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Nano Spray Inhaler Ashitaba Leaf Extract (*Angelica keiskei*) on Malondialdehyde, Catalase Enzyme Activity and Lung Tissue Damage in Mice Exposed to Cigarette Smoke

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Abstract

A total of 30 mice were divided into 5 treatment groups. K1: control (-) group not exposed to cigarette smoke and not given nanospray inhalers. K2: control (+) group exposed to cigarette smoke and not given nanospray inhalers. P1, P2, and P3 The treatment group exposed to cigarette smoke for 28 days and given nanospray inhaler ashitaba leaf extract (*Angelica keiskei*) at a dose of 50 g / KgBB / day, 200 g / KgBB / day and 500 g / KgBB / day. Day 29 was given nanospray inhalers for 3 weeks, then Malondialdehyde (MDA), enzyme catalase activity were examined from serum and lung organs for pulmonary histopathology. The administration of 200 g / KgBB / day nanospray inhaler can reduce malondialdehyde levels, activation of the enzyme catalase, emphysema, inflammatory cell infiltration and the number of erythrocytes exposed to cigarette smoke.

Key words: Nano Spray Inhaler, Ashitaba Leaf Extract (*Angelica keiskei*), pulmonary histopathology.

Exposure of cigarette smoke can be dangerous for the human body due to increase in dangerous radicals in body thus the endogenous antioxidants were unable to neutralize, then leading to oxidative stress (Kurutas E.B, 2016). One of the biomarkers that most generally used to measure the degree of oxidative stress is Malondialdehyde (MDA), it is end product of lipid peroxidation (Moselhy *et al.*, 2013). Endogenous antioxidant defense systems against these changes do not benefit the body through enzymatic enhancement mechanisms such as

catalase, SOD and glutathione peroxidase) and non-enzymatic (albumin, uric acid). This lack of factors triggers oxidative stress that underlies the development of a number of diseases (Wojcik M *et al.*, 2010).

One of the plants that have antioxidant properties is Ashitaba (Chavan *et al.*, 2016). Which contains chalcones, coumarins, flavonoids and polyphenols (Caesar and Cech, 2016). Chemical compounds of Ashitaba leaves have the potential as antioxidants 100 times potent than vitamin C and 25 times that of vitamin E.

Nanospray preparation from leaves of Ashitaba (*Angelica keiskei*) is given by inhalation to assess the influence of inhaler leaves of Ashitaba (*Angelica keiskei*) on Malondialdehyde (MDA), the activity of serum catalase and pulmonary histopathological changes due to cigarette smoke.

Materials and Methods

This study has got approval with certificate No. 1.KE.102.06.2018 by Animal Care and Use Committee on Veterinary Medicine Universitas Airlangga Surabaya Indonesia. Ashitaba (*Angelica keiskei*) is obtained from ashitaba garden in Trawas, Mojokerto, East Java, Indonesia. Ashitaba has got certificate No. 1297 from Overseas Merchandise Inspection CoLTD (OMIC).

The ashitaba leaves shade dried for 7 days by grinding, maceration and dried under shade for 3 days. Ashitaba leaf nanoparticle use comparison that extract: NaTPP: Chitosan is 1:1:6. 10 ml ashitaba leaf extract 5% mixed with 10 ml NaTPP 0,1% and then mixed 60 ml chito-

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Table I. Malondialdehyde and catalase enzyme levels of mice serum exposed to cigarette smoke and treated with asitaba nanospray inhalers (*Angelica keiskei*) (Mean \pm SD)

Groups	Malondialdehyde counts	Catalase Enzyme
K1	37.50 ^b \pm 0.50	32.83 ^a \pm 10.50
K2	59.00 ^c \pm 2.00	110.34 ^b \pm 40.34
P1	72.75 ^d \pm 8.75	71.17 ^{ab} \pm 31.50
P2	12.00 ^a \pm 2.00	13.67 ^a \pm 1.00
P3	68.25 ^{cd} \pm 23.59	42.50 ^a \pm 5.50

Different superscript in the same column indicated very significantly different (P<0.05)

Table II. The pulmonary histopathological changes of mice exposed to cigarette smoke treated with asitaba leaf nanospray inhaler (*Angelica keiskei*)

Groups	Mean scoring of emphysema	Mean scoring of Inflammatory cell infiltration	Mean scoring of Erythrocyte Amount
K1	3.80 ^d	11.40 ^{ab}	17.10 ^{ab}
K2	17.70 ^{ab}	20.50 ^{ab}	17.80 ^{ab}
P1	21.70 ^a	11.70 ^{ab}	11.00 ^{ab}
P2	10.70 ^c	6.50 ^c	7.80 ^{cd}
P3	11.10 ^{bc}	14.90 ^{ab}	7.88 ^{cd}

Different superscript in the same column indicated very significantly different (P<0.05)

san 0,2%. The material mixed and sonication with sonicator machine for 60 min at frequency 20 kHz and freeze dried (Stoica *et al.*,2013).

The mice were exposed to commercial smoke is Marlboro® cigarettes (13 mg tar and 1,0 mg nicotine) per day for 28 days using a smoking box. A smoking box has a size 30 cm long, 20 cm wide, and 15 high with 8 holes. The animals were maintained in this smoke-filled air condition (\pm 3%) for 6 min, this procedure was repeated daily (Triana *et al.*,2013).

A total of 30 animals were divided into 5 treatment groups, namely K1: the (-) control group that was not exposed to cigarette smoke and not given the nanospray inhaler. K2: (+) control group exposed to cigarette smoke and not given nanospray inhalers. P1, P2, and P3 are the treatment groups exposed to cigarette smoke for 28 days and given nanospray inhalers asitaba leaf extract (*Angelica keiskei*) with a dose P1. 50 g / KgBB /day, P2 = 200 g / KgBB / day, P.3 = 500 g / KgBB / day. On day 29 was given a nanospray inhaler for 3 minutes. Then intra-peritoneally treated with 0.1 ml of ketamine

injection, heart blood is collected to assess. Thiobarbituric acid reactive substance (TBARS) examination of Malondialdehyde (MDA) (Reilly, *et al* 1991; Konig, *et al loc. cit*) and catalase by the assay enzyme activity based on the rate of hydrogen peroxide / ammonium molybdate complex formation and pulmonary organs for histopathology with stained examination under a light microscope with 1000 times magnification to see changes in alveolar emphysema, inflammatory cell infiltration, and erythrocyte infiltration (Yang *et al*, 2008). Data on the results of the study were analyzed by one-way ANOVA. If there is a difference, continue with the test using SPSS® 22.

Results and Discussion

The results of malondialdehyde levels in mice exposed to cigarette smoke and without given Ashitaba leaf nanospray inhaler (K2) showed an increase compared to the control group without cigarette exposure treatment. Cigarette smoke contains free radicals that can oxidize lipids, proteins and carbohydrate molecules, damage cell membranes and DNA that affect

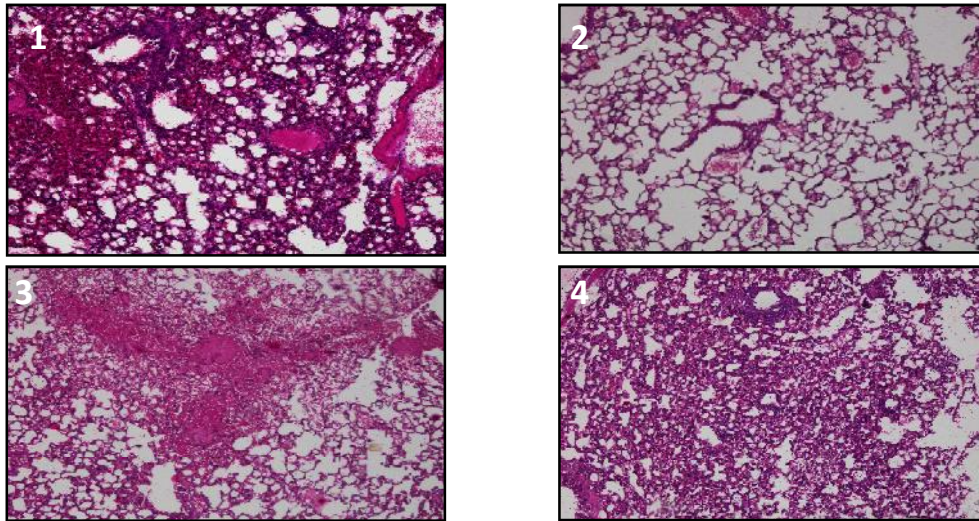


Fig 1. Results of lung organ histopathology changes of mice exposed to cigarette smoke 1) pneumonia, 2) emphysema, 3) hemorrhage, 4) atelectasis.

cell structure and function. Membrane cells rich in sources of polyunsaturated fatty acids that are easily oxidized by free radicals cause lipid peroxidation (Khushdeep *et al.*, 2013). MDA is the final product of lipid peroxidation by free radicals, the increase in MDA concentration shows an increase in free radicals in the body, so an increase in MDA levels as an oxidative stress marker in groups exposed to cigarette smoke (Kahnamoei *et al.*, 2014 and Safyudin and Subandrate, 2016). who were exposed to cigarette smoke and were given the Ashitaba leaf nanospray inhaler dose of 200 g / KgBB / day, showed a decrease in the level of malondialdehyde compared with all groups treated with exposure to cigarette smoke (P1, P2, P3). (Table I). Ashitaba leaves contain flavonoids, polyphenols, and carotenoids that can improve antioxidant status in humans, so as to reduce free radicals due to exposure to cigarette smoke.

The average results of catalase enzyme activity in the control group exposed to cigarette smoke that were not given Ashitaba leaf nanospray inhaler showed an increase compared to the treatment group exposed to cigarette smoke and given nanospray inhaler ashitaba leaf extract, while in the control group that was not exposed to cigarette smoke and not given nanospray inhaler ashitaba leaf extract (K1) with the treatment group exposed to cigarette

smoke and given nanospray inhaler ashitaba leaf extract dose of 200 g / KgBB / day, and 500 g / KgBB / day (P2, P3) showed a decrease (Table I). This result is consistent with the research of Ignatowicz *et al.*, 2013. Catalase is an important element in the antioxidant defense system and oxidative reaction reducer. Increased catalase activity is a parameter that shows organ reactivity in xenobiotics such as cigarette smoke.

The average score of histopathological changes in pulmonary emphysema, inflammatory cell infiltration and the number of erythrocytes showed a decrease in the K1 control group and the treatment was given nanospray inhaler ashitaba leaf extract dose of 200 g / KgBB / day. (Fig 1 and Table II) These results indicate that exposure to cigarette smoke causes oxidative stress that affects the cellular antioxidant defense system which can induce lung tissue apoptosis, inflammation and tissue damage that can be prevented by giving antioxidants (Al-Awaida *et al.*, 2014 and Nagaraj *et al.*, 2014).

Summary

The administration of a 200 g / KgBB / day nanospray inhaler can reduce malondialdehyde levels, the activity of the catalase enzyme also decreases emphysema, and infiltrates the lung inflammation cells of mice exposed to cigarette smoke.

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