Research Journal of Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Research Journal of Medicinal Plants

ISSN 1819-3455
DOI: 10.3923/rjmp.2020.149.155

Research Article
Hypoglycemic Activity of *Aloe vera* Powder and Gel Drink in Alloxan-induced Diabetic Rats

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Abstract

Background and Objective: *Aloe vera* leaves contain of flavonoid compounds which were used as an antioxidant. Previous research showed that the flavonoids of *Aloe vera* extracts could lower blood glucose. However, the use of *Aloe vera* extract is unfavorable. Therefore, processing of *Aloe vera* into an acceptable product such as a powder or *Aloe vera* gel drink was necessary to be carried out. The purpose of this research was to evaluate the hypoglycemic activity of *Aloe vera* powder and gel drink. **Materials and Methods:** The antioxidative activity of *Aloe vera* products was analyzed by using the 1,1-Diphenyl-2-picrylhydrazyl and ferry thiocyanate methods and the hypoglycemic test was determined with the *in vivo* method using diabetic Wistar rats induced with alloxan 25 mg/200 g b.wt., as experimental animals. The rats were fed with a standard feed combined with *Aloe vera* powder or gel drink. The intake of fed was equivalent to 600 IU antioxidant standard of α-tocopherol. **Results:** The research showed that *Aloe vera* powder had high antioxidative activity shown by the Radical Scavenging Activity (RSA) which was 26.15% and the inhibition of lipid peroxidation was 44.17%, whereas *Aloe vera* gel drink were 21.96 and 5.63%, respectively. These result related to the hypoglycemic effect. **Conclusion:** *Aloe vera* powder and gel drink had hypoglycemic effect which indicated by a decrease in blood glucose from after four weeks, so these products appropriate for diabetics.

Key words: Antioxidative-activity, flavonoid, hypoglycemic-effect, alloxan, antidiabetic activity, *Aloe vera*

Citation: Chatarina Wariyah and Riyanto, 2020. Hypoglycemic activity of *Aloe vera* powder and gel drink in alloxan-induced diabetic rats. Res. J. Med. Plants, 14: 149-155.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Aloe vera (*Aloe vera* var. *chinensis*) is a tropical or subtropical plant characterized by lance-shaped leaves with jagged edges and sharp points. *Aloe vera* contains two major liquid sources, a yellow latex (exudates) and the clear gel (mucilage)[1]. Traditionally, the yellow latex has been used as constipation treatment and the gel has been used as wound healing treatment and anti-inflammatory[2]. According to Kumar *et al.*[3], *Aloe vera* has been used in health foods, cosmetics and medicines and is also believed to function as an antitumor, antidiabetic and antibacterial. *Aloe vera* contains flavonoid compounds i.e., kaempeferol, quercetin and merycetin of about 257.70, 94.80 and 1283.50 mg kg^{-1}, respectively[4]. Flavonoids are a group of phenolic compounds believed to have antioxidative activity. This antioxidative activity is due to their ability to capture free radicals of DPPH (1,1-Diphenyl-2-picrylhydrazyl)[5]. Joseph and Raj[6] indicated that *Aloe vera* contains bio-active substances that can lower blood glucose. Whereas, Jasmine and Daisy[7] found that methanol soluble extract of *Eugenia jambolana,* which also contains flavonoids could lower blood glucose, so the hypoglycemic effect is estimated to be related to the flavonoids. Yagi *et al.*[8] showed that an *Aloe vera* fraction of about 10 ppm and consumed over a period of 6 weeks, could lower the glucose and blood lipid levels of the experiment animals.

However, the use of fresh *Aloe vera* was considered less practical and had low acceptability, due to its less preferred flavor. Therefore, the processing of *Aloe vera* into products such as powder and gel drink was necessary. The processing of *Aloe vera* powder and gel drink were done in stages i.e., peeling, slicing and heating and these stages allowed contact with oxygen, heat and light that could lower antioxidative activity[9]. Riyanto and Wariyah[10] stated that *Aloe vera* extract had high antioxidative activity shown by the percentage of Radical Scavenging Activity (RSA) was about 35.17% and inhibition of lipid peroxidation was 49.53%. Drying of *Aloe vera* gel could lower the antioxidative activity. The reduction of antioxidative activity could affect the hypoglycemic activity.

The hypoglycemic effect of *Aloe vera* was related to free radical neutralization by antioxidants as *Aloe vera* flavonoids. According to Barlett and Eperjesi[11], hyperglycemia and diabetes causes an increase in Reactive Oxygen Species (ROS), which is a term used to describe some types of free radicals i.e., superoxide, hydrogen peroxide and singlet oxygen. The ROS is capable of damaging lipid membranes, proteins, nucleic acids and carbohydrates via oxidation, resulting in the formation of cytotoxic chain reactions. Therefore, a substance which neutralized free radicals, namely an antioxidant is needed. The purpose of the study was to evaluate the hypoglycemic activity of *Aloe vera* powder and gel drink with the *in vivo* method using experimental animals.

MATERIALS AND METHODS

Research duration: This study was carried out in March-September, 2015 in the Food Processing Technology Laboratory, Mercu Buana Yogyakarta University and Center of Food and Nutrition Study Laboratory, Gadjah Mada University of Yogyakarta.

Materials and research tools: *Aloe vera* leaves (*Aloe vera* var. *chinensis*) were obtained from the Loano district in the Purworejo Regency of Central Java, Indonesia. The ingredients for making *Aloe vera* gel drink i.e., sugar, potassium sorbate, salt and lime were bought from the nearest traditional market in Yogyakarta and the reagents used in this research were from Merck, except the DPPH from Sigma-Aldrich Chemie. The rats for animal experiment were obtained from the Integrated Research Center Labs., Gadjah Mada University, Yogyakarta, Indonesia.

The equipments used in this research were a set of *Aloe vera* gel-drink processing equipment, oven (Memmert DIN 40050 IP 20) for making *Aloe vera* powder and UV-Vis spectrophotometer (Shimadzu UV mini 1240) for analysis of antioxidative activity, balance (OHAUS Pioneer PA214), lyophilized powder with Freeze Dryer ALPHA1-2/LD No. 101021, colour of *Aloe vera* powder and gel drink were determined with chromameter (Konica Minolta), texture test with Zwick test and glassware for chemical analysis from Pyrex Iwaki (Iwaki glass under LIC).

Research procedure: *Aloe vera* was processed into the powder and gel drink referred to Riyanto and Wariyah[10]. The powder and gel drink were analyzed for their antioxidative activity using the DPPH (1,1-Diphenyl-2-picrylhydrazyl) radical scavenging activity method[5] and inhibition of lipid peroxidation with FTC (*Ferry thiocyanate*) method[12], total phenolic content was determined by using Folin-Ciocalteu method[13,14] and the physical properties: colour test with chromameter.

Preparation of *Aloe vera* powder and gel drink for antioxidant activity assay referred to Hu *et al.*[5] with some modification. For *Aloe vera* gel drink, the gel was prepared by lyophilized thin pieces of gel for 2 days and then ground into fine powder for further use. Two gram of each powder was dissolved in 50 mL of 80% ethanol (v/v). The mixture was

shaken vigorously during 30 min and then filtered, the residue was washed twice with 10 mL of 80% ethanol. The filtrate was combined on to a flask, then added with ethanol into volume of 100 mL and used as the *Aloe vera* powder or *Aloe vera* gel drink solution.

Determination of *Aloe vera* powder and gel drink intake for the experimental animals: *Aloe vera* powder and gel drink for animal intake were determined based on their reducing power refer to Duh *et al.*[15] and 0.40 mg capsule of commercial vitamin E containing 100 IU was used as standard, in equal proportions with *Aloe vera* powder and gel drink. The relative reducing power stated by absorbance value. The higher the absorbance, the greater its reducing power or the higher its antioxidative activity. The *Aloe vera* powder and gel drink intake for rats were equalized with Adequate Daily Intake (ADI) of antioxidant to prevent degenerative disorder up to 600 IU/day/adult. About 0.40 g vitamin E used in this study equivalent to 100 IU α-tocopherol or 2.4 g equivalent with 600 IU α-tocopherol. Moreover, the value was multiplied with a conversion factor (0.018) to fit the animal feed.

Determination of hypoglycemic effect of *Aloe vera* powder and gel drink with animal experiment: The hypoglycemic effect was determined by the *in vivo* method[16] using Wistar rats with an age of 3-4 months and a weight of between 240-260 g. The *Aloe vera* powder was used as animal feed combined with standard feed[17] and the gel drink was fed by force-feeding and the rats continued feeding with standard feed. All rats received drinking water *ad libitum*. The animals (n = 6)[18] were observed for 4 weeks. The hypoglycemic effect was calculated by the change in blood glucose of diabetic rats (induced with alloxan at 100 mg kg^{-1} b.wt. and were used after 5 days induction) before and after being fed with the sample. The rats were fed with the treatment for a period of 4 weeks and blood glucose were analyzed each week using the GOD-PAP method[19].

Statistical analysis: This study used completely randomized design with the treatment time as a factor. Data were statistically evaluated by using one way ANOVA, the differences among the treatments were determined by F-test and the significant difference between samples was examined by Duncan's Multiples Range Test (DMRT) by using SPSS 13.0 for windows.

The regression equation of vitamin E, *Aloe vera* powder and gel drink were determined by using SPSS 13.0 for windows. These regression equations were used to calculate the *Aloe vera* gel drink or *Aloe vera* powder needed for human intake equivalent with 600 IU. The regression analysis was refer to Henderson *et al.*[20] study, the vitamin E reducing power regression equation was determined to calculate 600 IU of *Aloe vera* powder or gel drink. Based on the vitamin E regression equation $y = 9.357x+0.031$ ($R^2 = 0.974$) with $x = 2.4$ g, obtained $y = 22.6428$. Furthermore, this y was entered into *Aloe vera* powder reducing power regression equation $y = 12.583x+0.017$ ($R^2 = 0.997$) and gel drink $y = 0.026x+0.018$ ($R^2 = 0.9430$) to meet the equivalent needs of 600 IU of vitamin E.

RESULTS

***Aloe vera* powder and gel drink characteristics:** The characteristics of *Aloe vera* powder and *Aloe vera* gel drink are shown in Table 1. Data showed that the *Aloe vera* powder and gel drink had a high antioxidant activity indicated with a percentage Radical Scavenging Activity (RSA) of powder 26.15% and inhibition of lipid peroxidation 44.17%, RSA of gel drink was 15.79% and inhibition of lipid peroxidation about 19.25%, whereas the RSA value of fresh *Aloe vera* was 35.17% and inhibition of lipid peroxidation was 49.53%.

Reducing power of *Aloe vera* powder and gel drink: Capsule of commercial vitamin E (0.40 mg) containing 100 IU was used as standard, in equal proportions with *Aloe vera* powder and gel drink based on their reducing power. The antioxidative activities of vitamin E, *Aloe vera* powder and gel drink were compared based on their reducing power. The relative reducing power stated by absorbance value is shown in Fig. 1. Data showed the relationship between absorbance and sample weight (gel drink, powder and vitamin E). The higher the absorbance, the greater its reducing power or the higher its antioxidative activity.

Table 1: Characteristics of *Aloe vera* powder and gel drink

Characteristics	*Aloe vera* powder	*Aloe vera* gel drink
Radical scavenging activity (RSA %)	26.15±1.93	15.79±0.30
Inhibition of lipid peroxidation (%)	44.17±1.09	19.25±1.36
Colour		
Lightness	75.45±1.39	18.26±1.99
Yellowness	23.05±0.62	-0.84±0.09
Redness	-4.92±0.45	0.45±0.04
Total phenol (GAE μg g^{-1} dry matter)	1.09±0.04	0.0023±0.0001

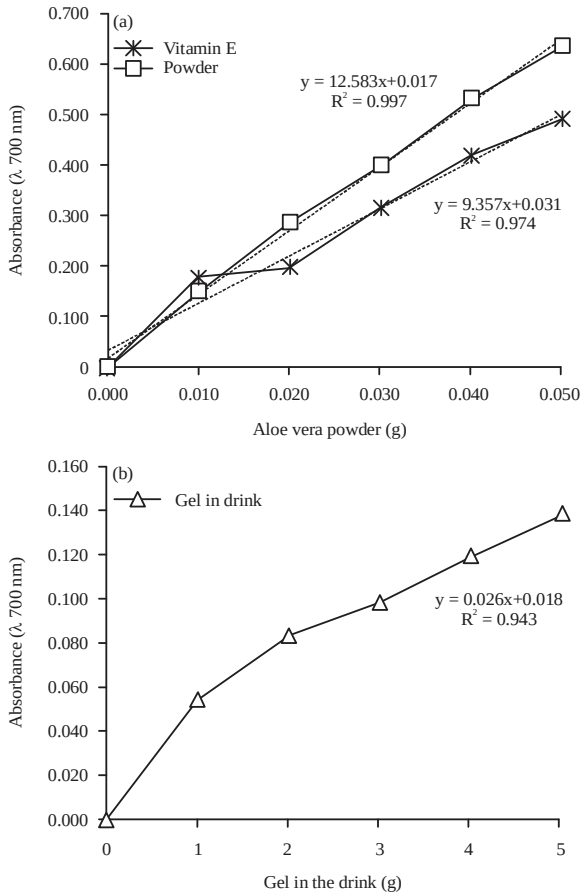

Fig. 1(a-b): Reducing power of vitamin E, (a) *Aloe vera* powder and (b) Gel drink

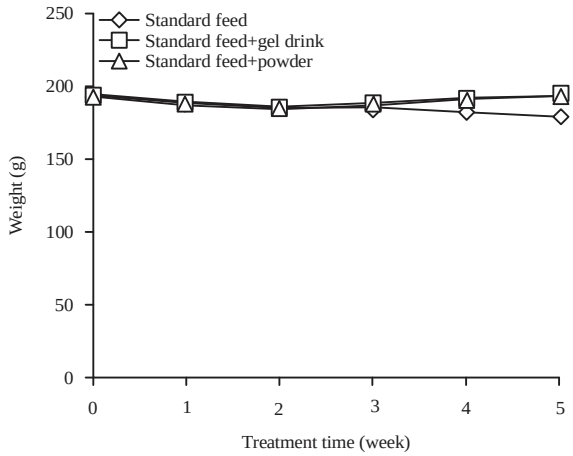

Fig. 2: Profile of rat body weight during treatment

Figure 1a-b showed the reducing power of vitamin E compared with *Aloe vera* powder and gel drink. The curves were separated into A and B, because the differences weight of the gel in the drink sample.

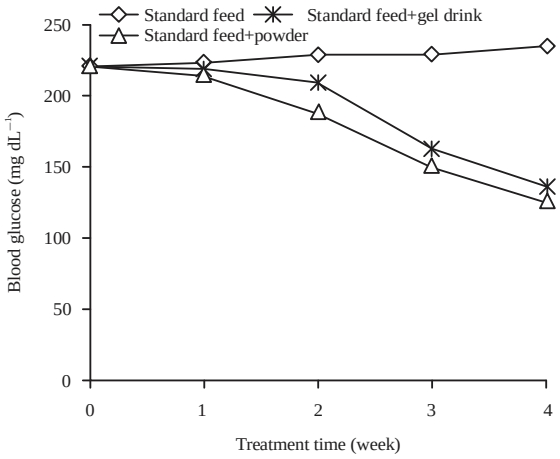

Fig. 3: Profile of blood glucose during 4 weeks treatment

***Aloe vera* powder and gel drink intake for rats:** The adequate daily intake of vitamin E is 600 IU/day/adult, so it is necessary for a human to consume about 864.0 g of gel drink or for a rat with a 200 g b.wt., to consume about 15.5 g, while for the *Aloe vera* powder it takes about 1.80 g of for a human and 0.0324 g for a rat with a 200 g b.wt.

Effect on body weight: Figure 2 showed the profile of rats weight during 4 weeks treatment. The body weight of diabetic rats fed with *Aloe vera* products remained relatively stable, whereas, the body weight of diabetic rats fed only standard feed tended to decrease.

Blood glucose profile: Figure 3 showed rat blood glucose profile with treatment of standard feed (control), standard feed combined with gel drink and standard feed combined with *Aloe vera* powder. Blood glucose profile of diabetic rats fed with or without *Aloe vera* powder or gel drink. The diabetic rats without an *Aloe vera* product showed high stable blood glucose (>200 mg dL^{-1}) during the 4 weeks of treatment.

DISCUSSION

Aloe vera powder and gel drink had high antioxidant activity, although they have been heated during processing. In this study, *Aloe vera* powder was dried at 60-70°C, therefore, its antioxidant activity was still high. As a source of antioxidant for humans, more *Aloe vera* gel drink was needed than powder, because (1) The antioxidative activity of the gel drink was lower than that of the powder (Table 1), (2) The moisture content of the gel was higher (97-98%) or total solid was lower than powder, so the concentration of bio-active

substances was also lower. Therefore, to obtain the required amount of antioxidant, humans must consume very large quantities of *Aloe vera* gel drink each day. So, it is practical to consume *Aloe vera* gel drink as a source of antioxidant with the ADI of 15 IU required for health care. According to Sultana and Anwar[4], the antioxidant effect of *Aloe vera* gel was caused due to its flavonoid content, such as; merycetin, quercetin and kaempferol. Hendrawati[21] found that *Aloe vera* powder made by added maltodextrin as a filler (with ratio 1:1) and dried with spray dryer at 120°C showed that all of phenolic compounds of *Aloe vera* powder can be maintained. Wariyah and Riyanto[22] also found that *Aloe vera* gel had antioxidative activity with RSA about 12.09±1.79% and inhibition of lipid peroxidation was 12.70±2.30%, while *Aloe vera* powder made with added 10% maltodextrin had RSA value 43.32±0.11% and inhibition of lipid peroxidation about 25.96±0.41%, with total phenol powder 2.64±0.105 μg g^{-1} dry matter.

According to Henderson *et al.*[20], the data of reducing power assay affected by the volume sample and composition. In the Ferric Reducing Antioxidant Power (FRAP) assay which free of volume effect, there was a correlation (R^2 = 0.982) between honey's Unique Manuka Factor (UMF) rating and total phenol. The higher the UMF rating, the higher the total phenol and reducing of FRAP or Fe^{2+}. It was mean that reducing power of sample established by total phenol content or its antioxidant capacity. Vitamin E is a phenolic compound which had antioxidative activity[23]. Figure 1a showed the reducing power curve of vitamin E increase with increasing of its concentration. There was a correlation between total vitamin E and absorbance (R^2 = 0.974) as well as *Aloe vera* powder and gel drink.

Hypoglycemic activity was indicated by the ability of the sample to decrease blood glucose. Hypoglycemic activity was determined by the *in vivo* method using diabetic Wistar rats as experimental animals. Figure 2 showed the profile of rats weight during 4 weeks treatment and Fig. 3 showed rat blood glucose profile with treatment of standard feed (control), standard feed combined with gel drink and standard feed combined with *Aloe vera* powder. The body weight of diabetic rats (Fig. 2) fed with *Aloe vera* products was significantly increased, whereas, the body weight of diabetic rats fed only standard feed was decreased. Kuzuya *et al.*[24] and Al Tera[25] described that decreasing body weight is one symptom of diabetes mellitus. Diabetic patients undergo weight loss when blood glucose can not be absorbed into the cells and the energy requirement is taken from body fat.

Figure 3 is the blood glucose profile of diabetic rats fed with or without *Aloe vera* powder or gel drink. The diabetic rats without an *Aloe vera* product showed high blood glucose (>200 mg dL^{-1}) and increased significantly during the 4 weeks of treatment. Whereas, normal fasting blood glucose was <110 and 140 mg dL^{-1} after meals[24]. The blood glucose of diabetic rats fed with *Aloe vera* powder and gel drink decreased significantly to normal levels by the fourth week of treatment. Aragao *et al.*[26] stated that *Cecropia pachystachya* containing flavonoid and was administrated into alloxan-induced diabetic rats resulted a significant reduction of blood glucose levels at 90 min (reduction of 60%). Jasmine and Daisy[7] stated that flavonoids are capable of stimulating insulin secretion from the pancreas and of excreting an insulin secretion inhibitor and it was indicated by the decreasing of the blood glucose of diabetic rats fed with flavonoid extract from *Eugenia jambolana* for 30 days decreased from 534.60-206.80 mg dL^{-1}. According to Hajiaghaalipour *et al.*[27], hyperglycemia caused by deficiency in insulin secretion in the pancreatic or insulin resistance in the body. One factor that can lead to insulin resistance is oxidative stress. In oxidative stress, condition reactive oxygen species such as; superoxide ($O._2^-$), hydroxyl (.OH), peroxyl ($.RO_2$), hydroperoxyl ($.HRO_2^-$) and reactive nitrogen species such as; nitric oxide (.NO) are responsible for lipid and protein modifications. Therefore, neutralizing free radicals by use of antioxidants such as; the flavonoids found in *Aloe vera* is an important step in decreasing the prevalence of diabetes mellitus. Therefore, it was important to produce *Aloe vera* products in the form of powder or gel drink which can be used for diabetics, so that the dependency on standard drugs can be reduced. However, consumption of this product should within the appropriate intake limit. Thus the results of this study have the potential to be developed commercially.

CONCLUSION

It was concluded that the diet of *Aloe vera* powder and gel drink had hypoglycaemic effect to reduce blood glucose on diabetics rats. Consumption of *Aloe vera* powder was more effective in smaller amounts than *Aloe vera* gel drink. However, it is important to consume *Aloe vera* products that are more practical and acceptable to replace standard drugs for diabetics.

SIGNIFICANCE STATEMENT

This study discovers the hypoglycemic effect of *Aloe vera* gel which had processed into *Aloe vera* powder and gel drink that can be beneficial for diabetic patients possible related with the antioxidative activity. This discovery proved *Aloe vera* as natural product that practically used for

diabetics. This study will help the researcher to uncover the critical area of the use of *Aloe vera* gel and powder on high blood glucose of diabetics that many researchers were not able to explore. Thus, a new theory on hypoglycemic effect of *Aloe vera* product may be arrived at.

ACKNOWLEDGMENT

We gratefully acknowledge to the Directorate General of Higher Education, Ministry of Education and Culture of the Republic of Indonesia, for providing financial support (grant number {No. 015/HB-LIT/III/2015} from the Competitive Research Grant Program 2015.

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