International Journal of Pharmacology and Pharmaceutical Research
www.pharmacologyjournals.com
Online ISSN: 2664-7192; Print ISSN: 2664-7184; Impact Factor: RJIF 5.42
Received: 05-05-2020; Accepted: 20-05-2020; Published: 06-06-2020
Volume 2; Issue 1; 2020; Page No. 07-13

Hypoglycemic and renoprotective effect of *Cichorium intybus var*, *Moringa oleifera* and *Olea europaea.*

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Abstract
Diabetes mellitus has a prevalence of 14% in the Mexican Republic, and the population continues to use medicinal plants in their treatment, of which their therapeutic properties must be corroborated. Objective: To study the hypoglycemic and nephroprotective properties of fresh leaves of *Olea europaea*, *Moringa oleifera*, and *Cichorium intybus var*. in a model of diabetes mellitus in rats. Methods: Diabetes mellitus was induced in rats by treatment with streptozotocin (55 mg/kg), intraperitoneally. From the fourth day, ethanolic extracts from fresh leaves of plants (100 and 200 mg/kg, orally) were given to diabetic rats for 2 weeks. Flavonoids, phenolic acids, and terpenoids were determined by HPLC in extracts. Blood glucose, proteinuria and renal hypertrophy were evaluated. Extracts of *O. europaea*, *M. oleifera*, and *C. intybus var* showed hypoglycaemic and renoprotective effects in diabetic rats.

Keywords: mellitus, prevalence, Hypoglycemic, Republic

Introduction

Diabetes mellitus (DM) is an alteration of carbohydrate metabolism characterized by elevated blood glucose levels or hyperglycemia [1], which is associated with damage and dysfunction of various organs including eyes, kidneys, nerves, blood vessels and heart [2]. Furthermore, this disease is a conditioning factor for the development of cardiovascular diseases such as heart attack and stroke [2, 3]. According to the 2018 National Health and Nutrition Survey, the prevalence represents just over 6.4 million people who were known to be affected by the disease in Mexico in 2012. This growing trend is consistent with projections for the prevalence of diagnosed DM, carried out [4], based on data from the referred national surveys; they estimated that, by 2030, this prevalence will reach 12 to 18%, and by 2050, from 14 to 22%. The increase in the prevalence of DM may be due to the aging of the population, to the increase in the prevalence of obesity related to changes in lifestyles (increase in caloric density of the diet, reduction in physical activity), as well as to changes in other factors related to diabetes. Some medications have been developed to control blood glucose levels, including drugs like glibenclamide and metformin [5-7], which are regularly prescribed for diabetic patients. However, these drugs produce side effects in the patient that include intrahepatic cholestasis, skin blisters, tissue anoxia and lactic acidosis, in addition to causing predisposition for the development of kidney and lung diseases [8]. Therefore, it is necessary to evaluate pharmacological alternatives of natural origin for the control of hyperglycemia that do not represent a toxicological risk for the diabetic patient. This last option can be of considerable benefit, especially during the early stages of the disease. In Mexico, the use and knowledge of medicinal plants has been developed since pre-Hispanic cultures and this practice is accentuated for 3 reasons: attention to their diseases, the extensive flora, as well as a wide number of indigenous groups that preserve their own traditions. As an example of plants with antidiabetic properties, there are fresh fruits of aguaymanto (*Physalis peruviana*) [9]; leaves of rue (*Ruta graveolens L.*) [10], and chaya (*Cnidoscolus chayamansa*) [11]. One of the main advantages attributed to naturopathic medical treatments is that they represent a lower toxicological risk for the patient compared to the administration or consumption of synthetic drugs [12], in addition to its nutraceutical contribution due to its content of phenolic compounds with antioxidant properties [13]. On the other hand, one of the traditional ways of preparing plant derivatives in naturist treatments consists of infusions or teas. However, there is limited information about the hypoglycemic and nephroprotective properties of fresh olive leaves (*Olea europaea*), fresh leaves of *Moringa oleífera*, and potential of root (*Cichorium intybus var*) for food and human health has an implication significant as a horticultural crop.

Materials and methods

Preparation and identification of the ethanol extracts of *O. europaea, M. oleifera and Cichorium intybus var*

The leaves of *Olea europaea* and *Moringa oleifera, for Cichorium intybus var* fresh vegetables were collected on March, 2019 at Xochimilco, Mexico, (N19°15″, W99°06′00″) and authenticated by Edith López Villafranco, Biologist. A voucher specimen of *O. europaea* (24813), *M. oleifera* (32415) and *C.*

intybus var (5476) has been deposited at the Herbarium of the Department of Botany, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma of México (UNAM).

For in vivo evaluation, powered leaves of *M. oleifera* y de *O. europaea* (5 kg) and *C. intybus var* fresh vegetable (5 kg) were extracted twice with ethanol (10 L) at room temperature for 14 days and evaporated in vacuo (50 °C). Ethanol extracts of *M. oleifera*, *O. europaea* and *C. intybus var* were stored at 4 °C.

Phytochemical profiling

For chromatographic analysis of ethanol extract was used a high-performance liquid chromatograph Hewlett Packard Mod. 1100, equipped with an automatic injector (Agilent Technologies Mod. 1200), a diode array detector (Hewlett Packard Mod. 1100) and quaternary pump HP Mod. 1100.

Chromatography for the analysis of phenolic acids in ethanol extracts of plants was performed on a nucleosil 100A 125 x 4 mm column, adjusted to 30º, using a linear gradient of 1 mL/min of water (pH 2.5 with trifluoroacetic acid) (Solution A) and acetonitrile (solution B). Initially, (0 to 0.1 min) 85% solution A and 15% solution B, (0.1 to 20 min) 65% solution A and 35% solution B and (20 to 23 min) 65% solution A and 35% solution B; injection volume: 20 ml; the phenolic acids were detected at 280 nm.

For the flavonoids in ethanol extracts of plants, the chromatography was performed on a Hypersil ODS 100A column of 123 x 4.0 mm, adjusted to 30º. The system was operated with gradient elution with solution A: water (pH 2.5) with trifluoroacetic acid and solution B: acetonitrile, with a linear gradient of 1 mL/min. Initially, (0 to 0.1 min) 85% solution A and 15% solution B, (0.1 to 20 min) 65% solution A and 35% solution B and (20 to 25 min) 65% solution A and 35% solution B; injection volume: 20 µL; flavonoids were detected at 254, 316 and 365 nm.

The terpenoid analysis was performed with a ZORBAX Eclipse XDB-C8 column (4 mm×125 mm, 5 µm). The major constituents were separated with gradient mobile phase; and the flow was adjusted to 1 mL/min for 21 min; that consists of water 20% and acetonitrile 80%; the detection wavelength of 215 and 220 nm; 20 ml injection volume [14].

Ethical consideration and animals used

The study was submitted to the Animal Use Ethics Committee of Facultad de Estudios Superiores Iztacala, UNAM. It was approved under Protocol No. CE/FESI/102017/1114). Thirty male Wistar rats were used, each with a weight of around 200–250 g.

During the study, the animals were kept in an air-conditioned environment, with a temperature of 25 ± 3◦C, and a humidity of 50 ± 10%, a photoperiod of 12 h of light.

Animals and Treatment In the present study, we used the well-established streptozotocin (STZ) induced diabetes model. Male Wistar rats were obtained from the rat colony of the Facultad de Estudios Superiores Iztacala. Animals aged 10 weeks, with initial body weight of 250 ± 20 g were studied. Rats had free access to standard rat chow (Rodent Laboratory Chow 5001, Ralston Purina, Richmond Indiana, USA) and tap water, with 12 - 12 h light-dark cycles throughout the experiment. Diabetes mellitus was induced by a single STZ intraperitoneal (ip) injection (55 mg/kg of body weight) in 10 mM sodium citrate buffer, pH 4.5. Control (C) rats received vehicle (10 mM sodium citrate buffer, pH 4.5) alone. Forty-eight hours after STZ injection, blood glucose concentration was determined in tail vein blood samples using a reflectance meter (One Touch; LifeScan, Milpitas, CA, USA). Only animals with blood glucose levels >300 mg/dL were included in the study. Diabetic rats were randomized into three groups: 1) untreated diabetic rats (DM) receiving vehicle (saline solution), 2) diabetic rats treated with glibenclamide (Gli), (5 mg/kg) and 3) diabetic rats treated with ethanolic extracts of *C. intybus var*, *O. europaea* y *M. oleifera* (100 y 200 mg/kg).

Once hyperglycemia with serum glucose levels ≥ 300 mg/dl was confirmed, treatment with glibenclamide or ethanolic extract was given daily during the 2 weeks of the experiment. Each group consisted of five animals. Two days before STZ injection and two days before the end of the experiment, the animals were placed in metabolic cages to measure food and water consumption, urinary volume, and to obtain urine samples to measure proteins. At the end of the study, the rats were anesthetized with sodium pentobarbital (45 mg/kg, ip). Animal care and procedures were performed in compliance with the Mexican Federal Regulations for Animals Investigation and Care (NOM-062 ZOO-1969, Ministry of Agriculture, Mexico), on care and use of laboratory animals, and the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) [15].

Hypertrophy Kidney

Weight/rat body weight ratio was used as a kidney hypertrophy index.

Analytical Methods

A 24 h urine sample was collected placing the animals in metabolic cages. Samples were immediately frozen and stored at −80˚C to measure protein concentration in urine by the Bradford method (Bio-Rad); we used bovine serum albumin (Sigma Chemical Co.) as protein standard [16]. For presence of leukocytes, urobilinogen, bilirubin, pH and ketone bodies were used test strips (Boehringer Mannheim GmbH, Mannheim, Alemania).

Statistical Analysis

The data are the mean ± SEM from 6 rats per treatment. All statistical analyses were performed using (GraphPad Software, La Jolla, California, USA). C, DM, DM + Gli, DM + ethanolic extract of *C. intybus var*, *O. europaea* and *M. oleifera* (100 and 200 mg/kg) groups were tested for effects of diet, treatment, and their interactions by two-factor analysis of variance (ANOVA). When the interaction and/or the main effects were significant, means were compared using Tukey´s multiple comparison post hoc test.

Results and Discussion

There are several models of DM induction in animals, the most reliable method, and therefore commonly used, is intraperitoneal administration of streptozotocin (STZ). So in this study we evaluate the hypoglycaemic activity and its association with the renal damage of ethanol extracts from *C. intybus*, *M. oleifera* and *O. europaea* using this model. Intraperitoneal injection of STZ (55 mg/kg) into adult Wistar rats has been shown to induce DM within the first 2 to 4 days. STZ enters the cells through the

GLUT-2 transporter, preventing glucose from passing into cell resulting in cell damage and a deficit in proinsulin expression generating a state of hyperglycemia [17].

Prior to administration of STZ, all experimental groups had a normo-glycaemic status (114 mg/dL), at 48 h after administration of STZ, showed a significant increase in plasma glucose concentration (hyperglycemia): DM, 403 ± 58 mg/dL compared to the 62 ± 2 mg/dL control rats. Throughout the experiment the glucose concentration in the diabetic rat group was increased in an interval greater than 400 mg/dL of glucose while the control group injected with citrate buffer had no significant changes in blood glucose levels.

Treatment with ethanol extracts of *C. intybus var*, *M. oleifer*a and *O.europaea* showed hypoglycaemic effect at doses of 100 and 200 mg/kg; *C. intybus* reduced plasma glucose concentration by 50% and 62%, for *M. oleifera* it was 77% and 64% and *O. europaea* reduced 50% and 61% (Fig 1).

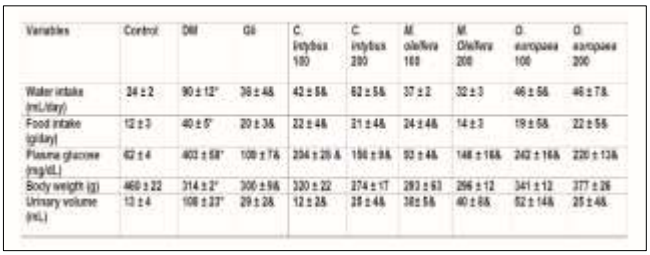

Fig 1: Effect of ethanol extracts treatment on rats. Control, diabetic (DM), DM + glibenclamide (Gli), DM + *Cichorium intybus var* (*C.intibus*), DM + *Moringa oleifera* (*M. oleifera*), DM+ *Olea europaea* (*O. europea*) 100 and 200 mg/kg). n = 6; * p < 0.05 control vs treatment, & p < 0.05 DM rats vs treatment.

The HPLC study of the ethanol extract of *C. intybus var* revealed the presence of 7 phytoconstituents: rutin, myrecitin, quercetin, galangin, gallic acid, caffeic acid and -amyrin (Fig. 1 and Table 2). With regard to *M. oleifera*, rutin, oleanolic acid, ursolic acid, galangin, -amyrin, carnosol and stigmasterol (Fig 2 and Table 3) was observed. For *O. europaea* presence of myrecitin, galangin, quercetin, phloretin and rutin was detected (Fig 3 and Fig 4).

Retention time (min)	Area (mAU*s)	Variables	%
2.67	35.751	Gallic acid	0.088
6.38	12.9	Caffeic acid	0.016
6.5	5.77	α-amyrin	4.3
21.35	17.39	Galangin	0.13
11.8	19.3	Quercetin	0.011
7.5	38.18	Myriecitin	0.094
4.48	59.5	Rutin	0.09

Fig 2: Phytochemicals detected in ethanol extract of *Cichorium intybus var*.

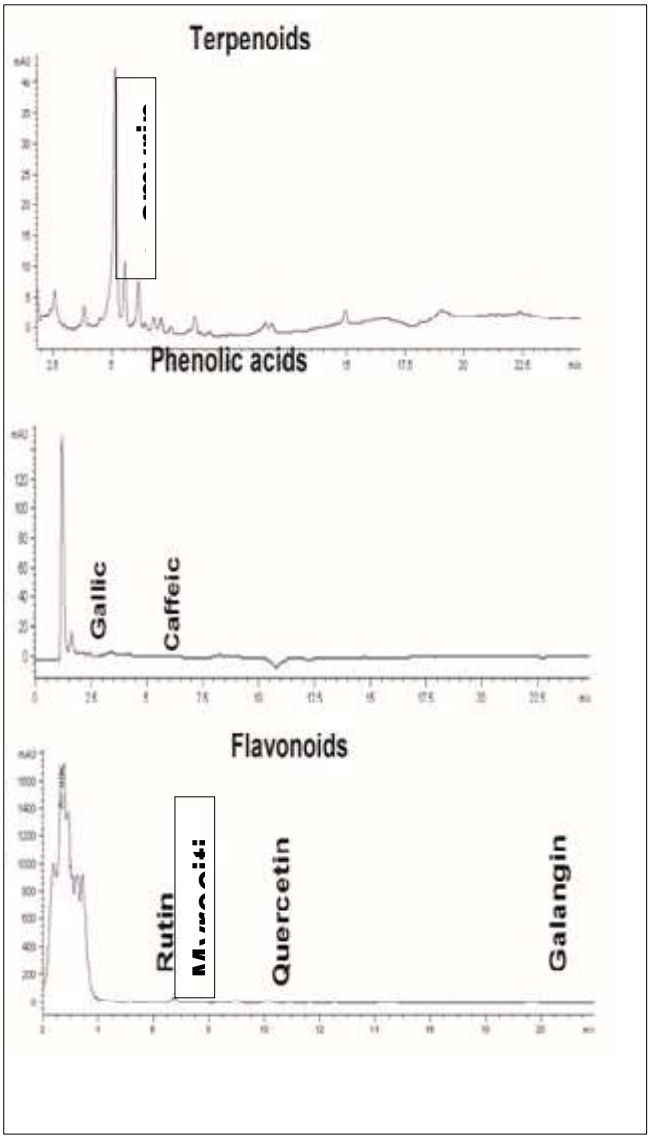

Fig 3: Chromatographic profile of ethanol extract of *Cichorium intybus var*.

Retention time (min)	Area (mAU*s)	Variables	%
2.67	35.751	Gallic acid	0.088
6.31	1973	Caffeic acid	0.03
9.18	9.21	Ferulic acid	0.09
2.28	453.23	Carnosol	1.7
2.8	11.64	Oleanolic acid	11.6
2.67	712.95	Ursolic acid	11.5
6.5	383.92	α-amyrin	5.2
21.68	312.56	Galangin	2.58
11.53	56.64	Quercetin	0.42
7.4	163.59	Myriecitin	0.54
4.7	24.69	Rutin	5.3

Fig 3: Phytochemicals detected in ethanol extract of *Moringa oleifera* leaves.

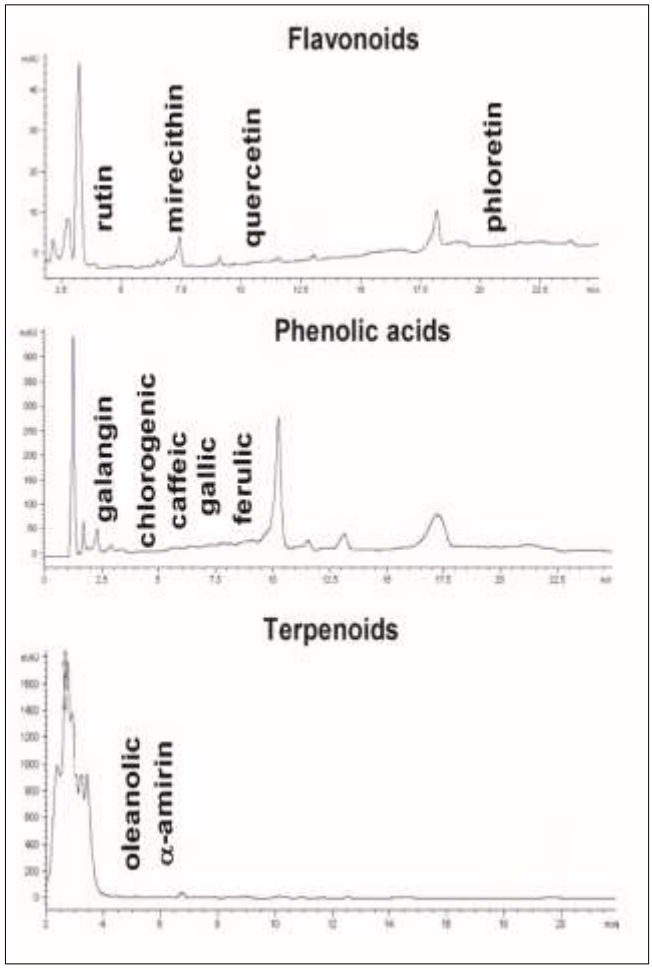

Fig 2: Chromatographic profile of ethanol extract of *Moringa oleifera* leaves.

Retention time (min)	Area (mAU*s)	Variables	%
2.88	146.66	Gallic acid	0.088
6.52	26.565	Caffeic acid	0.042
9.1	74.75	Ferulic acid	0.16
4.37	20.94	Chlorogenic acid	0.18
13.31	424.46	Phloretin	3.3
21.79	1924.32	Galangin	6
11.79	1776.27	Quercetin	5
7.67	2126.76	Myriecitin	13.8
4.506	1256.42	Rutin	2.2

Fig 4: Phytochemicals detected in ethanol extract of *Olea europaea* leaves.

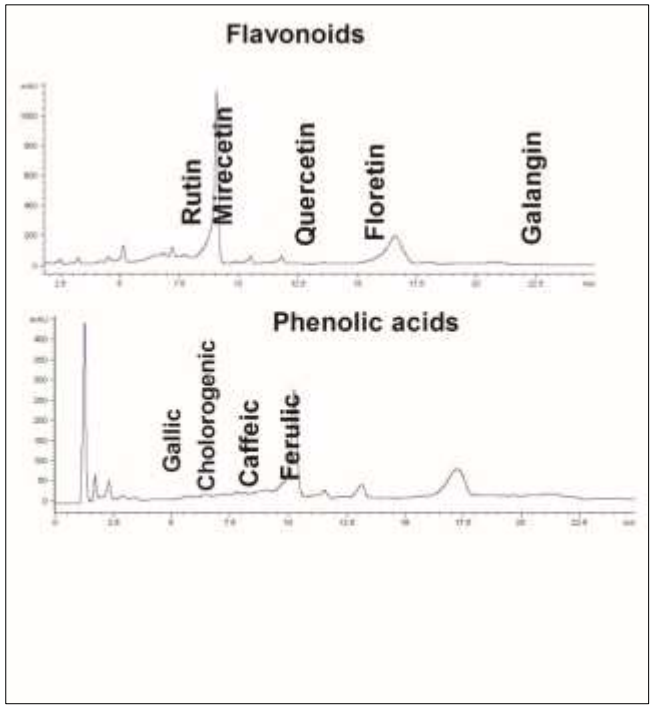

Fig 3: Chromatographic profile of ethanol extract of *Olea europaea* leaves.

The hypoglycemic effect possibly due to the rutin presence, some studies have shown that subacute administration of this flavonoid improves glucose tolerance, glycosylated hemoglobin and increases serum insulin and peptide C concentration [18, 19].

In *C. intybus* and *O. europaea* presence of myricetin and quercetin was found, with insulinomimetic effect on muscle tissue and induces the expression of the receptor for insulin and Akt [20, 21].

With regard to water intake and urinary volume measured at 24 h in the control group it was 24 ± 2 mL and 13 ± 4 mL, both parameters increased in the DM group (90 ± 12 mL and 108 ± 20 mL). In the group treated with glibenclamide (5 mg/kg), water intake and urinary volume were reduced (36 ± 4 mL and 29 ± 2 mL, respectively) (Table 1). With regard to food intake in the DM group was greater compared to the control (12 ± 3 g and 40 ± 5 g), in this group weight loss was also observed (Table 1); this effect is due to the lack of glucose in skeletal muscle cells and adipocytes, so to meet the energy demand, gluconeogenesis is induced by hydrolysis of proteins from muscle tissue and through the degradation of triglycerides from adipose tissue [22]. With regard to the different treatments, *C. intybus var*, *M. oleifera* and *O. europaea* reduced 50% or more, the water and food intake, in both doses (Table 1).

Polyuria is caused by excess glucose filtered by the kidneys that is not reabsorbed by the renal tubules, therefore it is excreted in the urine. Non-reabsorbed glucose generates water retention by osmosis in the proximal contoured tubule, eliminating a higher volume of water and electrolytes. The results showed that DM rats excreted a higher volume of urine compared to normoglycemic ones; while polydipsia is a compensatory mechanism that is generated against the volume of liquid lost as a result of the increase in water excretion [23], so in the presence of the different treatments a significant decrease in polyuria and polydipsia was observed, suggesting a possible renoprotective effect. One of the compounds found is quercetin administered for 4 weeks to DM rats reduces polyuria and polydipsia, improves renal damage through interacting directly with superoxide anion (O_2-) forming hydroxyl radicals through the quelation of iron ions, and by reacting directly with the lipid catchers of butoxy radical during lipid peroxidation [24, 25].

Several studies have shown that in early stages of the development of diabetic nephropathy there is an increase in the weight of the kidneys, as well as the area of proximal tubular cells; structural alteration is known as renal hypertrophy [26]. In this study, renal hypertrophy was determined through the renal weight/total body weight ratio and was associated with the presence of proteins in the urine, these are two indicators that allow us to infer the renal damage that is suffering from the glomerular filtration barrier due to the persistent hyperglycemic state, since this barrier prevents the passage of macromolecules such as plasma proteins, by their size, their shape and their negative electrical load [27].

The kidney weight of the rat group with DM increased in relation to the control group, (4.7 ± 0.44 vs 2.8 ± 0.04 mg/g) and was associated with a significant increase in urinary protein excretion (95 ± 5 vs 30 ± 4 mg/24 h) (Fig.4).

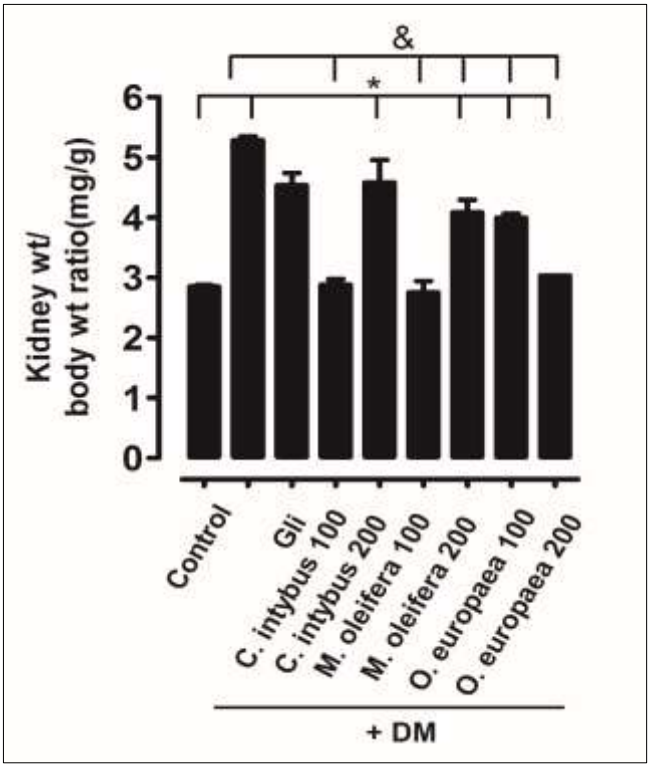

Fig 4: Effect of ethanol extracts of *Cichorium intybus var*, *Moringa oleifera* and *Olea eurpaea* on kidney weight/body weigth ratio (g/100g). All values are represented as mean ± SEM. n = 6; *p < 0.05 control vs treatment; & p < 0.05 DM vs. treatment. Diabetic (DM), Glibenclamide (Gli), ethanol extracts of *C. intybus var*, *M. oleifera* and *O. europaea* (100 and 200 mg/kg).

Kidney weight/body weigth ratio for groups treated with *C. intybus var* was (2.8 ± 0.08 mg/g), *M. oleifera* (2.7 ±0.19 mg/g) and *O. europaea* (3.9 ± 0.05 mg/g) with the dose of 100 mg/kg, so a preventive effect on renal hypertrophy induced by hyperglycemia was observed (Fig. 4), at both doses 100 and 200 mg/kg. Urinary protein excretion decreased, suggesting a renoprotective effect (Fig. 5). This effect may be due to the presence of rutin in ethanol extracts, which reduces the presence of tissue inhibitors of metalloproteinases and increases the activity of the metalloproteinases of the matrix, thus maintaining a balance between synthesis and degradation of the components of the extracellular matrix, thus reducing proteinuria [28].

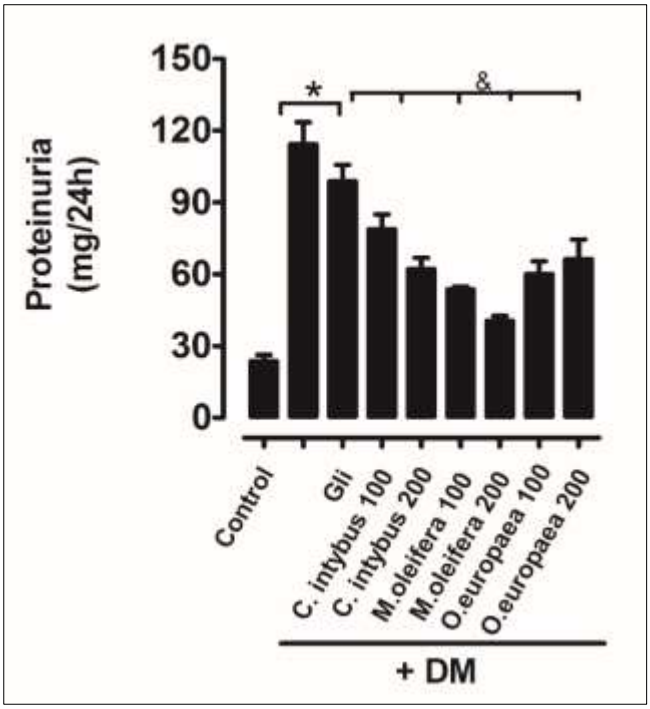

Fig 5: Effect of ethanol extracts of *Cichorium intybus var*, *Moringa oleifera* and *Olea europaea* on proteinuria (mg/ 24h). All values are represented as mean ± SEM. n = 6; *p < 0.05 control vs treatment; & p < 0.05 DM vs. treatment. Diabetic (DM), Glibenclamide (Gli), ethanol extracts of *C. intybus*, *M. oleifera* and *O. europaea* (100 and 200 mg/kg).

The results obtained in uroanalysis in presence of different treatments after two weeks of treatment, showed for the DM group: presence of ketone bodies, pH (7.3), urobilinogen (6.8), positive bilirubin and leukocytes. In the presence of the different treatments *C. intybus var*, *M. oleifera* and *O. europaea* were observed at the dose of 100 and 200 mg/kg was not observed the presence of ketones, pH was found in a range of 7.0-7.5, urobilinogen (0.2- 3.0), negative bilirubin and leukocytes.

The presence of traces of white blood cells in the DM group is a clear sign of renal and urinary tract inflammation. Urinary tract infections are linked to obstructive problems and alterations in

urinary tract function [29]. DM patients are twice as likely to develop complicated urinary tract infections compared to those who do not; acute pyelonephritis is 5 times more common in DM; in 60% of patients hospitalized with bacteremia and DM the source of infection is the urinary tract [30].

Conclusion

In the groups of diabetic animals to which different treatments were administered, we found a decrease in hyperglycemia significantly compared to diabetic control group. We observed that at 2 weeks of treatment, group that was administered ethanol extract of *C. intybus var* and *O. europaea* highest percentage reduction in hyperglycemia (50%). In phytochemical characterization presence of flavonoids myricetin, quercetin, galangin, phloretin and rutin. For terpenoids, □-amyrin, oleanolic acid, carnosol and ursolic acid was found. In the DM group, he had leukocytes in urine and proteinuria, suggesting the presence of a possible infection, as well as probable damage to glomerular function through the relationship between kidney weight / total body weight as an indicator of renal hypertrophy and its association with proteinuria. The ethanol extracts of *C. intybus var*, *M. oleífera* and *O. europaea* showed hypoglycemic and renoprotective effects.

References

1. Roden M. Diabetes mellitus: definition, classification and diagnosis. Wien Klin Wochenschr. 2016; 128(2):S37-40.
2. Sarwar N, Gao P, Seshasai SR, Gobin R, Kaptoge S, Di Angelantonio. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. Emerging Risk Factors Collaboration. Lancet. 2010; 26(375):2215-2222.
3. DeFronzo RA. Pathogenesis of type 2 diabetes mellitus. Med Clin North Am. 2004; 88(4):787-ix.
4. Meza R, Barrientos-Gutiérrez T, Rojas-Martinez R, Reynoso-Noverón N, Palacio-Mejia LS, Lazcano-Ponce E, *et al*. Burden of type 2 diabetes in Mexico: Past, current and future prevalence and incidence rates. Prev Med. 2015; 81:445-450.
5. Olmedo V, Olmedo-Canchola H, Enters-Altés J, Del Ángel-Cruz RE, Rosas-Heredia ML. Ensayo clínico para evaluar la efectividad de una combinación fija de glimepirida y metformina en el tratamiento de pacientes con diabetes mellitus tipo 2. Medicina Interna de México, 2013, 159-163.
6. Pavlovic M, Carvajal CJ. Hipoglicemiantes orales para el tratamiento de la diabetes mellitus gestacional. Rev. Chilena de Obstetricia y Ginecología. 2013; 78(3):167-178.
7. Carvajal SC, Miesfeld N, Chang J. Evidence for long-term impact of Pasos Adelante: using a community-wide survey to evaluate chronic disease risk modification in prior program participants. Int J Environ Res Public Health. 2013; 10(10):4701-4717.
8. Rasouli H, Yarani R, Pociot F, Popović-Djordjević J. Anti-diabetic potential of plant alkaloids: Revisiting current findings and future perspectives. Pharmacol Res, 2020; 24:155-104723.
9. Rodríguez-Fragoso L, Reyes-Esparza L, Burchiel S, Herrera-Ruíz-D, Torres E. Risks and benefits of commonly used herbal medicines in México. Toxicol Appl Pharmacol, 2008; 227:125-135
10. Figueroa-Valverde L, Díaz-Cedillo F, Camacho-Luis A, López-Ramos M. Efeitos induzidos por Ruta graveolens L., Rutaceae, Cnidoscolus chayamansa McVaugh, Euphorbiaceae, e Citrus aurantium L., Rutaceae, nos níveis de glucose, colesterol e triacilglicerídeos num modelo do rato diabético. Braz J Pharmacogn. 2009; 19(4):898-907.
11. Valenzuela Soto R, Morales Rubio ME, Verde Star MJ, Oranda A y Cárdenas, Preciado-Rangel P, González JA y Juan Ramón E. Cnidoscolus chayamansa hidropónica orgánica y su capacidad hipoglucemiante, calidad nutraceutica y toxicidad. Rev. Mex. Cienc. Agríc. 2015; 6(4) Texcoco may./jun.
12. Marín-Peñalver JJ, Martín-Timón I, Sevillano-Collantes C, del Cañizo-Gómez FJ. Update on the treatment of type 2 diabetes mellitus. World J Diabetes. 2016; 7(17):354-395
13. Loarca-Piña G, Mendoza S, Ramos-Gómez M, Reynoso R. Antioxidant, antimutagenic, and antidiabetic activities of edible leaves from Cnidoscolus chayamansa Mc. Vaugh. J Food Sci. 2010; 75(2):68-72.
14. Maldini M, Montoro P, Piacente S and Pizza C. Phenolic compounds from Bursera simaruba Sarg. bark: phytochemical investigation and quantitative analysis by tandem mass spectrometry, Phytochemistry. 2009; 70(5):641-9.
15. Malik S, Suchal K, Khan SI. Apigenin ameliorates streptozotocin-induced diabetic nephropathy in rats via MAPK-NF-κB-TNF-α and TGF-β1-MAPK-fibronectin pathways. Am J Physiol Renal Physiol. 2017; 313(2):F414-F422.
16. Ku HK, Lim HM, Oh KH, Yang HJ, Jeong JS, Kim SK. Interpretation of protein quantitation using the Bradford assay: comparison with two calculation models. Anal Biochem. 2013; 434(1):178-180.
17. Choudhari VP, Gore KP, Pawar AT. Antidiabetic, antihyperlipidemic activities and herb-drug interaction of a polyherbal formulation in streptozotocin induced diabetic rats. J Ayurveda Integr Med. 2017; 8(4):218-225.
18. Rauter AP, Martins A, Borges C, Mota-Filipe H, Pinto R, Sepodes B, *et al*. Antihyperglycaemic and protective effects of flavonoids on streptozotocin-induced diabetic rats. Phytother Res. 2010; 24(2):S133-8.
19. Kamalakkannan N, Prince PS. Antihyperglycaemic and antioxidant effect of rutin, a polyphenolic flavonoid, in streptozotocin-induced diabetic wistar rats. Basic Clin Pharmacol Toxicol. 2006; 98(1):97-103
20. Peng J, Li Q, Li K. Quercetin improves glucose and lipid metabolism of diabetic rats: involvement of Akt signaling and SIRT1. Journal of Diabetes Research, 2017, 10.
21. Hanhineva K, Törrönen R, Bondia-Pons I. Impact of dietary polyphenols on carbohydrate metabolism. International Journal of Molecular Sciences. 2010; 11(4):1365-1402.
22. Zari TA, Al-Thebaiti MA. Effects of Caralluma russeliana stem extract on some physiological parameters in streptozotocin-induced diabetic male rats. Diabetes. Metab Syndr Obes. 2018; 12(11):619-631.
23. Siddiqui S, Khan MR, Siddiqui WA. Comparative hypoglycemic and nephroprotective effects of tocotrienol rich fraction (TRF) from palm oil and rice bran oil against hyperglycemia induced nephropathy in type 1 diabetic rats. Chem-Biol Int. 2012; 188:651-8.

24. Gomes IB, Porto ML, Santos MC, Campagnaro BP, Pereira TM, Meyrelles SS, *et al*. Renoprotective, anti-oxidative and anti-apoptotic effects of oral low-dose quercetin in the C57BL/6J model of diabetic nephropathy. Lipids Health Dis. 2014; 6:13-184.
25. Pereira Braga C, Momentti AC, Barbosa Peixoto F, de Fátima Ferreira Baptista R, dos Santos FA, Fava FH, *et al*. Influence of treatment with quercetin on lipid parameters and oxidative stress of pregnant diabetic rats. Can J Physiol Pharmacol. 2013; 91(2):171-177.
26. Meza Letelier CE, San Martín Ojeda CA, Ruiz Provoste JJ, Frugone Zaror CJ. Pathophysiology of diabetic nephropathy: a literature review. Medwave. 2017; 17(1):e6839.
27. Dai H, Liu Q, Liu B. Research Progress on Mechanism of Podocyte Depletion in Diabetic Nephropathy. J Diabetes Res, 2017, 2615286.
28. Ghorbani A. Mechanisms of antidiabetic effects of flavonoid rutin. Biomed Pharmacother, 2017; 96:305-312.
29. Morrison K.; Laboratorio Clínico y Pruebas de Diagnóstico, 1ª. Edición, 97-106. El Manual Moderno, México, 1998.
30. Flores E, Parra I, Jiménez A, Fernández G. Pruebas presuntivas del análisis de orina en el diagnóstico de infección en vías urinarias entre diabéticos tipo 2. Salud Pública Méx, 2005; 47:376-380.