



The Effect of Cigarette Smoke Exposure with Bidara (Sidr) Leaf Powder Biofilter on Glucose Levels and Pancreas Histology in Mice Diabetes Mellitus

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ABSTRACT

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Cigarette smoke free radicals are chemical compounds that are harmful to the health of the body. Diabetics have high levels of oxidative stress due to excess free radicals and lack of antioxidants, so antioxidants are needed to stabilize free radicals in the body. The results of previous studies showed that 0.9 gram bidara (Sidr) leaf powder biofilter was able to capture free radicals in cigarette smoke. The purpose of this study was to examine the effect of exposure to cigarette smoke with a biofilter of bidara leaf powder on blood glucose levels and pancreatic histology in diabetic mice. The sample in this study used five treatment groups, previously induced with streptozotocin to become diabetic. Exposure to cigarette smoke was carried out for 3 weeks, every week checking blood sugar. After that the mice were dissected to observe the histology of the pancreas. Statistical results using One way ANOVA showed that there was an effect of exposure to cigarette smoke with a biofilter made from bidara leaf powder on blood glucose levels and pancreatic histology in diabetic mice.

1. INTRODUCTION

Cigarettes are familiar to most people. There are many pros and cons that discuss the problem of consuming cigarettes. The Indonesian standards body in its press stated that cigarettes are dangerous for the consumers and are considered to be the biggest problem because of the content of free radicals that can harm the health of the human body. Smoking is a means for people to establish friendship and strengthen brotherhood. Smoking can also encourage people who don't know each other to get to know each other more personally. Smoking not only provides individual benefits, including a sense of comfort, pleasure, calm, stimulates creative thinking, encourages imagination wandering and at certain times psychologically makes a person not feel alone because of smoking.

Cigarettes are one of the controversial products because of the pros and cons that arise in society. The many adverse effects such as the effects of addiction, the health problems caused to the increasing death rate due to excessive cigarette consumption are being challenged by people in the world. In fact, every May 30, the world community celebrates No Tobacco Day as a form of their protest against tobacco which is the main ingredient of cigarettes. On the other hand, from an economic point of view, there is no doubt about the prospect of the cigarette industry because it is very promising, especially regarding the amount of foreign exchange income to the country and providing employment opportunities for many people [1].

Smoking can cause changes in the structure and function of the airways and lung tissue. Cigarette smoke will accelerate the decline in lung function and have a greater effect than dust on the respiratory tract [2].

The biofilter composite membrane has a good ability to capture free radicals in cigarette smoke. Bidara leaf powder biofilter membrane 0.9 grams is proven to be effective in capturing free radicals of cigarette smoke [3].

Diabetes Mellitus (DM) is a group of metabolic diseases characterized by high blood glucose levels due to defects in insulin secretion, insulin action, or both [4]. The incidence of type 2 diabetes is increasing worldwide [5] and on a global scale, DM is one of the five main diseases that cause death [6]. Diabetes mellitus affects nearly 150 million people worldwide, and by 2025, the number of people with diabetes is expected to increase to 300 million.

Current research proves that oxidative stress plays a role in the development of DM. Oxidative stress is a condition caused by increased production of free radicals (ROS, reactive oxygen species) beyond the protective ability of natural antioxidants. Chronic hyperglycemia has been shown to increase oxidative stress which results in a reduced number of glucose transporters (GLUT) and has an impact on increasing insulin resistance, weakening insulin signaling and disrupting insulin secretion by pancreatic beta cells [7].

Smoking is one of the most important modifiable risk factors associated with diabetes mellitus [8]. Exposure to cigarette smoke is associated with vascular damage, endothelial dysfunction and activation of the blood coagulation cascade [9], so it can be argued that the combined harmful effect of increasing blood glucose with smoking accelerates vascular damage in people with diabetes who smokes. It is widely accepted that smoking substantially increases the risk of micro and macrovascular complications in patients with type 2 DM [10]. Quitting smoking substantially reduces this risk [11]. Even reducing exposure to secondhand smoke is a must for public health, even more so

for patients with DM, as reflected in most clinical guidelines [12].

Many studies have been conducted to reduce oxidative stress in DM patients by consuming foods that contain high antioxidants. But research that is specific to objects that are often exposed to cigarette smoke is rarely done. Moreover, there are very few studies on efforts to minimize the free radical content of cigarette smoke.

It has been proven that people with diabetes mellitus have higher levels of oxidative stress than normal conditions [13]. Oxidative stress is a condition in which the body has an excess of free radicals and a lack of antioxidants [14], so that by giving antioxidants it can bind to free radicals and can reduce the risk of diabetes and is useful in reducing insulin resistance. Bidara leaves have a strong antioxidant content [15, 16]. So that the use of bidara leaf powder as a basic ingredient in cigarette smoke biofilters is expected to be able to reduce the risk of free radicals from excess chemical content and turn them into free radicals that contain natural antioxidants as needed by DM sufferers as in the research objectives.

In summary, the results of this study indicate that the biofilter of bidara leaves can reduce blood glucose levels and can repair damage to the histology of the pancreas in mice with diabetes mellitus.

2. MATERIALS AND METHODS

Study of the effect of exposure to cigarette smoke with a biofilter of bidara leaf powder on glucose levels and pancreatic histology in mice with diabetes mellitus is an experimental study with 5 treatments and 4 replications. The treatment used is (C-) normal mice without exposure to cigarette smoke, (CD-) diabetic mice without exposure to cigarette smoke, (CD+) diabetic mice with exposure to cigarette smoke without a biofilter, (DBB) diabetic mice with exposure to biofilter cigarette smoke from bidara leaf powder, (NBB) normal mice exposed to cigarette smoke biofilter bidara leaf powder.

Making a biofilter is done by mixing bidara leaf powder with polyethylene glycol (PEG) adhesive, then stirring with a spatula until homogeneous. The composite was molded with a hose with a diameter of 0.7 mm and a height of 2 cm, and allowed to dry. After drying, the biofilter composite was removed from the mold and baked at 105°C for 20 minutes.

In these 5 treatments, testing was carried out for 21 days with a volume of smoke of 10ml per exposure and the administration of cigarette smoke for 15 exposures with an interval of 1 minute for each administration of cigarette smoke. Mice were previously induced by streptozotocin at a dose of 30mg/kgBW to develop Diabetes Mellitus. The steps in this research are as follows:

1. Experimental animals are put into cages.
2. The biofilter is attached to the base of the cigarette.
3. Installed a 10 ml hose and syringe for cigarette smoke.
4. Cigarettes were burned and cigarette smoke was exposed to experimental animals for 15 times with an interval of 1 minute.

For the sampling of experimental animals, the blood glucose levels and the histology of the pancreas were observed as follows:

1. Blood glucose levels were taken on days 7, 14 and 21.
2. Neck dislocation was performed on experimental animals to avoid stress conditions.

3. Pancreatic organ harvesting for histological observation.

Briefly, the research steps can be seen in Figure 1.

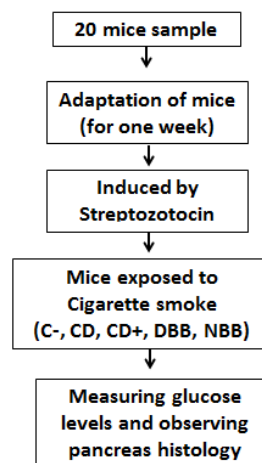


Figure 1. Research flow

2.1 Determination of blood sugar levels in mice

Determination of mice blood sugar levels was carried out on day 4, starting from the day of injection with streptozotocin (STZ), then the mice were exposed to cigarette smoke with a biofilter and without a biofilter for 21 days as treatment. Blood sugar levels were also checked once a week during exposure. The way to check blood sugar levels is to rub a cotton swab that has been given alcohol around the tail of the mouse, cut off a little at the end and pull it slowly. Touch the drop of blood on the test strip that has been installed on the advantage glucometer (Roche) to cover the surface of the reagent on the test strip, where the blood glucose level will be read within 26 seconds.

2.2 Preparation of preparations and observing the histological picture of the pancreas

Mice that had been treated with exposure to cigarette smoke for three weeks had previously been fasted for a day, then dissected and the pancreas was removed and preparations were made as follows:

1. The first stage is coating, starting with marking the glass object to be used with a glass file on the edge area, then soaking it with 70% alcohol for at least overnight, then drying the object glass with a tissue and soaking it in a 0.5% gelatin solution for 30-40 seconds per slide, then dried in a reclined position so that the gelatin coats the glass evenly.
2. The second stage, the pancreas organ that has been stored in a 10% formalin solution is washed with alcohol for 2 hours, then followed by a gradual washing with alcohol, namely with 90%, 95% absolute ethanol (3 times), xylol (3 times) each for 20 minutes.
3. The third stage is the infiltration process, namely by adding paraffin 3 times 30 minutes.
4. The fourth stage, Embedding, the material along with paraffin is poured in a container that has 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 until the blocks are completely firm.

5. Cutting stage with a microtome, the cutter is heated and affixed to the block so that the paraffin melts a little. The holder was clamped on a rotary microtome and arranged by adjusting the thickness of the slices, then the pancreas was cut to a size of 6 m, then the sliced ribbon was taken using a brush and put in cold water to open the folds, then put into warm water and the best slice recovery was performed. The selected slices were taken with a glass object that had been cut and then dried on a hot plate.
6. Deparaffinization stage, where the preparation is put into xylol 2 times 5 minutes.
7. In the Rehydration stage, the preparations were put in a graded ethanol solution starting from absolute ethanol (2 times), 95% ethanol, 80%, and 70% each for 5 minutes, then the preparations were immersed in distilled water for 10 minutes.
8. Staining stage, the preparations are dripped with Hematoxylin for 3 minutes or until the best color results are obtained, then washed with running water for 30 minutes and rinsed with distilled water for 5 minutes, after that the preparations are put into eosin alcohol dye for 30 minutes and rinsed with distilled water for 30 minutes.
9. The next step is dehydration by adding preparations in graded ethanol series from 80%, 90%, 95% to absolute ethanol (2 times).
10. Clearing stage is done by inserting the preparation in xylol 2 times for 5 minutes and dried.
11. The last stage of gluing with ethylene. The results were observed under a microscope and photographed, then observed and recorded the level of damage to the pancreas organ, from each treatment group.

- C-: Control Without Exposure to Cigarette Smoke;
- CD-: Diabetes Control Without Exposure to Cigarette Smoke;
- CD+: Diabetes Control Exposed to Cigarette Smoke Without Biofilter;
- DBB: Diabetes Exposed to Cigarette Smoke with Bidara Leaf Biofilter;
- NBB: Normal Exposure to Cigarette Smoke with Bidara Leaf Biofilter.

The value of blood sugar levels from week to week in the CD- and CD+ groups experienced the highest condition, while in the DBB group blood glucose levels decreased by 46% to a low or normal state. This is because the effect of giving a biofilter made from bidara leaf powder contains antioxidants so that it can neutralize free radicals contained in kretek cigarette smoke. Where the particle content of the bidara leaf powder biofilter that enters the body along with cigarette smoke particles, namely the antioxidant content of 20% in the bidara leaf powder biofilter can neutralize free radical compounds in cigarette smoke.

The results of the one way ANOVA analysis showed that there was a significant effect of exposure to cigarette smoke with a biofilter of bidara leaf powder on blood glucose levels (Table 1).

Table 1. Anova analysis of blood glucose level data

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	24328.175	4	6082,044	5.476	.007
Within Groups	15549.952	14	1110,711		
Total	39878.126	18			

2.3 Pancreatic damage determination technique

Microscopic observations of pancreatic histology were carried out by looking at the diameter of the islets of Langerhans using a compound microscope at 400X magnification. Then measured the diameter of the islets of Langerhans and the mass of beta cells in the islets of Langerhans, namely to determine the degree of insulinitis damage to the pancreas gland, by comparing the average graph between the diameter of the islets and the mass of beta cells from all treatment groups.

3. RESULT AND DISCUSSION

Effect of Cigarette Smoke Exposure with Bidara Leaf Powder Biofilter on Blood Glucose Levels in Mice (*Mus musculus*). (See in the Figure 2).

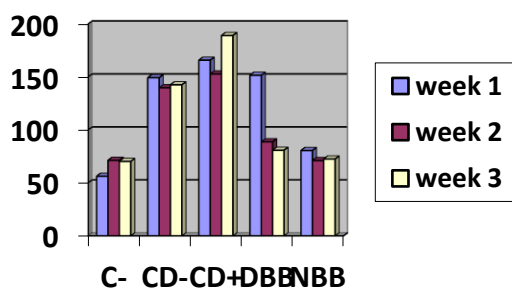


Figure 2. Mice blood glucose data from 5 treatments

Clove cigarette smoke contains 7 types of free radicals that can be detected by Leybold Haracus ESR (Electron Spin Resonance), namely Hydroperoxides, CO², C, Peroxy, O₂, CuGOx, CuGeO₃ [17]. Free radicals arise due to incomplete combustion of kretek cigarette smoke, free radicals are chemical compounds that are reactive (unstable) because they do not have paired electrons in their outer shell, so they will attack other compounds including cells in the body to stabilize themselves as shown. on the first interaction. While the second reaction is applied as in the use of cigarettes with a biofilter, the antioxidants contained in the biofilter will work to stabilize free radicals by donating their outer electrons to free radicals, so that free radicals are no longer reactive and antioxidants remain stable because they have double groups as a result, conditions cells and organs in the body in a safe condition without damage by free radicals.

Increased blood glucose or hyperglycemia is one of the early signs of disturbances in carbohydrate metabolism. Carbohydrate metabolism is influenced by insulin action. Insulin maintains the balance of glucose in the blood and acts to increase the uptake of glucose by the body's cells to be converted into energy. Failure of the body to produce insulin or insufficient amount of insulin will cause glucose not to enter the cells for metabolic processes, so that glucose in the blood will increase or hyperglycemia occurs [18].

Cell is a functional unit of life which is a living thing or a constituent of living things which is composed of protoplasm which is covered by a thin membrane and is able to reproduce both sexually and asexually. Normal cells are round in size and

have a cell nucleus in it. When cells are attacked by free radicals, they will experience DNA and mitochondrial damage so that ATP production decreases and causes cells to lack oxygen. Oxidative stress is a condition in which cells have excess free radicals and lack of antioxidants, causing cell damage. Free radicals can damage all cells in the body, including cells which are pancreatic endocrine cells [19], cells have an important role in the pancreas, 70% of the cells in the pancreas are cells and the rest are, and f cells. [20]. cells function to produce insulin which plays a role in converting glucose in the blood into energy, if insulin in the pancreas cannot be produced by cells, blood glucose levels in the body accumulate which is called a condition (hyperglycemia) so that the body becomes energy deficient as in people with diabetes mellitus [21].

3.1 Effect of cigarette smoke exposure with bidara leaf powder biofilter on histological pancreas of mice (*Mus musculus*)

Histological observations of pancreatic tissue were carried out with paraffin blocks using the -staining method Hematoxylen-Eosin. Islets of Langerhans is a collection of endocrine glands scattered throughout the pancreas, shaped like islands and is traversed by blood capillaries. On HE staining, the islets of Langerhans are paler than the surrounding acinar gland cells so that the islets of Langerhans are easily distinguished. DM patients will experience morphological changes on the islets of Langerhans, both in number and size [22].

In Figure 3 The condition of blood glucose levels in people with diabetes mellitus becomes high if insulin in the pancreas cannot be produced normally. Insulin is produced by pancreatic cells in the islets of Langerhans which are endocrine cells, insulin in the body works to convert glucose in the blood into energy so that energy needs are fulfilled. The results of the study on exposure to cigarette smoke with biofilter of bidara leaf powder showed a correlation between the increase in blood glucose levels and damage to the histology of the pancreas, most of the groups showed that if the glucose level was high, the level of damage to the pancreas was also high. According to the mechanism, cigarette smoke containing free radicals can damage cells in the pancreas, namely by damaging DNA and reducing mitochondrial oxygen conditions, resulting in a lack of oxygen in cells and impaired ability to produce insulin. Giving a biofilter is a safe way to neutralize free radicals in cigarette smoke, namely with antioxidants contained in the main ingredients of the biofilter, antioxidants stabilize free radicals by donating their outer electrons to free radicals so that the nature of free radicals is no longer reactive and antioxidants remain stable. insulin production in cells continues to run normally.

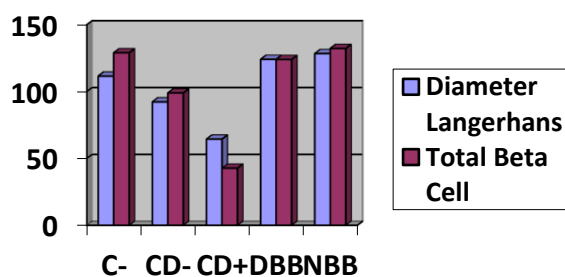


Figure 3. Levels of Pancreatic Histology Damage from 5 treatments

In adult mice, the pancreas contains approximately 1-2% islets of Langerhans with a diameter between 100-200 m [23]. Rats and mice are one family animal that has the same organ, structure and size of organs.

Beta cells secrete 70% insulin from the endocrine cells of the islets of Langerhans and are located in the center of the islets of Langerhans. Beta cells have large, round nuclei. The histology of the pancreas in group C- from four repetitions obtained an average value of the diameter of the islets of Langerhans of 111.57 m while the number of beta cells was 129, so it can be concluded that there was no pancreatic damage in the C- group as the control group.

Histology of the pancreas in the CD- and CD+ groups found necrosis in the islets of Langerhans. Another possibility of histology on CD- and CD+ is that the cell nucleus is destroyed while leaving fragments of chromatin scattered within the cell. So that in some circumstances the cell nucleus loses the ability to absorb the color of HE so that no pancreatic beta cells are seen.

Beta cells have a role in secreting insulin 70% from the endocrine cells of the islets of Langerhans where insulin will convert blood glucose into glycogen, so that if the cells in the pancreas are damaged and cannot produce insulin, glucose levels in the body will accumulate and the energy expended is reduced. This condition is called excess blood sugar levels.

The CD- and CD+ treatments showed that the mass of pancreatic beta cells and the diameter of the islets of Langerhans were abnormal when compared to histology in the C- group, but the CD+ group appeared to have more severe damage than the CD-, this is due to the effect of cigarette smoke given to the CD+ group. However, after being exposed to cigarette smoke with a bidara leaf biofilter, the DBB and NBB treatments showed that the histological picture of the pancreas was not damaged.

In general, exposure to cigarette smoke worsens insulin resistance, this is caused by the active chemical in cigarettes, namely nicotine which causes a decrease in insulin release due to the activation of catecholamine hormones, negative effects on insulin action, and disorders of pancreatic cells [24]. Nicotine affects insulin secretion through nAChRs on pancreatic cells. Insulin secretion can be regulated by an endogenous pancreatic ganglionic mechanism. Several in vitro studies have shown that nAChRs are present in the ganglion in the pancreas and affect insulin secretion through complex intraganglionic mechanisms. Not only long-term exposure to nicotine but also short-term exposure results in decreased insulin secretion. On short-term exposure to nicotine concentrations of more than 1µmol/L inhibit insulin release. These findings indicate that functional nicotinic receptors are present on pancreatic islets and cells and that the nicotine moiety negatively affects pancreatic cell function [25].

From this explanation, it is also suspected that the bidara leaf biofilter can also minimize the nicotine content in cigarette smoke (needs further research), it is shown that exposure to cigarette smoke with a bidara leaf biofilter can minimize damage to pancreatic histology.

4. CONCLUSIONS

From the results above, it is concluded that the use of biofilter of bidara leaf powder affects blood glucose levels and pancreatic histology in Diabetes Mellitus (*Mus musculus*) mice. Blood glucose levels of mice with diabetes mellitus

decreased after exposure to cigarette smoke with a biofilter, as well as the number of beta cells and the diameter of the islets of Langerhans using a biofilter was greater than that of mice with diabetes mellitus exposed to cigarette smoke without a biofilter.

These results indicate that cigarette smoke, which has been considered harmful to health, can actually be minimized by using a bidara leaf biofilter.

This research needs to be continued by testing other organs such as MDA levels, sperm quality, etc., to see the effect of cigarette smoke with a biofilter made from bidara leaves on diabetes mellitus sufferers. This research can also be developed to look for natural ingredients as biofilters in order to minimize free radicals in cigarette smoke.

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ETHICAL APPROVAL

This study and all of the experimental procedures in involving animals were conducted in accordance with the animal care guidelines of the State Islamic University of Maulana Malik Ibrahim Malang, Indonesia.

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