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The Effect of Anti Pollutant Gel from *Sansevieria trifasciata* on Malondialdehyde Level and Histopathology of Rats' Liver and Lungs Induced by Cigarette, Coal, and Mosquito Smoke

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ABSTRACT

Gas pollutants that accumulate in the room with restricted air circulation may cause respiratory system disorders. A pregnane glycoside compound from *Sansevieria* can reduce the pollutants. This study aims at producing gels from *Sansevieria* extract to neutralize indoor gas pollutants. Sansevieria extract is produced by maceration process with composition 8g simplicia and 100mL ethanol 96%. The extract was processed into the gel with 20% concentration. The gel was applied to rats (Rattus norvegicus) Wistar strain induced by cigarette, coal, and mosquito smoke with positive control and treatment groups. After 8 days, Gross examination and histopathology of lungs and liver were observed quantitatively. MDA levels were measured with TBARS method. Data were analyzed by independent sample T-test and Mann-Whitney test with p value considered significant if <0.05. Gross examination of lungs showed a significant difference between treatment and control group that was induced by coal smoke (p=0.031), and mosquito smoke (p=0.006) and liver's gross of cigarette smoke (p=0.040). Histopathology of lungs showed a significant difference in mosquito smoke (p=0.032) and no significant difference in histopathology of the liver. MDA levels showed significant difference in coal smoke (p=0.020) and mosquito smoke (p=0.000). In conclusion, anti pollutant gel reduces MDA levels and damage of the lungs induced by pollutants.

Key words: Sansevieria Gel, Pollutant, MDA, Liver, Lungs

INTRODUCTION

According to the latest World Health Organization report, deaths caused by air pollution reach 8 million people per year globally. Indoor air pollution is predicted to cause 4.3 million deaths (World Health, 2014) Indoor air pollution is the third most common cause of disability adjusted life years (DALYs) globally and a major cause of DALYs in Southeast Asia (Abubakar, *et al*., 2015). In Indonesia, indoor air pollution causes 452,800 deaths and causes 54 DALYs per 1000 population (WHO, 2018). Estimated percentage of main air pollution components in Indonesia especially in transportation and industry are carbon monoxide (CO) 70.50%, sulfur oxide (SOx) 0.9%, and nitrogen oxide (NOx) 8,9% (Handayani & Sugiarti, 2009). CO is absorbed through the lungs and is largely bound by reversible hemoglobin, forming carboxyhemoglobin (COHb). The CO affinity of hemoglobin is 200-250 times more than oxygen affinity that leads to hypoxia (World Health, 2000). The main effect of the SO_2 pollutants on human is respiratory system irritation. Throat irritation occurs at SO_2 levels of 5ppm or more (Depkes, 2014). NO_2 is four times more toxic than NO. NO_2 levels higher than 100ppm can kill most of the experimental animals and 90% of these deaths are caused by pulmonary edema. NO_2 levels of 800 ppm will result in 100% mortality in animals tested within 29min or less. Giving NO_2 at 5ppm for 10min to human causes difficulty in breathing (Wardhana, 2001). These pollutants often contaminate indoor air and mostly in the form of cigarette, coal, and mosquito smoke. Indoor air pollutants are more dangerous because it has poor air circulation and long duration of exposure.

Sansevieria trifasciata contains active compounds of pregnane glycosides that can change pollutants into organic acids, sugars, and some amino acid compounds (Purwanto, 2006). *Sansevieria trifasciata* can decompose formaldehyde, benzene by 53% and trichloroethylene by up to 13% within 24h (Sulianta & Yonathan, 2009). Pregnane glycosides can be extracted from the *Sansevieria trifasciata* and processed into an air freshener. However, there has been no research developing the extract of *Sansevieria* in the form of a gel. Gel was chosen to facilitate the implementation of *Sansevieria* extract as an anti pollutant and air freshener by adding fragrance. An anti pollutant gel is used to attenuete the damage of indoor air pollution to the lungs and liver. This study aims at determining the effect of anti pollutant gel to neutralize indoor pollutants assessed from levels of malondialdehyde (MDA), histopathology of rats' lungs and liver induced by cigarette, coal, and mosquito smoke.

MATERIALS/METHODS

The tools used in this study are beaker, spatulas, blenders, watch glass, filter paper, aluminum paper, stirring magnets, funnels, analytical balance, knives, fume hoods, thermometer, heater, erlenmeyer, thermostatic oscillator, syringe injection 3mL. The materials used in the study were snake plant, ethanol 96%, formalin buffer, distilled water, carrageenan kappa, glucomannan, propylene glycol, sodium benzoic acid, concentrated sulfuric acid, glacial acetic acid, thiobarbituric acid (TBA) 0.37% in 0.25N HCl, trichloroacetic acid (TCA) 15%, and male mice (Rattus norvegicus) Wistar strain.

Identification of pregnane glycoside in sansevieria extract

Sansevieria is extracted by maceration method. Grinded *Sansevieria* is dried in 70^{o}C and then extracted into 8g simplicia with 100mL ethanol 96% for 3 days. The pregnane glycoside in sansevieria extract identified using Liebermann-Burchard reaction. The blue-green color was determined by UV-VIS spectrophotometer at 420nm and compared to cholesterol standard.

Anti pollutant gel production

Distilled water is diheated until 75°C. The distilled water is added with 1.8g kappa carrageenan, 1.2 glucomannan, and 0.1 sodium benzoates (Fitrah, 2013). The temperature of the solution is decreased to 65°C. The solution is added with 10g propylene glycol, *Sansevieria* extract consisting of 8g simplicia and 100mL ethanol 96%, and 5g essential oil (Table I). The solution that is already well-mixed is poured into the gel mold.

Table I. Gel composition with 100g base

Composition	Weight (g)
Hydrocolloid	3
Kappa carrageenan	1.8
Glucomannan	1.2
Propylene glycol	10
Sodium benzoates	0.1
Sansevieria extract 20% from base	20
Essential oil	5
Aquadesh (s.d. 100 g)	Add. 100g

Animal examination

This is an experimental study using a posttest-only control group design. Thirty Wistar rats weighing 200-300g are acclimatized for 8 days. Samples are completely randomized into 6 groups with 3 treatment groups are given with anti pollutant gel and induced by pollutant and 3 control group are induced by pollutant. After 8 days of treatment, the rats are analyzed for their MDA level, macroscopic features and histopathology of lungs and liver.

Gross examination of lungs and liver are scored using criteria for color, black spot, and white membranes. Grading and staging for histopathology of rats' lung were assessed by the percentage of consolidation and vascular hyalinization in 3 power fields with 40x magnification. Grading and staging of rats' lung were also based on the presence (score 1) or absence (score 0) of necrosis and squamous metaplasia of bronchioles. Grading and staging for histopathology of rats' liver were done by METAVIR system. MDA level is measured by TBARS (thiobarbituric acid reactive substance) method with 545nm wavelength.

Statistical analysis

Independent Sample T-test and Mann-Whitney test are used for the data analysis with p value considered significant if <0.05. Researchers used SPSS 25.0 for windows according to process the data.

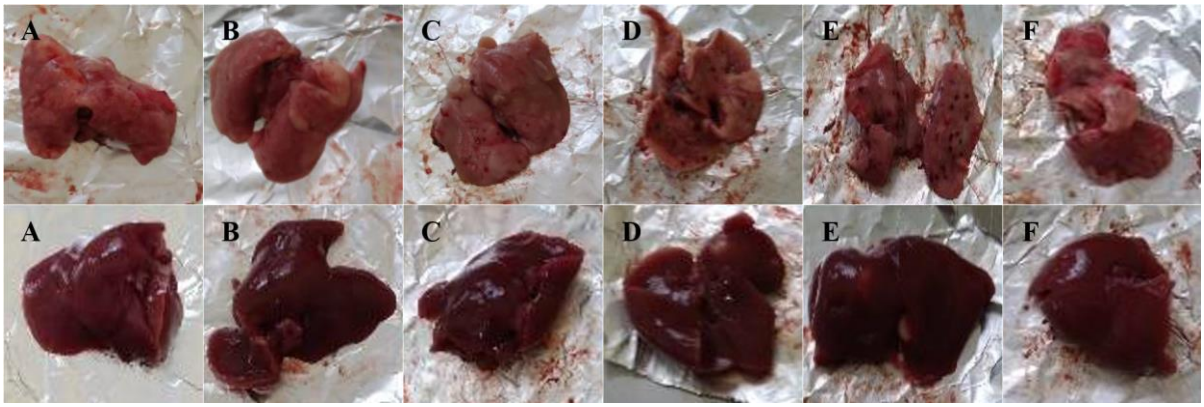

Figure 1. Gross examination of lung and liver. The untreated groups, the cigarette smoke (A), coal smoke (B), and mosquito coil smoke group (C) showed more visible and numerous lesions compared to the treated groups. The worst lesions was found in the mosquito coil smoke group (C). Mosquito coil smoke group (C) has the worst macroscopic view with darker color, many black spots, white membranes, and white mass found. The macroscopic lessions was lesser in the cigarette smoke with gel (D), coal smoke with gel (E), and mosquito coil smoke with gel group (F). The white mass was not found in the groups with gel treatment.

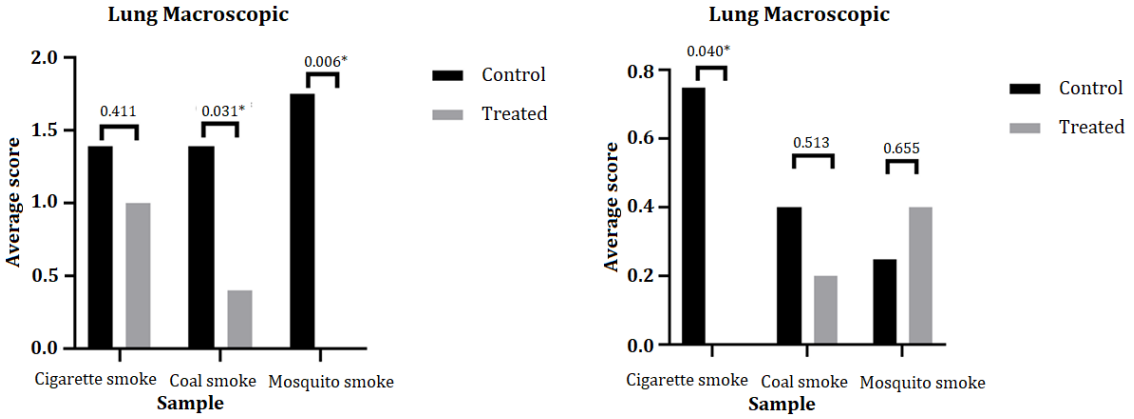

Figure 2. Gross examination scoring and statistical analysis of lung (A) and liver (B)

Ethical conduct

Ethical clearance was obtained from the Ethical Committee (Komisi Etik Penelitian Kesehatan) No. 93/EC/H/FK-RSDK/VII/2018.

RESULTS AND DISCUSSION

The blue color of Sansevieria extract after Liebermann-Burchard reaction indicated pregnane glycoside content of 318.26±20.99mg/dl. Gross examination of lungs and liver are measured by scoring based on the criteria of color, black spot, and white membranes. Lung gross examination showed that the treated group has a lower score than a control group. A significant difference was found in coal smoke and mosquito smoke. A histopathology examination showed significant difference in mosquito smoke. Liver gross examination and histopathology score showed that a treated group has lower score than a control group. A significant difference was found in cigarette smoke on gross examination. No significant difference was found in liver histopathology. Control group has higher MDA level than the treatment group in coal smoke and mosquito smoke. A significant difference was found in coal smoke and mosquito smoke.

Black spot in the gross examination of lungs indicated consolidation. Consolidation was caused by inflammation of lung tissue due to exposure to pollutants. Discoloration to blackish red was caused by hyperemia due to vasodilation of blood vessels (Reid, *et al*., 2011).

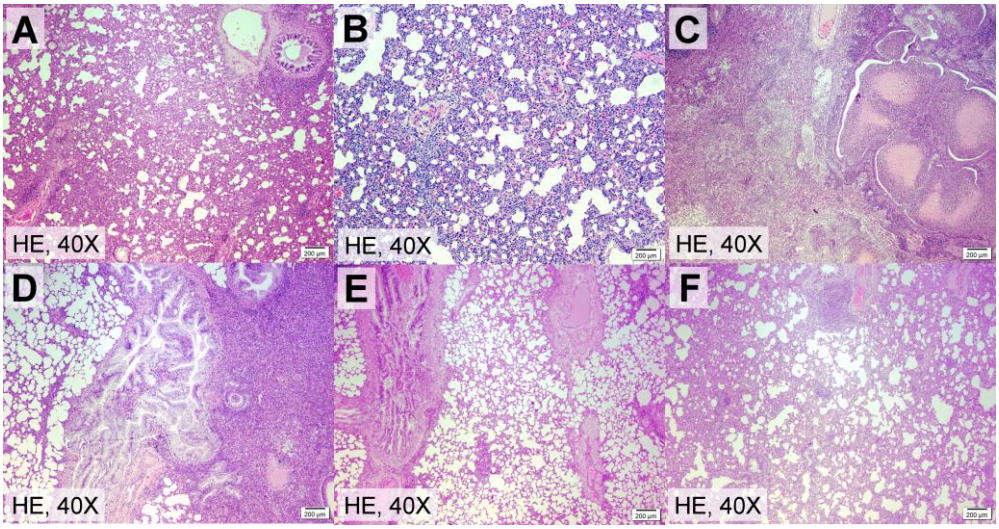

Figure 3. Histopathological lesion found in lung with HE routine staining with magnification of 40x. Groups without gel treatment showed more areas of alveoli filled with inflammation infiltrate. Among the cigarette smoke (A), coal smoke (B), and mosquito coil smoke group (C), the most wide spread lesion area was found in the latest group, as well as necrotic area and bronkhiolus metaplasia. Compared to the untreated groups, the groups with gel treatment showed better histopathologically features. Alveoli filled inflammation infiltrate area was far lesser in the cigarette smoke with gel (D), coal smoke with gel (E), and mosquito coil smoke with gel group (F). Although, the lesion in mosquito coil smoke with gel group still showed the worst histopathological lesion such as necrosis and bronkhiolus metaplasia between the three treated groups.

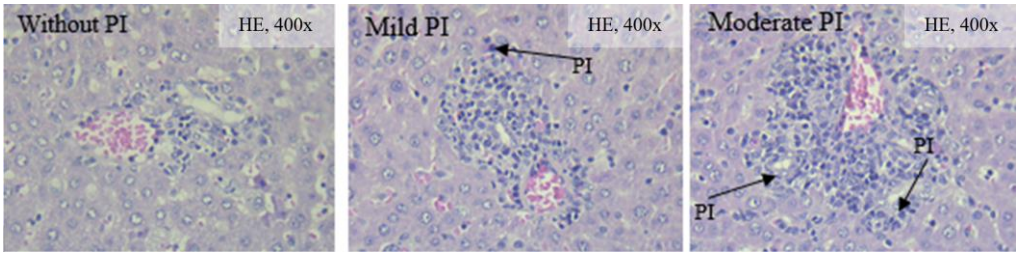

Figure 4. Histological section of portal triad in liver tissue stained with HE magnification 400x. Among untreated group, the mosquito smoke showed higher average of METAVIR score which indicate severe portal infalmation (PI). The PI was higher in cigarette and mosquito smoke among treated and untreated group.

White membrane was caused by pleural fibrous reaction to the underlying inflammation when the consolidation extends to the surface (Kumar, *et al*., 2017). Pleural space lacks of blood supply and lymphatic systems making exudates difficult to be cleaned. As a result, the pleural space will organize with fibrous tissue and produces white membranes (Reid *et al*., 2011). Significant differences were obtained from coal smoke and mosquito smoke.

Lung histopathology showed that all samples in the control group had a higher percentage of consolidation compared to treatment group. A treated sample induced by cigarette smoke had a grouping consolidation in one area and normal alveoli conditions in some areas. Meanwhile, a control sample induced by cigarette smoke has even alveoli consolidations. Sample induced by coal smoke also showed more severe consolidation in the control group. However, there was no significant difference between control and treatment group of cigarette and mosquito smoke. Control sample induced by mosquito smoke showed severe necrosis, squamous metaplasia of bronchioles, and consolidation.

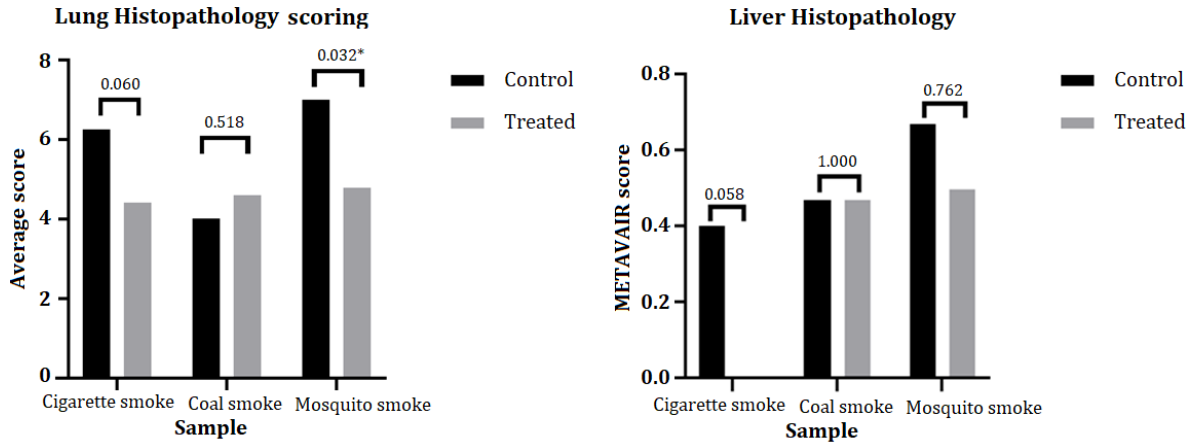

Figure 5. Histopathology scoring and statistical analysis of lung (A) and liver (B)

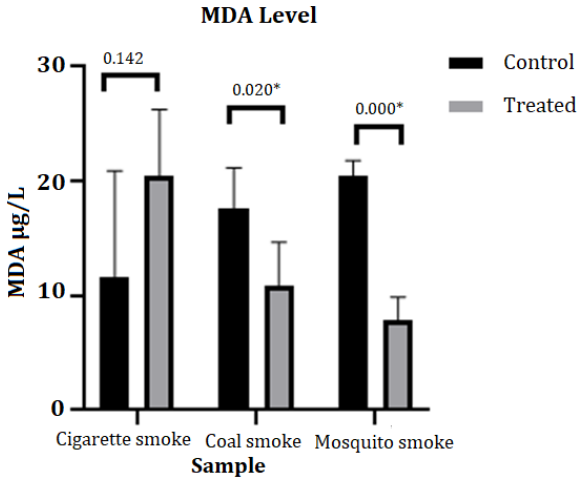

Figure 6. MDA level and statistical analysis

There was a significant difference between control and treatment group od mosquito smoke. The difference is not significant in the group exposed to cigarette smoke and coal due to the resolution of the alveoli. Alveolus showed a fast resolution mechanism due to its numerous blood capillaries and lymphatic systems to easily clean the exudates (Reid *et al*., 2011) Gross examination of the liver showed discoloration of blackish color in most control groups. This was caused by an inflammatory response in the liver. Control and treatment group of cigarette smoke showed significant differences in gross examination. Histopathological observations of liver exposed to cigarette smoke showed that control had inflammation in the portal triad that was more severe than treatment. Cigarette smoke that was not neutralized in C1 will induce cytochrome P450 2E1 (CYP2EI) enzyme in the liver. CYP2E1 will conjugate cigarette smoke into more polar compounds and produce reactive oxygen species (ROS) (Xu *et al*., 2017). ROS induced by CYP2EI was produced locally and stimulates inflammation in the liver (Panigrahy, Kaipainen, Greene, & Huang, 2010).

Gross and histopathology examination of liver exposed to coal smoke showed no significant difference. This was because exposure to pollutants can already be compensated by inflammation in the lungs. Gross examination of the liver exposed to mosquito smoke showed that treatment has a blackish red color more dominant than the control group. The smoke of mosquito coil contains MGK24 compounds and geranyl acetone which are inhibitors of the P450 enzyme group (Ramirez, *et al*., 2012). There was no significant difference on

mosquito smoke both in liver macroscopy and liver histopathology.

Examination of MDA levels showed that there were significant differences in coal smoke and mosquito smoke. This indicated that anti pollutant gel was effective to reduce oxidative stress. No significant difference was found in MDA level in the cigarette smoke exposure.

Gaseous air pollution is primarily removed by the plants through leaf stomata, absorbed gases diffuse into intercellular space react with the inner surface of leaves, maybe absorbed by water to form acid (Smith, 1990). Sansevieria, *Aloe vera*, *Epipremnum aureum,* and *Chlorophytum comosum* are examples of indoor antipollutant plant. The activity of *Aloe vera,* Epipremnum aureum and *Chlorophytum comosum* remove formaldehyde had been investigated through Formaldehyde dehydrogenase activity (Xu, *et al*., 2011). *Chlorophytum comosum* has higher formaldehyde reduction due to its C1 metabolism (Giese *et al*., 1994). Sansevieria neutralizes the pollutant through the metabolic breakdown mechanism (Lingga, 2008). An active compound of pregnane glycoside from Sansevieria plant transforms the dangerous pollutants into organic compounds. Compared to other antipollutant plants, Sansevieria can neutralize 107 pollutants and has abundant resources due to its larger leaves and it is easier to be cultivated (Wolverton *et al*., 1989). However, the mechanism of antipollutant gel neutralizes the pollutants remains unclear. Evaporated of pregnane glycoside compounds from anti pollutant gel presumably impregnated the dangerous pollutant. An impregnated pollutant has a higher density than that of the original pollutant that will cause the pollutant to drop and be exhaled less by the respiratory system. Once the impregnated pollutant is inhaled, it is presumably less dangerous. Moreover, the antioxidant content form Sansevieria extract could maintain the oxidative stress created from the damage of the pollutants.

CONCLUSIONS

Anti pollutant gel significantly reduces damage of mosquito coil smoke to the lungs, reduces damage of cigarette smoke to the liver, and reduces MDA level of coal smoke and mosquito smoke. Further research should be conducted to observe the effect of anti pollutant gel on human.

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