

The Effectiveness of Aloe-based Drink

by Heri Nugroho

Submission date: 10-May-2023 03:32PM (UTC+0700)

Submission ID: 2089328929

File name: The_Effectiveness_of_Aloe-based_Drink.pdf (329.68K)

Word count: 4370

Character count: 22294



The Effectiveness of Aloe-Based Drink in Reducing Glycated Albumin and Insulin Resistance of Metabolic Syndrome: A Randomized Clinical Trial

RIZKI WIDYAN AISYA¹, K. HERI NUGROHO H.S², NYOMAN SUCI WIDYASTITI³,
KUSMIYATI-TJAHJONO⁴ and DIANA NUR AFIFAH^{1*}

¹Department of Nutrition Science, Faculty of Medicine, Diponegoro University, Semarang, Indonesia.

²Department of Internal Medicine, Faculty of Medicine, Diponegoro University, Semarang, Indonesia.

³Department of Clinical Pathology, Faculty of Medicine, Diponegoro University, Semarang, Indonesia.

⁴Department of Biochemistry, Faculty of Medicine, Diponegoro University, Semarang, Indonesia.

Abstract

Insulin resistance (IR) has an important role in the pathology that forms the metabolic syndrome (MetS). Glycated Albumin (GA) has a role as an index of glycemic control associated with MetS. *Aloe vera* (*Aloe barbadensis* Miller) is a plant that has anti-diabetic and anti-hypercholesterolemic function. This study aims to investigate the effect of Aloe-based drink on GA and IR in MetS. This study was a true experimental using pre-post randomized control group design. Thirty-eight MetS subjects were divided into two groups: treatment group (n=19) which was provided by 165 g/d of Aloe-based drink for 4 weeks; and the control group (n=19). Both groups were given education regarding management of MetS. GA was measured by using an ELISA method and calculated by HOMA-IR of both group and statistically analyzed at baseline and the end of treatment. The data were analyzed using paired t-test and independent t-test. At the end of the study, the treatment group showed reduction of GA and HOMA-IR statistically significant (Δ GA=-4.3±2.35%; p<0.001; Δ HOMA-IR=-1.6±1.87; p=0.001). Compared to control group, the change of GA and HOMA-IR in intervention group were also significantly different (p<0.001; p<0.001). Aloe-based drink was proven to reduce GA and IR in the MetS.



Article History

Received: 16 April 2020

Accepted: 9 June 2022

Keywords

Aloe-Based Drink;
Glycated Albumin;
Insulin Resistance;
Metabolic Syndrome.

CONTACT Diana Nur Afifah ✉ d.nurafifah.dna@fk.undip.ac.id 📍 Department of Nutrition Science, Faculty of Medicine, Diponegoro University, Semarang, Indonesia.



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Doi: <http://dx.doi.org/10.12944/CRNFSJ.10.2.13>

Introduction

Metabolic syndrome (MetS) is a group of risk factors related to cardiovascular disease which consists of visceral obesity, hypertension, high level of fasting blood glucose, and dyslipidemia.¹⁻³ World Health Organization (WHO) data showed a high prevalence of obesity (26%), diabetes (9%) and hypertension (22%) in adults in the world which were included in the risk component of MetS.⁴

Insulin resistance, the main pathogenesis of type 2 diabetes mellitus (DMT2) and directly consequences of hyperglycemia by disrupting the action of insulin, has an important role in the pathology that forms MetS.^{5,6} Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) is one of biomarkers for IR assessment.⁷ Glycated Albumin (GA) has high accuracy of glycemic control over periods and is considered as a good indicator as GA is not influenced by the lifespan of erythrocyte.⁸

GA is a product of glucose formation which reacts non-enzymatically with reactive amino protein groups to form the Schiff base group. Schiff base then forms Amadori products to form intermediate compounds like α -oxalaldehyde.^{8,9} Further oxidation of the formation of Schiff base and Amadori result in advanced glycation end products (AGE) formation. The formation of AGE is a major contributor to the development of diabetes type 2 which can cause glycosylation process related to free radical formation.¹⁰⁻¹² Oxidative stress can be considered as a characteristic that unites the components of MetS during aerobic metabolism.^{13,14}

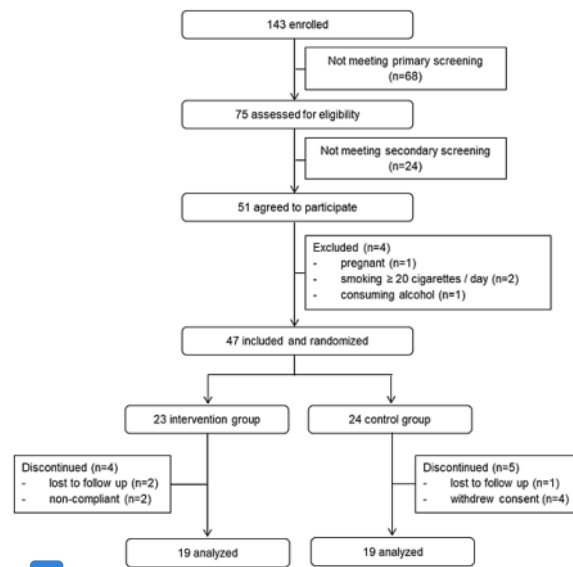
Aloe vera possesses a number of polyphenols, polysaccharides, and amino acids and has biological activities such as anticancer, antioxidant, anti-inflammatory, immunomodulatory, hepatoprotective, antiulcer, antihyperglycemic, antihypercholesterolemic and antidiabetic.¹⁵⁻¹⁸ The antioxidant contents of *Aloe vera* are flavonoids, tannins, α -tocopherol (vitamin E), ascorbic acid (vitamin C), and carotenoids.¹⁸ The previous study stated that extract of *Aloe vera* has antioxidant activities for phenolic 2.07-40.5 mg Gallic acid equivalent (GAE)/g.¹⁹ The main polyphenols in *Aloe vera* are quercetin (94.80 mg/kg), myricetin (1283.50 mg/kg), and kaempferol (257.7 mg/kg).²⁰

The study related to the administration of *Aloe vera* gel in women with MetS aged 46.8 ± 9.7 years stated that *Aloe vera* concentrated 5:1 (5 L *Aloe vera* to obtain 1 L of total *aloe*) in daily ingestion during 4 weeks can reduce blood glucose levels by reducing the pro-inflammatory state and reduction of glucose intolerance.²¹ Another RCT was performed in women with MetS aged ≥ 20 years administered the *Aloe vera* gel complex orally (two caps after breakfast and two after dinner, for 8 weeks) reduced body weight, body mass and insulin resistance.²² Another study in a double-blind randomized controlled trial investigated the effect of two *Aloe vera* doses (300 mg and 500 mg, 1 capsule twice a day over an 8-week period) on fasting blood glucose, HbA1C, and lipid profile in prediabetic subjects. They detected a significant decrease in the levels of fasting blood glucose and HbA1C in the treatment group.²³ Due to the unavailability of the data related to the effect of *Aloe*-based drink on the levels of GA and HOMA-IR values on MetS subjects, this study aims to investigate the effect of *Aloe*-based drink on the levels of GA and insulin resistance expressed by HOMA-IR values in MetS.

Materials and Methods

Study Design and Recruitment of Participants

This study was a true experimental using pre-post randomized control group design. The study was conducted in April – June 2018 on employees and members of the Central Java Regional police. Inclusion criteria, subjects were aged 35-56 years who met three or more risk factors for MetS according to NCEP ATP III criteria, not have hyperthyroidism history, hyperthyroidism, nephrotic syndrome, Cushing's syndrome, glucocorticoid supplementation, liver cirrhosis, pregnancy/lactation, taking drugs for blood glucose levels or hypertension, smoking ≥ 20 cigarettes / day, and consuming alcohol. Out of 143 employees in total, 47 met the selection criteria, all of whom agreed to participate and provided informed consent. Subjects were randomized into 2 groups: intervention group (n=23) and control group (n=24). There was a loss of 4 subjects in the intervention group and 5 in the control group due they could not be followed up, leaving 19 subjects in each intervention group and control group shown in Figure 1.



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Fig. 1: Study population and data collection algorithm

This experimental design was reviewed and approved by Faculty of Medicine of Diponegoro University-Dr.Kariadi Semarang Hospital research committee proved by ethical clearance certificate number 238/EC/FK-RSDK/IV/2018.

Laboratory, Anthropometric and Clinical Data Collection

Baseline data of body weight (assessed by Omron Digital Personal Scale HN289; to nearest 0.1 kg), body height (assessed by GEA Stature Meter SH-2A; to nearest 0.1 cm), blood profiles included fasting blood levels, triglyceride levels, and HDL-C levels (assessed by using enzymatic colorimetric techniques with automatic analyzer Bio Maxima Chemistry BM-100), blood pressure (assessed by Polygreen Automatic Upper Arm Blood Pressure Monitor KP-7550), GA concentration in the plasma sample (analyzed by ELISA method using *Elabscience* Catalog No: E-EL-H1993, USA kit), and fasting insulin (analyzed by ELISA method using *Calbiotech* ²¹ catalog No. IN374S, USA kit) were collected. The HOMA-IR was calculated using the following formula.²⁴

$$\text{HOMA-IR} = (\text{fasting plasma insulin } (\mu\text{IU/mL}) \times \text{fasting plasma glucose (mmol/L)}) / 22.5$$

All the compilation of the intervention period measures were repeated at the end of study. The outcome measures were GA and HOMA-IR. Serum samples were collected by qualified staff from Sarana Medika Laboratory. All subjects fasted overnight for 12 hours before the day of taking blood sample. Furthermore, the blood sample were stored at -25°C before examined and analyzed in the same day after all subjects had completed study days. The blood samples were analyzed twice in first day before being given intervention and a day after 4-weeks intervention period for blood glucose, triglyceride levels, and HDL-C levels in Sarana Medika Laboratory, Semarang while serum GA and insulin were analyzed in GAKY Laboratory Diponegoro University, Semarang.

Preparation Aloe-based Drink

Aloe vera were obtained in Kulon Progo farm, Yogyakarta, while the processing of *Aloe vera* was carried out by the researchers and assisted by *Aloe vera* cultivators in Yogyakarta. *Aloe vera* in a similar ripening stage were selected for the *Aloe*-based drink processing. First, the *Aloes* were washed and were peeled off to get the *Aloe vera* leaf extract (a transparent inner gel). Then they were cut into cubes of 1 cm size. The sufficiently 1 tablespoon

salt and ½ teaspoon citrate acid were added to the *Aloes* cubes then were washed after 10 minutes in sequence. The *Aloe* cubes were blanched for 3-5 minutes in warm water (60-70°C) which had been added by with two pieces of Pandan leaves for aroma. A portion of *Aloe* cubes (165 g) were added to 30 mL low calorie liquid syrup solution.

Study Design

The intervention group were provided with 165 g of *Aloe*-based drink to be taken each day in beverage form of 300 mL in addition to the normal diet and provided with an education to have a good diet for MetS. The control group was provided nutrition education to consume balanced, healthy diet, maintain body weight and follow active lifestyle for control of MetS. The intervention was given for 4 weeks. Adherence was confirmed through checking with the respondent and other family members.

Statistical Methods

The characteristics of the participants were analyzed by using Shapiro-Wilk test. Differences in anthropometry data (body weight, body height and waist size), triglyceride, food intake data (total energy, fat and carbohydrate) between groups were tested with Independent t-test. Meanwhile, Mann-whitney was used for analyzing differences of age, clinical examination (blood pressure systolic and blood pressure diastolic), fasting blood glucose levels, HDL-C and protein intake between groups. Paired t-test was used for bivariate analysis of HOMA-IR data, whereas Wilcoxon test was used for GA, fasting blood glucose levels, and insulin levels. The data were processed by SPSS 21 program. The data were considered as significant level at $p < 0.05$ two-tailed.

Results

Baseline Data

Seventy-five people were found to be above normal weight based on screening, out of them some subjects dropped out as they refused to provide blood samples. The remaining 47 respondents were randomized and divided into two groups: intervention ($n=23$) and control ($n=24$). In the end of study, 4 respondents of intervention group and 5 respondents of control group dropped out due they could not be followed up. The final respondent rate

was 80.85% which consisted of 19 respondents of intervention and 19 respondents of control group.

Table 1 provides the characteristics data of two groups.

There were no differences in the characteristics of the subjects between the treatment and control groups ($p > 0.05$) (age, body weight, body height, BMI, waist size, clinical/physical examination, fasting blood glucose, triglycerides, and HDL-C). It can be concluded that the subjects had the same characteristics at the baseline shown in Table 1 respectively.

Table 1 also showed that there were no significant differences in the energy intake ($p=0.176$), protein ($p=0.549$), and fat ($p=0.841$) between the treatment and control groups, while in carbohydrate intake MetS, there was a significant difference between the treatment and control groups.

Glycated Albumin and HOMA-IR

Table 2 describes the changes in glucose levels, fasting insulin level, GA, and HOMA-IR in both groups. There was a significant difference between the treatment and control groups on the changes of fasting blood glucose, fasting insulin level, GA, and HOMA-IR ($p=0.001$; $p<0.001$; $p<0.001$; $p<0.001$). Fasting blood glucose, insulin, GA, and HOMA-IR before and after the administration of *Aloe*-based drink showed a significant change ($p=0.001$; $p=0.006$; $p<0.001$; $p=0.001$). All of the fasting blood glucose of the respondent in the intervention group improved, but 2 respondents had steady fasting blood glucose. One participant in the intervention group exhibited the fasting blood glucose improvement by 100 mg/dL. None got worse in the control, across the 4 weeks there was variation of fasting blood glucose in control group.

Discussion

There was a significant difference in carbohydrate consumption at baseline, however this was considered not clinically significant. The screening results from this study in Central Java Regional Police were obtained that the subjects of treatment group and control group had the range age of 36-56 years and 35-55 years respectively. Age is one of

the factors that could possibly affect the tissue function related to change of metabolism in the body.²⁵ The study by Sihombing (2015) stated that 18.2% the proportion of MetS had the age range by

35-44 years old and had the risk factors by 1.84 times than the subjects with the age of range by 25-34 years old.²⁶

Table 1: The baseline characteristics of the subjects based on the age, anthropometry, clinical examination, biochemical parameters, and food intake in both groups

	Treatment (n=19)			Control (n=19)			p
	$\bar{X} \pm SD$	Median (Min – Max)	$\bar{X} \pm SD$	Median (Min – Max)	$\bar{X} \pm SD$	Median (Min – Max)	
Age (y)	47±7.67	52(36-56)	45±8.22	45(35-55)			0.508 ^b
Anthropometry							
Body Weight (kg)	76.9±9.41	75.60(62.00-99.20)	83.2±9.87	81.6(65.40-100)			0.560 ^a
Body Height (cm)	160.3±10.71	160.9(140.60-176.30)	164.5±7.70	165.6(150.1-180.2)			0.177 ^a
BMI	30±2.97	29.02(25.53-37.28)	30.5±2.68	30.39(26.25-36.47)			0.448 ^a
Waist Size (cm)	94.4±6.12	93.00(83-107)	95.5±7.75	94.9(81-109)			0.619 ^a
Clinical examination							
Blood Pressure systolic (mm Hg)	130±13	130(110-160)	127±16	130(100-150)			0.523 ^b
Blood Pressure diastolic (mm Hg)	85±6	90 (70-90)	85±6	90(70-93)			0.974 ^b
Biochemical Parameters							
Fasting blood glucose (mg/dL)	135±56.98	119(86-332)	121.84±45.06	109(84-268)			0.199 ^b
Triglyceride (mg/dL)	199.4±100.94	174(48-403)	193.7±69.39	192(53-337)			0.842 ^a
HDL-C (mg/dL)	52.8±9.27	54(39-70)	53.10±13.36	51(35-89)			0.568 ^b
Food Intakes							
Energy intake (kcal)	2539±300	2506(2079-3036)	2685±353	2787(2148-3487)			0.176 ^a
Protein Intake (g)	85±14	81(60-116)	91±15	89(73-122)			0.549 ^b
Fat Intake (g)	97±19	98(54-128)	99±27	92(54-162)			0.841 ^a
Carbohydrate Intake (g)	317±51	317(235-421)	362±65	358(256-475)			0.023 ^a

^aIndependent t-test, ^bMann-Whitney, significant if p-value<0,05

Table 2: The changes in FBG, fasting insulin, GA, and HOMA-IR before and after the administration of Aloe-based drink

Variables	Treatment (n=19)			Control (n=19)			P
	$\bar{X} \pm SD$	Med (Min – Max)	$\bar{X} \pm SD$	Med (Min – Max)	$\bar{X} \pm SD$	Med (Min – Max)	
FBG/fasting blood glucose (mg/dL)							
pre	135.7±56.98	119.00(86.00-332.00)	121.8±45.06	109.00(84.00-268.00)	121.8±45.06	109.00(84.00-268.00)	0.001 ^a
post	133.3±42.22	99.00(80.00-239.00)	125.7±40.28	118.00(72.00-254.00)	125.7±40.28	118.00(72.00-254.00)	
Δ	-22.5±24.08	-16.00(-93.00-19.00)	3.8 ±20.47	2.0(-37.00-51.00)	3.8 ±20.47	2.0(-37.00-51.00)	
p	0.001 ^b		0.316 ^b		0.316 ^b		
Fasting insulin level (μIU/mL)							
pre	10.7±4.87	10.95(0.14-18.72)	8.3±2.97	8.85(3.88-16.94)	8.3±2.97	8.85(3.88-16.94)	0.000 ^c
post	7.4±4.51	5.80(2.21-16.86)	9.9±4.12	9.04(4.27-20.54)	9.9±4.12	9.04(4.27-20.54)	
Δ	-22.5 ±24.08	-2.73(-9.17-7.48)	1.6 ±1.79	1.29(-0.90-4.98)	1.6 ±1.79	1.29(-0.90-4.98)	
p	0.006 ^b		0.003 ^b		0.003 ^b		
GA (%)							
pre	19.3±1.73	19.40(16.35-21.78)	19.2±1.79	19.32(16.67-22.27)	19.2±1.79	19.32(16.67-22.27)	0.000 ^a
post	14.9±2.22	15.11(11.22-19.77)	18.7±1.99	18.57(14.40-21.49)	18.7±1.99	18.57(14.40-21.49)	
Δ	-4.3±2.35	-4.32(-9.00-(-1.20))	-0.3±1.79	-0.18(-4.22-2.88)	-0.3±1.79	-0.18(-4.22-2.88)	
p	0.000 ^d		0.346 ^d		0.346 ^d		
HOMA-IR							
pre	3.6±2.04	3.47(0.04-7.92)	2.6±1.44	2.27(0.82-6.61)	2.6±1.44	2.27(0.82-6.61)	0.000 ^a
post	1.9±1.13	1.81(0.47-4.54)	3.2±1.81	2.64(0.90-6.81)	3.2±1.81	2.64(0.90-6.81)	
Δ	-1.6 ±1.87	-1.58(-4.66-1.77)	0.6 ±0.93	0.37(-0.77-3.22)	0.6 ±0.93	0.37(-0.77-3.22)	
p	0.001 ^d		0.014 ^d		0.014 ^d		

^aIndependent t-test, ^bWilcoxon, ^cMann-Whitney, ^dPaired t-test, significant if p-value<0,05

All participants in intervention group had insulin improvement, but 2 participants had steady insulin. The highest insulin improvement in intervention group was 9.17 μIU/mL. Look at the control

group, insulin increased significantly (p=0.003). All participants in intervention group exhibited GA reduction, except for one participant. GA of one participant in intervention group exhibited

the best improvement by 7.73%. There was no participant got worse of GA at the end of study either in intervention group or in control group. The intervention group showed a decrease of HOMA-IR, but three participants in intervention group had no change in HOMA-IR. At the end of study, control group had an elevation by 0.6 ± 0.93 of HOMA-IR.

³⁰ induces superoxide formation and stimulates NADPH oxidase activity. NADPH oxidase is the most major source of ROS production in non-phagocytic cells, including vascular smooth muscle cells.⁹ The decrease of GA in the treatment group showed that there was a hyperglycemic improvement, while insignificant decrease of GA was concomitant with insignificant improvement of FBG in the control group. At the hyperglycemic state, albumin will be glycated so that the function of albumin as anti-inflammatory is disturbed. The disruption of the inflammatory system in the body will stimulate the formation of ROS. In this study, the consumption of *Aloe*-based drink was assumed to increase antioxidant status that resulted oxidative stress reduction, consequences for decreasing GA.²⁷ The study of Cardenas-Ibarra's stated that the administration of *Aloe vera* could reduce fasting hyperglycemia in a new case of diabetes type 2.²¹ In another study conducted by Radha, the administration of *Aloe*-based drink made from *Aloe vera* gel has many benefits such as acts as an anti hypercholesterolemic and antihyperglycemic for patients with DMT2.¹⁸

This study revealed *Aloe*-based drink decreasing HOMA-IR. *Aloe*-emodin is an anthraquinone compound, extracted from *Aloe vera* gel that can reduce fat accumulation, suppress the pro-inflammatory cytokines induced inflammation and insulin secretion reduction.¹⁹ The administration of *Aloe*-based drink was in accordance with the study by Choi (2013) about the administration

of 700 mg *Aloe vera* that could reduce body weight, body fat mass, and insulin resistance in obesity with untreated pre-diabetes.²² *Aloe vera* gel improves insulin sensitivity and has hypoglycemic and hypolipidemic effects as well as decreases adipocyte size. *Aloe vera* can improve insulin sensitivity by activating AMP-activated muscle protein kinase, which is important in glucose regulation and lipid metabolism.²²

In this study, we obtained new finding that the administration of *Aloe* can reduce the levels of GA and improve the state of insulin resistance. Further study is needed to analyze the effectiveness in giving *Aloe*-based drink to patients with MetS who routinely consume the diabetic drugs with the composition and amount of diet that has been determined.

Conclusion

In conclusion, *Aloe*-based drink administration with a dose of 165 g / day for 30 days has proven to reduce glycated albumin and insulin resistance in people with MetS. *Aloe*-based drink can be used as an alternative functional food in people with MetS.

Acknowledgment

The authors would like to thank the Head of Community Resources of the Central Java Regional Police and the Members of the Central Java Regional Police who had participated in this study voluntarily. This study did not receive any grant from external funding agencies.

Funding

Thank you to Diponegoro University for helping with the cost of publishing this research.

²²

Conflicts of Interest

Authors declare that there are no conflicts of interest regarding the publication of this paper.

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