DOI: 10.21776/ub.jpacr.2022.011.01.618 *J. Pure App. Chem. Res.*, **2022**, 11 (1), 31-37
30 March 2022

The Effect of Klika Ongkea Extract (*Mezzetia parviflora* Becc.) on Pancreatic β-Cells Regeneration by Streptozotocin-Induced in Wistar Rats

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Received 14 September 2021; Accepted 30 March 2022

ABSTRACT

This study aimed to determine the effect of Klika ongkea (*Mezzetia parviflora* Becc.) extract on the regeneration of pancreatic β-cells on Wistar rats that were induced using streptozotocin. Streptozotocin (STZ) with a 40 mg/kg BW dose was used in this study. Eighty adult male Wistar rats were used for this study. The animals were divided into four groups, Group I, as a healthy controls; Group II as an STZ-induced, Group III, STZ-induced and treated with galvus (Vildagliptin) 0.9 mg/200 g BW; Group IV, STZ-induced and treated with Klika ongkea 100 mg/kg BW. This study was conducted for 28 days. Four animals each group on the 1st, 7th, 14th, 21st and 28th days were observed to analyze the number of pancreatic β-cells. On the last day, pancreases were isolated and stained with hematoxylin & eosin (H&E) to analyze the regeneration of pancreatic β-cells. The data analysis was performed using an Independent-Sample T-Test to compare the number of pancreatic β-cells. The results showed that the administration of Klika ongkea extracts affected the pancreatic β-cells regeneration. These findings suggest that Klika ongkea has an effect on the regeneration of pancreatic β-cells in streptozotocin-induced rats.

Keywords: Klika ongkea, pancreatic β-cells, streptozotocin

INTRODUCTION

Currently, the world of health discusses a lot about free radicals and antioxidants. It occurs cause most of the diseases are initiated by an excessive oxidation reaction in the body. Oxidation reactions happen all the time. These reactions trigger the formation of highly active free radicals, which can damage cell structure and function. However, antioxidants such as superoxide dismutase, catalase, and glutathione peroxidase can inhibit free radical reactivity [1]. One of the diseases related with excessive free radicals is diabetes mellitus (DM) [2].

The prevalence of diabetes has been steadily increasing rapidly. In 2019 suffering of DM in the world reached up to 463 million, and in 2045 was estimated will be increased by 700 million or more [3]. Data from International Diabetes Federation in 2021 showed that Indonesia was in 5th place in the most significant number of DM sufferers globally after China, India, Pakistan and the United States [4]. In South Sulawesi, the DM sufferers in 2018 as many as 1.8% [5].

Diabetes was related with hyperglycemia, it refers to high levels of glucose in the blood. This occurs due the pancreas is unable to produce enough insulin or insulin resistance. Insulin is produced by the β cells of the pancreas, so if there is damage to the β cells it will cause

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hyperglycemia. Prolonged hyperglycemia condition leads to the production of ROS or free radicals [6].

Recently, polyphenols compounds have been receiving much attention for healthy, such as for DM therapeutic. They are widely distributed in plants. Polyphenols have a role to control DM by enhancing the secretion of insulin, inhibiting the absorption of glucose from the small intestine, and controlling the release of glucose from liver [7]. Moreover, polyphenol have an effect as an antioxidant and anti-inflammatory [8]. One of the plants containing polyphenolic compounds is Klika ongkea (*Mezzetia parviflora* Becc.) [9]. Klika ongkea is an indigenous plant in Andaman, Sumatra, Kalimantan, Maluku Thailand peninsula, and Malaysia peninsula [10].

Empirically, the extract of Klika ongkea, mainly the wood bark of the plant is used by the Bau-Bau people of Buton Regency, Southeast Sulawesi, Indonesia as a traditional medicine for cholesterol-lowering, slimming, diabetes mellitus, and tumors [11,12]. The previous study shows that the ethanol extract of Klika ongkea has effectively as a scavenge free radical [11]. Phytochemical testing of Klika ongkea showed a 20.24% polyphenol compounds, flavonoids and tannins 1.76% 26.46%, respectively [12].

Considering the potential and high content of polyphenolic compounds in Klika ongkea, this study has been carried out to develop the Klika ongkea extract into a standardized herb with an anti-hyperglycemic effect. Previous study showed that the polyphenols Klika ongkea decreased the levels of blood glucose in rats induced by Streptozotocin [12]. The polyphenol has been known for its antioxidant and anti-hyperglycemia activities by capturing free radicals and inhibiting gluconeogenesis respectively [13]. This compound also has ability to regenerate pancreatic β-cells [14] that can repair the disturbance of glucose metabolism homeostasis as a result of pancreatic cell damage after streptozotocin (STZ) induction [15]. This experiment aims to investigate the effect of Klika ongkea extract (*Mezzetia parviflora* Becc.) on regeneration of pancreatic cells in Wistar rats induced by single-dose of STZ 40 mg/kg BW (i.p).

EXPERIMENT

Experimental Design.

The type of this study is experimental with a modified *pretest-posttest randomized controlled group design*. The sample was determined based on the number of groups, namely four groups, each group consisting of 20 rats so that the sample in this study was 80 individuals. As many as 60 rats were taken randomly as 20 healthy controls, 20 unhealthy controls, and 20 drug controls. The remaining 20 individuals were also taken at random to be put into treatment groups. This study was conducted for 28 days. Rats were divided into four treatment groups: Group I, as a healthy controls. A groups given a colloidal solution of Na. CMC 1% orally every day for 28 days. Group II as an STZ-induced at a single dose 40 mg/kg BW i.p. Group III, STZ-induced and treated with galvus (Vildagliptin) 0.9 mg/200 g BW. Group IV, STZ-induced and treated with Klika ongkea 100 mg/kg BW. All of the groups, on day 1, to 14, to 21 and to 28 fasted before and then observed each the 4 tails.

Plant Materials and exctract preparation

Fresh Klika ongkea were obtained from Buton regency, Southeastern Sulawesi province. The Klika ongkea was extracted by methanol 70% with maceration method. The solvent from the extract was evaporated by rotary evaporator then freeze dried.

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Animal Model:

The use of experimental animal in this study was conducted under the guidance of the basic standard, which has been approved by The Ethical Committee of Megarezky University, Indonesia (No. 002.E/07.091056/XII/2021). A total of 80 male rats (*Ratus novergicus*), strain Wistar were used in this study. The rats were maintained in standard cages at a free-pathogen facility and in the Laboratory of the Faculty of Pharmacy, Hospital technology and Informatics, Megarezky University, Makassar, Indonesia. All animals received the normal diet. 60 male rats were induced by Streptozotocin (STZ) at a dose 40 mg/kg BW.

Measurement of Blood Glucose:

Measurement of blood glucose levels is done 5 times, which is 1 time before STZ induction and 4 times after STZ induced, ie day 7, to 14, to 21 and to 28. it was done with taking blood intraorbital 0.5 ml and subsequent capillary pipette blood glucose levels were measured using a glucometer [16].

Histological observation of pancreas

Examination of the pancreatic β-cells number was conducted with Hematoxylin Eosin (HE) staining. The rats were sacrificed, then the pancreas was removed and fixed in formalin solution. The rat's pancreas was prepared and embedded in paraffin for histological observation according to Ariastuti et al. [17] with modification.

Data Analysis

Data analysis was performed using the Independent-Sample T-Test to compare the number of pancreatic β-cells between groups. The number of pancreatic β-cells were statistically analyzed using the SPSS program with one way of variance analysis (ANOVA) with p-values < 0.05.

RESULT AND DISCUSSION

The Effect on Rat Pancreatic Islets Histology

The Histomorphological observation of pancreatic sections with HE was done in all groups (Figure 1.). Histology of pancreatic in the Group I (Figure 1A) showed that the condition of Langerhans islet still intact, clearly defined cytoplasmic boundaries, and the cells in the islets of Langerhans are clearly visible. Histopathological examination in the Group II (Figure 1B) on STZ-induced, there were changes in several structures of Langerhans islet, including decreased number of Langerhans cells, reduced size of islets of Langerhans as compared to Group I, and atrophy. The Group III and Group IV (Figure 1C and 1D) are treatments group showed that the Langerhans islets close similarity to Groups I. Its evidence that the treatment of Galvus as a drugs control and Klika ongkea have an effect to cellular improvement or be able to regenerate the cell of Langerhans islet, characterized by increase of the cell on Langerhans islets and the location of nucleus cell is orderly.

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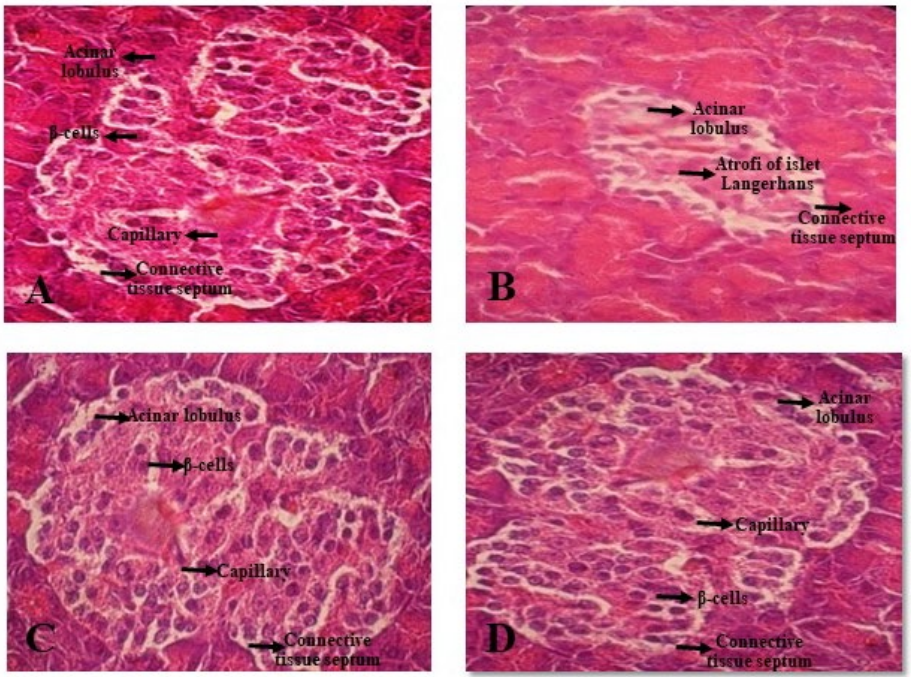

Figure 1. Histology of pancreas. A. Control groups (healthy groups); B. Groups of STZ-induced; C. Treatment galvus groups; D. Treatment Klika ongkea groups

Based on figure 1, this study shows that the induction of STZ causes damage to the pancreatic islet. Intraperitoneal injection at dose 40 mg/kg BW of STZ was able to destroy the pancreatic β-cells and cell destructions through DNA alkylation and ROS production. STZ is a substance that can cause alkylation. It renders directly methylates DNA, leading to DNA strand breaks, unscheduled DNA synthesis, DNA addition, chromosomal aberrations, micronuclei, sister chromatid exchanges, and pancreatic cell death [17]. Several studies declare that STZ has been used to induce an animal model of diabetes mellitus. STZ selectively destroys the pancreatic β-cells and resulting in diabetes mellitus [15].

The administration of Klika ongkea have an effect to cell regeneration of Langerhans islet were assumed due to the presence of polyphenolic compounds [9]. Flavonoids are one of the main groups of polyphenols that have a role in therapeutics found in natural products [18]. Flavonoids are involved in repairing pancreatic tissue damage caused by DNA alkylation due to STZ induction and, consequently, can improve the morphology of the rat pancreas. Flavonoids are reported to have an antidiabetic activity that can regenerate Langerhans islet cells [19].

The Effect on Number of Pancreatic β-cells

The analyzed result (Table 1) showed the average number of pancreatic β cells of Group I and Group II groups from treatment on days 21 were significantly different ($P<0.05$). Whereas Group I with Group III and Group IV were not significantly different. The data showed significant differences between Group II with Group III and Group IV groups ($P<0.05$). It shows that the treatment of Klika ongkea extracts from day 21 enhanced pancreatic β cells. The reduction number of the pancreatic β-cells on the Group II occurred from day 7 to day 28 with an average score of 86.45 ± 5.09 to 50.07 ± 3.30.

The number of pancreatic β-cells of group III and IV on days 21 and 28 increased significantly as compared to group II ($P<0.05$) caused by pancreatic β-cell regeneration (more

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number of β cells). Pancreatic β-cells play important role on insulin secretion that is responsible for regulation of glucose homeostasis [20]. Statistically, the number of pancreatic β-cells on group I, III and IV showed a significant difference against group II ($P<0.05$). The average of pancreatic β-cell increased on group I, III and IV were 89.10 ± 5.62; 86.05 ± 2.19; and 89.70 ± 5.65 respectively, comparing to group II with an average score of 50.07 ± 3.30. The decreased number of pancreatic β-cell in the Group II as compared to the normal group (Group I) were assumed due to high production of ROS after STZ induction. STZ can increase the levels of ROS [21]. ROS can cause the progression and complications of DM due to increased free radicals and decreased antioxidant enzymes [22]. Treatment groups with Galvus (Drug control) and Klika ongkea at 100 mg/kg BB dose, performed the regeneration of pancreatic β cells. It was demonstrated by histological preparations of pancreatic Langerhans islets in each treatment group. Galvus succeed to restore the β-cell mass and Klika ongkea also showed improvement of β-cell pancreas regeneration in the STZ induce. It is due to the polyphenol compound in this Klika ongkea.

Table 1. The number of pancreatic β-cells

Observations on Day-	The Number of Pancreatic β-cells			
	Groups I	Groups II	Groups III	Groups IV
1	87,35±5,50	87,75±5,21	87,77±8,43	88,07±4,67
7	87,12±5,57	86,45±5,09	84,62±4,17	84,72±5,02
14	88,20±5,19	79,57±6,80	84,55±6,17	85,57±1,36
21	83,50±10,48*	64,07±4,97**	84,00±2,59*	88,30±7,32*
28	89,10±5,62	50,07±3,30	86,05±2,19	89,70±5,65

Note : Values represent mean ± SE (standard error) at $P<0.05$.
*Significant different against group II; **Significant different against group I, III, IV.

The improvement of pancreatic β-cells from day 14 of Klika ongkea extract treatment was characterized by enhancing the pancreatic β-cell average score. Cell neogenesis can occur as a result of insulin-mediated normalization of blood glucose levels. Two types of cell precursors will appear regenerating islets of Langerhans. One type expresses glucose transporter-2 (GLUT 2), and another type represents insulin and somatostatin. Both cells then become monospecific cells containing insulin and filling the damaged islets of Langerhans [23].

The improvement of Langerhans islet on the group of Klika ongkea extract was characterized by the enhancement of pancreatic β-cell number. The treatment of Klika ongkea extract showed the enhancement of pancreatic β-cell count compared to group I (control) and similar with group III (drug control) that treated with 0.9 mg/200 g galvus drug. The enhancement of pancreatic β-cell number on group IV was expected to be caused by the bioactive compounds in Klika ongkea extract, such as polyphenol (flavonoid) tannin) and alkaloid that acts as an antioxidant. Antioxidant involved in the repair process of cell damage that caused by free radical. The antioxidant function is as reducing agents and reduce oxidizing

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agents before damaging cells so that cell damage can be reduced [24]. Polyphenol was known to involve in free radical scavenging or as a natural antioxidant [25]. This antioxidant activity allows polyphenols to capture or neutralize free radicals (such as ROS or RNS) associated with phenolic OH groups so that they can improve the state of damaged tissue. In other words, the inflammatory process can be inhibited [26].

CONCLUSION

In conclusion, this study demonstrated that the administration of Klika ongkea extracts at dose 100 mg/kg BW affected the number of pancreatic β-cells regeneration and succeeded to improve/repair the histomorphology of pancreatic islets of Langerhans. Based on these results, the Klika ongkea have a potential as an antidiabetic agent.

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