ORIGINAL ARTICLE

HYPOGLYCEMIC EFFECT OF *Moringa oleifera* (MORINGA) COMPARED WITH *Smallanthus sonchifolius* (YACON) ON *Rattus norvegicus* WITH INDUCED DIABETES MELLITUS

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ABSTRACT

Objective: To compare the hypoglycemic effect of the aqueous extract of *Moringa oleifera* (moringa), *Smallanthus sonchifolius* (yacon) and metformin on *Rattus norvegicus*, albino variety, with induced diabetes mellitus. **Materials and methods:** Preclinical, experimental, controlled and randomized study. Diabetes was induced intraperitoneally with a dose of alloxan at 130 mg/kg. A total of 24 male *Rattus norvegicus*, albino variety, Holfzman strain (6 per group) were used. They were divided as follows: control group (no treatment), metformin group (14 mg/kg), *M. oleifera* group (200 mg/kg), and *S. sonchifolius* group (140 mg/kg), treatments were administered via orogastric tube for 15 days. Glycemia levels were determined using an Accu-Chek® Instant electronic glycometer (Roche). **Results:** Decreased glycemia was observed in the treatment groups: *M. oleifera* ($p = 0.009$), *S. sonchifolius* ($p = 0.002$) and metformin ($p = 0.002$), by 313 mg/dL, 281.5 mg/dL and 415 mg/dL, respectively. When comparing glycemia in the treated and control groups, no difference was observed ($P > 0.05$) at 24 hours and four days of treatment; while at the eighth ($P < 0.05$) and fifteenth day ($P < 0.01$) the treated groups had lower glycemia than the control group, but it was similar among them. **Conclusion:** The aqueous extract of *S. sonchifolius*, *M. oleifera*, and metformin presented similar hypoglycemic effect in experimental rats with induced diabetes.

Keywords: Hypoglycemic Agents; Diabetes Mellitus; Moringa oleifera; Rats; Alloxan (source: MeSH NLM).

INTRODUCTION

The World Health Organization states that the number of people with diabetes *mellitus* (DM) worldwide has increased from 108 million in 1980 to 422 million in 2014 and will be the seventh leading cause of death by 2030 [1,2]. Type 2 diabetes *mellitus* (DM 2) is a growing problem, 371 million adults live with this type of diabetes in the world, of which 26 million (7%) reside in Latin America [3]. Peru's National Institute of Statistics and Informatics (INEI) reported that nationally, 3.9% of the population aged 15 years and older was diagnosed with DM in 2019 [4]. On the Peruvian coast, a larger population with diabetes was reported (4%) and a higher incidence in urban areas [5].

DM is a chronic degenerative disease, its most frequent chronic complications are nephropathy, neuropathy, retinopathy, ketoacidosis, and cardiovascular disease [6]. Treatment for DM 2 is multifactorial and personalized, and it's based on nutrition, physical activity, and medications, with 47.8% reported adherence [7]. International guidelines recommend metformin as the first line pharmacological treatment [8].

Medicinal plants (MP) constitute a viable therapeutic alternative due to their low cost and easy availability for many populations. More than 400 MP with great phytochemical diversity are studied for their antidiabetic potential and less or no side effects, so it is important to scientifically

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validate the effectiveness and safety to ensure their use [9-13]. *Moringa oleifera* (moringa) and *Smallanthus sonchifolius* (yacon) are easily accessible and low-cost MP in our environment.

Moringa oleifera is a tree native to the southern Himalayas [14] and part of the *Moringaceae* family [15]. In addition to proteins, minerals and vitamins, the leaves of *M. oleifera* contain phytochemicals, such as flavonoids, phenolic acids, alkaloids and carotenoids, isothiocyanates, glucosinolates and tannins, saponins, oxalates, and phytates [13]. Possible compounds with hypoglycemic and antioxidant effect have been identified in previous phytochemical studies [6], which act by different mechanisms, including inhibition of α-amylase and α-glucosidase activities, increased glucose uptake in muscle and liver, inhibition of glucose uptake from the gut, decreased gluconeogenesis in the liver, and increased insulin secretion and sensitivity [13]. In addition, toxicity studies in experimental animals have shown that aqueous and alcoholic extracts of *M. oleifera* do not have adverse effects [13]. *M. oleifera* protects tissues from oxidative stress [16], reduces free radical activity, lipid peroxidation, and prevents the development of chronic complications [6].

S. sonchifolius belongs to the family Asteraceae, native to the Andean valleys of South America, cultivated at 2,000 - 3,100 m above sea level and reaches maturity between 6 and 12 months after sowing [17]. The phytochemical composition of *S. sonchifolius* has been previously described and has revealed high concentrations of fats and oils, phenols and tannins, alkaloids, lactones, flavonoids and anthocyanidins [12]. Likewise, its high safety has been reported in acute toxicity tests in experimental models, as of its atomized extracts and its alkaloids [12]. *S. sonchilfolius* has hypoglycemic effects, induces insulin release, and increases its concentration in plasma in diabetic and normal rats [18]. This effect has been confirmed mainly by caffeic and chlorogenic acids together with 3 isomeric dicaffeoylquinic acids, which could satisfactorily contribute to an inhibition of α-glucosidase in the brush border of the cells from the small intestine. Decoction of yacon leaves has shown *in vitro* and *in vivo* to prolong glucose absorption time, delaying rapid digestion of sucrose; an isolated component of yacon, lactone sesquiterpene, significantly decreases postprandial glycemia levels in diabetic rats [19].

The hypoglycemic effect of these MP has been demonstrated *in vitro* [12,13,18,19]; however, it is not known which of the two plants has the greater effect in vivo, which would allow considering them as an alternative treatment for DM, after the subsequent and corresponding clinical trials. The aim of this study was to compare the hypoglycemic effect of the aqueous extract of *M. oleifera*, that of *S. sonchifolius*, and metformin in *R. norvegicus* with induced diabetes mellitus.

KEY MESSAGES

Motivation for the study: The increase in the Peruvian population diagnosed with diabetes and the search for new active ingredients with hypoglycemic effect that can be useful for treatment.

Main findings: The aqueous extract of *Smallanthus sonchifolius* (yacon) and *Moringa oleifera* (moringa), and metformin presented similar hypoglycemic effect in experimental rats with alloxan-induced diabetes.

Implications: Moringa and yacon had a similar hypoglycemic effect to metformin in an animal model. Preclinical studies with active principles derived from these plants and subsequent clinical studies are required.

MATERIALS AND METHODS

Study design and experimental animals

A randomized controlled experimental study was conducted with male albino rats (*Rattus norvegicus*, albino variety) that developed alloxan-induced diabetes *mellitus*. All those that did not raise their glucose levels (at least 250 mg/dL) and those that presented some pathology were excluded.

Twenty-four male rats of the Holfzman strain were selected, approximately 12-14 weeks old and weighing 180 ± 20 g. The animals were acquired from the biotherium of the Lambayeque Regional Hospital, where they had 10 days of adaptation with balanced food from the Universidad Nacional Agraria La Molina (UNALM). They received growth diet with the following nutritional value: 10% water, 13-14% proteins, 3-4% lipids, and 0.5% calcium; and according to the manufacturer's brochure, the food was elaborated with the following ingredients: yellow corn, soybean 48% cake, cotton pulp, sunflower cake, agro-industrial by-products, alfalfa hay, calcium carbonate, dicalcium phosphate, synthetic amino acids, organic promoters, vitamins, minerals, sodium chloride, antifungal, and antioxidants.

Procedure

Harvesting and drying of plant materials

The moringa leaves were collected from the Yurimaguas farmhouse, district of Jayanca, province of Lambayeque, approximately two months after planting. Yacon leaves were

collected from the village of Montegrande Bajo, district of Huarmaca, province of Huancabamba, Piura, approximately seven months after planting. The samples were deposited in the Lambayeque PRG Herbarium, of the Botany Department of the Universidad Nacional Pedro Ruiz Gallo (UNPRG) and identified by a qualified specialist.

The moringa and yacon leaves were washed and placed in a hot-air tray dryer for two hours at 56 °C, leaf stems were separated, and only the leaves were crushed using a mortar (each plant separately). The crushed leaves of each plant were stored in separate airtight containers, insulated from light and moisture.

Treatment preparation

Preparation of the aqueous extract of M. oleifera and S. sonchifolius at 100 mg/mL

At the Research Laboratory of the Lambayeque Regional Hospital (HRL), 10 g of moringa and yacon were weighed with an analytical scale; then, in a biosafety cabin, they were mixed with 100 mL of sterile distilled water, placed in 15 mL sterile tubes, and then taken to a bain-marie for 30 minutes at 90 °C. Subsequently, they were centrifuged at 3,500 RPM for ten minutes. In the biosafety cabin, the supernatant was extracted with the help of a 10 mL sterile syringe which constituted the aqueous extract. Aliquots were prepared and stored at 2-8 °C until use over the next four days.

Preparation of metformin at 10 mg/mL

At the Research Laboratory, 1 g of metformin (Sigma, USA) was weighed and diluted in 100 mL of distilled water [20], homogenized, and stored at 2-8 °C until being used, in the next four days.

Diabetes mellitus induction in Rattus norvegicus albino strain Holfzman

Experimental diabetes was induced with a single alloxan dose (Sigma, St. Louis, MI, USA) of 130 mg/kg liveweight (LW) via the intraperitoneal route. Glycemia levels were measured at 72 hours [21] and those with a level greater than 250 mg/dL were considered diabetic [22].

Study group distribution

The diabetic rats were distributed in four groups of six rats each. Each rat was numbered with an indelible marker and randomly assigned with the Epidat 4.1 program. The groups were labeled as follows: control group, which did not receive treatment, and drank water at will; metformin group, which received 14 mg/kg LW, standard treatment of DM 2 [20]; *M. oleifera* group, which received 200 mg/Kg LW of the aqueous extract of *M. oleifera* [16]; and *S. sonchifolius* group, which received 140 mg/Kg LW of the aqueous extract of *S. sonchifolius* [19].

The evaluation was carried out during 15 consecutive days, the duration of the experiment. Glycemia levels were measured at 7 hours. Likewise, the substances with hypoglycemic effect were administered at 8 hours, through a disposable orogastric tube (orogastric route), except for the control group which did not receive treatment.

All experimental animals were given a 14 g ration of balanced rodent food once a day after the corresponding treatment. The day before the glycemia measurement, the food was placed in the cages at 10 a.m. and 7 p.m., then removed for the 12-hour fast with water ad libitum.

Measuring glycemia in rats

The blood glucose concentration was measured with the Accu-Chek Instant Glucose Meter (Roche). During the experiment, blood glucose was monitored on the first, fourth, eighth, and fifteenth post-treatment days. To carry out this procedure, the specimens remained in fasting for approximately 12 hours, the blood sample was collected by puncture in the apex of the tails, after antisepsis of the area with 70% alcohol, discarding the first drop and receiving the next one on the test strip. The values obtained were expressed in milligrams per deciliter (mg/dL).

According to the manufacturer's report, the Accu-Chek® Instant system (meter and strips) is factory-calibrated from diabetics' capillary blood (comparison of methods and accuracy), venous blood (repeatability) and control solution (reproducibility). In addition, at the start and halfway through the evaluation of rats, measurements were made with the control solution administered by the manufacturer, which contained a glucose concentration of 100 mg/dL. The equipment complies with the requirements established by the ISO 15197:2013 and ISO 15197:2015 standards for in vitro diagnostic test systems, requirements of self-diagnostic systems for blood glucose monitoring in the management of DM [23].

Statistical analysis

The data was coded and registered in Microsoft Excel 2016, and processed with IBM SPSS 24 (IBM Corp., Armonk, N.Y., USA). After the analysis of the distribution curve and the result of the Shapiro-Wilks normality test, a descriptive analysis of the glycemia in all treatments and measurements was performed, by calculating medians and interquartile ranges.

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The comparative analysis of the hypoglycemic effect of the extracts of *M. oleifera, S. sonchifolius*, and metformin, was carried out through the non-parametric tests of Kruskall Wallis and Dunn test (multiple comparisons). Likewise, the Wilcoxon non-parametric test was used to measure the reduction of pre-treatment glycemia with respect to the fifteenth day of treatment. A value of $p < 0.05$ was considered significant.

Ethical aspects

Research was conducted in accordance with the following principles: reducing the number of experimental animals, replacing experimental animals with other methods, and refining techniques to lessen suffering. The protocol was reviewed and approved by the Committee on Ethics in Research for Animal Use (CEIPUA), of the HRL. Also, the principles and aspects related to the care and use of laboratory animals detailed in Directive 2010/63/EU of the European Parliament and Council, regarding the protection of animals used for scientific purposes, and the National Law for the Protection of Animals in Captivity [24] were respected. At the end of the experiment the rats were euthanized with sodium pentobarbital at a dose of 100 mg/kg LW.

RESULTS

The data had a non-normal distribution. Table 1 shows medians and interquartile ranges of glycemia of rats with induced diabetes mellitus, distributed according to experimental groups (control and treatments with *M. oleifera, S. sonchifolius*, and metformin).

A glycemia reduction of 415 mg/dL, 313 mg/dL and 281.5 mg/dL was observed in treatments with metformin, *M. oleifera* and *S. sonchifolius*, respectively (Table 2).

Figure 1 shows the trend in the experimental groups regarding glycemia levels, where it can be seen that as time passes, glycemia in the treated groups decreases when compared to the control group, which did not receive treatment.

When comparing glycemia levels in the treated and control groups, it was observed that at 24 hours and at four days of treatment there was no significant difference ($p > 0.05$); while at the eighth and fifteenth day, the treatment groups had lower glycemia with respect to the control one, this difference was statistically significant (Table 3 and 4).

DISCUSSION

The aqueous extracts of *S. sonchifolius* and *M. oleifera* were found to have significant hypoglycemic effect, like metformin. In this regard, previous studies have described that the aqueous extracts of yacon and moringa leaves are frequently used in humans because of multiple health benefits, among which the reduction of postprandial glucose [18-20] stands out.

Likewise, another study found that the decoction of yacon leaves contains phenolic compounds from caffeic, chlorogenic and dicaffeoylquinic acids, such as ferulic acid, p-coumaric acid, protocatechuic acid and quercetin, all of which contribute in the hypoglycemic effect inhibiting α-glucosidase, promoting glucose regulation [19,25]. Its hypoglycemic action is also attributed to the ability of binding to insulin receptors and to enhance the activity of the tyrosine kinase enzyme, whose purpose is to decrease glucose levels, as well as to protect against complications produced by diabetes [18].

The hypoglycemic effect of the aqueous yacon extract observed in our study was similar to the one reported by Dos Santos *et al*., near-normal decreases in glucose levels in rats treated with hydroethanolic extracts were obtained [25]. Likewise, Mejía *et al.* studied the hypoglycemic effect by using whole yacon root, obtaining a lower decrease in glucose levels (27.6 mg/dL in 34 days) [26], compared to our study where we obtained a decrease of 281.5 mg/dL in 15 days.

Table 1. Glycemia in rats with induced diabetes *mellitus*, 15 days post-treatment with 140 mg/kg LW of extract *Smallanthus sonchifolius* (yacon), 200 mg/kg LW of *Moringa oleifera* (moringa) and 14 mg/kg LW of metformin.

Groups (n=6)	Glycemia (mg/dL)				
	Pre Tx Me (IQR)	1 dTx Me (IQR)	4 dTx Me (IQR)	8 dTx Me (IQR)	15 dTx Me (IQR)
Control	439 (315-565)	402 (166-469)	445.5 (402-600)	586.5 (522-600)	389 (330-512)
Metformin	522 (471-600)	331 (191-476)	271 (77-412)	127 (115-251)	107 (99-119)
M. oleifera	417 (341-600)	451 (378-600)	427 (417-457)	246 (170-421)	110 (104-170)
S. sonchifolius	398 (353-471)	268 (249-435)	298 (182-361)	134 (121-346)	117 (101-232)

Tx: Treatment; dTx: days with treatment; Me: median; IQR: interquartile range

Tabla 2. Reducción de la glicemia en ratas con diabetes mellitus inducida y tratadas con extracto de 140 mg/kg PV *Smallanthus sonchifolius* (yacón), 200 mg/kg PV *Moringa oleifera* (moringa) y metformina 14 mg/kg PV

Groups (n=6)	Median		Decrease		Valor de p*
	Pre Tx	15 dTx	mg/dL	%	
Control	439.0	389.0	50.0	11.4	0.981
Metformin	522.0	107.0	415.0	79.5	0.002
M. oleifera	423.0	110.0	313.0	74.0	0.009
S. sonchifolius	398.0	116.5	281.5	70.7	0.002

Tx: Treatment; dTx: Days with treatment
*Wilcoxon test.

Similarly, the group treated with *M. oleifera* significantly decreased glycemia levels compared to the control, this result could have been due to its high content of polyphenols and flavonoid compounds [6,13], glucosinolates and isothiocyanates [27], terpenoids, quercetin and kaempferol [28] found in previous phytochemical studies. The compounds act as insulin secretagogues and contribute to attenuate diabetic complications [16,28] since, they improve the regeneration and viability of destroyed cells; another of their mechanisms is to reduce gluconeogenesis and glycogenolysis in the liver, which is attributed to the chlorogenic acid contained in *M. oleifera* leaves [28].

Our results are congruent with those obtained by previous studies where hyperglycemic experimental rats are treated with *M. oleifera* extract, resulting in a significant decrease in glycemia [6,20,29].

The aqueous extracts of *M. oleifera* and *S. sonchifolius* had similar hypoglycemic effect without significant difference, probably because both plants contain similar components, such as polyphenols, which are attributed with hypoglycemic properties [19,25,27,28], although the mechanism of action has not yet been exactly established, and which seems to be at several levels.

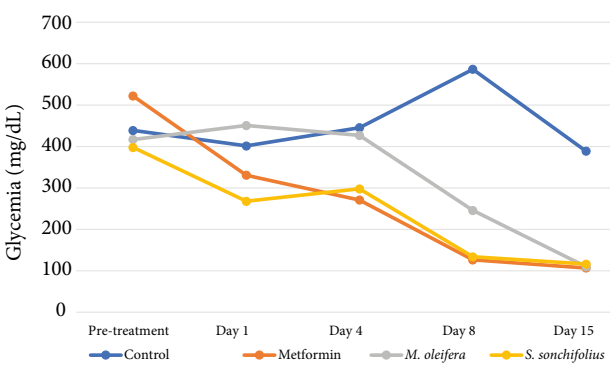

Figure 1. Glycemia in rats with induced diabetes mellitus, treated with 140 mg/kg LW of *S. sonchifolius* (yacon) extract, 200 mg/kg LW of *M. oleifera* (moringa) and 14 mg/kg LW of metformin.

Reduction of pre-treatment glycemia was observed when compared to the fifteenth day of treatment in the positive control (79.5%), treatments with *M. oleifera* (74%) and *S. sonchifolius* (70.7%). Previous studies report that groups treated with *M. oleifera* obtained similar values to the hypoglycemic effect produced by metformin [20,29]. But there are no similar reports about yacon, and no studies were found comparing both MP.

The small sample size was a limitation for this study; however, this is usual in this kind of designs due to ethical and logistic aspects. Despite this, we consider that the results are valid [19,20,29,30]. Another limitation could be the loss of an experimental unit of the moringa group halfway through the experiment. This is a preclinical study that proves hypoglycemic effects in experimental animals, but research in humans that prove these benefits and measure other risks must be carried out. Likewise, for logistical reasons, aspects of the alloxan physiopathology and the way treatments act, as well as the characterization of phytochemicals in the extracts, were not studied.

It is concluded that the aqueous extract of *Smallanthus sonchifolius* at 140 mg/kg LW and of *Moringa oleifera* at 200 mg/kg LW, and metformin at 14 mg/kg LW did not present significant differences in their hypoglycemic effect on

Table 3. Comparison of glycemia at day 8 of treatment with 200 mg/kg LW of *M. oleifera*, 140 mg/kg LW of *S. sonchifolius* and metformin, in rats with induced diabetes.

Group	n	Median	p value*	Comparison**
Control	6	586.5	0.015	A
Metformina	6	126.5		B
M. oleifera	5	246.0		B
S. sonchifolius	6	134.0		B

* Kruskall Wallis' p value.
**Equal letters indicate groups with similar data (Dunn test).

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Table 4. Comparison of glycemia at day 15 of treatment with 200 mg/kg LW of *M. oleifera*, 140 mg/kg LW of *S. sonchifolius* and metformin, in rats with induced diabetes.

Group	n	Median	p value*	Comparison**
Control	6	389.0	0.004	A
Metformin	6	107.0		B
M. oleifera	5	110.0		B
S. sonchifolius	6	116.5		B

* Kruskall Wallis' p value.
**Equal letters indicate groups with similar data (Dunn test).

rats with alloxan-induced diabetes, after 8 and 15 days of treatment. For this reason, it is recommended to carry out complementary safety studies, where toxicity and cytotoxicity are evaluated, *in vitro* and *in vivo*. Then, studies could be carried out to determine if the hypoglycemic effect found in this study is similar in humans. It is also recommended to study interactions and synergies between treatments, as well as the physiopathology of their effects.

Authors' contributions: DMSM, OWVT, JPAT and LZBG conceived and designed the manuscript; HSD analyzed and interpreted the data; DMSM, OWVT and HSD wrote the manuscript. DMSM, OWVT, HSD and LZBG participated in the collection of results. All authors participated in the critical review of the article, approval of the final version and assume responsibility for the contents of the article.

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