The effects of soursop leaves extract on the islets of \textit{langerhans} of the male \textit{wistar} rats exposed to high fat diet and \textit{streptozotocin}

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Abstract. The extract of soursop leaves possesses an ability to function as an antioxidant and is potentially capable of being radical scavenging, which allows it to reduce oxidative stress in the pancreatic beta cell and stimulate the endogen regeneration of the islets of \textit{Langerhans}. This research aimed to analyse the effect of the extract of soursop leaves on the diameters of the islets of \textit{Langerhans} within the male \textit{Wistar} rats exposed to high fat diet and \textit{streptozotocin}. Research design based on the concept of post-test only control group design. The total number of samples in the research was 50 male \textit{Wistar} rats, consisting of 2 control groups and 3 groups of different dosage treatments of soursop leaves extract. Several findings were discovered, in which there is an effect on the diameters of the islets following the utilization of 150 mg/kg extraction of soursop leaves, and a significant positive correlation between the extract dosage of soursop leaves and the diameters of the islets (r=0.462;p=0.005). It can be concluded that the higher the extract dosage is utilized, the more enhancement shown by the diameters of the islets, indicating a rapid cell regeneration performed by the pancreatic beta cell.

1. Introduction

The prevalence of diabetes mellitus (DM) type 2 has been increasing in recent years. Judging from the fact mentioned above, it is predicted to be increasing from 2010 until 2030 by 54% of the total number of people in the world [1]. It is predicted to be increasing from 2010 until 2030 by 69% in developing countries and 20% in developed countries [2]. Indonesia, on the other hand, sits on the seventh largest country with most diabetes patients in the world [3]. Ninety percent of the diabetes patients in 2010 was diagnosed to have diabetes mellitus type 2 [4]. Diabetes mellitus type 2 is one of complex multifactorial metabolic diseases, which is affected by lifestyle factor associated with obesity. This disease is indicated by the appearance of high level of blood sugar (hyperglycemia). There are two metabolic defects occurring in the diabetes mellitus type 2, which are as follows: (1) The decrease of peripheral response toward insulin secretion (insulin resistance) and (2) dysfunction and decrease of pancreatic beta cell mass which is in the form of weak insulin secretion caused by insulin resistance [5]. Several researches have shown that a therapy applied on the patients often fail to take effect as there is a dysfunction and mass decrease of the pancreatic \beta cell. Patients with dysfunctional pancreatic \beta cell are associated with poor glycemic control of the patients themselves following some certain continuous medications for 4 years [6].
A research experimented on Wistar rats with diabetes shows that there are morphological transformation and morphometrics in the pancreatic β cell. The transformations are in the forms of degenerations and necroses, as well as shrunken islets of Langerhans; β cell degranulation; β cell pyknotic nuclei; drastic density decrease of Islets of Langerhans, the occurrence of weighty vacuolation; the reduced number of islets of Langerhans in terms of diameters, area, and volume; and the reduced number of β cell. In the case of diabetes mellitus type 2, the insulin undergoes resistance and pancreatic β cell dysfunction, both of which worsen the hyperglycaemia situation. The mechanisms having been mentioned above play significant roles in the therapy. A therapy which utilizes anti-diabetic medication is yet to be focused on the recovery and restoration of the reduced mass of pancreatic of β cell, which is one of metabolic defect characteristics of DM type 2. The reduced mass of pancreatic of β cell is indicated by a shrinkage or decrease of diameters of islets of Langerhans [7].

Soursop (Annona moricata L.) is a green plant deriving from family Annonaceae, which can widely be found in Indonesia for traditional medications. The extract of metabolite from this plant is pharmacologically beneficial, particularly in terms of anti-inflammations, anti-ulcers, anti-helminths, anti-bacteria, as well as free radical scavenging. Soursop is ethno-medicinally used as diuretic, medication for hepar disorder, anti-parasite, low blood pressure stimulator, anti-hyperglycemia, and anti-diabetic in several countries such as Amazonia, Brazil, Malaysia, Peru, Togo, and West India. The parts of this plant which includes seeds, leaves, barks, and roots are generally beneficial in the form of extract and sometimes in the form water, powder or even raw fruits [8]. The extract of soursop leaves is known to have been able to restore several dysfunctions in the form injury in the pancreatic β cell. A research experimented on Wistar rats suffering diabetes mellitus type 2 shows that the extract of the soursop leaves is capable of reducing oxidative stress in the pancreatic β cell [9]. In addition, another research also revealed similar results on the pancreatic cell following the STZ-induced rats being fed the extract of soursop leaves, resulting to the β cell regenerating in the Islets of Langerhans in the pancreases [6]. Medication using the extract also shows significant increase of the anti-oxidant enzyme activity which has high concentration of non-enzymatic anti-oxidant. Both situations play important roles during the situation of oxidative stress [10,11]. Other researchers regarding the benefits of the extract from the soursop leaves to the DM type 2 are still limited, due to which a further research related to what soursop leaves can do against the DM 2 still needs to be conducted for further investigations.

2. Research Methods
This research was designed based on experimental laboratory research in which the concept of randomized post-test only control group design was applied. The soursop leaves were gathered in simplicial forms from Balai Materia Medica, Batu, East Java. They were then extracted at the phytochemical and pharmacognosy laboratory, Faculty of Pharmacy, Airlangga University. The extraction process was initiated by grinding the simplicial forms to get powder, which was then continued by soaking it in ethanol for 24 hours. Subsequently, a segregation process was performed in order to obtain separate powder and liquid which was then evaporated. The extract was then blended with Na-CMC (Carboxyl Methyl Cellulose) prior to being fed into the experimental rats.

The experimental objects were 5 groups of rat, consisting of 50 2-3-month old, Wistar strain, male white rats (Rattus norvegicus) which were 100-170 gram and physically healthy. The experimental rats were acclimated for 7 days and placed in random groups, which where 2 control groups (negative controls, C1; and positive control, C2) and 3 treated groups (T1, T2, T3) respectively. Prior to being exposed to high fat diet and streptozotocin (STZ), all the rats in their respective groups were examined to determine the level of fasting blood glucose 1 (FBG1). The control group 1 (C1) was put under standard diet (4% converted energy from fat) from day 1 until the last day of research, while the groups C2, T1, T2, and T3 were put under high fat diet (29% converted energy from lard) from day 1 until day 36 which was then continued to standard diet until the last day of research. The groups C2, T1, T2, and T3 were given intraperitoneal STZ 27.5 mg/kg injection on day 29. Following the
injection, all the rat groups were examined in order to determine the level of FBG2. The groups T1, T2, and T3 were subsequently given the extract of soursop leaves from day 37 until day 58. The group T1, T2, and T3 were given the extract of soursop leaves as much as 50 mg/kg, 100 mg/kg, and 150 mg/kg respectively.

The experimental rats were anesthetized by using ketamine HCL as much as 60 mg/kg through intramuscular method. Following the anaesthesia, an incision and decapitation were conducted in order to obtain the pancreatic organ out of the abdomen of the immolated experimental rats. Having been drawn out from the abdomen, the pancreatic organ was put under fixation and parafinization process which was then followed by colouring process by using haematoxylin eosin. The diameters of islets of Langerhans were then measured following the colouring process. Symmetrical diameters of islets of Langerhans were directly utilized, while the others from the longest to the shortest which are not symmetrical were summed up and divided by two.

Comparative test between the groups was conducted through one-way Anova test (α=0.05), while the correlation test between the dosage of the soursop leaves extracts and the diameters of islets of the Langerhans was performed through Pearson test (α=0.05)

3. Results
The experimental result of the Pearson correlation between the extract of the soursop leaves and the diameter of the islets of Langerhans showed there is a significant positive correlation between both of them (r=0.462; p=0.005). This finding indicates that the higher dosage of the extract of soursop leaves results in more enhancement the diameters of the islets of Langerhans. There is an effect on the diameters of the islets following the utilization of 150 mg/kg extraction of soursop leaves (Figure 1).

![Figure 1. The Diameters of Islets of Langerhans in each group. C1 and C2 is control groups; T1, T2 and T3 is treatment group. The red arrows indicate the islet of Langerhans.](image)

4. Discussion
Pharmacology activities having been conducted in several previous researches in regards to the extracts of soursop leaves showed that there are anti-hyperglycaemic activities, integrity restoration activities taking place in the islets of Langerhans within the pancreases, hepatoprotective activities, antinociceptive and anti-inflammation activities, anticancer, anti-herpes simplex virus, anti-depression, anti-hyperlipidemia, anti-microbes, antioxidant activities, and anti-hypertension [8]. The pancreatic beta cell is capable of undergoing regeneration through the existing beta cells replication or via neogenesis stem cell and cell progenitor inside or outside the islets of Langerhans. The neogenesis can also derive from other types of cell, such as the alpha cell, the delta cell, epithelium ducts, acinar
cell, and centrocinar cell. All these processes occur depending on the extra-pancreatic hormonal activator, growth hormone, etc. [12].

It was discovered in the research that there is a significant relation between the extract dosage of soursop leaves and the diameters of islets of Langerhans, in which the more the extract of the soursop leaves is given, the larger the diameters of the islets of Langerhans become. The enlargement of islets of Langerhans is associated with the regeneration development of the pancreatic beta cell. The results acquired in this research correspond to those of the research having been performed by Adeyemi in 2010, showing significant developments in terms of number, diameters, and the beta cell volumes in the Islet of Langerhans within the pancreases of the rats having been given and treated with the extracts of soursop leaves. It was shown through the research that there is β cell regeneration in the pancreatic islets of Langerhans of the rats having been treated with soursop leaves extracts [6,13].

The capacity of the intracellular antioxidant of the pancreatic beta cell is considerably low, due to which the beta cell is vulnerable against oxidative stress. The oxidative molecule is an important mediator which can induce glucotoxicity in the pancreatic beta cell of the DM type 2 patients. The regeneration mechanism of the pancreatic beta cell in the DM type 2 is enhanced by the antioxidant generated by the extracts of soursop leaves. The mechanism initiates through catalyzing oxidations of any electrons from free radicals such as H$_2$O$_2$ by which the oxidative stress in the pancreatic beta cell is reduced. The extract of soursop leaves possesses both enzymatic and non-enzymatic antioxidant activities. The enzymatic antioxidant activities are in the forms of catalyse, superoxide, dismutase, peroxidise, polyphenol oxidase, and glutathione reductase, which can give protective effects to the injured tissue ROS. Non-enzymatic antioxidant activities are in the forms of extraction of soursop leaves such as ascorbid acid, tannin, flavanoid, phenolic, vitamin C, caretoniod, lycopene, a-tocopherol, and reduced glutathione, which possess scavenging and protective effects against ROS. In addition, this extract is capable of inhabiting the formation of ROS [11,13].

The enzymatic antioxidant in the soursop leaves mediate protections against the apoptosis of pancreatic beta cell, prevent pancreatic beta cell degeneration, and stimulate the endogene regeneration of islets of Langerhans. In addition to being a non-enzymatic antioxidant, the regeneration mechanism performed by the flavonoid is also processed through the interaction with protein and modulation of intracellular cascade and gene expression respectively. Flavonoid can interact with the signalling insulin path of the other cells from which the function of beta cell, insulin secretion, and beta cell proliferation can be modulated [14,15].

The results of other researches also showed the occurrence of morphological transformation in the pancreases of the rats. Moreover, an occurrence of significant decrease in the blood glucose, MDA, and NO was also discovered. It was also discovered that there is a significant increase in the enzymatic antioxidant activities as well as in the pancreases. It was shown in the research that the extract of soursop leaves possess protection effects to the integrity of pancreatic beta cell [16].

5. Conclusions
The 150 mg/kg of soursop leaves extraction contributes significant effects to the diameters of islets of Langerhans of the male Wistar rats which are exposed to the high fat diet and STZ. The higher the dosage of soursop leaves effects is given, the more the diameters of the Islets of Langerhans develop. It is recommended that another in-depth research regarding the diabetes mellitus type 2 and the effects of the extract of soursop leaves be conducted in the future in order to identify its benefit to the other organs.

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