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Research Article

ANTIDIABETIC ACTIVITY OF ETHANOLIC EXTRACT OF *Kalanchoe pinnata* LEAVES IN ALLOXAN INDUCED HYPERGLYCAEMIC RATS

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ABSTRACT

Diabetes remains a major burden in health care both in developed and developing countries. *Kalanchoe pinnata* has been used as a traditional medicine to treat diabetes. We try to find scientific evidence of antidiabetic activity of *Kalanchoe pinnata* extract (KPE) through hypoglycemic effect using animal model of diabetes mellitus. Hyperglycaemia was developed in rats using alloxan 150mg/kgBW. Three days after alloxan injection, rats having fasting blood glucose (FBG) >200mg/dL were divided into six groups, namely HG (hyperglycaemia), HG+KPE high-dose (hyperglycaemia+KPE 33.2mg/kg), HG+KPE medium-dose (hyperglycaemia+KPE 11.6mg/kg), HG+KPE low-dose (hyperglycaemia+KPE 5.8mg/kg), standard drug 1 (hyperglycaemia+glibenclamidee 1.35mg/kg), standard drug 2 (hyperglycaemia+acarbose 13.5mg/kg). Then, FBG was measured every 5 days recorded as t1, t2, and t3 to determine fluctuations in blood glucose. At the end of the study, rats were sacrified, pancreas was collected and number of pancreatic beta cell langerhans was determined. KPE 11.6mg/kg showed best hypoglycemic effect and improvement of the number of pancreatic beta cell langerhans. KPE has hypoglycemic effect through improvement of the number of pancreatic beta cell langerhans but not in dose dependent manner.

Key words: Antidiabetic, *Kalanchoe pinnata,* hyperglycaemic, alloxan, pancreatic

INTRODUCTION

WHO (2015) reported 9% people aged 18+ worldwide suffered from diabetes, while in Indonesia the prevalence was only 1.5% (Kemenkes RI, 2013). It is worth noticing that this disease is a burden of health care in both developed and developing countries (Abdulazeez *et al.,* 2013). Diabetes develops from prolong glucose imbalance between intracellular and extracellular resulted in hyperglycaemia state in which blood glucose can not be utilized by living cells. These conditions lead to vascular complication through increasing oxidative stress and inflammation. Vascular complication is the leading cause of death in both diabetes type I and type II patient (Domingueti *et al.,* 2015).

Diabetes mellitus (DM) are classified as two major disease: type I DM and type II DM. Type I DM is an autoimmune disease that immune system of the body attack insulin producing pancreatic β cells results in low insulin production. This condition leads to insulin dependent DM. In the other hand, type II DM was caused by inadequate action of insulin in action site (Hossain *et al.,* 2016).

Pharmacotheraphy in DM patient does not eliminate the cause of the disease but targeting the symptom using insulin and hypoglicemic agent such as sulfonylurea and biguanids (Abdulazeez *et al.,* 2013; Verma et al, 2015). However, sulfonylurea exert several adverse effect such as hypoglicemia and weight gain, in addition to toxic effect on liver and renal, while metformin cause lactic acidosis (Verma *et al.,* 2015).

Alloxan is widely used in experimental pharmacology to induce diabetes in rat. The pathological mechanism is by destruction of insulin producing pancreatic β-cells. Alloxan undergo redox reaction resulted in increasing radical species. In this case, pancreatic β-cells is the most sensitive organ towards these radical attacks. Hyperglycaemia state does not occur immediately. At first, alloxan induce insulin

release independent of glucose level. However, this effect is followed by suppression of the islet respons to even high level of glucose leading to hyperglycaemia state (Szkudelski *et al.,* 2001).

Nowadays, the need for safe and effective drugs has brought natural product as the promising source of companion drug for diabetes (Sharma and Gupta, 2015). *Kalanchoe pinnata* has been traditionally used worldwide as medicinal plants, including to treat diabetes (Patil *et al.,* 2013). Our previous study reveals its antidiabetic activity through inhibition of α-glucosidase with IC_{50} 16.12ppm. Meanwhile, radical scavenger activity using DPPH method revealed IC_{50} 29.61ppm of ethanol fraction, 23.15ppm of buthanol fraction, and 17.27 ppm of ethyl acetic fraction (Indah *et al.,* 2013).

Therefore, current study aims to determine antidiabetic activity of ethanolic extract of *Kalanchoe pinnata* in alloxan-induced diabetic rats.

MATERIALS AND METHODS

Kalanchoe pinnata leaves were collected from Kawasan Puspiptek Serpong and were authenticated by Research Center for Biology, Indonesian Instititute of Sciences (LIPI).

Preparation of plant extracts

The leaves were dried using blower oven <50°C for 24h, crushed, and extracted using 70% ethanol (3x24h maseration). Then, ethanol extracts obtained were concentrated in rotary evaporator under vacuum. The yield of ethanolic extract of *Kalanchoe pinnata* (KP) dry leaves was 15.7%.

Animals

The Male Sprague Dawley rats (150-350g) were procured from Fakultas Kedokteran Hewan IPB, Bogor and housed under standard conditions of temperature and relative humidity with 12h light/dark cycle. Animals were fed on standard commercial pellet diet and water ad libitum. The ethical clearance of the expreriment have been approved by Health Research Ethics Commitee, University of Indonesia and Cipto Mangunkusumo Hospital, Indonesia (Ethical clearance certificate number: 663/UN2.F1/ ETIK/2016).

Induction of diabetes

Induction of diabetes was performed according to Misra and Aiman (2012), with modification. Base line FBG was measured before alloxan injection (t(-1)). Alloxan monohydrate (Sigma Aldrich; stored at 4°C) was dissolved in normal saline at room temperature (freshly prepared) and injected to 30 overnight fasted male Sprague-Dawley rats at a dose of 150mg/kg intraperitonially. The animals were then kept for the next 7 days on 10% glucose after alloxan administration. After 72h of alloxan injection (t0), FBG was determined using GlucoDr glucometer strips. Animals with FBG >200mg/dL were considered to have developed experimental diabetes as shown by hyperglycaemia state. Then, FBG was measured every 5 days recorded as t1, t2, and t3 to determine fluctuations in FBG. Blood was collected from tail vein. As standard reference, Glibenclamide was given at a dose of 1.35mg/kg orally per day, while Acarbose was given at a dose of 13.5 mg/kg orally per day.

Animal experimentation

In the present study the animals were distributed into 7 groups (n=5) as follows: normal control, HG (hyperglycaemia), HG+KPE high-dose (hyperglycaemia+ KPE 33.2mg/kg), HG+KPE medium-dose (hyper-glycaemia+KPE11.6mg/kg),HG+KPE low-dose (hyperglycaemia+KPE 5.8mg/kg), standard drug 1 (hyperglycaemia+ gliben-clamidee 1.35mg/kg), standard drug 2 (hyperglycaemia+acarbose 13.5mg/kg). The study was conducted for 15 days to evaluate the potential of the extracts to lower FBG level. Body weights of the rats were monitored weekly during the study period.

Pancreas histological observation

Pancreas histological observation was conducted on day 15, at the end of the experimental period. Rats were euthanized by ether and the pancreas were collected and fixed with bouin's solution for 24h. The pancreas was then stained with *Hematoxylin eosin* (HE) for observation of number of beta cell Langerhans.

Tabel I. Effect of KPE on FBG during study

Group	FBG± SD (mg/L)				
	t (-1)	to	t1	t2	t3
Normal	103.67±15.33	117.92±8.07	107.83±7.15	97.42±13.75	93.5±8.24
HG	115±2.45	429±106.88	267±106.10	207±104.24	130±16.85
HG+KPE (high dose)	112.2±12.76	384±137.48	174.6±111.84	111.2±24.22	116±13.93
HG+KPE (medium dose)	112±10.05	259.4±48.53	134.4±30.00	115.8±17.88	116.2±10.40
HG+KPE (low dose)	112.2±6.57	353.8±163.69	142.4±35.67	113.6±16.36	124.6±10.26
HG+ Acarbose	109.5±16.99	262±36.79	121.83±22.25	105.67±14.51	97±8.65

Statistical analysis

Numeric data were expressed as mean ± SD. Data were analyzed using one-way analysis of variance (ANOVA) ($p<0.05$) followed by Multiple LSD.

RESULTS DISCUSSION

Base line FBG (t(-1)) was determined before alloxan injection. All rats showed normal FBG with no significant difference among the groups (Table I, Figure 1).

Three days after alloxan injection (t0), hyperglycaemia state was confirmed by FBG > 200mg/dL in all the rats. However, FBG increase was not significant in HG+KPE medium dose group and acarbose group compare to normal group. Meanwhile, no significant difference was observed among groups that received alloxan injection.

FBG reduction were detected after 5 days (t1) alloxan injection in all groups. While 5 days treatment brought FBG to a level < 200mg/dL in all groups, HG groups remain suffered from hyperglycaemia as shown by FBG >200mg/dL. As a result, only HG group differs significantly from normal group. In the other hand, all treatment reduced FBG significantly, but not high-dose KPE.

Ten days after treatment (t2), all rats' FBG kept decreasing. However, HG group remain in hyperglycaemia state as shown by FBG >200mg/dL. As a result, only HG group differs significantly from normal group and all treatments reduced FBG significantly.

Fifteen days after treatment, all rats' FBG went back to normal as shown by FBG <200mg/dL. However, only standard treatment (glibenclamide or acarbose) reduced FBG similar to normal while KPE treatment did not reduced FBG significantly.

Histological observation using HE staining showed that all treatment, except KPE high-dose, improved the morphology of β cells in comparison to the HG group based on the number of cell (Figure 2). However, only KPE medium and low dose increased the number of β cells significantly.

K. pinnata has traditionally been used as medicinal plant to treat various ailments around the world, including diabetes (Patil *et al.,* 2013).

Therefore, several study have been conducted to evaluate its bioactivity, such as antidiabetics, anticonvulsant, antinociceptive, antiedematogenic, antiinflammatory, anticancer and antiHPV, antileishmanial (Patil *et al.,* 2013; Mora-Perez *et al.,* 2016; Ferreira *et al.,* 2014; Mahata *et al.,* 2012; Muzitano *et al.,* 2006).

K. pinnata leaves contain anthocyanins that considered responsible for its antidiabetic, anticancer, cardiovascular, and neurological activity (Cruz *et al.,* 2012). Anthocyanins are water soluble flavonoid compounds that gives color in plant (Cruz *et al.,* 2012). Phytochemistry study of the KPE used in this study revealed flavonoids and steroids compound and isolated quercetin glycosides (Fajriyah, 2011).

We suggest that the bioactivity of *K. pinnata* in reducing FBG and improving morphology of the islets of Langerhans and β cells were caused by its various natural compounds constituent, for example quercetin through antioxidant mechanism (Figure 3).

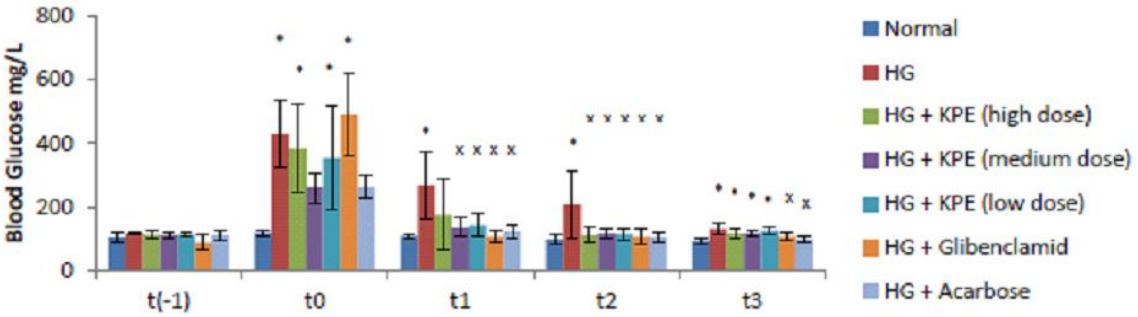

Figure 1. Effect of KPE treatment on FBG level in alloxan induced rat. HG=Alloxan, KPE= *Kalanchoe pinnata* extract, high dose = 33.2mg/kg BW, medium dose= 11.6mg/kg BW, low dose= 5.8mg/kg BW, Glibenclamide = 1.35mg/kg bw, Acarbose = 13.5mg/kg BW. t(-1) =base line, t 0= after alloxan injection, t1 or t2 or t3 = 5 or 10 or 15 days after test sample administration. Data are expressed as mean ± SD. * & ^{x}, $p < 0.05$ (* indicates comparison between normal and HG; ^{x} indicates comparison between HG and treatments).

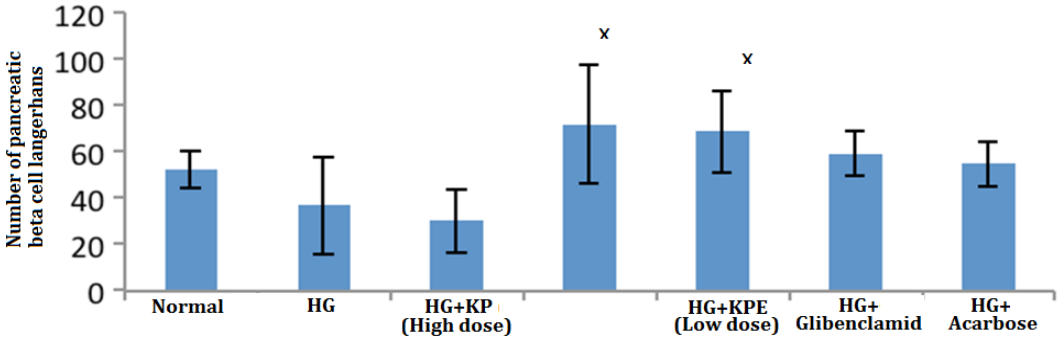

Figure 2. Effect of KPE treatment on number of pancreatic beta cell langerhans in alloxan induced rat. HG=Alloxan, KPE= *Kalanchoe pinnata* extract, high dose = 33.2 mg/kgbw, medium dose= 11.6 mg/kg bw, low dose= 5.8 mg/kg bw, Glibenclamide = 1.35 mg/kg bw, Acarbose = 13.5 mg/kg bw. ^{x}, $p < 0.05$. (^{x} indicates comparison between HG and treatments).

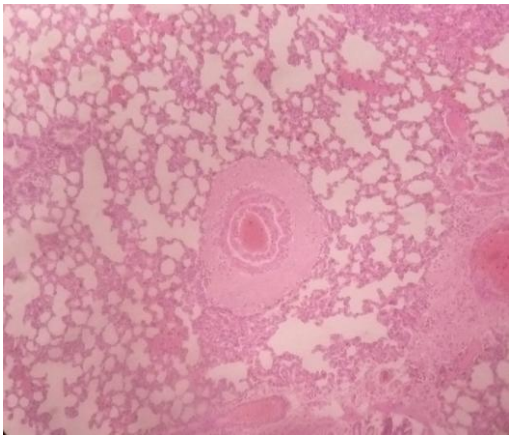

Figure 3. Pancreatic Beta cell Langerhans stained with *Hematoxylin eosin* (HE).

It is worth noting that oxidative stress plays a role in the pathogenesis of diabetes mellitus. Hyperglycaemia was characterized with increase oxidative stress leading to defect in insulin action and insulin secretion. Quercetin as antioxidants reduced oxidative stress leading to protection of β cells of the pancreas resulted in the increase of insulin production and decreased of FBG. Several studies reported quercetin mechanism of action in diabetes such as decreases lipid peroxidation, increases antioxidant enzymes activity, inhibition of insulin-dependent activation of phosphoinositol-3-kinase (PI-3K), reduces intestinal glucose absorption by inhibiting GLUT (Sunarwidhi *et al.,* 2014).

Based on pharmacological and histopathological studies, we suggested that the hypoglycemic effect of KPE was optimum at medium dose (11.6mg/kg bw).

However, it is difficult to conclude that hypoglycemic activity of KPE in this study is only caused by quercetin. Various active compounds in KPE may synergistically increase hypoglycemic effect.

Therefore, the mechanism action of each active compounds in the extract needs further investigation, including immunohistochemical observation on pancreatic insulin expression.

CONCLUSION

This study showed that KPE possesed bioactivity in lowering blood glucose and improving the morphology of Langerhans islet and β cells. KPE is potential to be developed as a blood glucose-lowering agent for diabetic patients.

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