The Effect of Antlion (Myrmeleon sp.) Extract Towards Histopathology Image of Pancreas in Diabetic Mice

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INTRODUCTION
Diabetes Mellitus (DM) is one of the main non-communicable disease characterized by various symptoms caused by high blood glucose level (hyperglycemia). Studies show that the global and national prevalence of DM increases each time.

RISKESDAS 2018 shows an increased prevalence from 1.5% to 20% (RISKESDAS, 2018). International Diabetes Federation (IDF) in 2017 estimated that there are 10,3 million people with DM and will be increased to 16,7 million in 2045 (International Diabetes Federation, 2017). Hyperglycemia that occurs in DM patient may...
leads to several complications if left untreated, such as heart attack, kidney failure, blindness, and nerve damage, that may increase morbidity in patients (Roglic, 2016; International Diabetes Federation, 2017). Some studies indicated that antlion (Myrmeleon sp.) treatment have been proven capable of reducing glucose concentration mice (Rahma et al. 2016; Maryati et al., 2019). However its effects on pancreas histological feature was not elucidated.

Pancreas is an organ that plays a major role in DM pathophysiology, as the organ produced insulin, a hormone that regulates blood glucose by decreasing the blood glucose level. Insulin is produced in the endocrine part of the pancreas called pancreas islet (Islet of Langerhans) by β cells (Mescher, 2016). Hyperglycemia that occurs in DM leads to increase of inflammatory cytokines such as IL-1, TNF-α, and NF-κB and Reactive Oxygen Species (ROS) that leads to inflammation and cell destruction, including pancreas islet (Walvekar et al., 2016; Volpe et al., 2018). The pathological condition where pancreas islet is infiltrated by inflammatory cell due to inflammatory state and cell damage is called insulitis (Jiang et al., 2017; Morgan and Richardson, 2018). If this continues, it may worsen the disease and increasing the likelihood of complications.

Antlion (Myrmeleon sp.) is one of traditional diabetes treatment many people use in Indonesia that has proven to be effective in lowering blood glucose in many studies (Mujahid et al., 2013; Rahma et al. 2016; Maryati et al., 2019). Recent study shows that antlion contain metformin, one of widely-used oral antihyperglycemic agent that has multifunctional activity such as anti-oxidant, anti-cancer, cardiovascular protective, and anti-inflammatory (Saisho, 2015; Cameron et al., 2016; Griffin et al., 2017; Heckman-Stoddard et al., 2017; Ren et al., 2017) Metformin also manage to ameliorates insulitis condition in diabetic mice that has been induced with Streptozotocine (Jiang et al., 2017). Another component in antlion are Alpha-Linoleic Acid (ALA) and Linolenic Acid (LA), parts of essential fatty acid known to ameliorate pancreatic damage in diabetic mice (Canetti et al., 2014). Research has not been done to find the effect of antlion in pancreas, one of the most important organ in DM pathophysiology.

Based on this background, aim of the study was to prove the effect of antlion (Myrmeleon sp.) extract towards histopathological feature of pancreas in diabetic mice.

METHODS

This study used true experimental study with Post Test Only Control Group Design. Twenty five male Swiss mice aged 3-4 months, weighing 20-25 grams, healthy and active. Twenty mice carried out the induction of diabetes with Streptozotocine. Mice were divided into 5 groups randomly, where each group consisted of 5 mice to be treated as follows; a. Nor-G: Normal control group, were administered standard diet. b. Neg-G: Negative control group, were administered Streptozotocine 200 mg/kg BW intraperitoneally. c. At-14: First treatment group, were administered Streptozotocine 200 mg/kg BW intraperitoneally, then daily administered by antlion extract 14 mg/

| Number | Markers |
|--------|---------|
| 0 | No sign of pathological changes in the pancreatic islets |
| 1 | Mild insulitis (infiltration and damage in less than 25% of islets area) |
| 2 | Severe insulitis (infiltration and damage in between 25 and 75% of the islets area) |
| 3 | Destructive insulitis (infiltration and damage in more than 75% of the islets area) |

Note: Every islets in each sample were assessed, and the score that appears the most (mode) was chosen to represent the histological image

| Variable | Groups |
|----------|--------|
|          | Nor-G  | Neg-G  | At-14  | At-28  | At-56  | P         |
|          | N=5, X | N=5, X | N=5, X | N=5, X | N=5, X | (Kruskall-Wallis) |
| Histological feature | 0.00±(0.000) | 1.80±(0.447) | 1.00±(0.707) | 0.80±(0.837) | 0.40±(0.548) | 0.008 |

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kg BW orally.

d. At-28: Second treatment group, were administered Streptozotocine 200 mg/kg intraperitoneally, then daily administered by antlion extract 28 mg/kg BW orally.

e. At-56: Third treatment group were administered Streptozotocine 200 mg/kg intraperitoneally, then daily administered by antlion extract 56 mg/kg BW orally.

This study was approved by the Research Ethics Commission (KEPK) of the Faculty of Medicine, Diponegoro University/RSUP Dr. Kariadi Semarang for all animal experiments with number of ethical clearance 62/EC/H/KEPK/ FK-UNDIP/V/2019.

Antlion Extract

The antlions were first dried with freeze drying method and extracted by using maceration method. Antlions were soaked in 96% ethanol for 72 hours at room temperature. The extract then filtered and evaporated to dryness in water bath at 50°C to remove the solvent. The extract was then stored in a refrigerator for future use. (Rahma et al. 2016; Prihatin et al., 2019).

Animal Preparation and Treatment

Before treatments, all mice have been acclimated for 7 days and have their blood glucose measured. Next day, Neg-G, At-14, At-28, and At-56 induced with Streptozotocine 200 mg/kg BW and given sucrose water for 3 days. After 10 days, their blood glucose measured to ensure the administered group have hyperglycemia. Afterwards, mice in At-14, At-28, and At-56 were daily treated with antlion extract 14, 28, and 56 mg/kg BW for 24 days. The blood glucose then measured and mice were terminated.

Histologic Preparation and Examination

After 24 days, the mice were terminated and each pancreas were removed and fixed in formalin solution. Samples then put in xylol solution for about 2 minutes. They then rehydrated in an alcohol series (70, 80, 90, 95, and 100%) for 30 min. They were cleared with xylene for 1.5 h until the color of xylene turned pale, and then cleared with xylene/paraffin substitute (3:1, 1:1, and 1:3, v/v, respectively) each for 30 min in an oven at 45-50°C. Then, they were infiltrated with hard paraffin twice daily and embedded in paraffin. Sections of these embedded samples were cut and stained with eosin.

Histological examination was observed at 400× magnifications under a light microscope. Histopathology image of pancreas was assessed by anatomical pathologist based on pathological changes or injury in the pancreatic islets using a scale of 0–3 as shown in table 1.

Statistical analysis

Data were analyzed using Kruskal-Wallis non-parametric test followed by post-hoc Mann-Whitney test and assessed by using statistical software SPSS version 25. The median and standard deviation (SD) were expressed as the measure of central tendency and dispersion, respectively. The median values were compared using non-parametric test followed by post-hoc Mann-Whitney test. The chosen significance level was 0.05.

Figure 1. Histological feature of Pancreatic islets injury following antlion treatment in each group. Microscopic investigation of pancreas from normal control group (Nor-G) showed the normal appearance of pancreatic islets, lightly stained than the surrounding acinar cells. Pancreas from the Neg-G mice showed collapsed islet architecture and tissue damage. Severe mononuclear cell infiltration were also found. On the other hand, histological images from At-14, At-28, and At-56 showed alleviation in the tissue damage and reduction mononuclear cell infiltration gradually. The lowest pancreatic islet damage was found in At-56, showing almost no tissue damage and infiltration.

P: pancreatic islet. Mann Withney test: * p<0.05; ns: not significant
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test if significant value was obtained. A P-value < 0.05 indicated statistical significance.

RESULTS

The results of the antlion extract treatment is summarized in table 2.

The highest score of pancreatic islet injury were found in the negative control group (Neg-G) and the lowest score were found in Nor-G. Meanwhile, the lowest score of pancreatic islet injury among the treatment groups (At-14, At-28, and At-56) was found in the third treatment group (At-56). Kruskall-Wallis statistical analysis showed that the pancreatic islet injury amongst groups were significant different, p<0.05. It can be stated that there are mean differences between at least two groups. Statistical analysis of Mann-Whitney indicated that the score of pancreatic islet injury in At-56 group was significant lower compared to that of Neg-G, p <0.05. Meanwhile the score of pancreatic islet injury in At-14 and At-28 were not significant lower compared to that to of Neg-G, p>0.05. Interestingly, the score of pancreatic islet injury in At-56 was not significant lower compared to that of Nor-G, p>0.05 (figure 1). This result indicate that antlion treatment with dose of 56 mg was the best among other dose.

DISCUSSION

This study shows that antlion extract is able to repair pancreatic islet damage. Pancreas histopathology in Neg-G showed that induction of Streptozotocine caused islet destruction and inflammatory cell infiltration. Intraperitoneal injection of a single dose, 200 mg/kg BW of Streptozotocine to mice is causing direct toxicity towards pancreatic β cells and cell destructions through DNA alkylation and ROS production (Furman, 2015; Nahdi et al., 2017). In both type of DM, β cell and pancreatic islet damage may caused by hyperglycemia, inflammatory process, cytokine secretion, and ROS production that induce several cellular changes, leading to cellular damage and apoptosis (Rojas et al., 2018; Volpe et al., 2018). These changes can be seen in histopathology image of pancreas, showing reduction in β cells with apparent collapse of islet architecture and inflammatory cell infiltration (Canetti et al., 2014; Jiang et al., 2017; Rojas et al., 2018). Administration of antlion extract was able to repair the damage in pancreatic islet as shown in histological image of At-14, At-28, and At-56. The lowest and significant result was found in At-56, the antlion extract administration at 56 mg/kg BW treatment group.

Antlion (Myrmeleon sp.) contains chemical compound such as metformin, ALA, and LA that can ameliorate cellular damages caused by either inflammatory process or ROS through various mechanisms (Canetti et al, 2014; Jiang et al., 2017; Muadifah et al., 2017; Malcicka, Visser and Ellers, 2018). Through its anti-inflammatory actions, Metformin was able to protect pancreatic islet damage caused by low-dose multiple injection of Streptozotocine in mice (Jiang et al., 2017). Metformin can also participated in the regeneration of β cells capacity to secrete insulin (Ismail et al., 2015). Meanwhile, ALA and LA compound was shown to protect pancreatic islets from Streptozotocine-induced damage and improves insulin level in Streptozotocine-induced diabetic mice (Canetti et al., 2014).

Recent study done by Prihatin et al., in 2019 aimed to investigate the effects of Myrmeleon formicarius (antlion) on liver and kidney histology in Streptozotocine-induced diabetic mice. The study showed that antlion extract treatment resulted in minimal damage in kidney and liver at 5 and 10 mg/kg BW respectively. In the study, another compound present in antlion larvae extract is a chaperone protein that can stimulate cell regeneration (Prihatin et al., 2019). Heat Shock Protein (HSP) HSP70 is the most common HSP in humans that could inhibit TNF-α-induced free radical formation, lipid peroxidation, and apoptosis (Ikwegbue et al., 2018). Thus, chaperone protein present in antlion extract can also take effect in its tissue-repair ability (Kumar et al., 2014; Prihatin et al., 2019).

CONCLUSIONS

Antlion (Myrmeleon sp.) extract at 56 mg/kg BW reduces pancreatic islet damage in Streptozotocine-induced diabetic mice. Further studies are required to confirm the compounds responsible for this effect and its mechanism in pancreas.

CONFLICT OF INTEREST

Authors declare there is no conflict of interest within this manuscript production.

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