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The Effects of Air Freshener Exposure at an Early Age on Histological White Rat (*Rattus norvegicus*) Liver Cells

Yuningtyaswari[1, a)] and Sofiana Athika Dwi[2)]

[1]*Departement of Histology, Faculty of Medicine and Health Science, Universitas Muhamadiyah Yogyakarta, Jl. Ring Road Selatan, Tamantirta, Kasihan, Bantul, Yogyakarta, 55183, Indonesia*
[2]*Student of Faculty of Medicine and Health Science, Universitas Muhamadiyah Yogyakarta, 55183 Indonesia*

[a)]Corresponding email: yuningtyas_fkumy@yahoo.com

Abstract. Air freshener can cause indoor air pollution if used unwisely. Air freshener contains a variety of volatile organic compounds such as formaldehyde with the potential to damage the liver. This study aims to determine the effects of air freshener exposure at an early age on histological liver cells. The subjects of this study were 30 rats [(*Rattus norvegicus* (Berkenhout, 1769)] at an early age which were divided into three groups: control (K), gel air freshener (P1) and spray air freshener (P2); each group consisting of 10 rats. Group P1 and P2 were exposed with air fresheners from the age of 8 d to 67 d. The duration of exposure for each day was started in 15 min in the morning and afternoon and increase of 15 min per wk for a total duration of 4.5 h in the last week of exposure. Histological preparations of liver cells by HE staining was observed with a microscope at a magnification of 40 × 10. The degree of liver cell damage was assessed using scoring Manja Roegnik. Data were analyzed by ANOVA followed by Tukey's test. The results showed histological of liver cell damage in the form of parenchymatous degeneration, hydropic degeneration and necrosis. There are differences in the effect of gel and spray exposure on liver cell histology of *Rattus norvegicus*. There is significant difference of the degree of liver cells damage: K < P1, K < P2 and P1 > P2. It was concluded that exposure of air freshener has a bad influence on the histological liver cells.

Keywords: Air freshener gel, air freshener spray, early age *Rattus novergicus* (Berkenhout, 1769), liver histology.

INTRODUCTION

Air is one component of the environment, which greatly affect our health. Poor air quality will affect the emergence of various health problems. At this moment, it is difficult to obtain clean and quality air. Air pollution encountered everywhere, both in the outdoors or indoors.

The World Health Organization has assessed the contribution of a range of risk factors to the burden of disease and revealed indoor pollution as the eighth most important risk factor and responsible for 2.7 % of the global burden of disease. Every year, indoor air pollution is responsible for the death every 20 s [1].

Concentrations of many volatile organic compounds (VOC) are consistently higher indoors (up to ten times higher) than outdoors. Household products contain VOCs, including paints, paint strippers and other solvents; wood preservatives; aerosol sprays; cleansers and disinfectants; moth repellents and air fresheners; stored fuels and automotive products; hobby supplies; dry-cleaned clothing [2].

Air freshener contains VOCs that can damage the health. Scientific Committee on Health and Environmental Risks [3] argued that the air freshener contains several chemicals, such as Volatile Organic Compound (VOC), allergens, benzene, formaldehyde, terpenes, styrene, toluene and diethyl phthalate.

According to a report from the National Institute of Occupational Safety and Health [4], hazardous chemicals in the air freshener is formaldehyde. Besides irritation to the eyes, nose, throat, skin; nausea; dizziness, bleeding; memory loss; cancer and tumors; liver damage, formaldehyde also causes irritation to the middle of the lungs, including symptoms of asthma [4].

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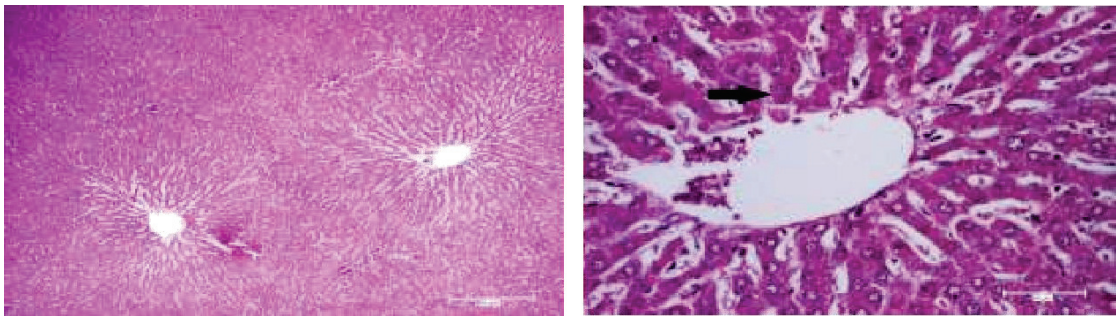
Exposure to formaldehyde appears to be associated with hepatoxicity in many species, including human, following injection, ingestion or inhalation. Macroscopic, microscopic and biochemical manifestations in the liver include alterations in weight, centrilobular vacuolization, focal cellular necrosis and increased alkaline phosphatase concentrations. Time-related changes in the pattern of the effects are suggested as one goes from acute exposure by inhalation at greater concentrations to repeated exposure at lesser concentrations. Although the hepatic changes are generally not extensive and can be reversible following acute exposure, repeated exposures causes more serious progressive damage. There are several possible mechanisms for the toxicity. Depending on the route, formaldehyde exposure could include direct effects on hepatocytes and/or indirect effects on the circulatory and immune systems [5]. This study aims to determine the effects of air freshener exposure at an early age on histological liver cells.

MATERIALS AND METHODS

The subjects of this study were 30 male rats *Rattus norvegicus,* (Berkenhout, 1769) with early age eighth days. Air freshener used was produced by one factory in Indonesia, with the gel and spray forms. They were obtained from Universitas Gadjah Mada, Faculty of Pharmacy and housed under standard conditions. Subjects were divided into three groups (n = 10): control (K) (untreated) group, the P1group (given a gel air freshener exposure) and P2 group (given exposure to spray air freshener). Exposure to the subject of the group P1 and P2 from the age of eighth days up to 67^{th} days, the initial dose of exposure 15 min every morning and afternoon. Subsequent doses increased by 15 min for each week, until the final dose of 4.5 h. On day 68^{th}, subjects were sacrificed and the liver tissues were removed for histological investigation. Liver histology preparations made by the method of paraffin blocks with Hematoxyline Eosin (HE) staining techniques. All histological preparations were observed under a binocular microscope at a magnification of 4 × 10 and 40 × 10, in the area around the vena centralis, in five visual fields. The degree of liver damage was assessed using a scoring system Manja Roenigk [6]. Observations were made on 100 cell liver, on each visual field. Histological observation of the cells was observed: normal cells (score 1), parenkimatosa degeneration/cloudy swelling (score 2), hydropic degeneration/ballooning (score 3) and necrosis (score 4) [7]. Degrees of liver damage were analyzed statistically using ANOVA, followed by Tukey's test at 95 % confidence level.

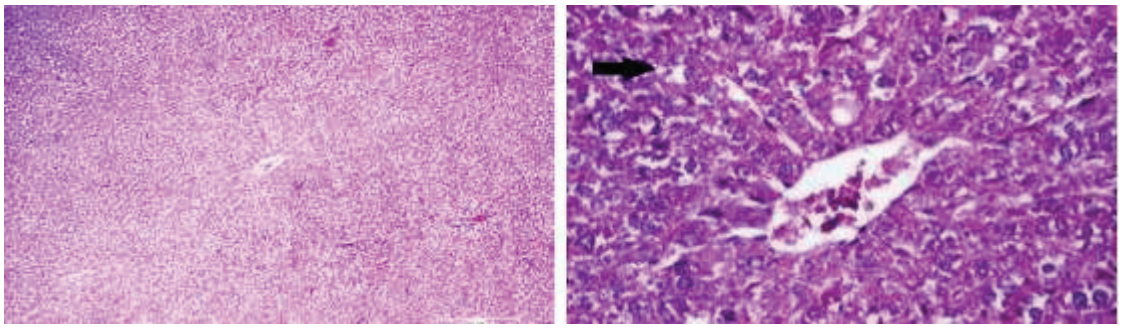
RESULT AND DISCUSSION

Histological observation of liver cell damage of this research can be seen in the Fig. 1 to Fig. 3 and Table 1 below. In general, the observations show the presence of differences in histological features of liver tissue among three groups of this research. In observation of histological preparations with weak magnification (4 × 10), it appears that the liver tissue of the control group has hepatic lobules with intact cells and neatly arranged in radier around the central canal of lobules; liver cells also generally appears normal. Different liver tissue histology is shown in group P1 and P2, particularly P1, where the lobules seem pale in some parts because most of cells degenerate.



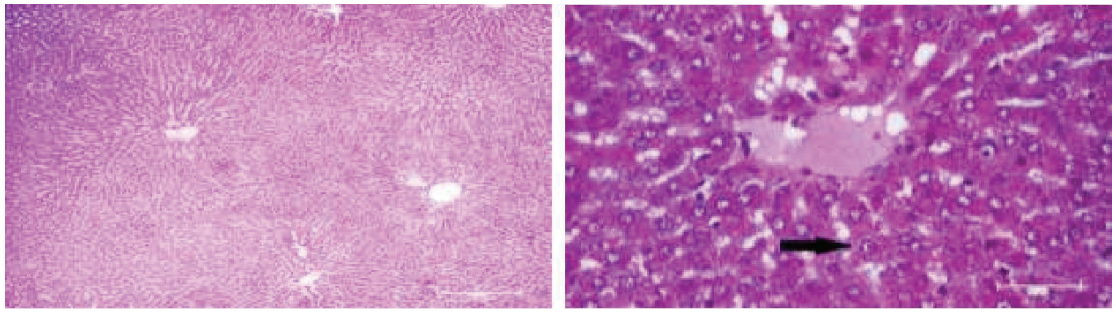
(a) (b)

FIGURE 1. (a) Liver histology of control group (K) (HE, 40×), (b) Figure liver histology of control group (K) (HE, 400×). Description: (→) showed normal cells with a score of 1



(a) (b)

FIGURE 2. (a) Liver histology of group of rat that was exposed to the gel air freshener (P1) (HE, 40×), (b) Liver histology of a group of rat that was exposed to the gel air freshener (P1) (HE, 400×). Description: (→) showed cell hydropic degeneration with a score of 3



(a) (b)

FIGURE 3. (a) Liver histology of a group of rat that was exposed to the spray air freshener (P2) (HE, 40×), (b) Liver histology of a group of rat that was exposed to the spray air freshener (P2) HE, 400×) Description: (→) showed degeneration cell parenkimtosa with a score of 2

In observation with stronger magnification (40 × 10), it showed that most of the cells in the liver of rats exposed to the air freshener (gel or spray) were damaged from the light damage (degeneration parenkimatosa), hydropic degeneration, even necrosis.

However, in this study, all groups of rats showed parenkimatosa and hydropic degeneration, but in the control group were very few in number. Parenkimatosa degeneration is reversible and only occurs in the mitochondria and endoplasmic reticulum because the stimuli may cause oxidation. This damage causes the accumulation of water in the cells, so the cells appear swollen and cloudy.

TABLE 1. The average score of histopathological changes in the cells of control group, group with gel air freshener exposure and group with spray air freshener exposure

Group of rats	**Average ± SD of Histopathological changes score Manja Roenigk**
Control Group (K)	1.7897 ± 0.15864^{a}
Gel air freshener (P1) Group	3.4414 ± 0.37409^{b}
Spray air freshener (P2) Group	3.0316 ± 0.26800^{c}

Description: $^{a, b, c}$ different letters indicate significant differences in the statistical test one way ANOVA post hoc Tukey with 95 % significance level

Necrosis indicates the presence of cell death, which is marked with an overview pyknotic nucleus, which can be divided into karyoreksis and karyolisis [8].

Results of assessment of the degree of liver cell damage using Manja Roenigk scoring showed that there were significant differences within three groups. Scores of damage was relatively high (> 3) in the two groups of rats that were exposed to the air freshener. This suggests that exposure to air freshener cause damage to the liver cells.

It has been known that the air freshener contains various volatile compounds, including formaldehyde that could potentially damage the tissue of the body. Strubelt [9] states formaldehyde is highly reactive substance which can interact with virtually every cellular constituent. Formaldehyde can possess systemic toxicity and may produce an organotrophic effect on remote tissue and organs. Results of investigation of the influence of this air freshener exposure (which contain formaldehyde) is also consistent with the explanation of Beall and Ulsamer [5] who states that formaldehyde exposure may associate with hepatotoxicity.

Liver tissue damage due to toxic compounds is associated with its detoxification function. One of the toxic compounds contained in air freshener is formaldehyde. Metabolism and detoxification of formaldehyde occur in the liver that produces toxic metabolites which can damage the liver. Formaldehyde will be metabolized into formic acid by the enzyme formaldehyde dehydrogenase. Formaldehyde dehydrogenase enzyme is oxidative enzymes located in the cytosol and mitochondria. The highest level of this enzyme is in the liver, kidney, lung and gastric mucosa consecutively. Formaldehyde exposure affects the liver cells by damaging the mitochondria that inhibit aerobic metabolism of cells [10, 11].

This study showed differences in the level of damage to liver cells of rats between groups P1 and P2. Group P1 (which was exposed to the gel air freshener) showed more damage than the P2 group. Significant differences between the groups P1 and P2 was possible because substances in spray air freshener exposure gradually decreased due to the air currents in the room, especially if there is ventilation. On the contrary, gel air freshener substance emits continuous exposure. Another factor that causes the different effect between gel air freshener and sprays air freshener is the differences in the composition of the constituent between spray air freshener and gel air freshener. Air freshener in gel form contains more formaldehyde than the liquid preparation/spray [3]. Therefore, it is acceptable to consider that gel air freshener has a more severe effect on liver cells damage than spray air freshener.

CONCLUSION

It was concluded that exposure of air freshener at an early age have a bad influence on the histological white rat (*Rattus norvegicus*) liver cells.

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