Effectiveness of administration haramounting leaf (*Rhodomyrtus tomentosa*) as antioxidant in preventing the damage pancreas mice (*Mus musculus* L.) after exposure of electric cigarette smoke

S Ilyas¹*, R S Tanjung²*, D Thahira² and J Wulandari²

¹ Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan
² Students of Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas of Sumatera Utara

*E-mail: syafruddin6@usu.ac.id.

Abstract. Cigarette Smoke is contain free radical was cause diabetes. Chemicals used to prevent or slow down free radical damage are antioxidants. Antioxidants in haramounting play a role to neutralize, free radicals. The study aims to determine the effectiveness of haramounting leaf extract (*Rhodomyrtus tomentosa*) as an antioxidant to histological pancreas mice (*Mus musculus* L.) exposed to electric cigarette. For research Methods, First, thirty six mice collected randomly and were assigned six groups including: control +, control -, Haramounting extract 100mg/kg for 28 days and 45 days, and Given Haramounting extract 200mg/kg exposured to smoke cigarette for 28 days and 45 days. For research started, first, preparation samples and extracts, exposure to cigarette smoke and extracticng, animal test surgery, preparation of a pancreatic surgery histology and analysis of data from weight loss, blood sugar levels and all sections of histology pancreatic organs of Mice. For research weight was weighed on the first day of treatment and weighed once a week on a regular basis. P3 is a haramounting group with a dosage of 200 mg / kg bw and exposure to smoke for 28 days only has a higher mean body weight compared to other groups. However, based on statistical tests, there is no real difference. Based on the test results obtained Ko (+) (exposed to cigarette smoke, without the extract) was not significantly different. This is because Ko (+) exposed to cigarette smoke without haramounting leaves extract as an antioxidant. *Rhodomyrtus tomentosa* have great potential as a natural antioxidant source. Haramonting leaf (*Rhodomyrtus tomentosa*) can prevent pancreatic damage seen from result of blood glucose value. Seeing the potential of haramounting leaf extract (*Rhodomyrtus tomentosa*) is good enough it is necessary to do further research for the development of haramounting leaf extract as an herbal medicine for the community.

1. Introduction
About 1 billion men in the world are cigarette smokers, 35% of them are from developed countries and 50% are from developing countries. An average of 435,000 people in the United States die from smoking-related illnesses each year causing 1 in 5 deaths. Based on the 2008 Asean Tobacco Control Report Card data, 30.1% of Southeast Asians are smokers. In Indonesia as many as 57,563,866 adults are smokers, making it the fifth highest cigarette consumer country in the world [1].

Cigarette is one of the pollutants in the form of gas containing various chemicals such as nicotine, carbon monoxide, tar and eugenol (in clove cigarettes). Cigarette smoke can affect macrophage metabolism by activating macrophages to release leukotrien B4, IL-8 and TNF-α leading to increased...
production of superoxide (O$_2^-$) and H$_2$O$_2$, also causing oxidative damage to macromolecules such as lipids, proteins, and DNA, can eliminate antioxidants. Forming free radicals such as nitric oxide (NO), nitrite peroxide (NO$_2$) in the gas phase as well as quinone (Q), semiquinone (HQ) and hydroquinone (HQ$_2$) in the tar phase [2].

Free radicals are very unstable molecular atoms (having one or more unpaired electrons), so to obtain an electron pair this compound is highly reactive and damages the tissue. Free radicals can come from various chemicals one of them alloxan. Alloxan is a cyclic urea derivative which is a potential agent widely used to cause Type I diabetes with a dosage of 150 mg / kg [3].

The chemicals used to prevent or slow down free radical damage are antioxidants. These endogenous antioxidants work to neutralize free radicals which, in the process, require exogenous antioxidants in the form of vitamins and minerals that can be obtained from food or supplements. Haramounting (Rhodomyrtus tomentosa) is native to Southeast Asia and spread in Indonesia. Camphor leaves contain steroids, terpenoids, alkaloids, phenols, flavonoids, tannins and saponins [4]. According to research conducted in vivo and in vitro [5]. In 2012, the acetone extract of kemunting leaf has a strong antioxidant activity. Based on the above description, this study was conducted to determine the effect of haramounting leaf extract (Rhodomyrtus tomentosa) against the prevention of damage to pancreas mice (Mus musculus L.) after exposure to electric cigarette smoke.

2. Methods

2.1. Adaptation of Mice

Before to the experiment, all the mice were adapted for 7 days in a cage made from a box-shaped plastic container with a little husk and ram bed sheets for cover. The body weight of the mouse was weighed at started of the study and then regularly every day until ending of the study. The design in the study used a complete randomized design test. This study used 36 mice divided into 6 groups of 6 mice each, with the combination of treatment as follows: Group I (control): not given haramounting extract and cigarette smoke. Group II: not given haramounting extract and exposed to cigarette smoke. Group III: haramounting extract 100 mg / kgBB, exposed to secondhand smoke for 28 days, Group IV: given haramounting extract 100 mg / kgBB, exposed to cigarette smoke for 45 days. Group V: given haramounting extract 200 mg / kgBB, exposed to cigarette smoke for 28 days. And Group VI: given haramounting extract 200 mg / kgBB, exposed to cigarette smoke for 45 days.

2.2. Sample preparation and preparation of extracts

According Rosmidah (2015), Sampling is done purposively. Simplicia Rhodomyrtus tomentosa is drying the leaves in a sun-protected condition for a week. After dry it is smoothed using a fining machine (grinder) up to a certain fineness size. Extraction method used is maseration with water solvent. Preparation of the extract was done by soaking simplicia for 24 hours with the composition of 100 grams simplicia in 1 liter water solution. Evaporation is then performed to separate the solvent by extracting at 60-70 °C.

2.3. Exposure of Cigarette Smoke and Extract Giving

Testing mice were fed pellets and drank in ad libitum, then given oral haramounting extract orally with doses of 100 mg / kgBW and 200 mg / kgBW each distinguished into two durations of 28 days and 45 days. On Mice Data Larasati (2010) study results illustrate that exposure to smoke per cigarette every day for 14 days has caused mild to severe lung organ damage. Therefore, in this research the exposure of secondhand smoke in group II, III and IV mice was done every day for 30 days according to Widodo procedure (2006). Provision of cigarette smoke is done with a dose of 1 stalk per group every morning after 1 hour of haramounting extract. Stages of exposure to cigarette smoke is done by first preparing the equipment used in the exposure of the smoking pump coupled with a smoking chamber. Smoking chamber has two holes, one hole on the side to be connected with the pump and the other one in front is used as ventilation / enables air exchange. Five mice from each group were inserted into the smoking chamber through the top of the smoking chamber, then closed again. One cigarette was placed in a pipe connected to the pump. Cigarettes that have been installed on the next pump an electric cigarette is mounted on the end of the hose so that cigarette smoke into the smoking chamber. Stopwatch / timer is installed to determine the time spent to spend a single stick of clove
cigarettes. Smoking chamber will be filled with cigarette smoke during the exposure of cigarette smoke and the behavior of mice can be observed in the smoking chamber. Every before giving cigarette smoke and harumonting extracts, the mice are empowered ± 5 hours to empty the stomach. Giving of cigarette smoke is done ± 1 hour after giving honey to absorbed honey first.

2.4. Surgery
On the 31st day, the mice were dissected, then the pancreas organ was fixed in 4% formalin, histologically prepared by paraffin block method with HE painting. This is done on the 31st day for the treatment effect to be noticeable.

2.5. Preparation of a Pancreas Surgery Preparation
Pancreatic incision making preparations done according to the procedure Nadzifa (2010): 1. The first stage is the coating, starting with the marking of glass objects that will be used with a file on the edge of the glass, and then soaked in 70% alcohol overnight minimum. Then the glass object is dried with a tissue and soaking in a solution of 0.5% gelatin for 30-40 sec / slide, and then dried by resting position until the gelatin coats the glass can be evenly 2. The second stage, the pancreas that has been stored in formaldehyde 4% washed with alcohol diving 2 hours, followed by washing in stages with alcohol is alcohol 90%, 95%, absolute ethanol (3 times), xylol (3 times), each for 20 minutes. The third step is a process Infiltration by adding paraffin 3 times for 30 minutes 4. Fourth stage, embedding. The ingredients along with paraffins are poured into cardboard boxes or containers that have been prepared and arranged so that no air is trapped near the material. Paraffin blocks are left overnight at room temperature then incubated in the freezer so the blocks are really hard. Cutting stage with microtom. Cutter is heated and affixed to the bottom of the block so that the paraffin slightly melts.

Holder clipped on a rotary microtome and arranged parallel to the blade or slicing microtom preceded by adjusting the thickness of the pancreas for μm cut to size, then tape the results of slices taken using a brush and put cold water to unfold and put warm water and do Selection of the best slices. The selected slices are taken with glass of the object that has been decoating then dried over the hot plate. The stages are neutralized, ie the preparations are included in xylol 2 times 5 min. 6. Phase rehydration, preparations included in graded ethanol solution ranging from absolute ethanol (2 times), 95% ethanol, 90%, 80% and 70% respectively 5 minutes. Then the preparations are soaked in aquadest for 10 minutes. 7. Staining stages, preparations dab with hematoxylin for 3 minutes or until the best color results. Then washed with running water for 30 minutes and rinsed with distilled water for 5 minutes. After the preparations included in the dye eosin alcohol for 30 minutes and rinsed with distilled water for 5 minutes. 8. Stages of dehydration, preparations immersed in ethanol with 80%, 90%, 95% and ethanol absolute (2 times) each for 5 min. 9. Clearing stage, in xylol solution 2 times for 5 minutes, then dried. 10. Stage mounting with ethylene. 11. The final results were observed by microscope, for each group of mice, photographed and then analyzed for existing pancreatic damage.

2.6. Data analysis
The data analysis used a complete randomized design (ANOVA) complete randomized design (RAL) at 95% confidence level, α = 0.5 with bootstrap analysis. All data is analyzed using SPSS 22 program.

3. Result and Discussion
Based on the results of research that has been done the results obtained research as shown in the picture and table below.

3.1. Extract Activity vs Body Weight
Mice weight was weighed on the first day of treatment and weighed once a week on a regular basis. The results of weight can be seen in the picture below. Based on Figure 1, P3 is a harumonting group with a dose of 200 mg / bb and exposure to secondhand smoke for 28 days only has a higher mean body weight compared to other groups. However, based on statistical tests, there is no real difference.
Figure 1. The weight of all treatments has no significant difference. K (+): Smoke Exposure. K (-): aquades. P1: Smoke Exposure and haramonting leaf extract 100 mg/kgBB for 28 days. P2: Cigarette Smoke Exposure and haramonting leaf extract 100 mg/kgBB for 45 days. P3: Cigarette Smoke Exposure and haramonting leaf extract 200 mg/kgBB for 28 days. P1: Cigarette Smoke Exposure and haramonting leaf extract 200 mg/kgBB for 45 days.

The greater the body mass, the more blood needed to supply oxygen and food to body tissues. The volume of blood in the blood vessels increases so the need for greater pressure on the artery walls. In addition, excess weight will also increase the frequency of heart rate and insulin levels in the blood [6]. That the decoction of *Piper crocatum* can increase the appetite of mice. Because Alkolid can also increase appetite in experimental mice performed [7].

3.2. Activity on Blood Sugar Levels
The results are data of blood glucose level, blood glucose examination done at the beginning of treatment, day 13, and last day of treatment. Differences in each blood sugar level in each treatment can be seen in Figure 2.

Figure 2. Blood Sugar Levels of all treatments were not significantly different. K (+): Smoke Exposure. K (-): aquades. P1: Smoke Exposure and haramonting leaf extract 100 mg/kgBB for 28 days. P2: Cigarette Smoke Exposure and haramonting leaf extract 100 mg/kgBB for 45 days. P3: Cigarette Smoke Exposure and haramonting leaf extract 200 mg/kgBB for 28 days. P1: Cigarette Smoke Exposure and haramonting leaf extract 200 mg/kgBB for 45 days.
Based on the test results obtained Ko (+) (exposed to cigarette smoke, without the extract) was not significantly different (p>0.05) with P1, P2, P3, and P4. This is because Ko (+) exposed to cigarette smoke without haramounting leaves extract as an antioxidant. While P1, P2, P3, and P4. Which was exposed to secondhand smoke, and given an extract, showed that the results of blood sugar levels were not significantly different (p>0.05) with Ko (-) (not treated).

Exposure to cigarette smoke causes blood sugar levels to rise. However, the treatment given apple skin extract before exposure to cigarette smoke has the ability to stabilize blood sugar levels in the body. Haramounting leaf extract as an antioxidant can inhibit pancreatic damage. One of the pancreatic constituent cells is the β cell. B cells function to release insulin. When blood glucose rises above the normal point, the release of insulin triggers the taking of glucose from the blood, thereby lowering the blood glucose concentration. When blood glucose falls below the normal point, the release of glucagon encourages the release of glucose into the blood, thereby increasing the blood glucose concentration.

Exposure to cigarette smoke causes blood sugar levels to rise. However, the treatment given apple skin extract before exposure to cigarette smoke has the ability to stabilize blood sugar levels in the body. Haramounting leaf extract as an antioxidant can inhibit pancreatic damage. One of the pancreatic constituent cells is the β cell. B cells function to release insulin. When blood glucose rises above the normal point, the release of insulin triggers the taking of glucose from the blood, thereby lowering the blood glucose concentration. When blood glucose falls below the normal point, the release of glucagon encourages the release of glucose into the blood, thereby increasing the blood glucose concentration.

Exposure to cigarette smoke causes blood sugar levels to rise. However, the treatment given apple skin extract before exposure to cigarette smoke has the ability to stabilize blood sugar levels in the body. Haramounting leaf extract as an antioxidant can inhibit pancreatic damage. One of the pancreatic constituent cells is the β cell. B cells function to release insulin. When blood glucose rises above the normal point, the release of insulin triggers the taking of glucose from the blood, thereby lowering the blood glucose concentration. When blood glucose falls below the normal point, the release of glucagon encourages the release of glucose into the blood, thereby increasing the blood glucose concentration.

Exposure to cigarette smoke causes blood sugar levels to rise. However, the treatment given apple skin extract before exposure to cigarette smoke has the ability to stabilize blood sugar levels in the body. Haramounting leaf extract as an antioxidant can inhibit pancreatic damage. One of the pancreatic constituent cells is the β cell. B cells function to release insulin. When blood glucose rises above the normal point, the release of insulin triggers the taking of glucose from the blood, thereby lowering the blood glucose concentration. When blood glucose falls below the normal point, the release of glucagon encourages the release of glucose into the blood, thereby increasing the blood glucose concentration.

Damage to insulin-producing β cells results in decreased production or insulin secretion. This condition can cause hyperglycemia conditions that lead to the occurrence of diabetes. Diabetes or high blood sugar is a disease that arises when interference occurs in the body's functions that regulate carbohydrates, fats, and proteins contained in food to produce energy. A condition when the blood glucose level is very high beyond normal levels is called hyperglycemia. Hyperglycemia usually occurs when the beta cells in Langerhans island are unable to produce insulin or have insulin deficiency. Hyperglycaemia is caused due to the failure of insulin secretion and or insulin work [9].

Free radicals from the environment due to exposure to cigarette smoke as occurs in passive smokers, causing antioxidants endogenous are no longer able to protect the body from oxidants, resulting in increased free radicals that trigger oxidative stress in pancreatic cells. Therefore, to prevent pancreatic damage due to free radicals of cigarette smoke, additional antioxidant intake is required. Antioxidants are compounds that can inhibit oxidation reactions, neutralize or capture free radicals [10].

Cigarette smoke depletes the intracellular antioxidants in the cell through mechanisms associated with oxidative stress, then oxidative stress causes lipid peroxidation that will cause cell membrane damage. Cell membranes help in and out of various substances through passive and active transport processes, as well as the enzyme attachment. The loss of cell membrane integrity causes the buildup of excess tissue fluid in cells called edema which is the phase leading to cell death (necrosis). Research on the effects of free radicals on pancreatic damage has been widely investigated by inducing chemicals such as alloxan (diabetogenic substances) as a source of free radicals that cause damage to pancreatic β cells, thus affecting the number and size of the langerhans island [11].

3.3 Microscopic Structure of Sample On Test
From the sample shows that the material contained in the existing sample into the body through fiili in the body to be delivered into the cells to be processed in delivering the juice of food. The food we eat can't be utilized by the body's cells when the food has not undergone digestive process (digest). The end result of the digestion process is the formation of molecules or food particles namely: glucose, fatty acids, and amino acids that are ready to be absorbed (absorption) by the digestive tract mucosa [8].
Figure 3. Microscopic samples use SEM (Scanning electron microscope) for *Rhomodytrus tomentosa* with 1500 x magnification

Vili has a function of expanding the absorption surface, so that food can be absorbed more efficiently. During the absorption process, food molecules will enter the bloodstream through the intestinal wall. Microscopic blood vessels or capillaries in the villi will absorb nutrients [12].

Acknowledgement

We gratefully thank the Ministry of Research, Technology and Higher Education Affairs Republic of Indonesia from 2017 creativity student program on research grant.

References

[1] Rachmadi, Afdol, Lestari, Yuniar and Yenita 2013 *Journal of Medicine Andalas* 2 6
[2] Soesilo and Novita Sari 2012 *The Influence of Noni Fruit Juice (Morinda Citrifolia L) Dosage Against Nitrile Oxidase (NO) Periontium Macrophage In Rats Exposed to Cigarette Smoke* University of Diponegeoro
[3] Mangkoewidjojo S 1988 *Maintenance, Breeding and The use of animal experiments in the Tropics* (Jakarta: University of Indonesia)
[4] Geetha KM, Sridhar C and Murugan V 2010 *International journal of PharmTech Research* 2 283
[5] Lavanya G, Voravuthikunchai SP and Towatana NH 2012 *Evidence-Based Complementary and alternative Medicine* 1
[6] Agnita and Efa 2011 *The Relationship Between Smoking and Eating Habits With Nutritional Status In Young Men* (Diponegeoro Univeristy)
[7] Safitri M and Fahma F 2008 *Journal of Biosciences* 15 45
[8] Campbell NA 2008 *Biology 8th Edition* (New York: Benjamin Cummangs)
[9] El-Soud, Neveen HA, Khalil MYH, Oraby JSFSH and Farrag ARH 2007 *Journal of Applied Sciences Research* 3 1073
[10] Murray RK, Granner DK, Mayes PA, Rodwell VW 2003 *Biokimia Harper* Edisi 25 Penerjemah: Andi Hartoko (Jakarta: EGC)
[11] Djuanda A 2011 *Skin Diseases and Sex Science Edition 6* (Jakarta: Faculty of Medicine University of Indonesia) 3
[12] Davani B 2003 *Diabetes* 53 851