ANTINEPHROPATHY EFFECT OF Aloe vera GEL TO PKC-β LEVEL ON WISTAR RAT KIDNEY IN DIABETES MELLITUS

Juliyatin Putri Utami¹, Umie Lestari², Nuning Wulandari²
Hendra Susanto², Yuda Handaya³

¹ Student of Biomedic Magister Programme, Medical Faculty, Brawijaya University, Veteran street 1, Malang Indonesia.
² Lecturer of Biology Department, Faculty of Mathematic and Science, State University of Malang, Semarang street 5, Malang Indonesia.
³ General and Laparoscopic Surgeon, Kepanjen Hospital
Corresponden Author: juliyatin_putriu@yahoo.com

ABSTRACT

Aloe vera gel has a large vitamins which has become potential candidate to supressed vascular damaged of organ which potentially lead to complications such as diabetic nephropathy. This process through down regulation of PKC-β level as a cascade activator of gene transcription such as contraction and permeability of cell. The research goal was to determinethe effect of Aloe vera gel on PKC-β level in the kidneys of diabetes mellitus (DM) wistar rats. Twenty four rats divided into eight treatments (negative controls, positive controls, Non DM with A.vera gel dosage 30, 60, and 120 mg/day, DM with A.vera gel dosage 30, 60 and 120 mg/day) with three replications each. All of the data was analyzed using one way Anova with Statistical Product and Service Solution 16 software. The research result showed that the A. vera gel has significant effect to decrease PKC-β level significantly (p < 0.000) in diabetes mellitus (DM) wistar rat kidney. In conclusion A.vera gel supressed diabetic nephropathy process via PKC-β and ROS activity in optimum dosage 30 mg/day.

Key words: Aloe vera Gel, PKC-β level, Diabetes mellitus.

INTRODUCTION

Diabetes mellitus is a major cause of kidney failure after hypertension. A total of 25% - 40% of kidney failure suffered by diabetics. According to data from the Ministry of Health of Indonesia, in 2010 the prevalence of diabetes was recorded 12.7 percent of the entire population and increased 2-3 times compared to developed countries. In epidemiology, it is estimated that by 2030 the prevalence of diabetes mellitus (DM) in Indonesia reached 21.3 million people (Departemen Kesehatan RI, 2011).

Diabetes mellitus is characterized by high blood sugar levels (hyperglycemia), due to the increased rate of metabolic disorders (Roy et al., 2005). DM can be divided into two types: type I insulin-dependent diabetes is caused by the autoimmune islet beta cells of Langerhans, and type II is not insulin-dependent diabetes is caused by insulin insensitivity in receiving cells (Corwin, 2009). DM type I and type II have in common that led to the development potential of microalbuminuria (albumin loss of 30-300 mg/hari) toward proteinuria (loss of albumin>300mg/hari) which is one of the stages of diabetic nephropathy (DN) (Sonkodi & Mogyorosi, 2003 ). Diabetic nephropathy is caused by hyperglycemia-induced changes in capillary blood vessels in the kidneys (Koya et al., 2003).

Early phase of diabetic nephropathy, characterized by glomerular hyperfiltration and increased glomerular filtration rate (glomerular filtration rate, GFR). GFR depends on the
surface area of glomerular filtration (Corwin, 2009). An increase in glomerular surface area (expansion) will increase the GFR. It is associated with increased cell growth (structural) and expansion of the kidney caused by hyperglycemia (Sunaryanto, 2010). Structural changes such as an increase in glomerular extracellular matrix deposition, and functional changes such as increased permeability of the glomerular basement membrane (Schena et al., 2005) which involving multiple pathway. Some of them are the cellular mechanisms, increased activation of the polyol pathway, advanced glycation end products lines (AGEs), triggering the production and action of active oxygen (oxidative stress), and activation of protein kinase C (PKC) (Geraldes et al., 2010).

Protein Kinase C (PKC) is a serine protein binding / threonine kinase that affect signal transduction pathways that play in many cellular functions (in Geraldes Newton et al, 2011). PKC consists of 12 isoforms, one of which is PKC-β. PKC-β isoforms are the predominant isoform in glomeruli, retina, aorta and liver (Ohshiro et al., 2005). PKC-β cause glomerular hyperfiltration and albuminuria in diabetic nephropathy. PKC-β isoforms affect cells function such as β cell activation, apoptosis induction, endothelial cell proliferation and glucose absorption (Su, 2002). Joy et al. (2005) study showed that administration of ruboxistaurin mesylate (pharmacologic agents) can inhibit PKC-β isoforms (1 and 2), thus potentially reducing the burden of microvascular complications in diabetic patients.

The activation of PKC caused by the accumulation of diacylglycerol (DAG) in the cell. In addition, oxidative stress is capable of inducing prolongation reported PKC activation in cells, either through reactive oxygen species (ROS) caused by hyperglycemia or by advanced glycation end products “(AGEs) which indicates the activation of PKC directly (Meier et al., 2007). Reactive oxygen species (ROS) contribute to trigger PKC responsibility mesangial cells in the kidneys with diabetes (Whiteside et al., 2002).

Treatment for diabetes has been widely cultivated by pharmacologists. Herbal treatment is chosen by people as alternative therapies most because of the lower cost and quite significant effect. Herbs that have potential to reduce blood sugar levels are basil and celery. Based on previous research (Ricky, 2007), ethanol extract of basil leaves can lower blood sugar levels (GDP) and the levels of malondialdehyde (MDA), which increases the occurrence of ketosis with the optimal dose of 300 mg / kg body weight of diabetic rats.

One of the herbal remedies that can treat kidney failure is Aloe vera. Aloe vera gel contains vitamins are included in important antioxidants such as vitamins A, C, and E, vitamin B (thiamine), minerals such as zinc, chromium and manganese as well as 20 amino acids and various active substances such as lignin, saponins and potentially treat accemann disease (Joseph et al., 2010). Antioxidant supplementation for diabetic patient is a supportive therapy in reducing free radicals that play a role in the activation of PKC-β pathway which make changes in the structure and function of the DN kidney. Antioxidant content of Aloe vera gel suggested to be antinephropathy which capable to inhibit the activation of PKC isoforms. This study aim to determine the effect of Aloe vera gel on PKC-β levels in the Wistar rat kidneys diabetes mellitus (DM).
MATERIALS AND METHODS

Aloe vera extraction

A. vera leaves were collected from Batu-Malang Plantation, East Java. After being washed, it was crushed with juicer followed by adding 96% ethanol (in 1:4 ratio). The mixture was precipitated for 10 h at 10°C. The precipitate is separated from the solution by using a suction filter next to vacuum oven dryer at a temperature of 50°C.

Animal Treatment

Male Rats Wistar strain were used as animal model. Animals were fasted for 18 hours before the experiment begins. Animals were divided into five groups: Positive control (C+), Negative Control (C-), diabetes mellitus group treatment (DM I, DM II and DM III) were injected with intraperitoneal 0.05 ml streptozotocyn in 1.5% and non diabetes mellitus treatment (NDM I, NDM II, and NDM III) while negative control group was treated by 1% PBS. DMI, DMII, DMIII group were fed A. vera gel with variation dosages: DMI (30 mg/day), DM II (60 mg/day), and DM III (120 mg/day). NDM I, NDM II, and NDM III group were fed A. vera gel with those variations too. The treatment was performed for 7 days to produce Diabetes Melitus animal model. A. vera gel with various dosage treated for 14 days. On the 14th day, rats were dissected and their kidney were taken. Fresh kidney extract were prepared for Enzym Linked Immunosorbent Assay (ELISA) to evaluate the PKC-β level.

RESULT AND DISCUSSION

![Graph showing PKC-β levels across different groups.]

Description:
A = Negative control (normal)
B = DM with A. vera gel dose 30 mg/day
C = DM with A. vera gel dose 60 mg/day
D = DM with A. vera gel dose 120 mg/day
E = Positive control
F = NDM with A. vera gel dose 30 mg/day
G = NDM with A. vera gel dose 60 mg/day
H = NDM with A. vera gel dose 120 mg/day

Figure 1. Relation between Dose of Aloe vera gel on PKC-β Level. Different notation shows a significant different between all of the groups by statistical analysis (p < 0.05).
The average value of the kidney levels of PKC-β in Figure 1 after being given Aloe vera gel are sorted from highest to lowest value of the NDM 60, NDM 30, NDM 120, positive control, DM 120, DM 60, DM 30 and negative control with an average value of 1854 mg / ml, 1801.778 ug / ml, 1699.556 ug / ml, 1691.778 ug / ml, 1665.111 ug / ml, 1645.11 ug / ml, 1535.111 ug / ml, and 1507.333 ug / ml, respectively. PKC-β positive control was higher than the negative control (normal). Based on the data supposed influence of gel A. vera against PKC-β levels in diabetic rat kidney (DM) and non diabetic rat kidney (NDM). PKC-â levels in the state of DM decreased when treated with Aloe vera gel but statistically is not significantly different.

Aloe vera gel dose of 30 mg / day (1535.1 mg / ml)giving effect to PKC-β level in diabetic rats kidney which approaching normal levels, it means A. vera gel dose 30 mg/day can reduce PKC-β levels in diabetic rat kidneys, but it was not significantly different. A decrease in levels of PKC-β in the DM group 30 mg/hari dose therapy suggests that the administration of gel A. vera can reduce PKC-β levels which acts as an activator of transcription factors. This is presumably because of the content of Acemannan on gel A. vera which act as antioxidants. Acemannan can increase the production of nitric oxide (NO) in the cells (Ramamoorthy et al., 1996) which have no direct relationship with high levels of ROS (reactive oxygen species) in diabetes.

NO is produced by nitric oxide synthase bond (NOS) with the substrate L-arginine (Steiner, 2002). L-arginine may reduce oxidative stress through reduction of superoxide anion. L-arginine also has a protective effect on cells exposed to free radicals (Lass, 2002 in El-Missiry et al., 2004). ROS levels are balanced in the network is expected to provide a stimulus to the reduced activation of PKC-β.

Dose of gel A. vera by 60 mg / day and 120 mg / day given in diabetic rats showed higher PKC-β levels. This is possible because of the toxicity of the reaction A. vera gel that appears when the administered dose exceeds tolerance dose for the body. This is supported by statements Ramamoorthy and Tizard (1998) that Acemannan has the ability to induce cell apoptosis through a mechanism of macrophage activation. PKC-β levels in NDM (normal) rat kidney shown by all three groups of non-diabetic (NDM) treated with A. vera with a dose of 30 mg / day, 60 mg / day and 120 mg / day has a value of 1801.778 mg / ml, 1854 mcg / ml and 1699.556 ug / ml higher than the positive control. PKC levels in the normal state remains activated to support cellular metabolism. Giving Aloe vera is thought to act as additional nutrients for the cells. The content of A. vera consists of many components such as vitamin E, vitamin C, amino acids and other active compounds which act to support the metabolism of cells, but these results do not indicate real differences.

CONCLUSION

Aloe vera gel gives no effect on the alteration levels of PKC-â in renal of wistar rat kidney with DM and without DM. Aloe vera gel dose at 30 mg / day led to PKC-β levels in DM group decreased approaching the normal group, but statistics showed that the difference was not noticeable. Further testing is needed to Aloe vera antioxidants, measured levels of other molecules involved in the PKC-β pathway and counting levels of PKC-β by immunohistochemistry, NanoDrop, western blotting and spectrophotometer.
REFERENCES


