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The Effect of Aloe Vera Peel Extract on Histopathology of Rat Pancreas Induced by Alloxan

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Abstract

The Extract of Aloe Vera Peel (EAVP) contains bioactive compounds (flavonoids, alkaloids, tannins, saponins, and phenolics) that it thought to improve pancreas histopatology on rat with diabetes mellitus, therefore it has potential for diabetes mellitus treatment. This research aimed to figure out the effect of EAVP on improving the histopathology of rat pancreas induced by alloxan. This research was an experimental study using a completely randomized design with a randomized post-test design. A total of 25 rats were divided into 5 groups: C(-) was a normal group, fed and drinking standard; C(+) was positive control group, induced by alloxan 120 mg/kgBW; PI, PII and PIII were groups that were induced by alloxan 120 mg/kgBW and were given a full-dose of EAVP of 87.5, 175 and 350 mg/kgBW respectively. The data was assessed using the Kruskal-Wallis and Mann-Whitney statistical analysis. The results of the statistical test showed that the histopathology of rat pancreas of the C(+) group were significantly different compared with the treatment group. Meanwhile, the representation of histopathology of pancreas between PIII and C(-) were not significantly different. It can be concluded that treating hyperglycemia rats with the EAVP for 28 days can improve the representation of histopathology of rat pancreas. At the laboratory level, EAVP has been shown to repair rat pancreatic damage. With this result, *Aloe vera* has the potential to be developed as a phytopharmaca for the prevention or treatment of diabetes mellitus.

How to Cite

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder due to inability of pancreas to produce insulin adequately. Therefore, produced insulin can not be used effectively by the body, with the result that glucose concentration in the blood will be increasing (hyperglycemia). Insulin is hormone that is responsible to regulate the balance of blood sugar levels. Type 2 diabetes mellitus patients produce insulin in normal amounts, but it does not work properly either the insulin resistance occurs caused by structural changes or reduction of insulin receptors on the cell membrane (Stumvoll et al., 2005). Jung et al. (2006) reported that insulin resistance contributes in increasing glucose release in the liver and decreasing glucose uptake into adipose tissue. It causes hyperglycemia and failure of glycogen formation. Gradually, it causes high blood sugar levels (Widowati, 2008).

Herbal medicine may be achievable alternative to control blood glucose levels. Recently, herbal medicine is preferred by the public since it contains natural compounds and few side effects compared to synthetic medicine. Nevertheles, WHO recommends herbal medicines utilization for cancer prevention, as well as for chronic and degenerative diseases (Sari, 2006).

Aloe vera is one of the plants that has potential as an antidiabetic drug (Grover, 2002). Another study revealed that ethanolic and water extract of *Aloe vera* had hypoglycemic effect on diabetic experimental mice (Erdiansyah et al., 2015). Phytochemical screening analysis discovered that ethanolic extract of *Aloe vera* contains secondary metabolites such as flavonoids, alkaloids, tannins, saponins, and sterols (Gibson et al., 2014). Furthermore, flavonoids, terpenoids, and saponins were also reported found in the extract (Aria et al., 2014).

To address the issues above, the present study was carried out to investigate the use of the extract of aloe vera peel (EAVP) to improve the histopathological condition of rat pancreas induced by alloxan. This research was developed to obtain scientific evidence related to the role of *Aloe vera* as phytopharmaca in Indonesia, especially for diabetes mellitus.

METHOD

This study was conducted experimentally using a Post Test Control Group Design. A total of 25 white rats (*Rattus norvegicus*) Wistar, male, aged 2-3 months, weighing 150-200 grams and in good condition were randomly divided into 5 groups. Each group consisted of 5 rats. Group C(-) is a negative control group (not induced by alloxan and not administered by EAVP). The C(+) group was the positive control group that was induced by alloxan 120 mg/kg without EAVP. PI group consisted of alloxan-induced rats (120 mg/kgBW) and administered by EAVP with the dose of 87.5 mg/kgBW. PII group consisted of alloxan-induced rats (120 mg/kgBW) and administered by EAVP with the dose of 175 mg/kgBW. PIII group consisted of alloxan-induced rats (120 mg/kgBW) and administered by EAVP with the dose of 350 mg/kgBW.

Alloxan induction (120 mg/kgBW) was done via IntraPeritoneal (IP) (Abbasi et al., 2014) to trigger hyperglycemia. After 4-7 days of alloxan induction treatment, blood glucose levels were measured. The indicator of hyperglycemic occurs if the blood glucose level are ≥126 mg/dl (Rahmawati, 2014).

EAVP was made by maceration method with 70% ethanol. The antioxidant activity of EAVP was measured using the DPPH method. The extract was administered orally once a day for 28 days at a dose of 87.5 mg/kgBW for the PI group, 175 mg/kgBW for PII and 350 mg/kgBW for PIII.

Examination of a pancreas histopathology

Treated rats were dissected and the pancreas tissue was examined for its histology using Hematoxylin-Eosin staining method. Pancreatic histopathology preparations were observed under a microscope with 400x magnification and recorded microscopic changes were found in 5 visual fields. Pancreas histopathology preparations were observed and scoring based on the following categories: score 0 if there is no pancreatic cell necrosis, score 1 if ¼ of total pancreatic cell undergo necrosis, score 2 if ½ of total pancreatic cells undergo necrosis, score 3 if ¾ of total pancreatic cells undergo necrosis and score 4 if all cells in preparations undergo necrosis (Dharma et al., 2015).

Histopathological observation data were collected and analyzed using the Kolmogorov-Smirnov test to find out whether the data obtained were normally distributed or not, the data was normally distributed as if P> 0.05. Whereas if the data was distributed abnormally and was not homogeneous, the analysis was continued by using non-parametric statistical methods (Kruskal-Wallis Test and Mann-Whitney Test). However, if the data was normally distributed and homogeneous, the analysis was continued by using

analysis of variance method (ANOVA) followed by Tukey HSD analysis (Akrom et al., 2014). The result was defined significant as if P <0.05 (Dahlan, 2014).

RESULT AND DISCUSSION

The results showed that EAVP had an antioxidant activity of 152.87 ppm. DPPH method is widely used to measure the ability of a compound in inhibiting free radicals or as a hydrogen donor (Pratama et al., 2013). The working principle of the DPPH test is a bioactive compound as an antioxidant which reduces DPPH free radicals (2,2-diphenyl-1-picrylhydrazyl) to diphenyl picryl hydrazine (Purwaningsih, 2012).

Histopatological result indicated that the C(-) group had the lowest mean of pancreatic histopathology score (0). The C(+) group had the highest mean of pancreatic histopathology score (3.6) (Table 1).

Based on the score analysis of pancreas histopatology, it was known that the PI, PII and PIII group has mean score of 2.4, 1.6, and 0.9 respectively (Table 1). While, PIII group is almost similar to C(-) group (normal). Histopatology analysis of C(-) group or negative control showed the morphology and structure of normal langerhans island, the cells were distributed homogeneously in the cover, no damage to the cell or the structure of the Langerhans island, they also had normal structure and size (Figure 1) that indicated that necrosis were not occurred in langerhans island.

Figure 2 represents the histology of Langerhans island cell in C(+) group. Figure 2 shows the high degree of damage from the island of Langerhans, characterized by nucleus fragmentation (a), cell hypertrophy (b), pyknosis (nucleus shrinkage) (d), and cytoplasmic vacuoles (e) on the langerhans islet. The number of pancreatic β cell clusters was reduce in middle part of Langerhans island along with the increase in connective tissue (Figure 2c). In consequence, the structure and Langerhans island cell morphology are irregular and the cells were not distributed homogeneously. Atrophy occurred in the langerhan of C+ rat group, so that its size is smaller than langerhans of the C-group rat. .

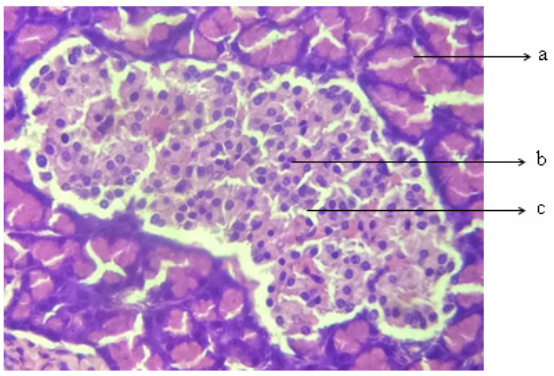

Figure 1. Histological structure of C(-) rat pancreas with a magnification of 10×40. a. Pancreatic acini serous, b. Pancreatic beta cells, c. Intercellular cavity.

Data indicate that cytoplasm vacuolation caused by apoptosis initial stages due to increased production of stress oxidations as a result of endoplasmic reticulum activity in β cells as a sign of insulin resistance. Increasing amount of connective tissue is thought to be caused by activation of mitogenous IL-1β in fibroblast activity. This condition is in line with the high levels of inflammatory cytokines produced when lipids are highly deposited in the body (Tedgui & Mallat, 2006). The results of the research by Boudreau et al. (2006) showed that during diabetes, beta cell nucleus undergo karyolysis, the cytoplasmic component is disintegrating, cell boundaries are unclear, and there are periods of debris containing core fragments and necrosis.

Table 1. The mean score of the histopathology of rat pancreas induced by alloxan and administered by extract of *Aloe vera* peel

Group (s)	Rat					Average
	1	2	3	4	5	
C(-) Control negative	0	0	0	0	0	0
C(+) Control positive	3.4	3.8	3.8	3.6	3.6	3.6
PI Dosage 87,5 mg/kgBW/day	2.4	2.4	2.6	2.6	2.2	2.4
PII Dosage 175 mg/kgBW/day	1.8	0.8	2.6	1.8	1.2	1.6
PIII Dosage 350 mg/kgBW/day	1.0	1.0	0.4	1.2	0.4	0.9

Note: Score of 0 if there are no pancreatic cell necrosis, score 1 if ¼ of total pancreatic cells undergo necrosis, score 2 if ½ of total pancreatic cells undergo necrosis, score 3 if ¾ of total pancreatic cells undergo necrosis and score 4 if all pancreatic cells undergo necrosis (Dharma et al., 2015)

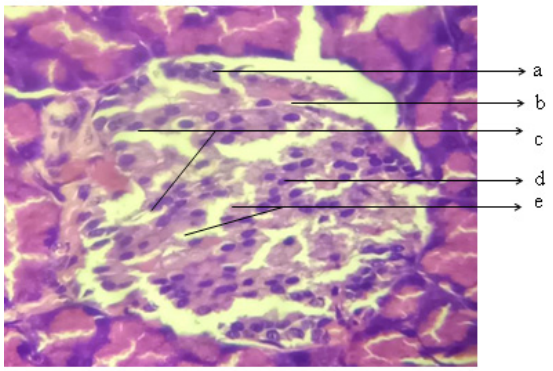

Figure 2. Histological structure of C(+) rat pancreas with a magnification of 10×40. a. β cell necrosis, β cell nucleus undergoes karyorrhexis (nucleus fragmentation), b. β cells undergo hypertrophy, c. Reduced β cell clusters and increased connective tissue, d. β cell necrosis, β cell nucleus undergoes pyknosis (nucleus shrinkage), e. Cell degeneration in the form of cytoplasmic vacuole.

The mean score of the histopathology of rat pancreas induced by alloxan and administered by extract of Aloe vera peel with a dose of 87.5 mg / kgBW (PI group) was lower than the C(+) group. Figure 3 represents histology data in P1 group that show a few tissue damage. EAVP treatment in the P I group with a dose of 87.5 mg/ kgBW was able to repair the damage of rat pancreas tissue. Damage levels appear to be reduced compared to the group C(+). It is indicated by the absence of a vacuole in the cytoplasm and the distribution of cells that are more homogeneous compared to the group C(+). Although the structure and shape of the Langerhans island of the PI rat group is irregular, but not as much as in the C (+) group. The use of EAVP can improve the conditions of Langerhans Island but it still cannot reach normal conditions.

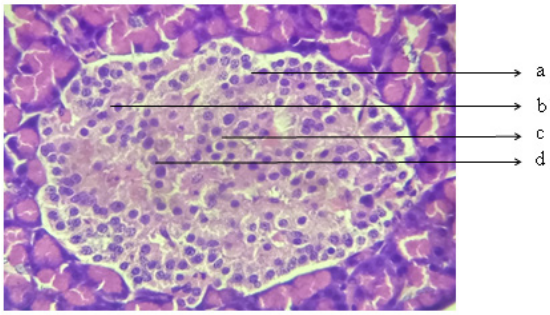

Figure 3. Histological structure of rat pancreas in group PI with a magnification of 10×40. a. β cells undergo hypertrophy, b. Reduced β cell clusters and increased connective tissue, c. Nucleus of necrotic β cell undergoes pyknosis (nucleus shrinkage). d. Nucleus of necrotic β cell undergoes karyorrhexis (nucleus fragmentation).

Figure 4 represents the histology of pancreas tissue in P II group. Histopathological picture of the pancreas, the intercellular cavity in the Langerhans island begins to improve, the cell distribution are more homogeneous compared to the group (C+). However, the condition of the Langerhans island is still not considered normal. Improvement of Langerhans island in group P II is followed by cell regeneration which is characterized by the presence of cells that colonized and the distribution of cells are more homogeneous.

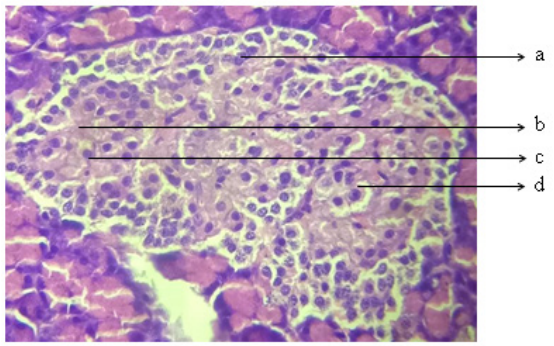

Figure 4. Histological structure of rat from PII with a magnification of 10×40. a. β cell necrosis, β cell nucleus undergoes karyorhexis (nucleus fragmentation), b. Reduced β cell clusters and increased connective tissue, c. β cell necrosis, β cell nucleus undergoes pyknosis (nucleus shrinkage), d. β cells undergo hypertrophy.

Group PIII have the best picture of histopathological observation compared to the other treatment groups. In this group, the pancreatic histopathology is close to normal with the Langerhans that are microscopically improving as can be seen in Figure 5.

Pancreatic histopathology with the treatment of EAVP of 350 mg/kgBW shows that the condition of the Langerhans is close to normal. Necrotic cells in the β cell nucleus were found to undergo karyorrhexis (core fragmentation) (a). However, it less than other treatment groups. Cell degeneration did not occur in this group, while endocrine cells were distributed homogeneously across the entire Langerhans island. Furthermore, the amount of connective tissue decreased, while the structure of Langerhans seen approaching the normal group, that indicated cell regeneration in the Langerhans. The reduction of necrotic cells was in line with cell regeneration, where the pancreatic cells of this treatment group showed an increase in the number of pancreatic β cells marked by the presence of colonized β cells.

This is thought to be influenced by the increasing number of bioactive compounds as the dosage increased (Prameswari & Widjanarko, 2014). The increase in dosage gives results in an increase in the number of bioactive compounds contained in the extract

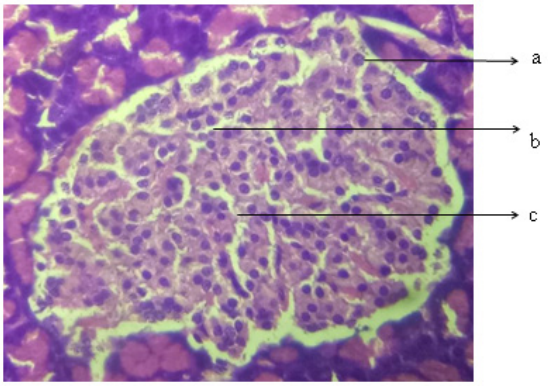

Figure 5. Histological structure of rat pancreas in group PIII with a magnification of 10×40. a. β cell necrosis, β cell nucleus undergoes karyorrhexis (nucleus fragmentation), b. Normal pancreatic β cells, c. Intercellular cavity.

Based on the results of the Kruskal Wallis calculation, the value of Xcount = 21.612 with sig 0.000<0.05. In other words, it can be concluded that there is a difference in damage to histopathological images of rat pancreas induced by alloxan between all groups C(-), C(+), PI, PII and PIII.

Further tests were carried out to find out which groups had different pancreatic histopathology. Based on Table 2, there were differences in the histopathological picture of rat pancreas induced by alloxan with the administration of EAVP.

Alloxan treatment had an effect on the degradation of β cells on the Langerhans island, the organ that responsible to synthesis the insulin in the body (Akrom et al., 2014). Pancreatic β cells are damaged by induction of alloxan which works specifically. The mechanism of alloxan occurs by the formation of reactive oxygen compounds that form superoxide radicals through the redox cycle. Through the redox cycle, very reactive hydroxyl will form which can cause damage to pancreatic β cells rapidly (Dipa et al., 2015). In addition, alloxan interferes with the process of cell oxidation due to the release of calcium ions from the mitochondria resulting in homeostatic disorders that cause the death of cells of the pancreas (Nugroho, 2006).

EAVP treatment in rat induced by alloxan can improve pancreatic histopathology due to the presence of bioactive compounds contained in EAVP. Bioactive compound can prevent oxidation of pancreatic β cells. Consequently, damage can be minimized. Bioactive compounds of EAVP has mentioned before in recent study by Gibson et al. (2014) that stated that ethanolic extract of *Aloe vera* contains secondary metabolites including flavonoids, alkaloids, tannins, saponins, and sterols. Scalbert et al. (2005) stated that compounds belonging to the polyphenol group has antioxidants activities and biological functions for improving glucose metabolism.

Flavonoids have antidiabetic activities that are capable of regenerating cells on the Langerhans Island. Flavonoid compounds can overcome insulin deficiency, therefore, the presence of flavonoids has a beneficial effect on the state of diabetes mellitus caused by the absence of

Table 2. The Result of Futher Test with Mann-Whitney

Test	Mann-Whitney U	Wilcoxon W	Z	Asymp. Sig. (2-tailed)	Criteria
C(-) - C(+)	0	15	-2.795	0.005	Significant
C(-) - PI	0	15	-2.825	0.005	Significant
C(-) - PII	0	15	-2.795	0.005	Significant
C(-) - PIII	0	15	-2.795	0.005	Significant
C(+) - PI	0	15	-2.652	0.008	Significant
C(+) - PII	0	15	-2.627	0.009	Significant
C(+) - PIII	0	15	-2.627	0.009	Significant
PI - PII	6	21	-1.392	0.164	Not Significant
PI - PIII	0	15	-2.652	0.008	Significant
PII - PIII	4.5	19.5	-1.687	0.092	Not Significant

Description: C(-): negative control group, C(+): Positive control group, P I: Treatment group 1, P II: Treatment group 2, P III: Treatment group 3. $\alpha < 0.05$.

insulin and damage to insulin receptors (Dipa et al., 2015). Alkaloids had been shown to have the ability to regenerate damaged pancreatic β cells (Arjadi & Susatyo, 2010). Antioxidant activity is able to capture free radicals resulted in the improvements of pancreatic β cell damage that causes DM 1 (Suryani et al., 2013).

The present study shows that EAVP with doses of 87.5 and 175 mg/kgBW, is able to improve the histopathology of the pancreas in alloxan induced rat compared with the positive control group. The effect of pancreatic tissue repair in diabetic rats (alloxan-induced) was more significantly seen in the administration of EAVP at a dose of 350 mg/kgBW. Whereas, 350 mg/kgBW of EAVP treatment (PIII group) had a lower mean of pancreatic histopathology score compared with treatment group I and II, and not significantly different from the negative control group. This means that the treatment of EAVP at a dose of 350 mg/kgBW is the most effective for improving the histopathology of the pancreas compared to other groups.

The experimental data indicate that EAVP in diabetic model rats can improve histopathological of pancreatic Langerhans island cells. This is presumably due to the presence of bioactive compounds namely flavonoids and polyphenols in EAVP. The activity of flavonoids and polyphenols acts as antioxidants (Jian et al., 2002). The antioxidant activity possessed by *Aloe vera* leaf extract had moderate antioxidant activity with an IC50 value of 152.87 ppm, that assumed to repair pancreatic beta cells damaged. Coskum et al. (2004) stated that the addition of antioxidants compound can reduce free radicals and protect the pancreatic islet from the effects of diabetogenic agents.

Aloe vera as one of the ingredients of traditional medicine, needs to be researched, developed and optimized for its use. At the laboratory level, *Aloe vera* peel extract has been shown to repair rat pancreatic damage. With this result, *Aloe vera* has the potential to be developed as a phytopharmaca for the prevention or treatment of diabetes mellitus disease.

CONCLUSION

In conclusion, our present study indicates that *Aloe vera* peel extract can improve the pancreatic histopathology for 28 days experiment treatment. While a dose of 350 mg/kgBW shows no significantly different condition compared to the normal group in pancreatic histopathology.

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