

Effect of a Polyphenol-Rich Extract from *Aloe vera* Gel on Experimentally Induced Insulin Resistance in Mice

Yolanda Y. Pérez,^{*,†} Enrique Jiménez-Ferrer,^{*} Alejandro Zamilpa,^{*}
Marcelino Hernández-Valencia,[‡] Francisco J. Alarcón-Aguilar,[§] Jaime Tortoriello^{*}
and Rubén Román-Ramos[§]

^{*}*Centro de Investigación Biomédica del Sur, Instituto Mexicano del Seguro Social
Xochitepec, Morelos, México*

[†]*Programa de Doctorado en Ciencias Biológicas, Universidad Autónoma Metropolitana (UAM)
Mexico City, Mexico*

[‡]*Laboratorio de Investigación en Enfermedades Endocrinológicas
Centro Médico Nacional Siglo XXI (CMN-SXXI), IMSS, Mexico City, Mexico*

[§]*División de Ciencias Biológicas y de la Salud
Universidad Autónoma Metropolitana-Iztapalapa (UAM-I), Mexico City, Mexico*

Abstract: Insulin resistance, which precedes type 2 diabetes mellitus (T2DM), is a widespread pathology associated with the metabolic syndrome, myocardial ischemia, and hypertension. Finding an adequate treatment for this pathology is an important goal in medicine. The purpose of the present research was to investigate the effect of an extract from *Aloe vera* gel containing a high concentration of polyphenols on experimentally induced insulin resistance in mice. A polyphenol-rich *Aloe vera* extract (350 mg/kg) with known concentrations of aloin (181.7 mg/g) and aloe-emodin (3.6 mg/g) was administered orally for a period of 4 weeks to insulin resistant ICR mice. Pioglitazone (50 mg/kg) and bi-distilled water were used as positive and negative controls respectively. Body weight, food intake, and plasma concentrations of insulin and glucose were measured and insulin tolerance tests were performed. The insulin resistance value was calculated using the homeostasis model assessment for insulin resistance (HOMA-IR) formula. Results showed that the polyphenol-rich extract from *Aloe vera* was able to decrease significantly both body weight ($p < 0.008$) and blood glucose levels ($p < 0.005$) and to protect animals against unfavorable results on HOMA-IR, which was observed in the negative control group. The highest glucose levels during the insulin tolerance curve test were in the negative control group when compared to the *Aloe vera* extract and

pioglitazone treated mice ($p < 0.05$). In conclusion, *Aloe vera* gel could be effective for the control of insulin resistance.

Keywords: *Aloe vera* Gel; Aloin; Aloe-Emodin; Insulin Resistance; Medicinal Plants.

Introduction

Insulin resistance (IR), defined as an impaired biological response to the action of insulin (ADA, 1998), is a precursor of type 2 diabetes mellitus (T2DM) with pleiotrophic effects, including several metabolic syndrome features such as dyslipidaemia and the direct promotion of atheroma formation (Jadhav *et al.*, 2004). High IR prevalence increases with age (Marques-Vidal *et al.*, 2002) and is present in approximately 50% of persons with hypercholesterolemia and/or hypertension (Bonora *et al.*, 1998).

IR predicts and proceeds by 10 years T2DM (Warram *et al.*, 1990; Hanley *et al.*, 2005). Reaven (1988) proposed that this pathological condition is the foundation of the metabolic syndrome, the most wide-spread pathology of new millennium (Reaven, 1988; IDF, 2005). In addition, IR is associated with myocardial ischemia (Serné *et al.*, 1999) and polycystic ovary syndrome (PCOS) (Dunaif, 1997) and is a major risk factor for cancer development (Reaven, 2005).

The pharmacological treatment of T2DM manages IR using peroxisome proliferator-activated receptor (PPAR) gamma and alpha agonists. Since it is difficult to quantify insulin resistance in daily practice (IDF, 2005), there are several methods to estimate it, such as the homeostasis model assessment for insulin resistance formula (HOMA-IR) (Matthews *et al.*, 1985). The HOMA model yields an estimate of insulin sensitivity and beta cell function (HOMA- β) from fasting plasma insulin and glucose concentrations (Matthews *et al.*, 1985). Recently, this model has also been used in laboratory animals (Tomie-Furuya *et al.*, 2005).

Aloe vera (L.) Burm. is a perennial plant of the Liliaceae family. The internal part of the leaf, called gel, is colorless, mucilaginous, and has a slightly bitter flavor (WHO, 1999a). It is composed mainly of large polysaccharide chains (Yaron, 1993). It also contains proteins (Winters and Bouthet, 1995) and anthraquinones in low quantities (Vázquez *et al.*, 1996; Reynolds and Dweck, 1999). The yellow-brown, bitter-flavored exudate obtained after cutting the leaves is known as juice, and has a high content of polyphenolic compounds, mainly aloin (15–40%) and aloe-emodin (WHO, 1999b).

Reports suggest that *Aloe vera* has been used for T2DM control since the Middle Ages (Riddle, 2004). At present, there are many reports of its use by Mexican-Americans in the United States of America (Brown *et al.*, 2002), as well as in Middle Eastern and Asian countries such as Saudi Arabia (Riddle, 2004), India (Grover *et al.*, 2002), and Thailand (Yongchaiyudha *et al.*, 1996). There are some investigations confirming the beneficial properties of *Aloe vera* gel and its polyphenolic compounds in the control of T2DM and its complications (Beppu *et al.*, 1993; 2003; 2006; Chintra *et al.*, 1998; Can *et al.*, 2004). Clinical trials in humans have also been conducted (Bunyapraphatsara *et al.*, 1996; Yongchaiyudha *et al.*, 1996), showing promising results with little side-effects.

The objective of this study was to investigate the effect of a polyphenol-enriched *Aloe vera* gel on IR, which is the pathophysiological basis on which T2DM and the metabolic syndrome have their origin (Reaven, 1998). The effect of *Aloe vera* gel was tested on insulin resistant ICR male mice by measurements of body weight, food intake, insulin resistance, fasting glucose, and insulin levels in plasma as well as the insulin tolerance test.

Materials and Methods

Plant Material

Whole fresh leaves of *Aloe vera* were collected from a controlled crop in Xochitepec, Morelos State, Mexico. Plant material was identified and authenticated at the Medicinal Plant Herbarium, Instituto Nacional de Antropología e Historia (INAH-Herbarium), where a voucher specimen was stored for reference under the code number 2029.

Preparation of the Polyphenol-Rich Extract from Aloe vera (PEAv)

Aloe vera leaves were washed and cut from the base, and the gelatinous pulp was extracted with a metallic spoon. The juice containing the polyphenolic compounds was collected after cutting and was added to the pulp and then homogenized, filtered, and evaporated to dryness at 30°C by rotary evaporation. The powder obtained (soluble solids) was kept in 3 ml containers at –20°C until use.

Anthraquinones Quantification

Samples were analyzed using high performance liquid chromatography (HPLC). The HPLC equipment consisted of a Waters 2695 Separations Module System equipped with a Waters 996 PDA UV detector and Empower Chromatography Manager version 1 software (Waters). Analysis was performed with a Merck Chromolith RP-18 column. The mobile phase consisted of linear water/acetonitrile gradient with an initial concentration of 20% acetonitrile that increased to 30% in 3 min; this concentration was maintained for 5 min and then continued to increase to 50% acetonitrile in 2 min, which was followed by a re-equilibration step (to 20% acetonitrile) during 2 min prior to the next injection. The flow rate was maintained at 1 mL/min, the column temperature was maintained at 30°C, and the injection volume was 70 µL. Identification of chromatographic peaks was estimated using a PDA 230–600 nm with a 356 nm detection wavelength. All solvents were HPLC grade (Merck). AloinA of 97% purity by HPLC and aloe-emodin of 95% purity by HPLC were supplied by Sigma Chemical Co. AloinA and aloe emodin showed retention times of 3.91 min and of 6.33 min, respectively.

For the quantitative determination of aloinA and aloe-emodin, commercial anthraquinones aloinA and aloe-emodin were employed as standards. Calibration curves based on the

peak areas of the HPLC chromatograms were constructed using 4-point dilutions of each compound: 125, 250, 500, and 1000 µg/mL in methanol ($R^2 = 0.99$ and 0.98 for aloinA and aloe-emodin, respectively). All compounds were detected at 356 nm, using injection volumes of 70 µL. Experiments were performed by triplicate, and values were expressed in terms of dry weight in grams. *Aloe vera* sample injections (70 µL, 4 mg/ml) displayed the concentrations of aloinA and aloe-emodin present in the sample. The total anthraquinone amount was obtained by addition of aloinA and aloe-emodin quantities.

Animals

Male ICR mice with low birth body weights induced experimentally by intrauterine malnutrition (pregnant females received a 50% restriction in food intake) were used (Hernandez-Valencia *et al.*, 2005). These mice were provided by the CIBIS animal house. Animals were kept in an air-conditioned room with a 12-hour light–12-hour dark cycle, with free access to water and food. To select insulin resistant animals, an insulin tolerance test was performed on fed animals at the age of 6 months. Mice with less than 30% diminished glycemia were considered insulin resistant and were included in the experiment. Handling of the studied mice was conducted in agreement with the statutes of the Institutional Committee for the Care and Use of the Animals (CICUAL) and by the Official Mexican Rule (NOM-062-ZOO-1999, revised in 2001).

Treatments

Twenty five animals were included and randomly assigned to three different treatment groups. Group 1 ($n = 9$), the control, received 5 µl/g of drinking water. Group 2 ($n = 8$), the positive control, received 50 mg/kg of pioglitazone (Zactos, 15 mg tablets, Takeda Chemical Industries, Ltd., Japan, and Eli Lilly and Company, Lilly Corporate Center, USA). Group 3 ($n = 8$), the experimental group, received 350 mg/kg of polyphenol-rich *Aloe vera* gel extract (PEAv). All treatments were administered daily by gastric tube at 9 am for 4 weeks. The extract and pioglitazone were dissolved in drinking water to reach a volume of administration equal to 5 µl/g of body weight.

Physical and Biochemical Analyses

The body weight of animals was measured three times per week, on alternate days, while food intake was measured during 3 continuous days each week. To obtain biochemical analyses, blood samples (200 µL) were obtained from the tail vein, in 12-hour fasted mice, prior to and at the end of treatment. Glucose was determined immediately, and the remaining blood was centrifuged at 3,500 rpm for 7 min; the serum was separated and stored at -20°C until the insulin level determination.

Insulin was measured with the ELISA method specific for mice using Linco Research Lab (USA) reagents. Glucose was quantified by means of the enzymatic glucose-oxidase-

peroxidase method using reactive strips and an Ascencia Elit glucometer (Bayer, Germany). The index of insulin resistance and β -cell function were calculated with the homeostasis model assessment formulas (Matthews *et al.*, 1985); for insulin resistance, $\text{HOMA-IR} = \text{fasting insulin } (\mu\text{U ml}^{-1}) \times \text{fasting glucose (mM)}/22.5$, and for β -cell function, $\text{HOMA-}\beta = (\text{fasting insulin } (\mu\text{U ml}^{-1}) \times 20)/(\text{fasting glucose (mM)}-3.5)$.

After 4 weeks of treatment, an insulin tolerance test (regular insulin 0.75 UI/kg, Regular Humulin, Lilly France, S.A., France) was performed on fed animals. Glucose levels were measured on tail vein blood (20 μl) at 0 min (basal) and at 15, 30, and 60 min after intraperitoneal (i.p.) insulin injection.

Statistical Analysis

All results were expressed as mean \pm standard error of mean (SEM). Differences among the groups were calculated using one-way ANOVA. The Tukey *post-hoc* test was applied to determine the magnitude of these differences. Statistical significance was obtained when p values were < 0.05 . All statistical tests were carried out utilizing the SPSS program for Windows version 11.0.

Results

The content of aloinA and aloe-emodin in PEA_v were 181.5 and 3.6 mg/g, respectively. The total content of anthraquinones was 185.1 mg/g.

As illustrated in Table 1, from the beginning of the treatment, PEA_v was able to reduce body weight. This effect was statistically different ($p = 0.008$) from that produced by pioglitazone alone in the first week. The control group displayed inconsistent changes in body weight. As illustrated in Table 2, food intake was reduced in the PEA_v group, mainly at the fourth treatment week.

Fasting blood glucose before treatment was 5.4 ± 0.1 mM/dL; after 4 weeks of treatment, this value was significantly ($p < 0.005$) increased, reaching 7.3 ± 0.45 mM/dL in the control group. Treatment with pioglitazone did not result in a significant change in this parameter (6.2 ± 0.1 mM/dL), while treatment with the PEA_v extract produced a significant ($p < 0.005$) reduction in glycemia (4.7 ± 0.1 mM/dL) (Table 3). Final insulin levels demonstrated no statistically significant differences.

While insulin resistance increased in the control group from 5.7 ± 0.70 to 7.7 ± 0.78 ($p < 0.05$), the pioglitazone and PEA_v groups did not exhibit changes in this parameter (5.7 ± 0.52 [$p > 0.05$] and 5.5 ± 0.50 [$p > 0.05$], respectively). These results demonstrate the ability of PEA_v to protect mice from the impairment observed in the control group (Table 3). β -cell function calculated with the HOMA- β formula increased significantly ($p = 0.001$) in the PEA_v-treated group when compared with the final data of both the pioglitazone-treated and the control groups (Table 3).

The insulin tolerance test showed that the glucose levels, at times 0, 15, and 30 min, were similar in the PEA_v (127 ± 5.3 , 96 ± 6 , and 87 ± 7.7 mg/dL) and pioglitazone

Table 1. Effect of PEAv (350 mg/kg) and Pioglitazone (50 mg/kg) Treatment on Body Weight (g) of Insulin Resistant Mice

Treatment\Body Weight Differences	Week 1	Week 2	Week 3	Week 4
Control	-1.53 ± 0.58	1.04 ± 0.23	-2.65 ± 0.31	-0.65 ± 0.56
Pioglitazone	-0.09 ± 0.74	-1.62 ± 0.55	-0.78 ± 0.32	-0.92 ± 0.17
PEAv	$-3.09 \pm 0.6^*$	-1 ± 0.34	-1.02 ± 0.25	-0.93 ± 0.28

PEAv: Polyphenol-rich extract from *Aloe vera* gel. Data are means \pm SEM of differences on body weight. * $p = 0.008$ vs. control.

Table 2. Effect of PEAv (350 mg/kg) and Pioglitazone (50 mg/kg) Treatment on Food Intake (g) in Insulin Resistant Mice

Treatment\Food Intake	Week 1	Week 2	Week 3	Week 4
Control	4.31 ± 0.26	4.59 ± 0.22	4.3 ± 0.2	5.07 ± 0.25
Pioglitazone	4.12 ± 0.4	4.13 ± 0.22	3.98 ± 0.17	$4.21 \pm 0.15^*$
PEAv	3.63 ± 0.36	4.05 ± 0.26	3.98 ± 0.24	$3.96 \pm 0.2^*$

PEAv: Polyphenol-rich extract from *Aloe vera* gel. Data represents means \pm SEM. * $p = 0.001$ vs. control.

Table 3. Effect of PEAv (350 mg/kg) and Pioglitazone (50 mg/kg) Treatment on Blood Glucose, Insulin, HOMA-IR and HOMA- β of ICR Insulin Resistant Mice

Treatment	Glucose ^a Media \pm SEM	Insulin Media \pm SEM	HOMA-IR Media \pm SEM	HOMA- β Media \pm SEM
Control	7.3 ± 0.10	23.6 ± 2.2	7.7 ± 0.78	125 ± 10
Pioglitazone	6.2 ± 0.45	21.7 ± 2.9	5.7 ± 0.52	212 ± 63
PEAv	$4.7 \pm 0.10^*$	26.7 ± 2.6	$5.5 \pm 0.50^{**}$	$481 \pm 70^*$

PEAv: Polyphenol-rich extract from *Aloe vera* gel. ^aIn 12-hour fasted mice. * $p \leq 0.005$ vs. pioglitazone and control groups; ** $p < 0.05$ vs. control.

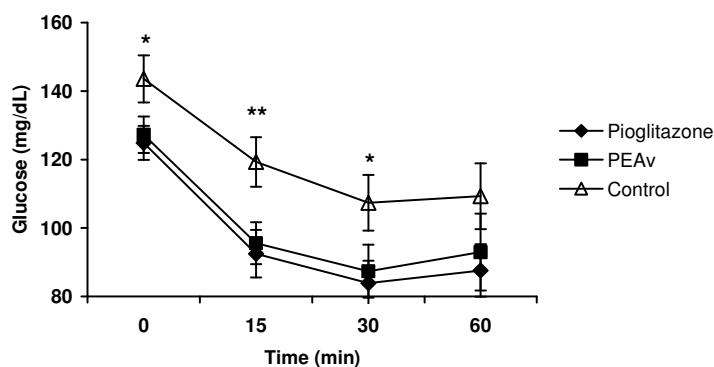


Figure 1. Effect produced by 4 weeks of daily administration of PEAv (350 mg/kg), pioglitazone (50 mg/kg), or drinking water (control) on the insulin tolerance curve (0.75 U/kg, i.p.) in insulin resistant mice. * $p < 0.05$; ** $p < 0.01$. Data are means \pm SEM.

(125 ± 5 , 93 ± 7 , and 84 ± 6.6 mg/dL). However, the control group had higher measurements of glucose (144 ± 6.9 , 119 ± 7.2 , and 107 ± 8.2 mg/dL), with a $p < 0.05$; < 0.01 , and < 0.05 , respectively (Fig. 1).

Discussion

The objective of this research was to investigate the effect of a polyphenol-enriched *Aloe vera* gel extract (PEAv) on insulin resistance. Insulin resistance was produced by the intrauterine malnutrition of ICR mice, which is in agreement with results obtained by Hernández-Valencia *et al.* (2005), where ICR mice with low birth body weight had, among other alterations, insulin resistance.

In the present investigation, the ability of PEA_v to decrease fasting glucose levels was confirmed and was compared to that of pioglitazone. A similarity was found between these two compounds; for example, both pioglitazone and PEA_v protected mice from the metabolic imbalances observed in the control group. However, PEA_v alone was able to raise the HOMA- β value. The evident improvements produced by PEA_v could be explained by a reduction in blood glucose, a slight increment in plasma insulin levels, and a protective effect on pancreatic β -cells. These results are in agreement with other reports regarding polyphenolic compounds from *Aloe vera* (Beppu *et al.*, 1993; 2006).

Aloe gel could be aiding mice with IR by acting as a dietary fiber. The beneficial effect of dietary fiber on body weight and blood glucose levels has been proven previously and was observed in this study. Fiber acts as a delaying agent in food digestion and intestinal absorption (Wolever *et al.*, 2004). Aloin, the glycosylated phenol compound found both in *Aloe arborescens* gel and in high concentrations in PEA_v, reduces glucose absorption in the small intestine (Beppu *et al.*, 2006). In addition, the high molecular weight polysaccharides present in the gel could also produce a fiber-like effect.

The beneficial effect of *Aloe vera* gel on the different manifestations of IR could be related to its anti-inflammatory capacity. It has been established that there is a relationship between a pro-inflammatory state and IR. There is growing evidence that IR in liver, muscle, and adipose tissue is not only associated with pro-inflammatory cytokines, but also is a direct result of this condition. Thus, it is reasonable to suggest that reducing the pro-inflammatory state has beneficial effects on IR.

The antioxidants present in *Aloe vera* gel could also be of benefit in decreasing IR. The beneficial effect on antioxidants on diabetic rats has been demonstrated (Beppu *et al.*, 2003; Can *et al.*, 2004). It has also been suggested that the action of antioxidants is important in medicinal plants used in the control of T2DM (Rajasekaran *et al.*, 2005).

The present investigation is the first to treat experimental IR with *Aloe vera* gel. Nevertheless, because a complete extract was used, it is not possible to define which components of the *Aloe vera* gel are responsible for the effect, since all polysaccharides and polyphenols administered orally can be absorbed. Because an immunomodulatory effect has been verified in nearly all *Aloe vera* gel components, it is possible that several compounds act synergistically through several different mechanisms. However, reports

regarding fractionated *Aloe vera* gel point towards polyphenols, such as aloin and aloe-emodin, as the most powerful agents for diminishing glucose absorption and modifying glucose and insulin levels in diabetic mice (Beppu *et al.*, 2006); in addition, they are also free-radical scavengers (Beppu *et al.*, 2003). Further pharmacological and phytochemical studies are required to uncover the bioactive compounds that protect mice from insulin sensitivity impairment.

In conclusion, the treatment of insulin resistant mice with a polyphenol-enriched *Aloe vera* gel extract (PEAv) diminished their insulin resistance, as is shown by the decrease in blood glucose levels without a significant increment in plasma insulin levels. This herbal extract may be beneficial in combination with dietary measures and medications for the control of T2DM patients with IR.

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