Evaluation of Blood Glucose Level and Microscopic Pancreatic Islets of Langerhans Treated with Lawsonia Inermis Linnaeus Leaves Ethyl Acetate Extract in Streptozotocin-induced Diabetic Rat

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Abstract: *Lawsonia inermis Linnaeus* leaf, is one of alternative medicine that used to treat diabetes mellitus (DM) in Indonesia. We investigated the effect of ethyl acetate extract of *Lawsonia inermis Linnaeus* (EAE) on blood glucose level (BGL) and the histopathological alterations of pancreatic islets of Langerhans (iL) in streptozotocin (STZ)-induced diabetic rats. A number of 24 rats were divided into 6 groups, Normal control (NC) was feed ad libitum, while STZ-induced diabetic rats (SDR) groups were treated with normal saline 10 ml/kg (P1), glibencamide 10 mg/kg (P2), EAE 250 (P3), 500 (P4) and 1000 (P5) mg/kgbw, daily orally for 14 days. Data were analyzed using one way ANOVA followed by Dunnett t test. BGL of NC-(79.75 ± 4.95); P2 (77.5 ± 3.8); P3 (79.5±6.9); P4 (78.5 ± 4.5); P5 (83.25 ± 4.3) were lower than P1-treated groups (292.5 ±4.63) mg/dl, significantly (P<0.01). The histopathological evaluation showed that the perimeter of the islets of Langerhans P1 were shrinked (8.59 ± 1.8)µm, while P2(12.71 ± 5.4); P3 (11.42 ± 2.9); P4 (1679 ± 11;4) and P5 (14.37±3.5)µm were larger and closed to NC (19.27 ± 3.5)µm. The present study concluded that EAE have antihyperglycemic activy and improve the pancreatic islet of Langerhans structure.

1 INTRODUCTION

In spite of knowledge, there are great efforts that have been made in the understanding and management of diabetes. Today, disease related complications are increasing day by day without any reduction in strength (Tiwari, 2002). In spite of the presence of known antidiabetic medicine available in the pharmaceutical market, remedies derived from medicinal plants are successfully used in the treatment of this disease (Bhattaram *et al.*, 2002; Choubey *et al.,* 2010)

However search for new Antidiabetic drugs continues. The mechanism of most of the herbals used to treat diabetes has not been defined. It has been attributed that the antihyperglycemic effect of these plants is due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. Hence treatment with herbal drugs has an effect on protecting â-cells and smoothing out fluctuation in glucose levels (Jia *et al.,* 2003; Elder, 2004)

Lawsonia inermis Linn. commonly known as henna, is a finely ground brown or green powder originating from dried leaves of the plant *Lawsonia inermis* which is grown in dry tropical and subtropical zones, including North Africa, India, Sri Lanka, and the Middle East. (Borade *et al.,* 2011).

Lawsonia inermis Linnaeus is one of the plants commonly used in Indonesian community for the treatment of different diseases (Widyawati *et al*, 2016). Previous study has been well investigated phytochemically by various researchers such as β-sitosterol, lawsone, esculetin, fraxetin, isoplumbagin, scopoletin, betulin, betulinic acid, hennadiol, lupeol, lacoumarin, laxanthone, flavone glycosides, two pentacytic triterpenes glucoside, flavonoids, quinoids, naphthalene derivatives, gallic acid, coumarins, and xanthones (Chaudhary *et al.*, 2010; Kamal and Jawaid, 2010; Borade *et al.*, 2011; Musa and Gasmelseed, 2012) in *Lawsonia* leaves has been reported. Earlier work establishes the use of henna as

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an alternative vegetable retanning agent (Musa and Gasmelseed,2012).

Several study showed that effect of *Lawsonia inermis* Linn ethanolic extract 500 mg/kg of body weight was found to be better then Glibenclamide (10mg/kgbw). These results suggest that the ethanolic extract possess significant antidiabetic effect (Choubey et al., 2010). Widyawati et al., 2016 showed that EAE is the most active extract as antihyperglycemic than with n-hexane (HE), ethylacetate (EAE), ethanol (EE), water1(WE1) and water2 (WE2). Hence the aim of the study is to investigate hypoglycemic effect of ethyl acetate extract of *Lawsonia inermis Linn* in streptozotocin induced diabetic rats and evaluated microscopic pancreas of islets Langerhans.

2 MATERIAL AND METHODS

2.1 Chemical and Reagents

Streotozotocin, formalin buffer 10%, paraffin wax, TBA reagent, heparin sodium, sodium chloride, cell lysis buffer, aquabidest, 70% and 80% aqueous alcohol and 96% absolute alcohol, xylol, glyserin, Mayer"s haematoxylin, eosin, canada balsem. All other chemical were of analytic grade.

2.2 Animals

Healthy male Wistar rats (150-200 g) were obtained from animal house of Universitas Sumatera Utara. The study was conducted after approved by Animal Research Ethics Committees (AREEC), Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Sumatera Utara (No. EC: 115/KEPH-FMIPA/2017).

2.3 Plant Material and Preparation of EAE

Lawsonia inermis Linn leaves were collected from Titi Kuning, Medan, North Sumatera, Indonesia and was authenticated by Department of Botany, Universitas Sumatra Utrara. The fresh leaves were dried under shade and ground into powder. The powdered leaf then was extracted serially by maceration in n-hexane and ethyl acetate (EAE).

2.4 Induction of Diabetes

Diabetic rats were obtain by induction STZ (55 mg/kg) intraperitoneally. Diabetic rats with fasting blood glucose level more than 200 mg/dl were included to the study. BGL was confirmed using glucometer (®Accu check), after 72 hours of STZ injection.

2.5 Experimental Design

The animals were divided randomly into six groups of four rats each and treated as follows:

- Group I (NC): Normal control rats (standard pellets and water *ad libitum*) for 14 days.
- Group II (P1): Diabetic control rats were administered with STZ, were treated with normal saline 10 ml/kg
- Group III (P2): Diabetic rats were treated with glibencamide 10 mg/kg
- Group IV (P3): Diabetic rats were treated with EAE 250 mg/kgbw daily orally for 14 days.
- Group V (P4): Diabetic rats were treated with EAE 500 mg/kgbw daily orally for 14 days.
- Group VI (P5): Diabetic rats were treated with EAE 1000 mg/kgbw daily orally for 14 days.

2.6 Preparation Pancreatic for Histopathological Analysis

At the end of the stipulated 14 days feeds were withdrawn, the rats were subjected to a 12 hours fast but had access to water. Sacrificed using chloroform vapour. Rats were positioned on the surgical board using pins or pin needles. The surgery started in rat stomach by using surgical scissors. The pancreas organ were carefully dissected out, trimmed of all fat and connective tissue blotted dry to remove any blood.

Within a 30 minute interval after excision, the pancreas was immersed in buffered 10% formaldehyde for 24 hours. The samples were fixed in buffered 10% formaldehyde for 24 hours, followed by dehydration
in: 1) 70% alcohol for 60 min, 2) 96% alcohol for 45 min, 3) absolute alcohol for 2 h. The clearing phase of the samples was made by repeated xylene immersions, followed by paraffin wax infiltrations.

The samples were automatically processed with tissue processor Thermo Scientific STP 120-3 and paraffin embedding was done with modular tissue embedding center Thermo Scientific Microm EC 350-1. Next,the resulting blocks were cut at 5 μm using the Leica RM 125RTS microtome and then carefully placed on the microscope slides. In order to distinguish between tissue types the sections were stained with Haematoxylin and Eosin (H&E) staining techniques, after which they were passed through ascending grade of alcohol, cleared in xylene and mount in DPX mountant, allowed to dry at room

temperature and observed histopathologically under digital light microscope

2.7 Photomicrography

Records of the Histopathological results were obtained by photomicrography using digital photomicrographic microscope was made with an Olympus BX 41 microscope coupled to an Olympus DP25 video camera at the Anatomic Pathology Laboratory, Department of Anatomic Pathology, Universitas Sumatera Utara.

2.8 Image Analysis

Morphometric measurements of the digitalized images of immunostained sections were carried out using the Image J IJ 1.46r plus image analyzer computer system (Wayne Rasband, Maryland, USA). The average area of the islets was determined by measuring the area of 3 islets in each section of one rat, and in total for 12 islets from each group (Ferreira and Rasband, 2012)

2.9 Statistical Analysis

Data were analyzed and presented as means ± SD. Differences between continuous data were analyzed using one way Annova followed by Dunnett t test. $p < 0.01$ was considered significant.

3 RESULT AND DISCUSSIONS

3.1 Blood Gucose Level (BGL)

BGL concentration increased following STZ injection in all groups compared with the diabetic group during the duration of the experiment. The rats in group treatment which received glibencamide 10 mg/kg (P2), EAE 250 (P3), 500 (P4) and 1000 (P5) mg/kgbw showed a significant decrease ($P < 0.01$) as compared with the rats of the untreated diabetic group (P1) (Table 1).

Table 1: Effect of EAE on blood glucose level in STZ-induced diabetic rats

Group	Blood Glucose Level (mean ± SEM)
NC	79.75 ± 4.95***
P1	292.5 ± 4.63
P2	77.5 ± 3.8***
P3	79.5 ± 6.9***
P4	78.5 ± 4.5***
P5	83.25 ± 4.3***

Data was expressed as mean ± SEM. ***$p<0.01$

The decrease of BGL in the treatment group with EAE caused by bioactive compounds of *Lawsonia inermis* Linn leaf can prevent the occurrence of oxidation in pancreatic β cells so the damage can be reduced. The bioactive compounds contained in previous study has been well investigated phytochemically by various researchers such as polyphenols, flavonoids, alkaloids and tannins. The role of polyphenols is thought to be capable of protecting pancreatic β cells from the effects of free radical toxicity produced (Chaudhary *et al.*, 2010; Kamal and Jawaid, 2010; Borade *et al.*, 2011.

In line with previous studies showed the that the feeding of 0,8mg/kg/bw of Lawsonia inermis Linn extract ethanol decreased the glucose concentration to normal condition after the 14th day (Syamsudin et al., 2008). The study of Choubey et al., showed the effect of ethanolic extract of Hena 500 mg/kgbw was found to be better then Glibenclamide (10 mg/kgbw). (Choubey et al., 2010)

This result so in agreement with Ojewunmi et al., showed that ethanol extract of *Lawsonia inermis* leaves was significantly reduced fasting blood glucose *(P<0.001)* compared to the untreated diabetic control. (Ojewunmi et al., 2014). Antika et al., showed the group treated ethanol extract of *Lawsonia inermis* a dose of 400mg/kgbw had the lowest decrease blood glucose levels. (Antika et al., 2017)

3.2 Evaluation of the Islets of Langerhans (iL)

Morphometric measurements showed changes in the mean values of iL in figure 1. The average area of iL P1 were shrinked (8.59 ± 1.8)μm^2, while P2 (12.71 ± 5.4); P3 (11.42 ± 2.9); P4 (1679 ± 11;4) and P5 (14.37±3.5)μm^2 were larger and closed to NC (19.27 ± 3.5) μm^2.

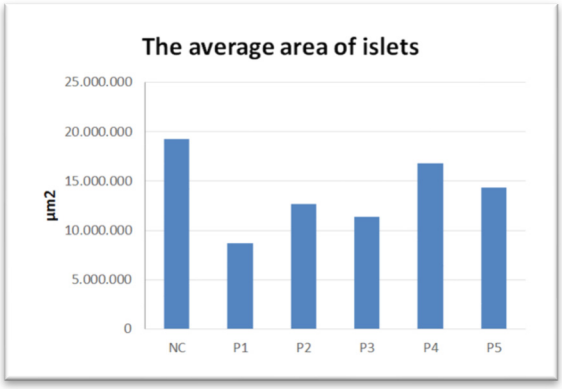

Figure 1: The average area of islets of Langerhans (iL)

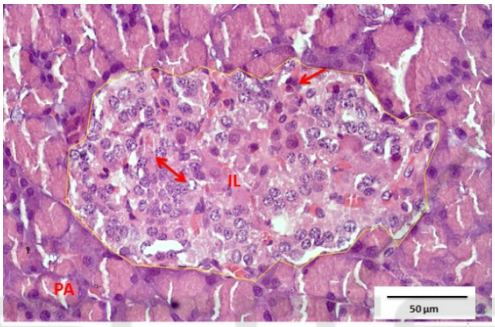

Figure 2: Photomicrographs of sections of the pancreas from control group showing pancreatic acinar (PA) and islets of Langerhans (IL) with granulated cytoplasm of islet cells with small, dark nuclei on the periphery (alpha-cells) (arrow), or with light and large nuclei (beta-cells) (duble arrow); H&E staining, scale bar = 50 µm

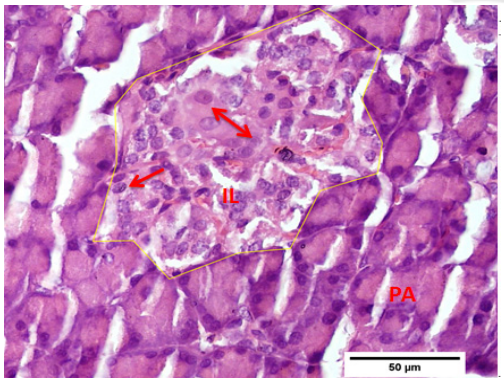

Figure 3: Photomicrographs of sections of the pancreas from diabetic group showing pancreatic shrinked with degenarative change in IL espescially in center of islet (double arrow). Irregular outlining of the islet. H&E staining, scale bar = 50 µm

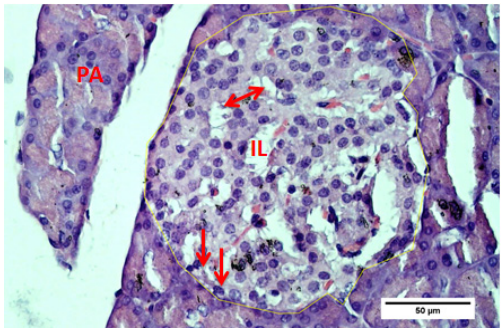

Figure 4: Photomicrographs of sections of the pancreas from EAE group showed the nearly regular outline of islet with apparently normal appearance of most cell. H&E staining, scale bar = 50 µm

The examination of H&E stained sections from the control group showed the pancreas to have a normal histological structure. The islets of Langerhans appeared as noncapsulated pale stained rounded or oval areas inside the pancreatic acinar lobules, which were formed of groups of cells arranged in irregular, branching, and anastomosing cords separated by blood capillaries (Figure 2). In diabetic group (P1), STZ caused degenerative changes in the pancreatic islets, mainly at the center of the islets. An apparent reduction in the size and number of islets was noticed (Figure 3). Sections from the iL of group P4 (given 500 mg/kg bw EAE) showed islets with nearly regular outlines and almost normal cell morphology (Figure 4).

Streptozotocin (STZ) is an antibiotic produced by *Streptomyces achromogenes*. It has been widely used for inducing experimental diabetes mellitus in a variety of animals, it stimulates the naturally occurring metabolic disorder DM by causing degeneration of pancreatic β cells. (Coskun, 2005). The selective β cell toxicity of STZ is related to the glucose moiety in its chemical structure, which enables STZ to enter the cell via the low affinity glucose transporter Glut2 in the plasma membrane which induces an increased release of reactive oxygen species, subsequently causing DNA damage (Szkudelski, 2001).

This study indicated that treated of EAE *L. inermis* extract caused improved destruction of iL with increases size and number of islets caused STZ-induced diabetic rats.

4 CONCLUSIONS

Lawsonia inermis Linn EAE have anti-hyperglycemia effect and protective microscopic

changes of islets of Langerhans in STZ-induced diabetic rats.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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