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EFFECT OF *Aloe vera* GEL ON SUPEROXIDE DISMUTASE (SOD) LEVEL IN STREPTOZOTOCIN (STZ)-INDUCED DIABETIC WISTAR *Rattus novergicus* LIVER

Nia Lukita Ariani[1], Abdul Gofur[2)], Dwi Listyorini[2], Hendra Susanto[2], and Yuda Handaya[3]

[1] Postgraduate Program of Basic Medical Sciences & Biomedical, Faculty of Medicine, Gadjah Mada University
[2] Department of Biology, Faculty of Mathematics and Natural Science, State University of Malang
[3] General Hospital Kepanjen, Laparoscopic Surgeon Division, Indonesia
Correspondence author: nia.ariani@rocketmail.com

ABSTRACT

Diabetes mellitus is a metabolic disorder characterized by the increasing levels of blood glucose as a result of either impaired of insulin secretion, insulin action, or both. Persistent hyperglycemia causes oxidative stress. *Aloe vera* is known of having an antioxidant activity. One of intrinsic antioxidant enzyme is superoxide dismutase (SOD) which eliminates superoxide radical. This research aims to determine the effect of *Aloe vera* gel on SOD level in STZ-induced diabetic Wistar rat liver. Wistar strain of *Rattus novergicus* used in this research were grouped into eight groups, consist of negative control (K-), non diabetic groups (NDM) consist of NDM 1 (dose of 30 mg/day), NDM 2 (dose of 60 mg/day), NDM 3 (dose of 120 mg/day), positive control (DM), and the DM group consists of DM 1 (dose of 30 mg/day), DM 2 (dose of 60 mg/day), and DM 3 (dose of 120 mg/day), with three replications each. Diabetic rats were induced using an intra-peritoneal injection of 60 mg/kg BW STZ. Three milliliters of *Aloe vera* gel were given intragastrointestinally for 14 days started from three days after injection of STZ. The level of liver SOD was measured with spectrophotometer.The results of One-Way Anova showed that *Aloe vera* gel has a significant effect on SOD levels ($p< 0.05$). Further analysis using LSD showed that only the treatment of NDM with 60 mg/day of *Aloe vera* gel giving a significant decreasing of SOD level compared to (K-). The conclusion is that *Aloe vera* has potency as a natural antioxidant.

Key words: *Aloe vera*, diabetes mellitus, superoxide dismutase (SOD), liver, streptozotocin (STZ)

INTRODUCTION

Diabetes mellitus is one of metabolic disorder which has found worldwide and has increasing significantly. Patients are classiûed as having a diabetic type if they meet one of the following criteria: (i) fasting plasma glucose level of e”126 mg/dl (e”7.0 mmol/l); (ii) 2-h value of e”200 mg/dl (e”11.1 mmol/l). Normal type is deûned as fasting plasma glucose level of <110 mg/dl (<6.1 mmol/l) or 2-h value of <140 mg/dl (<7.8 mmol/l) (Seino *et al.*, 2010).

Persistent hyperglycemia causes oxidative stress which production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) exceed local antioxidant capacity (Forbes *et al.*, 2008). Increased ROS production will increase free radicals, one of them is superoxide ($\bullet O^{2-}$) (Johansen *et al.*, 2005). Superoxide is very reactive free radical and not easily diffuses through the cell. Increased production of superoxide is offset by an increase in the internal antioxidant superoxide dismutase (SOD), which convert superoxide ($\bullet O^{2-}$) into hydrogen peroxide (H_2O_2) and then into water (H_2O) by GSH peroxidase (Dröge, 2002).

Patients with diabetes mellitus having an imbalance between the production of free radicals and antioxidant capacity. External antioxidants are used to help internal antioxidants work in hyperglycemia condition. Previous studies found out some natural antioxidant resources, such as *Averrhoa blimbi*, leaf of *Syzygium polyanthum*, leaf of *Piper betle*, etc. *Aloe vera* is also well known as herbal medicine which has high antioxidant activity

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(Rajasekaran *et al*., 2006; Miladi *et al*., 2008). The ethanol extract of Aloe gel is also reduces blood glucose level and prevents free radical production excessively (Rajasekaran *et al*., 2005). Many of the medicinal effects of Aloe leaf extracts have been attributed to the polysaccharides found in the inner leaf parenchymatous tissue but it is believed that these biological activities should be assigned to a synergistic action of the compounds contained there in rather than a single chemical substance (Dagne *et al. in*: Hamman, 2008). Our research aims to determine the effect of *Aloe vera* gel on SOD level in STZ-induced diabetic Wistar rat liver.

MATERIALS AND METHODS

Animals

Wistar strain male rats (150-200 g BW) were fed normal daily intake and water ad libitum, divided into eight groups, consist of negative control (K-), non diabetic groups (NDM) consist of NDM 1 (dose of 30 mg/day), NDM 2 (dose of 60 mg/day), NDM 3 (dose of 120 mg/day), positive control (K+), and the DM group consists of DM 1 (dose of 30 mg/day), DM 2 (dose of 60 mg/day), and DM 3 (dose of 120 mg/day), with three replications each. For untreated group (placebo) was injected with PBS pH 7.4. Diabetic animal model was induced by an intraperitoneal injection of streptozotocin in saline solution (65 mg/kg BW). The animals were considered as diabetic only if their blood glucose concentration was more than 200-300 mg/dl after 3 days post injection. The experiment was carried out fourteen days after the confirmation of diabetes (Gokhale *et al.,* 1998; Kaneez et al., 2003; Wei *et al.,* 2003; Haddad *et al.,* 2005).

Aloe vera Gel Extract

Fresh *Aloe vera* Linn leaf was collected from Batu, East Java. Aloe leaf was washed with nontoxic desinfectan (hipoclorit calcium 98%) and aquadest. The leaf was placed perpendicular until mucous were left out for 30-60 minutes. The base of Aloe leaf cut about 1 cm, then skinned over parenchyma cell. Flesh leaves of *Aloe vera* (gel) was rinsed with running water several times, peeled, cut in to small slices, and juiced. Juice was added with etanol 96% (1:4). Mix juice of *Aloe vera* and ethanol was stirred for 10 minutes at 30^{o}C. Deposited for 10 hours at 10^{o}C. The juice was filtered and the residue was removed. The extract was concentrated under vacuum to get solid yield at 50^{o}C (Padmadisastra *et al*., 2003; Kusmawati & Pratiwi, 2009). Three milliliters of *Aloe vera* gel were given intragastrointestinally for 14 days started from three days after injection of STZ with three variation dosages (30 mg/day, 60 mg/day, and 120 mg/day) (Handaya, 2011).

Determination of SOD Level in Liver Tissue

After two weeks treatment, the liver samples were taken from the animals and extracted. 100 µl liver extract was then added with 100 µl xantine, 100 µl xantine oxidase, 100 µl NBT and 3100 µl PBS and incubated for 30 minutes at 30^{o}C (in dark condition). Its absorbance was measured at 500-600 nm using spectrophotometer.

Data Analysis

One-way analysis of variance (ANOVA) (SPSS 16.0) and LSD post hoc test were used for data analysis. All data were expressed as mean ± SD and $p<0.05$ were identified as significantly different.

RESULTS AND DISCUSSION

The results of One-Way Anova shows that *Aloe vera* gel has a significant effect on SOD levels ($p< 0.05$) (Table 1).

Table 1. Result of One-way Anova

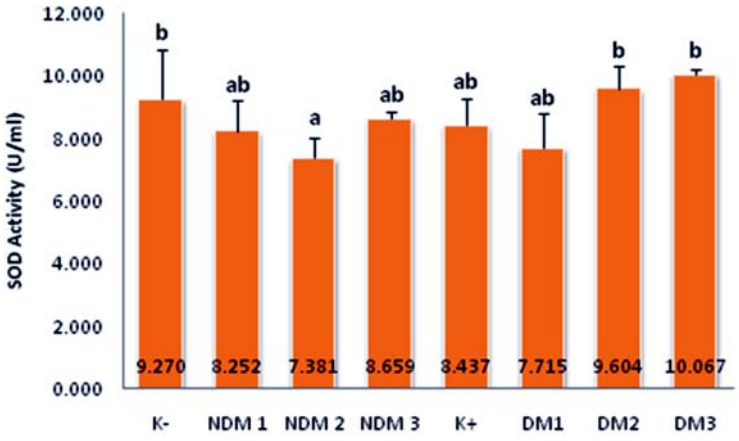
Tests of Between-Subjects Effects

Dependent Variable:Kadar SOD (U/ml)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	18.666[a]	9	2.074	2.452	.064
Intercept	1805.389	1	1805.389	2.134E3	.000
Perlakuan	17.951	7	2.564	3.032	.037
Ulangan	.715	2	.358	.423	.663
Error	11.842	14	.846		
Total	1835.897	24			
Corrected Total	30.508	23			

a. R Squared = .612 (Adjusted R Squared = .362)

Further analysis using LSD showed that only the treatment of NDM with 60 mg/day of *Aloe vera* gel giving a significant decreasing of SOD level compared to negative control (K). Other treatment in both DM and NDM do not give a significant difference to both control (Figure 1).



K- : normal (non diabetic) group
NDM1 : non diabetic group treated with *Aloe gel* dosage 30 mg/day
NDM2 : non diabetic group treated with *Aloe gel* dosage 60 mg/day
NDM3 : non diabetic group treated with *Aloe gel* dosage 120 mg/day
K+ : untreated diabetic group
DM 1 : diabetic group treated with *Aloe gel* dosage 30 mg/day
DM 2 : diabetic group treated with *Aloe gel* dosage 60 mg/day
DM 3 : diabetic group treated with *Aloe gel* dosage 120 mg/day

Figure 1. The SOD Level of *Rattus novergicus*'s Liver

The body has mechanisms to maintain a state of homeostasis when exposed to free radicals in a given period. The main mechanism that occurs when there is a signal increased production of free radicals is increasing production of antioxidative enzymes as a response (Dominguez *et al.,*1998; Dröge, 2002). In hyperglycemia, increased superoxide production will be offset by increased production of antioxidant enzymes such as SOD to convert superoxide radicals into hydrogen peroxide which is more stable. Increased production of SOD is thought to protect cells from free radical exposure (Erejuwa *et al*., 2011). Increased antioxidant enzyme production will occur in a certain period until concentration of free radicals in the cells back to normal.

In the present research, SOD level were not significantly decreased in the DM control group (K +) compared with the normal group (K-). In some studies, it was reported that an increase in antioxidants occur in the early stages of diabetes, but in a longer duration will cause a decrease in the production of antioxidant enzymes due to the accumulation of H_2O_2, so hyperglycemia leads to reduced cellular antioxidant defense (Venkateswaran *et al*., 2002). Increased glucose oxidation produces H_2O_2 which can inactivate SOD or in other word accumulated H_2O_2 be one of explanation why SOD activity decreased in the control group DM (K +) (Nobar *et al*., 1999; Pi *et al.,* 2007). In addition, hyperglycemia can decrease the activity of SOD enzyme through glycation (Akinola *et al*., 2010). Hyperglycemia causes increased production of free radicals through the oxidation of glucose, followed by protein glycation which produces advanced glycation end products (AGEs). AGEs when binding to its receptor RAGEs can cause inactivation of enzymes, including the antioxidant enzymes and alter the structure and function, and unable to detoxify free radicals (Maritim *et al.,* 2003).

Administration of *Aloe vera* gel at a dose of 30 mg / day in the DM group led to decreased levels of SOD were not significant compared with the control group DM (K +). Decreased level of SOD is suggested because of the *Aloe vera* gel is able to compete with ROS production until ROS levels return to normal. As a result of declining levels of ROS, the production of SOD also decreased.

Decreased levels of SOD at a dose of 30 mg / day showed that *Aloe vera* has potential as an antioxidant. Antioxidant potential of *Aloe vera* extract is made possible through a reduction in sugar levels that can prevent excessive free radical production through various metabolic pathways and reduce glycation of antioxidant enzymes (Rajasekaran *et al.,* 2005; Ramachandraiahgari *et al*., 2012). Mechanism of decreased glucose levels caused by various compounds of *Aloe vera* that have antioxidant activity, including acemannan polysaccharides, flavonoids, vitamins, minerals, antioxidants and enzymes contained in the gel (Hu *et al*., 2003; Ammar *et al.,* 2010; Anilakumar *et al.,* 2010; Saritha *et al.,* 2010; Jain *et al*., 2011).

Aloe vera could be expected to prevent β cell death and or repair β cells that have been damaged partially (Noor *et al*., 2008; Ramachandraiahgari *et al.,* 2012). The antioxidant mechanism is suggested to repair the β cells through decreased activity of superoxide radicals in β cells as a cause of DNA damage in STZ-induced β cell (Szkudelski, 2001). The repaired β cells will return to normal function so that the levels of insulin produced is also back to normal and can lower blood sugar levels. Acemannan is a highest level of polysaccharide content of *Aloe vera* gel (Hamman, 2008) thought to play a role in β cell repair. In

previous research, β cell repair mechanism analogous to the glucomannan, which is also polysaccharides by increasing levels of proinsulin gene mRNA. Increase in mRNA levels is apparently due to an increase in the number of β cells and therefore contributes to increased cell activity results in the form of mRNA transcription and insulin translation (Fatchiyah, 2011).

Administration of *Aloe vera* gel is suggested to help SOD activity in the group of diabetic rats. Increased levels of SOD occurred after administration of *Aloe vera* gel at a dose of 60 mg / day and 120 mg / day, but the increase did not occur significantly when compared with the control group DM (K+) and normal controls (K-). Ethanol extract of *Aloe vera* gel contains several compounds, one of which is the enzyme SOD (Hamman, 2008). Increased levels of SOD in gel administration with doses of 60 and 120 mg / day is thought to due to excessive dosage thereby increasing the quantity of internal SOD cells.

Aloe vera gel administration in non-diabetes mellitus group at a dose 30 mg/day decreased SOD levels not significantly and at a dose of 60 mg / day also resulted in a significant decrease in SOD compared with the normal group (K-). This is presumably because the administration of *Aloe vera* gel can help internal SOD activity so as to detoxify free radicals that normally is produced in the body. Decreased levels of free radicals followed by decreased levels of SOD. Giving *Aloe vera* gel at a dose of 120 mg / day can increase levels of SOD not significantly compared with the normal group (K-), but this increase did not occur significantly than the second dose earlier. An increased level of SOD at this dose is apparently due to the excessive accumulation between SOD derived from *Aloe vera* gel and internal liver SOD.

In conclusion, our findings in this work showed the beneficial effect of *Aloe vera* in alteration of SOD level of STZ-induced diabetic Wistar rats. Further analysis from the other antioxidant enzymes and more specific type of SOD is needed to complete this finding.

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