KnE Life Sciences
ISSN 2413-0877 Volume 2 (2015) 45-50
The 3rd International Conference on Biological Science 2013
(The 3rd ICBS-2013)

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

Juliyatin Putri Utami[1], Umie Lestari[2], Nuning Wulandari[2]
Hendra Susanto[2], Yuda Handaya[3]

[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*gelhas a large vitamins which has become potential candidate to supressed vascular damaged oforganwhich 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* gelhas significant effectto 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

Selection and Peer-review under responsibility of the 3rd ICBS-2013
Doi http://dx.doi.org/10.18502/kls.v2i1.115

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 occurence of ketosiswith 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 accemannan 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

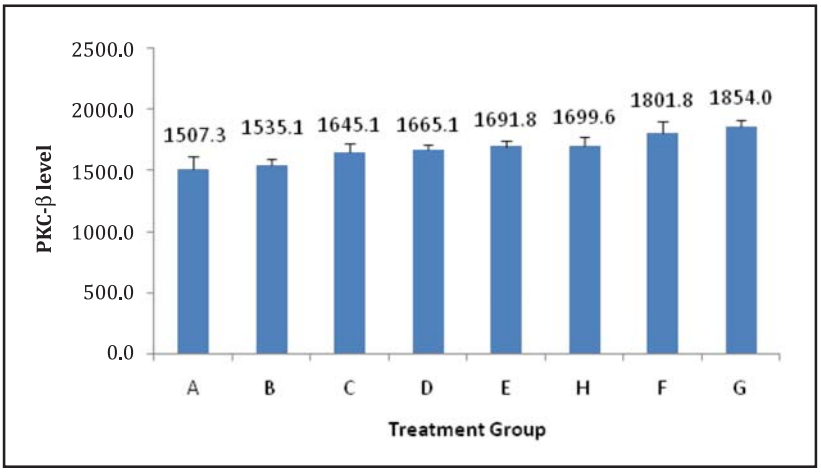
Aloe vera extraction

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 10^0C. The precipitate is separated from the solution by using a suction filter next to vacuum oven dryer at a temperature of 50^0C

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



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 = Positif 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 ratkidneys, 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

American Association of Diabetes Educators. 2003. *AADE core curriculum: Diabetes and Complications 5th ed*. Chicago, Illinois; American Association of Diabetes Educators. pg:158-159.

Corwin, E. J. 2009. Departemen Kesehatan Republik Indonesia. 2011. Tahun 2030 Prevalensi Diabetes mellitus di Indonesia Mencapai 21,3 Juta Orang. *http://www.depkes.go.id/index.php/berita/press-release/414-tahun-2030-prevalensi-diabetes-melitus-di-indonesia-mencapai-213-juta-orang.html,* accessed 11 September 2011.

El-Missiry, M.A., and A.I. Othman. 2004. AmerL-Arginine Ameliorates Oxidative Stress in Alloxan-induced Experimental Diabetes Mellitus. *J. Appl. Toxicol*. 24, 93–97.

Geraldes, P., and G.L. King. 2011. Activation of Protein Kinase C Isoform and Its Impact on Diabetic Complication. *Circ. Res.* 106; 1319-1331.

Joseph, B., and Raj. 2010. *Pahrmacognostic and Phytochemical Properties of Aloe vera Linn*. Malankara Catholic College.

Joy, S.V., A.C. Scates, S. Bearelly, M. Dar, C.A. Taulien, J.A. Goebel, and M.J. Cooney. 2005. Ruboxistaurin, a protein kinase C beta inhibitor, as an emerging treatment for diabetes microvascular complications.*Ann Pharmacother.* Oct;39(10):1693-9.

Koya, D., K. Hayashi, M. Kitada, A. Kashiwagi, R. Kikkawa, and M. Haneda. 2003. Effect of Antioxidants in Diabetes-Induced Oxidative Stress in the Glomeruli of Diabetic Rats. *J Am.SocNephrol* 14: S250-S253.

Meier, M., J. Menne, J-K. Park, and H. Haller. 2007. Nailing down PKC isoform specificity in diabetic nephropathy- two's company, three's a crowd. *Nephrol Dial Transplant*. 22: 2421-2425.

Ohshiro, Y., Y. Lee., and G.L. King. 2005. Mechanism of Diabetic Nephropathy : Role of Protein Kinase C Activation. *Advanced Studies in Medicine*. Vol. 5 1 (A).

Ramamoorthy, L., M.C. Kemp, and I.R. Tizard. 1996. Acemannan, a beta-(1,4)-acetylated mannan, induces nitric oxide production in macrophage cell line RAW 264.7. *Mol Pharmacol*. 50(4):878-84.

Ramamoorthy, L., and I.R. Tizard. 1998. Induction of apoptosis in a macrophage cell line RAW 264.7 by acemannan, a beta-(1,4)-acetylated mannan. *MolPharmacol.* 53(3):415-21.

Ricky, D. 2007. Pengaruh Ekstrak Etanol Daun Kemangi (*Ocimum basilicum*) terhadap Kadar Malondialdehid dan Glukosa Darah Tikus Jenis Wistar yang Diinduksi Aloksan Monohidrat (online). *http://jurnal.pdii.lipi.go.id/admin/jurnal/61073338.pdf*, accessed 24January 2011.

Roy, K., F. Harris, S.R. Dennison, D.A. Phoenix, and J. Singh. 2005. Effect of Streptozocin-induced type 1 diabetes mellitus on protein and ion concentrations in ocular tissues of the rat. *Int J Diabetes & Metabolism*. 13: 154-158.

Sonkodi, S., and A. Mogyorosi. 2003. Treatment of diabetic nephropathy with angiotensin II blockers. *Nephrol Dial Transplant*. 18 [Suppl 5]: v21–v23.

Steiner, D., R.S. Norberto, C. Gonzalez, and J.G. Wood. 2002. Interaction between reactive oxygen species and nitric oxide in the microvascular response to systemic hypoxia. *J ApplPhysiol* 93: 1411–1418.

Sunaryanto, A. 2010. *Penatalaksanaan Penderita dengan Diabetik Nefropathy.* Denpasar: FK UNUD.

Su, T.T. 2002. PKC beta controls I kappa B kinase lipid raft recruitment and activation in response to BCR signaling. *Nature.Immun*. 3: 780-786.

Whiteside, C.I., and J.A. Dlugosz. 2002. Mesangial Cell Protein Kinase C Isoenzyme Activation in The Diabetic Milieu. *Am J Physiol Renal Physiol* 282: F975-F980.