Histological Change of Pancreatic Islands Following Administration of *Saurauia vulcani* Korth Leaves Extract in Alloxan-induced Diabetic Mice

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Abstract: The importance of plant-based ingredients in traditional diabetes therapy has been well known. In North Sumatra, one of the local species used was *Saurauia vulcani* Korth. plant (pirdot). The study was intended to investigate the effect of *S. vulcani* leaves extract on the histology of pancreatic island in alloxan-induced diabetic mice. Thirty male mice were divided into three groups of ten mice, namely: Normal Mice (NM) group, Non-Treated Diabetic Mice (NTDM) group, and Treated Diabetic Mice (TDM) group which is given 200 mg/kg bw leaves extract of S. vulcani daily for 21 days by oral gavage. Diabetes was induced in mice by alloxan injection. Blood sugar levels (BGL) and body weight were measured on day 0 (baseline, time of alloxan injection), 72 hours, and on 7, 14, and 21 days after diabetes induction. On the 21st day, the mice were sacrificed and the pancreatic organ was isolated and 8 micron tissue sections were made and stained with Haematoxylin and Eosin. The results showed that BGL in NTDM group increases until the end of the experiment, while in TDM it increases first but then decreases to the level that similar to NM control group which is relatively stable around 100 mg/dl during the study. In histological examination, irregular island forms, loose cell population, and cells with pyknotic nucleus were found, as an indication of a damage to the pancreatic island. These changes were not found in NM groups and TDM groups. Our result indicated that *S. vulcani* extract promotes cell regeneration in pancreatic islands. The results indicated that S. vulcani leaves extract promotes cell regeneration in pancreatic islands.

1 INTRODUCTION

Diabetes (Diabetes mellitus, DM) is a metabolic disease with a high prevalence rate. Globally, the number of people with diabetes in the year 2000 was 171 million (2.3%), this figure is predicted to increase to 368 million (4.4%) in 2030 (Wild, 2004). The main characteristic of diabetes is high blood glucose levels (BGL). High BGL can be caused by impaired insulin secretion in the pancreas, impaired insulin action in pheripheral tissue, or a combination of both. The continued effects of chronic diabetes can cause damage, dysfunction, and failure in various organs (Saikh, 2016). The treatment of diabetes includes injecting insulin, using drugs that can increase pancreatic secretion activity, and drugs that can increase tissue response to insulin. Diabetes medications such as sulfonylurea analogues, alpha-glucosidase inhibitors, and biguanides have side effects, such as lowering BGL to a very low levels (hypoglycemia effect), hepatotoxicity, lactic acidosis, and diarrhea. (Fowler, 2007). Therefore, there is a need to look for new agents that meet the requirements as an ideal antidiabetic compound. The candidates compound which is ideal as an anti-diabetic drug is an agent that can reduce blood sugar levels and simultaneously increasing the pancreatic beta cell population. One strategy that can be done is to test the antihyperglycemic activity and the effect on pancreatic organ of plants extract that have traditionally been used by people as a drug for diabetes.

In one province in Indonesia (North Sumatra province), the leaves of pirdot plants (*Saurauia vulcani* Korth.) have long been used as a drug for diabetes (Situmorang., 2015). This article reports the effect of *S. vulcani* leaf extract on the histology of pancreatic island in alloxan-induced diabetic mice.

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2 MATERIALS AND METHODS

2.1 Preparation of Plant Extract

The leaves of S. vulcani were obtained from the forest of Sintongmarnipi, Toba Samosir, North Sumatra Province, Indonesia. The leaves are picked, cleaned, and separated from the petiolus, then dried in open air for about one week. The dried leaves are powdered into fine flour using an electric blender. The extraction process was carried out by maceration method using ethanol as a solvent. The maceration results obtained are then filtered and the solvent was evaporated using a rotary evaporator at 40 ° C (Sitorus, 2015).

2.2 Experimental Animals

The experimental animals used were mice (*Mus musculus* L.), male, healthy, aged ± 3 months, weighing 25-30 grams. Animal experiments were obtained from and maintained in animal cages Department of Biology FMIPA USU Medan. Mice are kept in a special cage for experimentation, given pellet feed and drinking water (tap water) on an ad libitum basis. The animals were adjust to a new environment by kept them in the cage for two weeks before experimentation.

Induction of diabetes in mice was carried out by giving a single injection of alloxan (100 mg/kg body weight) intraperitoneally. Mice were fasted 12 hours before the injection. Selection of mice was done 72 hours after the alloxan injection. Only mice that have a blood glucose level (BGD) >200 mg/dl are included in the experiment.

The experiment was designed in completely randomly designed (CRD).Thirty diabetic mice (BGL >200) were randomly assigned to three treatment groups of 10 mice each. The treatment groups were: normal mice as control (NM groups), Non-Treated Diabetic Mice (NTDM groups), and Treated Diabetic Mice (TDM groups) that received S. vulcani leaves extract 200 mg/kg body weight. The treatment was given daily by oral gavage for 21 days.

2.3 Blood Glucose Level and Body Weight

Blood glucose level was measured on blood removed from the tail of the mouse. BGL determination is performed with a glucometer (EasyTouch). BGL measurements were carried out on day 0 (baseline) at the time of alloxan injection, then 72 hours after the injection, and then on the 7th, 14th, and 21st days. About 5 mm the tip of the mouse's tail was cut with scissors, then the blood was left to drip to a special test strip for glucose and after about 10 seconds the BGL number will appear on the glucometer screen.

The body weight was weighed on the same day as the BGL determination day by using an electronic scale.

2.4 Tissue Preparation

Animals were sacrificed at the end of the experiment. Pancreatic organs were isolated and fixed in formaldehyde. Tissue section was prepared by the paraffin method. Eight μm thick tissue sections were stained with HE and used for pancreatic island observation.

2.5 Statistic Analysis

Parametric data were analyzed with ANOVA, the Duncan 'test post hoc was apllied for all ANOVA significant result. The differences between means were considered significant at $p<0.05$.

3 RESULT AND DISCUSSION

Results from ANOVA showed that at the time of alloxan injection (baseline, day 0) blood glucose level (BGL) between treatment groups was not significantly different ($p>0.05$). In the group of normal mice (NM) the BGL baseline was around 100 mg/dl. This level was stable in NM group until the end of the experiment.

Significant differences in BGL between treatments began to be seen in observations 72 hours after alloxan injection, whereas mice injected with alloxan increased their blood glucose levels up to> 200. In figure 1, BGL in the NTDM group continued to increase to> 250 mg/dl. The increasing level of BGL in NTDM group was believed due to the development of diabetic condition. In contrary, the BGL in the TDM group was dropped after the animal received S. vulcani leaves extract. The BGL decreases was detected on day-7, but the significant different to NTDM group ($p<0.05$) started on day-14. The level continues to decrease until it reaches a level that is not significantly different from the control (NM group) at the end of the study ($p>0.05$).

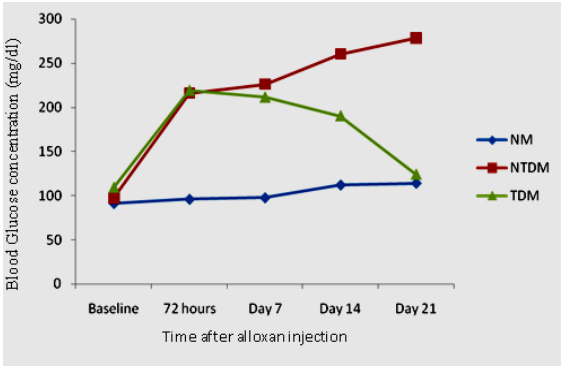

Figure 1. The change in mean of blood glucose level in normal mice group (NM) group, Non-Treated Diabetes Mice (NTDM), and Treated Diabetes Mice (TDM) at different time of observation. The TDM mice received 200 mg/kg body weight leaves extract (Saurauia vulcani Korth).

The effect of treatments on body weight is shown in figure 2. There is no significant difference in body weight between treatments both at baseline and at 72 hours after alloxan injection, but on day 7 the body weight in NTDM and TDM groups is lower than body weight in control (NM) ($p<0.05$). These conditions, especially in the NTDM group, continued until the end of the study, while in the TDM group the body weight improved to levels that were not different significantly from controls (NM).

These results indicated that alloxan induces diabetes in mice characterized by an increase in BGL and then followed by weight loss. The results obtained was agree with the previous studies (Ewenighi,., 2015; Hutahaean., 2018). Uncontrolled diabetes causes an increase in glycogenolysis, lipolysis, and gluconeogenesis. Biochemical activities through these three pathways are basically the use of a new energy source for the body to compensate for the low glucose entering the cell. The use of energy sources from body fat and protein in these processes is believed to be the pathway for the mechanism of weight loss in diabetes.

The mechanism of diabetes induction by alloxan is by damaging the pancreatic beta cells through the resulting free radical effects. Alloxan is a glucose analog which is specifically accumulates in the pancreatic beta cells. By intracellular thiol activity, especially glutathione, alloxan produces reactive oxygen species (ROS) in a cyclic redox reaction with its product, dialuric acid. Dialuric acid autoxidation produces superoxide radicals, hydrogen peroxide and, hydroxyl radicals. Hydroxyl radicals are responsible for the death of beta cells that have very low antioxidant defense abilities. As a thiol reagent, alloxan also selectively inhibits glucose-induced insulin secretion through its ability to inhibit glucose sensor in beta cells (Szkudelski, 2001; Lenzen, 2008).

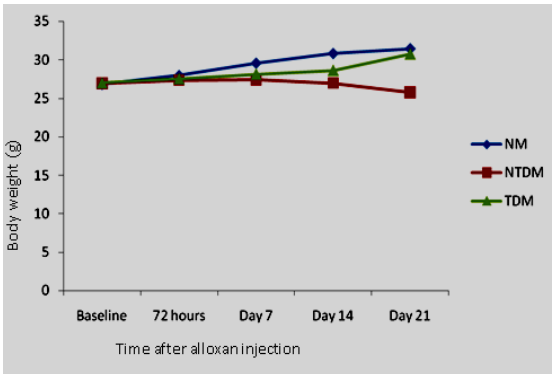

Figure 2. The change in mean body weight in normal mice group (NM) group, Non-Treated Diabetes Mice (NTDM), and Treated Diabetes Mice (TDM) at different time of observation. The TDM mice received 200 mg/kg body weight leaves extract (Saurauia vulcani Korth).

Histological observation showed the damage of pancreatic cells in the NTDM group (figure 3).

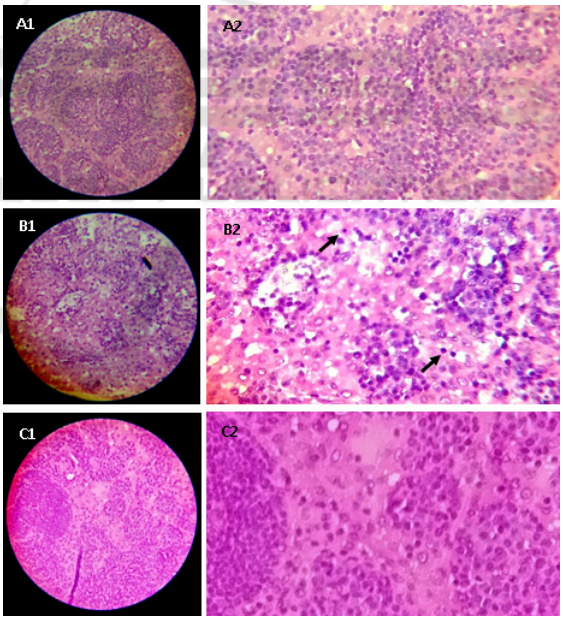

Figure 3. The change of pancreatic island feature after Saurauia vulcani leaves extract treatment in alloxan-induced diabetic mice. A1 and A2: normal mice (NM); B1 and B2: Non-Treated Diabetes Mice (NTDM); C1 and C2: Treated Diabetes Mice (TDM). The TDM mice received 200 mg/kg body weight leaves extract (Saurauia vulcani Korth). Staining HE; 400 X.

The damages were cells with a pyknotic nucleus, cell populations reduction which was characteristics of a more loose group of cells in the island (figure 3, B1 and B2). In NTDM group, island cell population reduced, the boundaries of the islands appear irregular, and cells damage of pyknotic type found (black arrow). Those were not found in control (NM) and in mice treated with Saurauia vulcani leaves extract (TDM group).

In addition, the forms of islands also appear irregular compared to the control group (NM). In the TDM group, there were indications of improvement in the morphological structure of the pancreas which was characterized by the denser features of island cells, no more pyknotic cells, and more regular island boundaries. The morphological changes obtained was supporting the findings of BGL repair due to the treatment of S. vulcani leaves extract.

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