived that stress and decreased appetite can reduce the production of the enzyme ADH5, which plays a role in formaldehyde metabolism. The hormone adrenaline can also be metabolized into methyamine and converted into formaldehyde by the body.21 In this study, there were 3–4 rats in one cage, which increased the chances of fighting between rats and causing stress. Placing more than one rat in one cage maintenance also causes differences in diet, although the researchers have minimized it by weighing every week. The two things above may be confounding factors that cannot be fully uniformized and are one of the reasons that no significant data differences were found between groups.

In this study, it was found that there were significant differences in alveolar

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**Table 1.** Post-intervention in blood formaldehyde levels in each study group.

<table>
<thead>
<tr>
<th>Study Groups</th>
<th>Mean Formaldehyde Level (µM) ± SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>1.6767 ± 1.91116</td>
<td></td>
</tr>
<tr>
<td>CC2</td>
<td>3.6133 ± 2.08753</td>
<td></td>
</tr>
<tr>
<td>EC2</td>
<td>3.0283 ± 1.59885</td>
<td>0.248</td>
</tr>
<tr>
<td>CC4</td>
<td>4.2033 ± 2.97261</td>
<td></td>
</tr>
<tr>
<td>EC4</td>
<td>4.335 ± 2.18914</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** C, control; CC2, conventional cigarette for 2 weeks; EC2, electronic cigarette for 2 weeks; CC4, conventional cigarette for 4 weeks; EC4, electronic cigarette for 4 weeks

**Table 2.** The degree of histopathological damage to alveoli in each study group.

<table>
<thead>
<tr>
<th>Study Groups</th>
<th>NSD</th>
<th>VL</th>
<th>L</th>
<th>M</th>
<th>H</th>
<th>VH</th>
<th>Mean Score ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>6</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>CC2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>7</td>
<td>3.16±0.75</td>
</tr>
<tr>
<td>EC2</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>5</td>
<td>0</td>
<td>2</td>
<td>2.67±0.82</td>
</tr>
<tr>
<td>CC4</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>5</td>
<td>0</td>
<td>2</td>
<td>2.67±0.82</td>
</tr>
<tr>
<td>EC4</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>2.83±0.75</td>
</tr>
</tbody>
</table>

**Abbreviations:** NSD, no specific damage; VL, very light damage; L, light damage; M, moderate damage; H, heavy damage; VH, very heavy damage; C, control; CC2, conventional cigarette for 2 weeks; EC2, electronic cigarette for 2 weeks; CC4, conventional cigarette for 4 weeks; EC4, electronic cigarette for 4 weeks
Table 3. The degree of alveolar histopathological damage based on the Mann-Whitney test.

<table>
<thead>
<tr>
<th>Study Groups</th>
<th>C</th>
<th>CC2</th>
<th>CE2</th>
<th>CC4</th>
<th>CE4</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>-</td>
<td>0.002</td>
<td>0.002</td>
<td>0.002</td>
<td>0.002</td>
</tr>
<tr>
<td>CC2</td>
<td>0.002</td>
<td>-</td>
<td>0.268</td>
<td>0.268</td>
<td>0.434</td>
</tr>
<tr>
<td>CE2</td>
<td>0.002</td>
<td>0.268</td>
<td>-</td>
<td>1</td>
<td>0.665</td>
</tr>
<tr>
<td>CC4</td>
<td>0.002</td>
<td>0.268</td>
<td>1</td>
<td>-</td>
<td>0.665</td>
</tr>
<tr>
<td>CE4</td>
<td>0.002</td>
<td>0.434</td>
<td>0.665</td>
<td>0.665</td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviations: C, control; CC2, conventional cigarette for 2 weeks; EC2, electronic cigarette for 2 weeks; CC4, conventional cigarette for 4 weeks; EC4, electronic cigarette for 4 weeks.

The degree of alveolar histopathological damage based on the Mann-Whitney test.

histopathology between the control group and the week two conventional cigarette group, the control group and the week two electronic cigarette group, the control group with the conventional cigarette group at week 4, and the control group with the electronic cigarette group at week 4 (p < 0.05).

In the conventional cigarette group at week 2, one rat had mild damage, three with moderate damage, and two with severe damage. The significant difference found in the control group was following a previous study by Glynos et al., which compared the inflammatory effects of conventional cigarette smoke and e-cigarette vapor for three days (acute) and four weeks (sub-chronic). This study found that rats exposed to conventional cigarette smoke for three days experienced wall thickening and lung interstitial inflammation. When using bronchoalveolar lavage (BALS), it was found that the number of macrophages in mice exposed to e-cigarette vapor for three days showed an increase in macrophages (90% of total cells). Cytokine examination found an increase in the value of IL-1β and IL-6. Increased macrophages and inflammatory cytokines can trigger an inflammatory process in the lungs, causing damage to the alveoli.

In rats exposed to conventional cigarette smoke for four weeks, the alveolar damage was not more prominent than in the conventional group exposure for two weeks, and the results of statistical tests were not significant. In exposure to conventional cigarette smoke for four weeks, there were three rats with a mild degree of damage, two with a moderate degree of damage, and one with a severe degree of damage. Glynos et al., also found that the damage scores increased on day three compared to week four. When compared with the control group, lung damage in the conventional cigarette smoke exposure group was significantly different. According to research by Yan et al., the lungs have a self-limiting ability to respond to chronic exposure to cigarette smoke. Lung inflammatory reactions and pathological damage did not increase with prolonged exposure to a specific period, which is caused by a partial decrease in the inflammatory response in the lung in chronic exposure.

In the intervention group with exposure to e-cigarette vapor for two weeks, there were three rats with mild damage, two with moderate damage, and one with severe damage. The effect of damage to the alveoli in this group follows a previous study by Glynos et al., where exposure to e-cigarette vapor had caused significant damage to the alveoli in just three days. Short-term exposure to e-cigarette vapor increases respiratory airflow impedance and resistance due to the irritant effect of propylene glycol. With more prolonged exposure, this effect will diminish, which causes no significant difference between the groups with cigarette exposure for two weeks and four weeks.

This study showed no significant difference between the two-week and four-week exposure duration in the conventional and electronic groups. This founding follows research by Rohmani et al., which proved no significant difference in alveolar damage between groups of rats exposed to conventional cigarette smoke and electronic cigarette vapors. This is because both conventional cigarette smoke and electronic cigarette vapor contain nicotine. Nicotine will trigger the release of fibronectin so that it can trigger fibrosis in the lung parenchyma. In addition, the carbon monoxide in both can inhibit the proliferation of fibroblasts and damage the elastin in the alveolar walls so that the alveoli lose their elasticity and are prone to destruction.

The limitation of this study was the inability to uniform the diet and stress experienced by rats thoroughly. Further research is recommended to extend the duration of exposure to conventional cigarette smoke and e-cigarette vapor for more than four weeks to obtain meaningful results. It is also suggested to reduce stress more significantly in rats, such as by placing one rat in a rearing cage to reduce the risk of quarreling and equalize eating patterns. It is recommended to examine the parameters of liver function.

CONCLUSION

Exposure to conventional cigarette smoke or electronic cigarette vapor significantly affects the histopathological picture of the alveoli but not the level of formaldehyde in the blood.

ACKNOWLEDGMENTS

We want to thank the Faculty of Medicine and Health Science Universitas Katolik Atma Jaya and all those who contributed to this research.

CONFLICT OF INTEREST

There is no conflict of interest regarding this article.

FUNDINGS

None.

AUTHOR CONTRIBUTION

All authors contributed equally in this study.

ETHICAL CLEARANCE

This study was provided ethical clearance number 14/10/KEP-FKIKUAJ/2020 by the Research Ethics Committee, Faculty of Medicine and Health Sciences, Universitas Katolik Atma Jaya, Jakarta.

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6. Available from: https://doi.org/10.1136/bmj.k5429


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