EFFECT of *Aloe vera* EXTRACT TO THE INSULIN-LIKE GROWTH FACTOR-1 (IGF-1) LEVELS FROM VISCERAL FAT TISSUE IN *Rattus norvegicus* WISTAR DIABETES MELLITUS

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ABSTRACT

Diabetes mellitus is a metabolic disorder indicated by hyperglycaemia due to lack of insulin production, insulin activity, or both. It causes oxidative stress that may damage the receptor of Growth Hormone Releasing Hormone (GHRH) in the pituitary. As the result, Growth Hormone (GH) and Insulin-like Growth Factor-1 (IGF-1) production are decreased and glucose uptakes to the cell is inhibited. *Aloe vera* has a high antioxidant activity. It is possible to counter oxidative stress in unregulated the production of IGF-1. This study aimed to determine the effect of *A. vera* extracts to the level of IGF-1 from visceral fat tissue *Rattus norvegicus* wistar diabetes mellitus. This study used 24 *R. norvegicus* wistar male. *R. norvegicus* STZ-induced diabetes mellitus *R. norvegicus* were grouped into positive control (C+), 30 mg/day, 60 mg/day, 120 mg/day. Fasting blood glucose test was performed to determine the successful induction of diabetes mellitus (DM) three days after induced. Non-DM rats (NDM) were grouped into negative control (C-), 30 mg/day, 60 mg/day, and 120 mg/day. Each group was consisting of three replications. Each animal, except the controls, were treated 3 ml suspension of *A. vera* extract for 14 days by gavage. IGF-1 levels were measured using indirect ELISA. The result showed that the extract of *A. vera* significantly influenced the IGF-1 levels in DM group. The dose of 60 and 120 mg/day significantly raised the level of IGF-1 compared to C+. In NDM group the dose of 60 mg/day significantly raised the level of IGF-1 compared to C-.

Key words: *Aloe vera*, *Rattus norvegicus*, IGF-1, visceral fat, diabetes mellitus

INTRODUCTION

Diabetes mellitus is a chronic disease and known as Non-Communicable Diseases (NCD) which commonly occurs in various regions of the world. Death due to NCDs globally increased by 15% from 2010 to 2020. The largest increased about 20%, was observed in African region, East Mediterania, and Southeast Asia (WHO, 2010). WHO reported that the incidence of diabetes mellitus by 364 million people around the world. Based on the final diagnosis, a total sample of 24,417 from Indonesia's population aged over 15 years had shown that as many as 10.2% were having diabetes mellitus and 5,3% at a young age (age 15-24 years). There are two types of diabetes mellitus, diabetes mellitus type 1 is characterized by insulin deficiency and diabetes mellitus type 2 is due ineffective function of insulin. A person suffering from diabetes mellitus characterized by the level of fasting plasma glucose (FPG) of 125 mg/dl which equal to 200 mg/dl two hours after eating. Long-term chronic hyperglycaemia can lead to various organs complications, such as blindness, kidney failure, ulcers, sexual dysfunction, cardiovascular disease, and liver disorders (WHO, 2011[a]; WHO, 2011[b]).

Hyperglycaemia causes oxidative stress which produces more free radicals than anti-oxidants in the body. Oxidative stress may cause damage to the Growth Hormone Releasing Hormone (GHRH) receptor in pituitary which affects the secretion of Growth Hormone (GH) and reduced the production of Insulin-like Growth Factor-1 (IGF-1). When production of IGF-1 is low, so does the formation of receptor glucose and it will aggravate the condition of hyperglycaemia because the glucose that enters the cell is low and the energy needed will be slightly gained. In order to fulfill the energy requirement, body will acquire it from fat. Fat molecules are broken down into fatty acids and caused ketoacidosis due to the rising of acid levels in blood (Forbes *et al.,* 2008; Bedard *et al.,* 2008; Riddell & Perkins, 2006).

In order to return the production of IGF-1 to normal, the GHRH receptor damage must be minimized by reducing the oxidative stress. The stress condition can be improved if the production of free radicals and antioxidants are well-balanced. Antioxidant are substances that can scavange the oxidized substrate and inhibit the oxidation of certain substrates (Hlliwell & Gutteridge *In* : Droge, 2002). There are many different kinds of plants contains high anti-oxidant, such as *Aloe vera* which is commonly used as a remedy for various diseases and can be used as astringent, hemostatic, antiseptic, antibacterial, anti-inflammatory, anticancer, and antidiabetic (Rajasekaran *et al.,* 2006; Afaf *et al,.* 2008; Joseph & Raj, 2010).

Aloe vera extract can lower glucose levels in diabetic rats and rabbit blood. The decreasing glucose level indicates that the extract *A. vera* stimulates insulin secretion and the regeneration of pancreatic β-cells (Saif-Ur-Rehman *et al.*, 2011; Sujono & Wahyu, 2005; Solomon et al. within Yeggnisetty *et al.,* 2012). *Aloe vera* contains about 200 different substances benefits for human health including vitamins, enzymes, minerals, amino acids, and antioxidant (Joseph & Raj, 2010). Natural antioxidants that are found in leaf gel of *A. vera* are vitamin A, vitamin C, vitamin E (Miladi & Damak, 2008). Thus, *A. Vera* might be a potential source for antioxidant that reduce the ROS level in the body and suppress the occurence of oxidative stress.

MATERIALS AND METHODS
Animal Preparations

A total of 24 male of *R. norvegicus* Wistar (150-200 g) were divided into 12 nondiabetic mellitus (NDM) and 12 streptozotocin (STZ)-induced diabetic mellitus (DM) by 60 mg/Kg BW via intraperitoneal injection. Three days later, the fasting blood glucose test was performed to determine the successful induction of diabetes mellitus. NDM group were divided into negative control (C-), NDM1 (30 mg/day), NDM2 (60 mg/day), NDM3 (120 mg/day). DM group was divided into positve control (C+), DM1 (30 mg/day), DM2 (60 mg/day), DM3 (120 mg/day). Each group consisted of three replicate. In each group except the control were given 3 ml suspension of extract *A. vera* with doses of 30, 60, and 120 mg/day by gavage for 14 days.

Aloe vera Extract Preparations

Leaves of healthy *A. vera* obtained in the area of Malang/Batu were cleaned using calcium hipoclorite and distilled water. After no mucous left, the leaves were cut on the base about 1 cm and the flesh was taken. The flesh was rinsed under the running tap water then

cut into small pieces and blended. After that, the gel soaked using ethanol 96% (1:4 v:v) and stirred for 10 h at 30°C, allowed for precipitation for 10 h at 10°C. The sediment was separated from the solution by filtration and dried with vacuum dryer at 50°C (Padmadisastra *et al.,* 2003; Kusumawati & Pratiwi, 2009).

Determination of IGF-1 Level in Visceral Fat Tissue

Two weeks after the treatment, all of the *R. norvegicus* were decapitated and the visceral fat tissue was taken. A total of 50 mg tissue were grinded and added with 500 µl Radioimmuno Precipitation Assay (RIPA) buffer lysis. The suspensions were then vortexed and incubted at 4°C for 30 mins. After 30 mins incubation, the fat tissue suspensions were centrifuged at 12,000 rpm for 20 mins. The supernatant was taken and moved to a new microtube to measure the IGF-1 level using ELISA reader at 492 nm (Titer Assay Enzyme Design EIA Kit, Catalog No. 900-101).

Statistical Analysis

The data was in the form of absorbance values and determination of the IGF-1 level measurement   used standard curve $y = 0.0003x + 0.0008$. The statistic analysis was performed using One way ANOVA ($p < 0.05$) and followed by LSD.

RESULT AND DISCUSSION

The result of IGF-1 measurements on visceral fat tissue of *R. norvegicus* showed that the extract of *A. vera* increased the levels of IGF-1 (Figure 1). ANOVA test was able to show that the treatment using *A. vera* extract was significantly affect IGF-1 levels ($p < 0.05$). LSD test showed that the levels of IGF-1 on visceral fat tissue *R. norvegicus* diabetes mellitus which were treated with 60 mg/day and 120 mg/day were significantly different compared to the positive control (C+) and 30 mg/day on *R. norvegicus* DM group whereas in NDM group there was showed that 60 mg/day *A. vera* extract treatment was significantly different compared to negative control (C-), but not significantly different compared to 30 mg/day and 120 mg/day dosage.

Streptozotocin is a compound that can cause a quick damage to pancreatic beta cells so that insulin production is reduced (Pi *et al.,* 2007) and based on the data STZ administration was succesfully induced diabetes mellitus. Decreased insulin production can lead to hyperglycemia. Hyperglycemia causes oxidative stress conditions and may cause damage
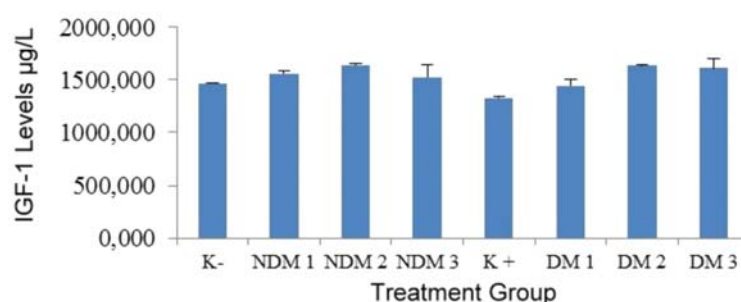

Figure 1. IGF-1 Levels on DM and NDM *Rattus norvegicus* Visceral Fats Tissue. Data present the mean of three replicates and the standard error.

in the pituitary GHRH receptors that results in the decreased secretion of GH. When GH secretion decreases, so does the IGF-1 production in the liver (Forbes *et al.,* 2008; Bedard *et al.,* 2008). The extract given to *R. norvegicus* DM was able to increase the levels of IGF-1. Perhaps, it was possible because *A. vera* has a high antioxidant activity which reduces the concentration of free radicals by 50% and can inhibit the release of ROS from phorbol 12-myristate 13-acetate (PMA). The other content is enzyme SOD which is thought that the increased production of SOD leads to cell protecting mechanism against free radical exposure (Hart *et al.,* 1990; Hamman, 2008; Khaing, 2011; Erejuwa *et al.,* 2011).

The presence of antioxidants can reduce oxidative stress so that the pituitary GHRH receptor synthesis can be returned to normal and the production of GH and IGF-1 will be improved. The other mechanism that can improve the production of IGF-1 comes from polysaccharides Acemannan which acts as an antioxidant and expected to repair damaged pancreatic β cells (Chun-hui *In* : Cock, 2011; Hamman, 2008). In this study the extraction process was using ethanol 96% as precipitant which will generate a lot of acemannan polysaccharides according to Kusmawati & Pratiwi (2009).

The mechanism of β-cell repairing is analogous to glucomannan polysaccharides that can increase the mRNA gene of proinsulin levels in rats with diabetes mellitus. An increase in mRNA level is characterized by an increase in the number of β-cells and so does the the production of insulin (Fatchiyah, 2011). Increased insulin production can improve the conditions of hyperglycemia and pressing the oxidative stress. The oxidative stress can be reduced if the production of IGF-1 can be returned to normal. The biological activity can occur not only one compound that works, but a whole compounds contained in *A. vera* extract that work synergistically (Dagne *et al.*, *In*: Hamman, 2008)

Based on the result, *A. vera* can be used as a candidate to treat diabetes mellitus due to the increased levels of IGF-1 and the hyperglycemia seems to be inhibited as well so that the danger of cell damage caused by oxidative stress can be avoided. In addition, the glucose is able to enter the cells and can be used as energy and the use of fat catabolism can be minimized so that ketoacidosis can be avoided. The dose extract of 30 mg/day didn't significantly affect the levels of IGF-1 on *R. norvegicus* normal compared to C- treatment, but the dose of 60 mg/day was significant. In both DM and NDM groups, the levels of IGF-1 on *R. norvegicus* given with 120 mg/day were lower than treated rats with 60 mg/day. It is possible that the highest dose (20 mg/day) has been overeffective so that it can disrupt the physiology of the body. Mali *et al.* (2011) mentioned that *A. vera* overdose can result in side effects such as lack of electrolytes, fluid imbalance, and intestinal cramps. Therefore the provision of *A. vera* as much as 120 mg/day are not recommended.

CONCLUSION

The extract of *A. vera* significantly increased the IGF-1 levels on *R. norvegicus* visceral fat tissue DM via oral administration with dose 60 mg/day and 120 mg/day compared to C+ whereas on normal *R. norvegicus* visceral fat tissue, the extract of *A. vera* seemed to increase the IGF-1 levels in dose 60 mg/day compared to C-. Thus, dose of 60 mg/day *A. vera* extract is suggested to be an alternative dose treatment for DM.

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