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Randomized double blind crossover trial of *Aloe vera*, *Cnidoscolus chayamansa and placebo* for reducing hyperglycemia in women with early metabolic syndrome

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SUMMARY

Background: There have been antidiabetic claims for *Aloe vera* (AG) Barbadensis Mill. gel and infusion of *Cnidoscolus chayamansa* (CC) McVaugh.
Objectives: To determine if the ingestion of total process AG concentrated 5:1 (TA), AG, CC or placebo can reduce hyperglycemia in women with early metabolic syndrome (EMS).
Methods: One hundred-twenty five women from two outpatient university clinics were randomly assigned to a three assay double-blind crossover procedure. Subjects were adult women with EMS by ATP III criteria assigned to assay 1: AG&CC vs P1&P2; assay 2: AG&P2 vs P1&CC; or assay 3: TA vs P3. All assays included the ingestion of one, then zero (washout period), then two gelatins/day, for 4, 1, 4 weeks, respectively. The expected outcome was an HbA_{1c} decrease $\geq$4.2 mmol/mol or lower but sustaining euglycemia.
Results: Participants had a mean age of 46.8 ± 9.7 years and a mean HbA_{1c} of 47.8 ± 12.7 mmol/mol at the start of the study. The least tolerated combination was AG&P2. Patients complained of bad taste and mild stomach pain because of the double dose of this treatment; this caused withdrawals: 4/25 vs. 9/21, respectively, Chi square = 4.1, $df = 1$, $P < 0.05$. Changes in HbA_{1c} (mmol/mol) were assay 1, −1.8 ± 7.5 vs −1.6 ± 6.9, $P > 0.05$; assay 2, −1.3 ± 6.6 vs −1.4 ± 7.6, $P > 0.05$; assay 3, −4.9 ± 8.3 vs 0.44 ± 5.4, $P < 0.01$,

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respectively. TA concomitantly reduced high-sensitive C-reactive protein (hs-CRP) ($P < 0.05$).
Conclusions: Data suggest that TA decreases blood glucose levels by reducing the proinflammatory state. The infusion of microwave dehydrated CC leaves did not reduce blood glucose or HDL and triglyceride levels.
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1. Introduction

Metabolic syndrome is defined as the presence of at least three of the following: hypertension, central obesity, and high blood levels of glucose, cholesterol, and inflammatory markers. A genetic predisposition, bad dietary habits, and a sedentary life style are also present in these patients. In the United States, there is a prevalence of metabolic syndrome of 34.5% in individuals over 20 years of age [1]. In Monterrey, Mexico, the prevalence of high blood pressure, diabetes mellitus and glucose intolerance in adults was 26%, 14% and 19%, respectively. Most of these individuals had central obesity [2]. Weight loss is key to treating this highly extended condition. However, people often fail to lose weight or regain the weight soon after it is lost [3].

Functional foods are defined as foods that are consumed as part of a usual diet but have physiological effect and/or reduce the risk of chronic disease beyond basic nutritional functions [4]. Reports show that *Aloe vera* (Barbadensis Miller) gel (AG) contains nutrients and functional activities such as decreasing hyperglycemia [5,6]. Perez et al. [7] also reported that a polyphenol extract from *A. vera* gel decreased glucose levels in induced insulin resistance in mice. Another plant with antidiabetic effects is *C. chayamansa* (McVaugh) (CC) used as an infusion [8]. González-Laredo et al. [9] described that the antidiabetic properties of CC could be due to its high content of flavonoids, which are most commonly known for their antioxidant activity [10]. Although their active principals have not been well characterized and the scarcity of clinical studies hamper an informed decision, people still use them [11].

We undertook this study to determine if the daily ingestion of concentrated 5:1 AG by total process (TA), AG or CC infusion vs. placebo reduces high blood glucose levels in women with early metabolic syndrome.

2. Materials and methods

Three controlled clinical procedures were performed. Each with two treatment sequences, crossover, and double blind randomization as follows: assay 1: AG&CC vs P1&P2; assay 2: AG&P2 vs P1&CC; assay 3: TA vs P3.

The study took place in two outpatient clinics: one at a university hospital (Monday to Friday) and another at a low-income community health center (Saturdays). Enrollment took place from Oct 15, 2008 to December 20, 2008; follow up ended on March 26, 2009. Subjects were adult women with early metabolic syndrome (EMS); i.e., with at least three of the ATP III clinical identification criteria [12]: waist circumference $\geq$88 cm, fasting capillary blood glucose $\geq$100 mg/dL, known arterial hypertension, or arterial blood pressure $\geq$130/$\geq$85 mm/Hg (two out of three measurements in the morning), triglycerides $\geq$150 mg/dL, and an HDL <50 mg/dL. Women who were pregnant or nursing, had hyperglycemic symptoms, were on hypoglycemic agents, or with a previous diagnosis of diabetes melllitus (DM), had advanced DM complications, dementia, a non-compensated endocrinopathy, or severe behavioral problems were excluded.

The protocol was previously approved by the University Hospital Ethics Committee and Research Committee (registration no. EN08-027). The study complied with the Helsinki declaration and all participants provided signed informed consent.

Sample size was calculated assuming a standard deviation difference of 0.6 of the outcome variable among treated groups and a power index of 2.8 (two-tiled alpha 5% and one-tiled beta 20%). Thus, a sample of 44 subjects per group was needed [13]. There were 44 in the AG group, 44 in the CC group, and only 22 in the TA group, since it is concentrated 5:1 AG. Fifteen more were added to compensate for possible dropouts, giving a total of 125 subjects.

Randomization was carried out using 125 sequentially numbered envelopes enclosing the code treatment with a computer-generated random number. To ensure balance, every ten envelops had 2 assignments for each of the treatments in assay 1 and 2, but only one each for TA and P3 in assay 3 (Fig. 1). To reveal the assignment code, the corresponding envelope was opened after the identified eligible subject had signed the informed consent provided by the coordinator. Decoding only took place in patients with suspected moderately severe side effects and after data analysis. Treatments were disguised in 230 ml lemon gelatins. These could contain AG&CC = 30 ml of *A. vera* gel and 200 ml of CC infusion; P1&P2 = 30 ml of placebo 1 and 200 ml of placebo 2; AG&P2 = 30 ml of *A. vera* gel and 200 ml of placebo 2; P1&CC = 30 ml of Placebo1 and 200 ml of CC infusion; for TA = 30 ml of concentrated 5:1 AG (5 L of AG to obtain 1 L of total aloe) and 200 ml of water; lastly, for group P3, 30 ml of stabilizers and a colorant without TA and 200 ml of water.

2.1. Plant material

Whole fresh leaves of *A. vera* (AG) were obtained from an internationally certified organic farm (Aloe Jaumave, S.A de C.V., Jaumave, Tamaulipas, Mexico); manually obtained crystalline gel without the yellow latex of the leaves. The gel was liquefied and aliquoted 30 ml per gelatin. Patented Totaloe (TA) concentrated 5:1 AG by total process was obtained from the same organic farm, also in aliquots of 30 ml per gelatin. TA was chromatographically defined as aloeride 2.56%, acemannan 8.77%, manapol 35.49%, aloemannan 19.30%, and small molecular species 33.88% by the North Texas Research Lab (Nov. 2009, Danhof, Ivan E.)

CC leaves were obtained from a local garden. The plants satisfied the morphology description by Kuti et al. [8] and Stephens [14]. To ensure a sanitized supply, CC leaves were washed and rinsed with tap water and then air dried and later microwave dehydrated. The infusion was prepared as follows: 10 g of dry leaves per 11.5 L of water heated at ~80 °C to reduce 1.5 L for a final 10 L of CC infusion. The gelatin dose was 200 ml.

Placebo 1 was one milliliter of green food colorant per 10 L of purified water to be used 30 ml per gelatin. Pacebo 2 was one milliliter yellow food colorant per 10 L of purified water utilized 200 ml per gelatin. Placebo 3 consisted of the same stabilizers and colorant for TA but without TA (Aloe Jaumave, S.A de C.V.). Production records and coded gelatin labels were used.

The assigned treatment was one lemon gelatin per day for four weeks. During the fifth week no gelatin was supplied (washout period), then a crossover to the opposite group was performed for a second treatment period of 4 weeks, increasing the dose to two lemon gelatins every day (Fig. 1). Three blood samples were taken, one at baseline and one at the end of each treatment period. A diet of 1500 calories was prescribed to each participant, along with a written guide to remember diet instructions. Seventy-four participants underwent a third treatment period. They were single blinded and assigned to either: AG&CC, AG&P2, P1&CC, or TA. Two gelatins per day were provided. One more blood sample was drawn after the 4th week. Clinical data and lab reports were handled independently. Participants learned their health status at the time of recruitment; however, subsequent lab test results were withheld until the data analysis was done.

A reduction of an elevated glycohemoglobin A_{1c} fraction ($\geq$4.2 mmol/mol) was considered success. Likewise smaller variations that sustain HbA_{1c} under 42.0 mmol/mol were also considered a success. The secondary outcome was a reduction of the BMI, proinflammatory hs-CRP levels, and HDL and triglyceride levels.

Safety was monitored by a complete blood cell count and liver function tests at baseline and after each treatment. Tolerance was evaluated by weekly questioning the effort to take the treatment (gelatin), the sense of wellbeing, energy, and satiety level, gastrointestinal (bowel movements), and general complaints. Any complaints of malaise were considered potential side effect unless another diagnosis was made.

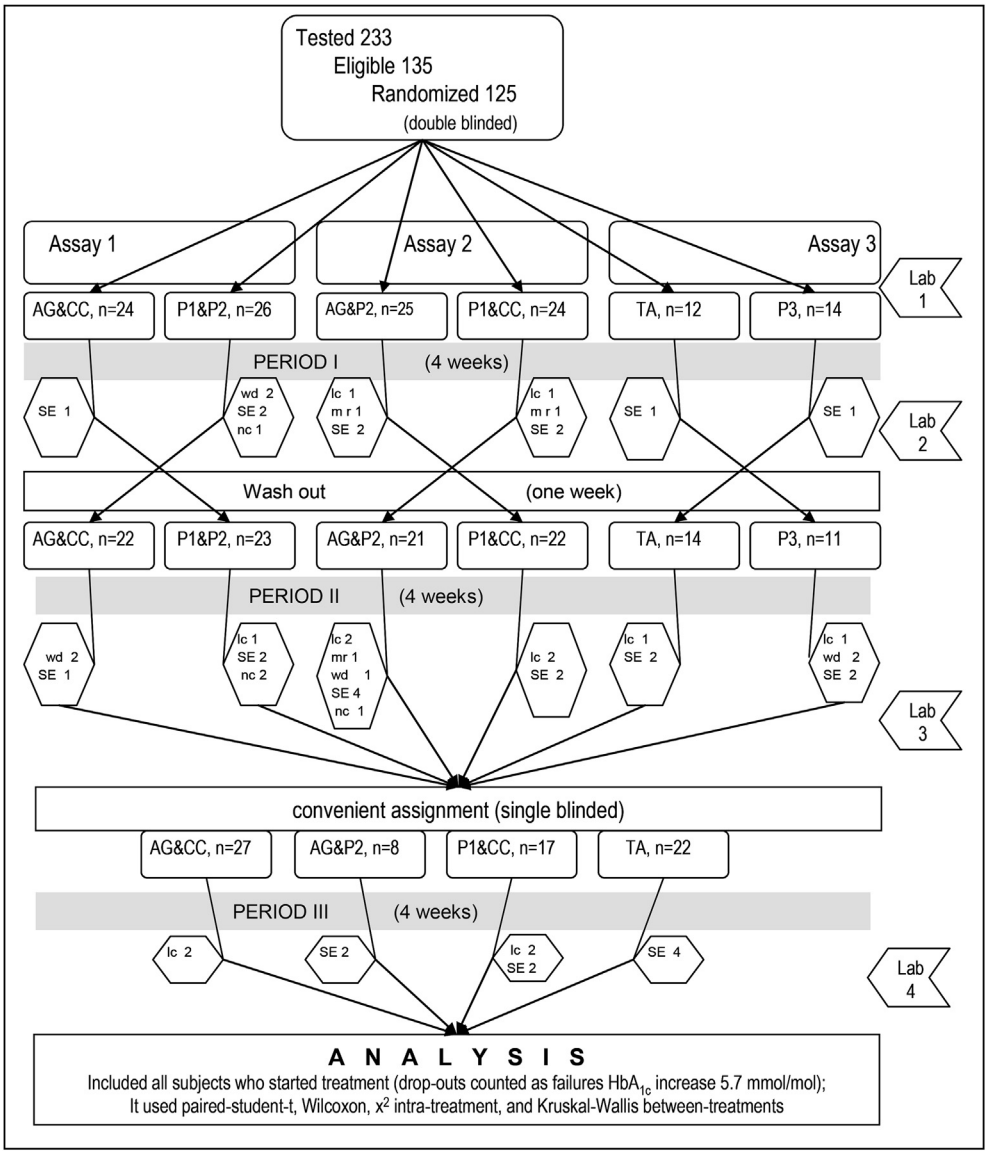

Fig. 1. Work flow algorithm: AG = *Aloe vera* gel; CC = *Cnidoscolous chayamansa* infusion; TA = concentrated 5:1 AG by total process; P = placebo (1, 2 and 3). Hexagons show drop out reason: lc = lost contact, mr = medical reason, wd = withdraw consent, SE = side effect, nc = non compliant. All SE were mild, no requiring medication but one subject took a butyl bromide tablet; 61% of subjects with any SE did not drop out (see Table 1). Lab determinations were blinded.

Laboratory determinations included glycated hemoglobin A_{1c} fraction by an immunoturbidimetric test (One-HbA_{1c} FS) via Star-Dust MC15 (both from DiaSys Diagnostic Systems, Wixom, MI). Their coefficient of variation was 1.6%. High sensitive C-reactive protein (hs-CRP) was measured using an IMMULITE immunoassay analyzer (Siemens Healthcare Diagnostics, Erlangen, Germany). Triglycerides and HDL cholesterol levels were determined manually with RANDOX kits (Randox Laboratories Ltd. London, UK) using the GPO-PAP method and precipitation with phosphotungstic acid/$MgCl_2$, respectively.

Wellbeing, energy, and satiety level were evaluated by the Kruskal–Wallis χ^2 test. Compliance and malaise complaints were analyzed with Fisher's exact test. Recurring HbA_{1c} was tested by the non-parametric Friedman procedure. Next, an intraassay comparison of groups was performed by independent *t*, for treatment effect. Paired *t* and/or the Wilcoxon test were used to assess treatment change of HbA_{1c}, hs-CRP, DHL-C, triglycerides, body weight in kg and body mass index (BMI) kg/m^2. A baseline comparison within assays and the six treatments used were made with the Kruskal–Wallis test and a one-way ANOVA. Dunnett's procedure was used for multiple comparisons versus a selected treatment with one side significance. Linear regression was performed for period I, parameterization: aloe dose (0 = none [placebo], 1 = AG, and 2 = TA), CC (0 = none [placebo], 1 = 200 or 400 ml of CC infusion). Two-sided alpha was considered (unless otherwise specified); significance was set at 0.05, and a trend, between 0.05 and 0.1. The intention-to- treat analysis drop outs were handled in two ways: 1) counted as a failure (A_{1c} increase 5.7 mmol/mol) in their corresponding group, and 2) last observation carried forward (LOCF) [13].

3. Results

The flow chart of the 125 recruited women with EMS is shown in Fig. 1. Baseline characteristics were similar across the groups. Collectively mean age ± SD was 46.8 ± 9.7 years, BMI was 34.0 ± 5.2 kg/m^2, body weight was 82.1 ± 14.8 kg, waist circumference was 101 ± 11.1 cm, HbA_{1c} was 47.8 ± 12.7 mmol/mol, hs-CRP 6.3 ± 6.5 mg/L, HDL 47.5 ± 10.3 mg/dl and triglycerides 133.1 ± 94.8 mg/dl. Overall, after the first and second treatment periods, and LOCF for dropouts, HbA_{1c} improved to 43.8 ± 9.9 and 42.4 ± 10.1 mmol/mol, respectively (Friedman's $\chi^2 = 72.2$, df = 2, $P < 0.001$).

3.1. Tolerance and safety

Subjects' compliance and side effects (SE) are shown in Table 1. A double gelatin dose (second treatment period) increased the number of subjects dropping out collectively reaching significance for the AG&P2 and P3 treatments; likewise for any SE reported ($P < 0.05$). Sleep disturbances and a peculiar sweat odor were reported for CC and was dose independent. Colonic malaise and unpalatable gelatin treatment predominated with *A. vera* (AG&P2 and TA). The main complaint with placebo was upper gastric discomfort (heartburn, more hunger, etc.). AG&CC had the fewest dropouts and SE, and also showed the best rank of wellbeing and energy level sensed by the patients in both treatment periods (Table 2); but this decreased in the second period for all treatments reaching significance for P3 and AG&P2. Safety surveillance, blood cell counts and liver function tests, were normal across assays (data not shown). SE (malaise complaints) improved without medication in all subjects except in one who took a butyl bromide tablet. Of those with any malaise complaints, 61% did not drop out.

3.2. Effect on blood glucose level

In an intra-assay comparison, the HbA_{1c} mmol/mol change for AG&CC vs P1&P2, AG&P2 vs P1&CC, and TA vs P3 was −1.8 ± 7.5 vs −1.6 ± 6.9, t = 0.1, df = 93, $P > 0.1$; −1.3 ± 6.6 vs −1.4 ± 7.6, t = 0.07, df = 90, $P > 0.01$; −4.9 ± 8.3 vs −0.4 ± 5.4, t = 2.7, df = 49, $P < 0.01$, respectively. Data by assay, treatment and period are shown in Table 3. TA had a significant improvement and its HbA_{1c} change was divergent from the one shown for P3 in both periods ($P < 0.05$). AG&CC and AG&P2 tended to improve ($P < 0.1$) in the first period only. Indeed, LOCF for drop outs, AG&CC results change insignificantly but AG&P2 improvement reached significance in both periods; however, it was not statistically divergent from its counterpart (P1&CC). Comparison for the six treatment sequences, using LOCF for drop outs, revealed a trend by Kruskal–Wallis $\chi^2 = 10.5$, df 5, $P < 0.08$ with TA −5.72 ± 7.4 mmol/mol producing the greatest improvement; its differences (±SE) versus AG&CC, P1&P2, AG&P2, P1&CC, and P3 were: -3.5^{*} ± 1.6, $-3.0^{\S}$ ± 1.5, −2.9 ± 1.6, $-3.4^{\S}$ ± 1.6, and -4.8^{*} ± 1.8 respectively; one sided $^{\S}P < 0.1$; $^{*}P < 0.05$ using Dunnett's multiple comparison test.

The results of period III with a single-blinded convenient assignment are displayed in Table 4. The groups containing CC worsen as they show a significant increase of HbA_{1c} (both $P < 0.05$), while aloe groups had a non-significant variation sustaining a mean in the euglycemia range. Between groups

Table 1
Protocol compliance and side effects (SE).

Period	All treatments		Assay1				Assay2				Assay3			
			AG&CC		P1&P2		AG&P2		P1&CC		TA		P3	
	I	II	I	II	I	II	I	II	I	II	I	II	I	II
n	124 (100)	113 (100)	24 (100)	22 (100)	26 (100)	23 (100)	25 (100)	21 (100)	24 (100)	22 (100)	12 (100)	14 (100)	14 (100)	11 (100)
Abandon	15 (12.1)	**29(25.7)****	1 (4.2)	3 (13.6)	5 (19.3)	5 (21.7)	4 (16.0)	**9(42.9)***	4 (16.7)	4 (18.2)	1 (8.3)	3 (21.4)	1 (7.1)	**5(45.5)***
Any SE	24 (19.4)	**33(29.2)**§	2 (8.3)	**6(27.3)**§	4 (15.4)	5 (21.7)	5 (20.0)	**10(47.6)**§	7 (29.2)	4 (18.2)	4vs3	3 (21.4)	3 (21.4)	5 (45.5)
Insomnia	5 (4.0)	7 (6.2)	1 (4.2)	2 (9.0)	0 (0.0)	1 (4.3)	0 (0.0)	1 (4.8)	4 (16.7)	3 (13.6)	0 (0.0)	0 (0.0)	0 (0.0)	1 (9.1)
Nocturia	2 (1.6)	**7(6.2)**§	0 (0.0)	2 (9.0)	0 (0.0)	1 (4.3)	0 (0.0)	**3(14.3)**§	1 (4.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (7.1)	1 (9.1)
Headache	4 (3.2)	7 (6.2)	0 (0.0)	2 (9.0)	0 (0.0)	1 (4.3)	1 (4)	2 (9.5)	1 (4.2)	0 (0.0)	1 (8.3)	1 (0.7)	1 (7.1)	1 (9.1)
PSO	1 (0.8)	1 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.2)	1 (4.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Dyspepsia	8 (6.5)	8 (7.1)	1 (4.2)	0 (0.0)	4 (15.4)	3 (13.0)	0 (0.0)	1 (4.8)	1 (4.2)	2 (9.0)	1 (8.3)	1 (0.7)	1 (7.1)	1 (9.1)
Colitis	5 (4.0)	9 (8.0)	0 (0.0)	2 (9.0)	0 (0.0)	0 (0.0)	3 (12.0)	2 (9.5)	1 (4.2)	0 (0.0)	1 (8.3)	2 (14.3)	0 (0.0)	**3(27.3)**§
Unpalatable	3 (2.4)	5 (4.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	**3(14.3)**§	0 (0.0)	0 (0.0)	3 (25.0)	1 (0.7)	0 (0.0)	1 (9.1)

AG = Aloe gel; CC = *Cnidoscolous chayamansa* infusion; TA = concentrated 5:1 AG by total process; P = placebo (1, 2 and 3).
PSO = peculiar sweat odor. Boxed data mean predominance of SE regardless of period (double dose in period II).
Data are n(%). I vs. II period Fisher's exact test, one side probability: §**P < 0.1;** ***P < 0.05;** ****P < 0.01**.

Table 2
Mean rank of subjects' reported wellbeing, energy, and satiety level.

Period	Assay1				χ^2	Assay2				χ^2	Assay3				χ^2
	AG&CC		P1&P2			AG&P2		P1&CC			TA		P3		
	I	II	I	II		I	II	I	II		I	II	I	II	
N	24	22	26	23		25	21	24	22		12	14	14	11	
Wellbeing	65	58	41	30	**25.0****	48	40	43	54	3.7	28	30	28	17	5.1
Energy	64	60	41	27	**28.3****	48	38	44	55	4.7	27	32	27	16	**7.7**§
Satiety	63	47	41	41	**10.6***	51	43	47	43	1.5	30	29	25	20	3.7

AG = Aloe gel; CC = *Cnidoscolous chayamansa* infusion; TA = concentrated 5:1 Aloe gel by total process; P = placebo, 1, 2 and 3. Data = Mean rank. The largest rank decrease is underlined. Intra-assay test were Kruskal–Wallis χ^2, with 3 degrees of freedom. Wellbeing, energy and satiety = sensed level. Significant values in bold **§P < 0.1; *P < 0.05; **P < 0.01**.
TA vs. P3 increase level of wellbeing = 19/26 vs. 11/25; and for energy: 22/26 vs 14/25, each **χ^2 > 4.4, df 1, P < 0.05**.

Table 3
Mean ± SD of pre and post treatment HbA_{1c} mmol/mol, by period and assay.

Period I	Assay 1		KW χ^2	Assay 2		KW χ^2	Assay 3		KW χ^2
	AG&CC	P1&P2		AG&P2	P1&CC		TA	P3	
	N = 24	N = 26		N = 25	N = 24		N = 12	N = 14	
PreTx HbA_{1c}	47.45 ± 11.13	46.6 ± 9.1	0.30	46.3 ± 7.9	46.2 ± 9.3	0.45	49.4 ± 16.4	51.2 ± 23.7	0.01
PosTx HbA_{1c}	44.22 ± 8.2	44.2 ± 6.1	0.31	43.9 ± 9.9	45.1 ± 9.2	1.1	42.7 ± 11.8	50.6 ± 18.9	**3.0**§
Diff. low-up 95% CI	**−0.3 to 6.8**§	−0.9 to 5.8	0.1	**−0.5 to 5.3**§	−2.2 to 4.4	0.1	**0.3 to 13.0***	−2.9 to 4.1	**3.8***
Period II	Crossover			Crossover			Crossover		
	N = 22	N = 23		N = 21	N = 22		N = 14	N = 11	
PreTx HbA_{1c}	42.48 ± 4.84	43.84 ± 8.3	0.01	42.9 ± 6.0	43.1 ± 10.7	0.36	49.3 ± 19.2	43.4 ± 12.2	1.2
PsTx HbA_{1c}	42.33 ± 8.10	43.13 ± 7.7	0.94	42.9 ± 8.2	41.43 ± 8.4	0.43	45.9 ± 19.1	45.2 ± 13.6	1.6
Diff. low-up 95% CI	−2.5 to 2.8	−1.4 to 2.8	0.22	−2.7 to 2.6	−1.6 to 5.0	1.24	**−0.4 to 7.1**§	−4.6 to 1.0	**4.3***

AG = Aloe gel; CC = *Cnidoscolous chayamansa* infusion; TA = concentrated 5:1 Aloe gel by total process; P = placebo (1,2 and 3). Pre–posTx = pre and post treatment. Drop outs count as failure (HbA_{1c} increase 5.7 mmol/mol). Data: median, sum −/+ of ranked pos subtract pre treatment HbA_{1c}. Analysis used paired-t by column; and intra-assay used Kruskal–Wallis by raw. Significant values in bold **§P < 0.1; *P < 0.05; **P < 0.01**.

Table 4
Period III convenient assignment HbA_{1c} mmol/mol.

	P1&CC	AG&CC	AG&P2	TA	Respective mean rank	Kruskal–Wallis χ^2
	n = 17	n = 27	n = 8	n = 22		
PreTx HbA_{1c}	43.1 ± 11.0	40.3 ± 6.9	36.8 ± 2.3	41.2 ± 8.1	39, 39, 22, 40	5.02
PosTx HbA_{1c}	46.1 ± 11.5	42.2 ± 8.2	39.1 ± 3.5	41.2 ± 7.8	46, 37, 26, 36	5.5
Diff. low-up 95% CI	**−3.2 to −0.5***	**−3.2 to −0.5***	**−4.9 to 0.3**§	−3.0 to 2.8	46, 35, 40, 33	4.4

AG = Aloe gel; CC = *Cnidoscolous chayamansa* infusion; TA = concentrated 5:1 Aloe gel by total process; P = placebo (1 and 2). PreTx = pretreatment; PosTx = post treatment. Data: mean ± SD, and between groups mean rank.
Drop out: 4, 2, 2, 4 subjects, respectively; these counted as failure (HbA_{1c} increase 5.47 mmol/mol). Paired *t* and Wilcoxon test CI were significant (bold); **§P < 0.1; *P < 0.05; **P < 0.01.** LCOF for missing, TA vs the rest, change A_{1c} were: −0.94 ± 6.0 vs 1.5 ± 4.6, Diff. Low-Up 95% CI **−0.03 to 5.1,** t = 2.0, **P = 0.053**.

assessment post-treatment and HbA_{1c} change show a higher mean rank for CC than aloe groups with neither reaching significance. Still, using LOCF for missing data, TA (n = 22) vs the rest combined (n = 52) was better in achieving euglycemia: −0.94 ± 6.0 vs 1.6 ± 4.6 mmol/mol, F = 0.7, t = −2.0, df = 72, P = 0.053 (95% CI − 5.11 to 0.03.mmol/mol).

Table 5
hs-CRP (mg/dl) Mean ± SD by treatment for period I and II. In the lower part period III.

Period I and II	AG&CC n = 46	P1&P2 n = 49	KW χ^2	AG&P2 n = 46	P1&CC n = 46	KW χ^2	TA n = 26	P3 n = 25	KW χ^2
PreTx	5.4 ± 4.1	5.3 ± 4.9	0.28	5.4 ± 4.5	5.5 ± 5.4	0.31	7.9 ± 6.6	6.2 ± 5.8	1.17
PostTx	6.2 ± 5.5	5.1 ± 4.1	0.38	5.4 ± 4.3	5.8 ± 5.6	0.01	5.2 ± 4.2	7.1 ± 5.5	1.23
Diff. low-up 95% CI	−2.0 to 0.5	−0.6 to 0.9	1.9	−1.4 to 1.3	−1.4 to 0.9	1.31	**0.5 to 5.0***	−3.3 to 1.5	**9.38****
Period III	AG&CC n = 27			AG&P2, n = 8	P1&CC, n = 17		TA n = 22		KW χ^2
PreTx	4.5 ± 4.9			5.0 ± 2.8	5.7 ± 4.2		4.8 ± 4.3		3.0
PostTx	4.1 ± 4.0			4.8 ± 3.2	5.3 ± 4.0		3.6 ± 3.6		3.0
Diff. low-up 95% CI	−1.2 to 1.9			−2.2 to 2.6	−0.8 to 1.7		**0.2 to 2.0***		1.27

AG = Aloe gel; CC = *Cnidoscolous chayamansa* infusion; TA = concentrated 5:1 AG by total process; P = placebo (1, 2 and 3); PreTx = pre-treatment level and PostTx = post-treatment level. Period III had a single blinded convenient assignment. LOCF for missing data. Data: median, sum of (−) and (+) ranked treatment difference. Analysis: Paired *t* by column, and between groups intra-assay Kruskal–Wallis. Significant values in bold **§P < 0.1; *P < 0.05; **P < 0.01**.

3.3. Concomitant changes

hs-CRP changes are shown in Table 5. A data point to significant improvement was found only for TA and were statistically divergent for its counterpart; replicating significant hs-CRP reduction in period III. For AG&P2 there were no significant hs-CRP changes. For comparison with Zhang Y et al. [15], HbA_{1c} was transformed to average blood glucose values in mg/dl using the equation of Nathan et al. [16]. This is described in Table 6, combining the three periods, along with hs-CRP, body weight, BMI, Triglycerides, and HDL. Body weight and BMI improved only in the AG&CC and AG&P2 groups. All triglyceride changes were insignificant, while HDL decreased significantly across all treatments. Mean blood glucose was significantly reduced for TA and AG&P2. A one-tailed Dunnett's procedure shows significantly better reduction for TA than AG&CC and P1&CC but no significant deviation from AG&P2. These differences were (mean ± SE) 7.2 ± 3.2, P < 0.05*; 6.1 ± 3.2, P < 0.1§; 3.3 ± 3.4, P > 0.3, respectively.

Table 6
Concomitant changes by treatment in 3 periods pooled.

Treatment	n		hs-CRP mg/dl	Bodyweight, kg	BMI kg/m^2	Triglycerides mg/dl	HDL mg/dl	BG mg/dl
P1&CC	63	PreTx	5.5 ± 5.0	82.1 ± 14.3	34.1 ± 5.3	113.3 ± 58.5	44.8 ± 8.0	131.4 ± 26.7
		PosTx	5.6 ± 5.2	81.9 ± 14.4	34.0 ± 5.4	120.3 ± 65.7	42.5 ± 5.9	128.2 ± 24.5
	Diff	Low-up 95% CI	−0.9 to 0.8	−0.2 to 0.6	−0.1 to 0.2	−17.2 to 2.8	**0.6 to 4.2***	−1.5 to 8.0
AG&CC	73	PreTx	5.1 ± 4.4	79.1 ± 12.2	33.0 ± 4.3	126.2 ± 78.9	43.8 ± 7.2	128.8 ± 22.2
		PosTx	5.4 ± 5.1	78.5 ± 12.3	32.8 ± 4.4	130.4 ± 102.6	41.6 ± 5.9	127.0 ± 21.3
	Diff	Low-up 95% CI	−1.3 to 0.6	**0.2 to 0.9****	**0.1 to 0.4****	−16.3 to 7.7	**0.5 to 3.9***	−1.8 to 5.9
AG&P2	54	PreTx	5.4 ± 4.3	80.7 ± 12.7	33.5 ± 5.2	112.7 ± 63.1	45.8 ± 9.8	129.3 ± 19.4
		PosTx	5.4 ± 4.1	80.3 ± 12.9	33.4 ± 5.2	113.7 ± 59.1	43.4 ± 6.3	123.0 ± 18.5
	Diff	Low-up 95% CI	−1.2 to 1.1	**−0.05 to 0.8**§	**−0.01 to 0.3**§	−8.5 to 6.4	**0.1 to 4.6***	**2.2 to 9.7****
TA	48	PreTx	6.5 ± 5.8	79.1 ± 16.0	32.6 ± 5.2	113.3 ± 61.8	44.4 ± 6.1	134.7 ± 38.2
		PosTx	4.5 ± 3.9	79.5 ± 16.2	32.8 ± 5.4	115.2 ± 75.0	42.4 ± 6.0	125.4 ± 32.4
	Diff	Low-up 95% CI	**0.7 to 3.3****	−0.95 to 0.1	−0.4 to 0.02	−11.4 to 7.7	**0.4 to 3.7***	**3.8 to 14.7****

AG = Aloe gel; CC = *Cnidoscolous chayamansa* infusion; TA = concentrated 5:1 AG by total process; P = placebo (1, 2 and 3). LOCF used for missing data. Paired t significant values in bold, **§P < 0.1; *P < 0.05; **P < 0.01.** One side Dunnett's TA best lowering vs table order diff (difference) ± SE of hs-CRP **1.7 ± 0.8*, 2.1 ± 0.7**, 1.7 ± 0.8***; and BG mg/dl: **6.0 ± 3.3§, 7.3 ± 3.2***, 3.3 ± 3.4. About BMI, TA was indifferent to P1&CC, but higher than AG&CC and AG&P2 diff (difference) ± SE: 0.25 ± 0.13, **0.43 ± 0.12*, 0.35 ± 0.13*** k/m^2. Lipids had insignificant differences.

Linear regression for period I with post-treatment HbA_{1c} (mmol/mol) as dependent variable, drop outs were counted as failures ($R = 0.829$, adjusted $r^2 = 0.67$, $F = 63.3$, $P < 0.001$). The stepwise selected predictors were: constant, pre-treatment HbA_{1c} level, post-treatment hs-CRP, SE (0 = none, 1 = any) and aloe dose (0 = none, 1 = AG and 2 = TA); beta coefficients (±SD) were 15.8 ± 2.2, 0.58 ± 0.05, 0.36 ± 0.12, 4.0 ± 1.4 and −2.0 ± 0.81, respectively; each $P < 0.05$. Non predictors were CC (0 = none, 1 = 200 or 400 ml of CC infusion), change of BMI kg/m^2, triglycerides and HDL mg/dl. Using LCOF for missing data, adjusted $r^2 = 0.7$, and the side effects stopped being a predictor variable.

4. Discussion

These three double-blind randomized 2-sequence, 2-periods, and 2-treatment crossover assays in women with EMS show a clinical and statistically significant reduction of hyperglycemia and hs-CRP levels with TA (the concentrated 5:1 aloe gel) in each of two treatment periods and it sustained euglycemia in Period III. The tendency for improvement with AG&P2 faded in period II. Doubling the dose generated a bad taste and mild stomach pain and caused patients to drop out; however, 57% managed to tolerate it and using LOCF for drop outs, a small but significant hyperglycemia reduction was seen ($P < 0.05$). On the other hand, most subjects with CC treatments felt an increase in wellbeing and their energy level without significant HbA_{1c} changes ($P > 0.1$). This significantly worsened in period III ($P < 0.05$). It seems that CC counteracts the AG hypoglycemic effect and the intolerance observed in AG without CC. In spite of the fact that AG&CC was the most tolerated treatment (highest rank of sensed wellbeing and energy levels), it failed to reduce hyperglycemia. Indeed, LOCF for dropouts, AG&CC ($n = 73$) was statistically worse than placebo (P1&P2, $n = 49$), mean rank 67.0 vs 53.3, respectively, K–W $\chi^2 = 4.4$, df1, $P < 0.05$.

The HbA_{1c} change was influenced by the prescribed 1500 calorie diet, participant eagerness for herbal remedies, pretreatment extent of hyperglycemic level and treatment effect. The observed change in trend suggests that the first two predominate with CC and placebo treatments, while the last two predominate with TA and AG&P2. Regarding the dose, a comparison of both significant improvements, in period I, TA vs AG&P2 were similar; corresponding to a mean blood glucose change −18.7 ± 25.1 vs −8.6 ± 16.5 mg/dl respectively, $t = 1.5$, $df = 35$, $P > 0.15$; but two gelatins/day were not tolerable because of bad taste and mild stomach pain for AG and did produce a greater reduction in glucose levels for TA. Thus, the greatest reduction in glycemia and the best tolerance was with the 30 mL dose of TA.

Our aloe findings are in agreement with the report by Yongchaiyudha et al. [17] reducing fasting hyperglycemia in new cases of diabetes mellitus. Choudhary et al. [18] also reported a reduction of hyperglycemia and hyperlipidemia after 3 months of intake of aloe gel powder plus diet in male patients with newly diagnosed non-insulin dependent diabetes. Also, Choi et al. [19] reported a lowering of body weight, insulin–resistance, and almost hypoglycemic effects with 600 mg/day of aloeQDM-complex for 8 weeks in obese prediabetic patients. Similar to them, AG&P2 in this study, with shorter treatment periods, showed a reduction of BMI and hyperglycemia, while TA exhibited hs-CRP lowering and twice the improvement of hyperglycemia than AG with an insignificant change in BMI. This agrees with the mechanism proposed by Yagi et al. [20] who reported an anti-hyperglycemic effect after 12 weeks of treatment with aloe, possibly by activation of the immune system. Also, a glycoprotein from aloe (verectin) with anti-oxidative, anti-thromboxane A2 synthase inhibition, and cyclooxigenase-2 inhibiting activities was isolated. In 2008, Hamman [5] described the anti-inflammatory and immunomodulatory effects of acetylated mannan (acemanan). The chromatography of TA used in this study, indicates that it contains 8.77% acemannan, and almost 34% of molecular species (where “verectin”, a glycoprotein of 14 kDa, could be found). Adipocyte cytokine insulin resistance has been linked to hs-CRP pro-inflammatory levels [21,22]. Other Authors [23,24] link impaired insulin sensitivity with oxidative stress, thus a new approach could be medical food. Aloe polysaccharides have shown potential to modulate the oxidative-immflamatory cascade in diabetes [25].

This study favors TA, and to a less extent, AG as anti-hyperglycemic agents. This could strengthen the meta-analysis by Zhang et al. [15] although insignificant changes of the lipid profile are probably due to insufficient treatment duration. In fact, the HDL decline across treatments is probably related to the 1500-calorie diet plan prescribed to all than to CC or aloe.

The improvement of bowel movements reported by our participants could be due to 1) a cathartic effect of gelatin, and 2) in the case of aloe through the intact polysaccharides (mannan) reaching the colon and acting as prebiotics, promoting healthier bacterial flora and ease in bowel movements as has been suggested by Gullón et al. [25] and Suez et al. [26].

This study did not show a reduction of hyperglycemia, lipids or hs-CRP by the gelatins containing an infusion of microwave dehydrated CC leaves (200 or 400 ml), contrary to the observations of Kuti and Torres in an animal model [8]. However, our subjects reporting increased alertness, vivid dreams, and sleep disturbances (both categorized as insomnia in Table 1) that suggest brain stimulating compounds with 200 and 400 ml of the CC infusion; thus it may be unwise to increase the dose. Kuti and Torres provided fresh leaves *ad libitum* [8]. Loarca-Piña et al. [27] reported a hypoglycemic effect in diabetic rats with intra-gastric 70 mg/kg of methanolic CC extract. Also, Ramos-Gomez et al. [28] with a 2% w/v aqueous extract of CC in diabetic rats reported a reduction of hyperglycemia and lipid levels but the extract caused microalbuminuria after long-term intake. The CC infusion with microwaved dehydrated leaves had a color and taste like an infusion with naturally dehydrated CC leaves (the way most people use it), both hide similarly in gelatin; but the microwave damaging CC anti-diabetic effect cannot be disproven.

Some of the limitations of the study were 1) the short treatment period, which did not allow a desirable demonstration of obesity and dyslipidemia reduction; also 3 months are needed to observe a full hemoglobin exchange, since the A_{1c} fraction is otherwise irreversible; 2) all participants were sedentary with no physical activity and no attempt was made to change this; 3) a calorie restriction diet was prescribed for all with this being pivotal to manage metabolic syndrome; to not prescribe it would be unethical, and 4) a longer wash-out period is needed. One week was not enough to return to basal levels. Nonetheless, our findings encourage continuing aloe studies and discarding microwave dehydration of CC leaves.

Keeping in mind the above mention limitations, the results can be safely applicable to other obese subjects with EMS. Here, only women were enrolled but Choudhary et al. [18]. studied only men successfully. Longer observations are needed to reveal a sustained effect in reducing levels of hyperglycemia and hs-CRP.

5. Conclusions

Data propose that 30 ml of TA has an anti-hyperglycemic and CRP reduction effect and is well tolerated as functional food. AG had a less significant anti-hyperglycemic effect but did not diverge from the rest, due to a large dropout rate. The gelatins with microwave dehydrated leaves for CC infusion tended to be worse than placebo for reducing hyperglycemia. Thus, TA concentrated AG could help decrease glucose intolerance by lowering CRP, and warrants in-depth studies to determine if this functional food can keep HbA_{1c} normal to prevent progression to diabetes and to clarify aloe mechanisms in reducing hyperglycemia.

Author specific activities on this work

LC: Conception, plan, coordination, assignment, analyses, interpretation, ms writing.

JZV: Over-all surveillance, resources approval, data interpretation, ms edition

JCL: Literature review, consent informer; sample preservation, database, discussion of results and ms drafting.

AN: Elaboration of sequential envelopes with randomization codes; instrument plan, data collection, discussion of results, sample preservation, ms drafting.

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Conflicts of interest

The authors declare no competing financial interest.

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