EFFECT OF ADMINISTRATION OF *GYMNEMA SYLVESTRE* ON GLYCEMIC CONTROL, INSULIN SECRETION AND INSULIN SENSITIVITY IN PATIENTS WITH IMPAIRED GLUCOSE TOLERANCE

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1. INTRODUCTION

Impaired glucose tolerance transition state to type 2 diabetes mellitus (T2DM). IGT is related to insulin resistance in the liver and normal insulin sensitivity in the muscle, in addition to a severe decrease in the secretion of insulin (1,2).

Several studies have linked IGT with a significant increase in the risk of developing T2DM, as well as in the prevalence of cardiovascular diseases compared with normoglycemic individuals (3).

One of the most recommended medications to treat IGT is metformin; however, new strategies are needed to improve insulin resistance and limit the secretory demand of insulin in beta cells to stop or postpone the conversion of prediabetes to T2DM (4).

Complementary and alternative medicine has attracted attention in the treatment of diabetes with several therapeutic agents that modulate glucose levels because they are relatively accessible and many of them have been used for decades or centuries without showing serious side effects. It is estimated that *35%–48% of the world's population use this type of treatment (5).

Gymnema sylvestre is an ancient medicinal plant belonging to the Asclepiadaceae family, and it is used in Ayurvedic medicine as well as in complementary and alternative medicine. Its leaves exhibit a wide range of therapeutic effects due to gymnemic acids (main compounds). Furthermore, G. sylvestre is widely used as a naturopathic treatment for diabetes, and it has also demonstrated other important uses, such as hypolipidemic, antiviral, diuretic, antiallergic, antibiotic, and as a weight loss supplement (6,7). However, these findings have not been studied in patients with IGT; although it has been used in diabetes, its effect on insulin is not clear, either through improving insulin sensitivity or insulin secretion. Therefore, due to the mechanisms attributed to improve glucose metabolism, the main objective of this study will be to evaluate the effect of G. sylvestre administration on glycemic control, insulin secretion, and insulin sensitivity in patients with IGT.

2. BACKGROUND

Gymnema sylvestre and glucose alterations

In relation to IGT, a OGTT was performed in obese mice, those given 500 mg/kg *gymnema sylvestre,*30 minutes before intraperitoneal glucose administration, a significant decrease in baseline glucose concentration,, as well as minutes 90 and 120 with a $p<0.05$ compared to the group of obese mice treated with vehicle were observed in the results (8).

Shridhar and Cols. (9) administered 50 or 100 mg/kg of *Gymnema sylvestre* extract (75% purity) in Wistar rats over a 45-day period, in which diabetes mellitus was previously induced with streptozotocin administration. Determinations were made on days 3, 15, 30 and 45. They found a significant decrease in serum glucose levels in both groups (50 and 100 mg/kg) on day 45 compared to day 3 (401.83 ± 6.14 *vs* 216.50 ± 2.06 mg/dL, $p<0.05$ for the 50 mg/kg group) and (399.50 ± 6.09 *vs* 194.50 ± 2.83 mg/dL, $p<0.05$ for the 100 mg/kg group).

Li and Cols. (10) conducted a study in 8 patients to evaluate the control of T2DM with the use of *Gymnema sylvestre* at a dose of 1 g/day divided into two doses for 30 days. Blood samples were collected to determine fasting glucose levels on days 0, 10, 20, 30 and 40. Serum glucose figures for day 0 were 219 ± 41 mg/dL of fasting glucose *vs.* 138 ± 17 mg/dL on day 30, $p<0.05$. After the intervention, on day 40, an increase in serum levels offasting glucose was observed,181 ± 38 mg/dL.

Al-Romayian and Cols. (11) 500 mg of *Gymnema sylvestre* was administered orally twice daily before food in 11 patients (7 women and 4 men) with T2DM for 60 days. Clinical and laboratory determinations were made before the intervention (day 0) and at the end (day 60). They found a significant decrease in fasting glucose (162 ± 23 *vs.* 119 ± 17 mg/dL, $p<0.05$) and postprandial glucose (291 ± 10 *vs* 236 ± 30 mg/dL, $p<0.02$). Improvement in glycemic control after administration of *Gymnema sylvestre* was associated with increased circulating insulin levels (24 ± 9 *vs.* 32 ± 6 U/mL, $p<0.001$) and the corresponding increase in C peptide (298 ± 42 *vs* 447 ± 48 pmol/L, $p<0.05$) in patients.

On the other hand, Shanmugasundaram and Cols. (12) conducted a study in 27 patients with type 1 diabetes mellitus (DM1); 23 (9 women and 14 men) between 10 and 31 years of age and 4 patients (1 female and 3 men) between 44 and 50 years of age with pre-study insulin monotherapy of 2 to 3 months. They were given 400 mg/day of *Gymnema sylvestre* over a period of 30 months as a complementary treatment for insulin therapy. Five evaluations were carried out during the intervention. All patients were free of kidney disease, heart disease or CVD. During the intervention, the patient was trained to monitor their capillary glucose and recognize symptoms of hypoglycaemia to adjust the insulin dose. They found significant decrease after the intervention of *Gymnema sylvestre* in TG (134.4 ± 4 *vs* 107 ± 6 mg/dL, $p<0.01$), FFA 84 ± 2.5 mg/dL, $p<0.01$), TC (206 ± 14 *vs* 176 ± 5 mg/dL, $p<0.01$) in addition *Gymnema sylvestre* increased concentrations of peptide C, compared to the insulin monotherapy group (0.105 ± 0.005 *vs.* 0.185 ± 0.003 pmol/mL, $p<0.01$).

The effects of *Gymnema sylvestre* on insulin pattern and rate of secretion were studied by al-Romaiyan and Cols. (11) used human islets *in vitro.* Infusion of islets with a buffer supplemented with 0.125 mg/dL of *Gymnema sylvestre* with a stimulating concentration of 2 mM glucose and an increase of approximately 2 times in insulin secretion (217%) baseline insulin concentration.

In another study, Bhansali and Cols (13), administrated gymnemic acids to 3 groups of 6 rats at doses of 50, 100 and 200 mg/kg orally for 20 days; previously, the rats were fed a high fructose diet (HFD) for 20 days. They found a decrease in estimated insulin resistance using the homeostatic insulin resistance assessment (HOMA-IR) at the end of the study (40 days) compared to data obtained at the end of the period of the administration of HFD (20 days) for groups receiving 100mg/kg (2.7 vs 1.7) and 200mg/kg (2.6 vs 1.3).

3. HYPOTHESIS

Administration of *Gymnema sylvestre* modifies glycemic control and/or insulin secretion and/or insulin sensitivity in patients with impaired glucose tolerance.

4. OBJECTIVES

Overall objective

Evaluate the effect of Administration of *Gymnema sylvestre* on glycemic control, insulin secretion and insulin sensitivity in IGT patients.

Specific objectives

- Determine glycemic control, using FG, 2h-PG and A1C, before and after placebo or *Gymnema sylvestre* administration.
- Calculate the first phase of insulin secretion, using the Stumvoll index, before and after placebo or *Gymnema sylvestre* administration.
- Calculate total insulin secretion, using the Insulinogenic index, before and after placebo or *Gymnema sylvestre administration.*
- Calculate insulin sensitivity, using Matsuda index, before and after placebo or *Gymnema sylvestre administration.*

Secondary goals

Measure the effect of placebo or *Gymnema sylvestre* administration on:

- Body weight.
- Waist circumference (WC).
- Body Mass Index (BMI).
- Blood pressure.
- Lipid profile: TG, HDL-C, TC, low-density lipoprotein cholesterol (LDL-C) and very low-density lipoproteins (VLDL).

Report adverse events and tolerability to *Gymnema Sylvestre.*

5. METHODOLOGY

A randomized, double-blind, placebo-controlled clinical trial will be performed in 30 patients of both genders between 30 and 59 years old, with a diagnosis of IGT according to the American Diabetes Association (8) (defined as a 2-h postload plasma glucose [2-h PG] concentration between 7.8 and 11.1 mmol/L). In both study groups, subjects will be considered sedentary (they usually perform light or moderate physical activity as much as 15 min a day and with less than three sessions per week during the last 3 months), non-smokers, and with a body mass index (BMI) ranging between 25 and 39.9 kg/m2 and a stable body weight (BWt), considering up to 5% changes in BWt for at least 3 months before the study.

None of the included patients had used any pharmacological treatment, medicinal herbs, or supplements with an effect on glycemic control for at least 3 months before enrollment in the study. Exclusion criteria: prior T2DM diagnosis; hypertension; renal, heart, thyroid, or hepatic disease; and women who were pregnant or breastfeeding.

Enrolled subjects will take assessments at baseline and at the end of the study (12 weeks). Weight and height will be measured with the subject standing barefoot and with the head aligned in the Frankfort horizontal plane with an electric bioimpedance scale (Model TBF-300 A; Tanita Corporation of America Inc., Arlington Heights, IL). BMI will be calculated as BWt (kg) divided by the square of body height (m^2). Waist circumference will be measured using a flexible tape at the midline between the lowest rib and the highest point of the iliac crest in the midaxillary line at the end of a normal expiration. All anthropometric measurements will be performed with the individuals wearing light clothing without shoes and after evacuation of the bladder.

Blood pressure will be evaluated after a 15-min resting period with the individual sitting using a digital sphygmomanometer (OMRON model HEM-7130), and a bracelet was adjusted 3 cm above the fold of the elbow of the left arm. The mean of three systolic and diastolic blood pressure measurements will be considered.

Blood samples will be collected from an antecubital vein after insertion of a catheter to determine fasting plasma glucose, glycated hemoglobin A1c (A1C), triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and fasting insulin concentrations. Subsequently, a 2-h oral glucose tolerance test (2-h OGTT) by consuming 75 g of a dextrose load will be performed, and two blood samples will be obtained at 30, 60, 90, and 120 min after glucose administration. The blood will be centrifuged, and the first sample will be used to determine plasma glucose immediately, and the second sample will be frozen at -20°C for insulin determinations within the next 30 days.

Before clinical and laboratory evaluations, all subjects will be instructed to maintain their usual physical activity, which consists of light or moderate physical activity as much as 15 min a day and with less than three sessions per week during the last 3 months. To ensure proper insulin secretion, patients will receive an isocaloric diet 3 days before the 2-h OGTT, containing a minimum of 250 g of carbohydrates per day. All females will be tested during the first phase of their menstrual cycle (days 3–8).

Glucose, TG, TC, and HDL-C levels will be measured by colorimetric methods using an automated analyzer (Erba XL 100®), with an intra- and inter-assay coefficients of variation (CV) of <1% and 2%, respectively. The A1C percentage will be measured using ion-exchange high-performance liquid chromatography (Bio-Rad Laboratories, Hercules, CA), with an intra- and inter-assay CV of 0.4% and 1.6%, respectively.

Insulin concentrations will be measured using a chemiluminescent immunoassay technique (DRG International, Inc.,), with an intra- and inter-assay CV of 2.6% and 2.88%, respectively. The areas under the curve (AUC) of glucose and insulin will be obtained using the trapezoidal integration (14).

Total insulin secretion will be calculated with the insulinogenic index [Δ AUC insulin/(Δ AUC glucose)],(15) the first phase insulin secretion using the Stumvoll index (1283 + 1.829 · insulin 300 - 138.7 · glucose 300 + 3.772 · insulin 00),(16) and insulin sensitivity with the Matsuda index [10,000/square root of (glucose 00 · insulin 00) · (mean glucose · mean insulin during 2-h OGTT)] (17). Low-density lipoprotein cholesterol (LDL-C) levels will be calculated with the Friedewald equation: LDL-C (mmol/L) = TC (mmol/L) - HDL-C (mmol/L) - [TG (mmol/L)/2.2], and the very low-density lipoprotein with the proportion of TG (mmol/L)/2.2.

Pharmacological administration

Simple randomization will be performed by a random number table. Fifteen individuals per group will receive either oral capsules of G. sylvestre (Swanson Superior Herbs) or homologated placebo, 300 mg b.i.d. before breakfast and dinner (for a total 600 mg/day) for 12 weeks. Presence of adverse events and adherence to the intervention will be evaluated monthly. Adherence will be evaluated by capsule countbacks (adherence of at least 80% was considered good). All patients will be asked to register the appearance of adverse effects in their daily treatment diary, and they were instructed to maintain their habitual physical activity. Finally, patients also will receive general nutritional recommendations during the study period.

Statistical analysis Plan

Sample size will be calculated using a formula for mean differences (18) for each of the primary variables with a statistical confidence of 95% and a statistical power of 80%. According to the largest sample size calculated from 2-h PG with a standard deviation (SD) of 0.8 mmol/L (19) and an expected difference between groups of at least 1.0 mmol/L, a total of 15 subjects per group will be obtained, including 20% of expected loss. Data will be analyzed using SPSS software (ver. 25; SPSS, Inc., Chicago, IL).

Continuous variables are presented as mean ± SD, and categorical variables are presented as frequencies and percentages. Values are presented according to the International System of Units. After assessing normality with the Shapiro–Wilk test, continuous data were compared using nonparametric tests; Wilcoxon signed-rank test and Mann–Whitney U test were used to evaluate intra and intergroup differences, respectively, and chi-square or Fisher's exact test was used to assess the differences in nominal variables. Intention-to-treat analysis was performed. Dropout cases were not replaced, and a P-value < 0.05 was considered statistically significant.

Ethical considerations

The present study will be performed in accordance with ethical principles for medical research involving humans described in the international guidelines for Good Clinical Practices and the Declaration of Helsinki. Informed consent will be obtained from all participants before the intervention and after being accurately informed regarding the nature, purpose, risks, and benefits of the study by the principal investigator.

$$n = 2\left(\frac{(Z_{\alpha} - Z_{1-\beta})(\delta)}{d}\right)^2$$

Table 2. Sample size

	FG	2h-PG	A1C	Insulin	
				Secretion	Sensitivity
Confidence level (%)	95	95	95	95	95
Statistical power (%)	80	80	80	80	80
Standard deviation	9 mg/dL	16 mg/dL	0.80 %	0.85	0.38
Expected difference	11 mg/dL	19 mg/dL	0.37 %	1.1	0.5
N	11	12	8	10	9
Losses 20%	14	15	10	12	11

n. Sample size, FG: Fasting glucose, A1C: Glycated Hemoglobin A1c, 2h-GP: Glucose 2 hours postload.

Value substitution was performed as follows: The level of statistical confidence was represented by z. It was calculated with a 95% confidence interval with a value of 1.96 two-tailed, for a type I error of 5% that corresponds to a value of 0.05. The statistical power is represented by Z. It was calculated at 80% with a value of 0.842 to two-tailed, for a type II error of 20% that corresponds to a value of 0.20.

For each of the variables (20-23) 20% possible losses were also calculated during the study. The final sample size was taken with the *n* largest of the calculated variables with what was obtained an *n* of 15 patients per group (see Table 2).

6. FINANCING

The financing of this study protocol is provided by the University of Guadalajara.

7. CONFLICT OF INTEREST

This study does not present a conflict of interest of any kind, such as agreements with the pharmaceutical industry or proper of the principal investigator or personnel involved in the study. The interest of this research is the search for new knowledge in the field of therapeutic options for patients with IGT.

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24. ANNEXES

Annex 1. Nutritional recommendations

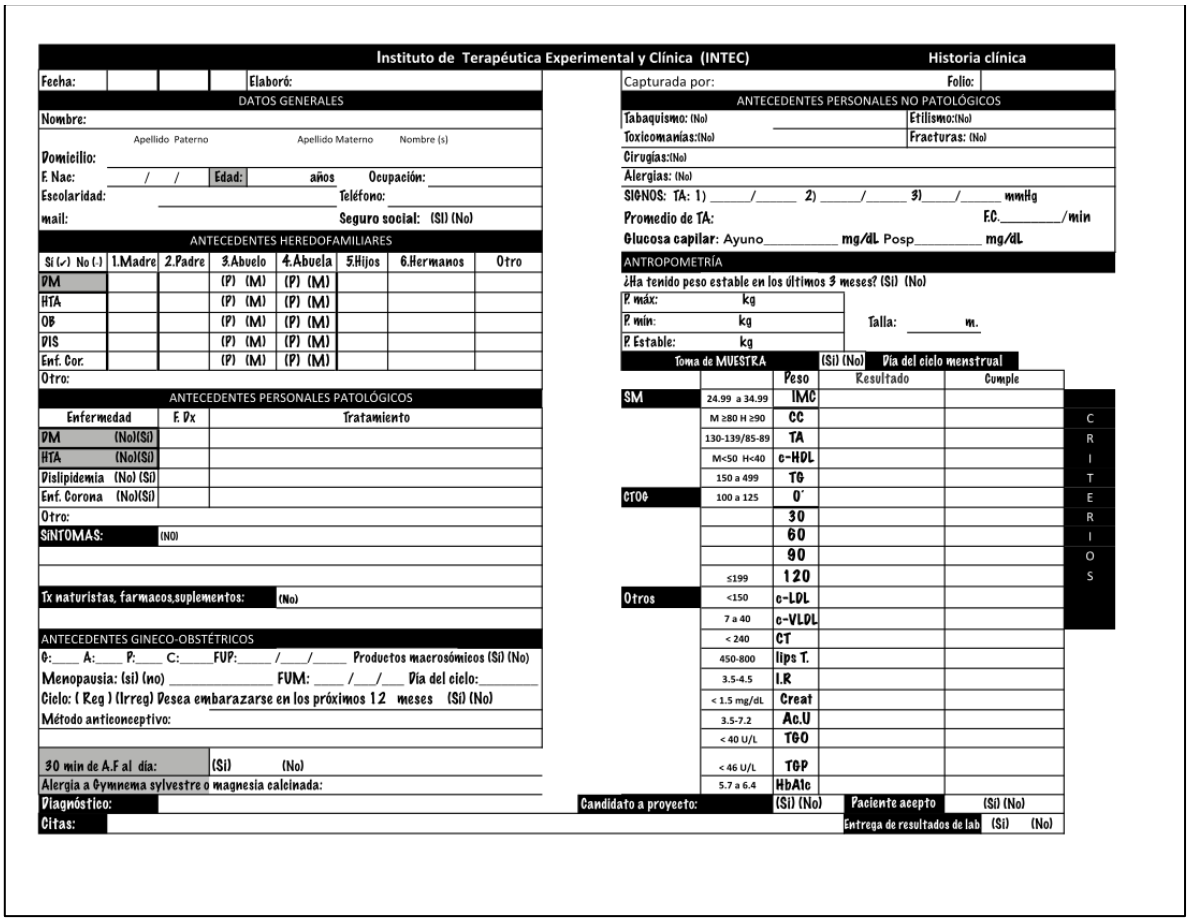
To ensure that you are fed well, remember the following points:

- **Complete**: This means that it contains all food groups and therefore all nutrients. This is achieved by including at least one food from each group in each meal.
- **Balanced**: The nutrients will keep the proportions to each other, when integrating the menus of the meals.
- **Sufficient**: The nutritional needs of each person must be met according to age, sex, stature, physical activity or physiological status.
- **Varied**: Include different foods from the three groups at each meal time.
- **Hygienic**: Be prepared, served and consumed with cleanliness.
- **Suitable**: For different tastes, customs and availability thereof.

General nutrition recommendations:

- Set regular mealtimes that include 3 meals and two servings or failing to go no more than 5 hours without feeding.
- Remember to cover at least 2 liters of natural water during the day.
- Avoid fried or breaded foods.
- Prefer lean fat-free meats.
- Integrate a piece of fruit or vegetables into every mealtime
- Use the dish of good to eat as a guide to combine your food.

Annex 2. Medical history



Instituto de Terapéutica Experimental y Clínica (INTEC) — **Historia clínica**

Fecha: ____ Elaboró: ____ Capturada por: ____ Folio: ____

DATOS GENERALES

Nombre: ____
Apellido Paterno / Apellido Materno / Nombre (s)
Domicilio: ____
F. Nac: ____/____/____ Edad: ____ años Ocupación: ____
Escolaridad: ____ Teléfono: ____
mail: ____ Seguro social: (SI) (No)

ANTECEDENTES HEREDOFAMILIARES

Si (✓) No (-)	1.Madre	2.Padre	3.Abuelo	4.Abuela	5.Hijos	6.Hermanos	Otro
DM			(P) (M)	(P) (M)			
HTA			(P) (M)	(P) (M)			
OB			(P) (M)	(P) (M)			
DIS			(P) (M)	(P) (M)			
Enf. Cor.			(P) (M)	(P) (M)			

Otro: ____

ANTECEDENTES PERSONALES PATOLÓGICOS

Enfermedad		F. Dx	Tratamiento
DM	(No)(Si)		
HTA	(No)(Si)		
Dislipidemia	(No) (Si)		
Enf. Corona	(No)(Si)		

Otro: ____
SINTOMAS: (NO)
Tx naturistas, farmacos,suplementos: (No)

ANTECEDENTES GINECO-OBSTÉTRICOS

G:____ A:____ P:____ C:____ FUP:____/____/____ Productos macrosómicos (Si) (No)
Menopausia: (si) (no) ____ FUM: ____/____/____ Dia del ciclo: ____
Ciclo: (Reg) (Irreg) Desea embarazarse en los próximos 12 meses (Si) (No)
Método anticonceptivo: ____

30 min de A.F al dia: (Si) (No)
Alergia a Gymnema sylvestre o magnesia calcinada: ____
Diagnóstico: ____ Candidato a proyecto: (Si) (No) Paciente acepto (Si) (No)
Citas: ____ Entrega de resultados de lab (Si) (No)

ANTECEDENTES PERSONALES NO PATOLÓGICOS

Tabaquismo: (No) ____ Etilismo:(No)
Toxicomanías:(No) ____ Fracturas: (No)
Cirugías:(No)
Alergias: (No)
SIGNOS: TA: 1) ____/____ 2) ____/____ 3)____/____ mmHg
Promedio de TA: ____ F.C.____/min
Glucosa capilar: Ayuno____ mg/dL Posp____ mg/dL

ANTROPOMETRÍA

¿Ha tenido peso estable en los últimos 3 meses? (Si) (No)
P. máx: ____ kg
P. mín: ____ kg
P. Estable: ____ kg
Talla: ____ m.

Toma de MUESTRA (Si) (No) Dia del ciclo menstrual

		Peso	Resultado	Cumple	
SM	24.99 a 34.99	IMC			
	M ≥80 H ≥90	CC			C
	130-139/85-89	TA			R
	M<50 H<40	c-HDL			I
	150 a 499	TG			T
CTOG	100 a 125	0'			E
		30			R
		60			I
		90			O
	≤199	120			S
Otros	<150	c-LDL			
	7 a 40	c-VLDL			
	< 240	CT			
	450-800	lips T.			
	3.5-4.5	I.R			
	< 1.5 mg/dL	Creat			
	3.5-7.2	Ac.U			
	< 40 U/L	TGO			
	< 46 U/L	TGP			
	5.7 a 6.4	HbA1c			

Annex 3. Diary of attachment and monitoring

INTEC

DIARY OF ATTACHMENT AND MONITORING

EFFECT OF ADMINISTRATION OF *GYMNEMA SYLVESTRE* ON GLYCEMIC CONTROL, INSULIN SECRETION AND INSULIN SENSITIVITY IN PATIENTS WITH IMPAIRED GLUCOSE TOLERANCE

PATIENT'S DATA

Intervention	
Consecutive number	
Patient initials	
Patient's name	

DO NOT FORGET:

- Keep your appointments fasting when you have blood tests (fasting for 10 to 12 hours).
- Wear light clothing to take weights and measurements.
- Bring your treatment diary to every appointment.
- Take the capsule bottle that was given to you to exchange it for another.
- Phone numbers at your disposal: 10585200 ext. 34211 and 3318037131 with doctor Luis Alfonso Gaytán Martínez

CAPSULE TAKING RECORD **Week 0**

Date:

Vial-day	Day	With the first bite		Changes in health condition
		Breakfast	Dinner	
1-1		Hour	Hour	
1-2				
1-3				
1-4				
1-5				
1-6				
1-7				

Comments: _____

Annex 4. Informed Consent Form

INFORMED CONSENT FORM

EFFECT OF ADMINISTRATION OF *GYMNEMA SYLVESTRE* ON GLYCEMIC CONTROL, INSULIN SECRETION AND INSULIN SENSITIVITY IN PATIENTS WITH IMPAIRED GLUCOSE TOLERANCE

Introduction

This form of informed consent may contain words that you do not understand, please ask the research team staff to explain any words you do not understand clearly. Do not sign this document unless you have received satisfactory answers to all your questions.

Purpose of study

This study aims to evaluate the effect of *gymnema sylvestre* administration on glycemic control, insulin secretion and insulin sensitivity in people diagnosed with glucose intolerance.

Glucose intolerance is a state in which people have high glucose after 2 hours of having taken a drink with 75 g of glucose. People with glucose intolerance are more at risk of cardiovascular problems and predisposed to the development of type 2 diabetes mellitus, because of the above, you have been invited to participate in this study because you have glucose intolerance, this state like altered glucose in fasting are components that make up prediabetes.

Insulin has a very important role in glucose control. It is secreted by the pancreas and the body's cells introduce glucose with the help of insulin. A decrease in insulin or cell failure to pick up insulin can cause glucose intolerance.

Because you, according to the results of the studies you were conducted, have glucose intolerance, you have been invited to participate in this study. Like you, 29 more eligible people will be invited to participate in this study. Your participation in this study is completely voluntary. Please read the information we provide and ask the questions you want before deciding whether or not to participate.

Gymnema sylvestre can act at different levels, such as decreased absorption of glucose in the gut, increased insulin secretion, increased muscle glucose uptake and decreased glucose production by the liver, inhibition of α-glucosidase activity, alteration of gastric inhibitor polypeptide concentration, so there is wide chance that it may be a treatment alternative for glucose intolerance.

If you wish to participate, you and two trusted witnesses must sign this consent under information. Subsequently, an appointment with staff will be arranged to carry out its clinical assessment and the corresponding laboratory determinations.

The schedule and requirements for attending your evaluation appointment are as follows:

Appointment hours: 8:00 to 10:00 am, Monday to Friday.

Requirements: Fasting for 10-12 hours.

The following activities and procedures shall be carried out during their evaluation:

- Preparation of medical history:

A medical history will be made that includes:

Anthropometric rating: Size, waist and weight. You will be asked about your current health status as well as a history of personal illnesses and your closest family members and previous or current treatments.

Vital signs: Heart rate and blood pressure will be measured.

- Laboratory procedures:

You will have a venous blood sample taken from one of your arms for some laboratory studies. These studies include measuring your sugar, fats and other substances. For your safety and hygiene, all material used in this study is sterile and disposable, and at the end of the planned analyses, the rest of the sample will be destroyed.

The purpose of clinical and laboratory studies is to learn more about your general health conditions. It will take approximately 30 minutes for you to have these clinical and laboratory tests. We will deliver the results of your laboratory studies no later than 3 days after the blood sample is taken.

Dating

Appointments will be scheduled in advance with an adjustment period of plus minus 3 days of the given date. The total of 7 appointments.

Appointment 1. Scrutiny

You will be given the weight, size, waist, blood pressure and a few questions from the medical history to know your personal and family health history.

The first blood sample will be taken to assess your health status.

Appointment 2. Basal measurements

Blood studies from the first appointment will be repeated to confirm the diagnosis and also an oral glucose tolerance curve to assess insulin secretion and sensitivity. This study involves taking a blood sample (at zero minute) before you drink a drink prepared with 75 g of glucose diluted in water. Once you drink the preparation every 30 minutes you will be taken a sample i.e. in the minutes: 30, 60, 90 and 120. To avoid punctuation several times, a catheter will be placed in your vein.

Appointment 3. Confirmation of diagnosis and delivery of treatment.

On the part of a person outside the research project, a treatment group, group A or B will be randomly assigned by an envelope containing the treatment group. Treatment will be delivered and you will start treatment the next day, 1 capsule in the morning with the first bite of breakfast and another in the evening with the first bite of dinner. You will be given the indication to continue with your usual diet as well as your physical activity without any change during treatment. It may be up to you to take placebo *or wild Gymnema, but* it will not be known that it was your turn until the end of the study.

Appointments 4 and 5. Monitoring

You will be quoted 30 days (per month) and 60 days (2 months) from starting treatment. In each of these appointments you will be taken the weight and circumference of the waist. The empty bottle will return or with the capsules not taken (if any).

The researcher will ask if you had any discomfort and then be given treatment for the next month. You will also be asked the day before, in order to monitor your caloric intake, nutritional habits and physical activity. There will be no blood sample on these visits, but if you need to come fasting so you don't gain weight for breakfast.

Appointment 6. Term of drug treatment

At 90 days (3 months), treatment is terminated, in addition to repeating the baseline appointment tests mentioned above. You will be given a brief explanation of the evolution you had during the 3 months of treatment.

Appointment 7. Tracking

30 days after the end of the intervention period. This appointment will assess whether you had any effects of treatment after you have stopped surgery. You will be given the evaluated changes and a personalized diet. If required, you will be told of pharmacological treatment for your condition.

Study description

It is a clinical trial, double-blind with random assignment. A so-called double-blind design is used because neither you nor the researcher will know which treatment you will receive, however, the researcher can obtain this information in case any unwanted effects occur. Pharmacological treatment will last 90 days (3 months) and one appointment per month of completing drug treatment.

You will choose a random envelope that will contain the number of your treatment that can be either group: placebo (calcined magnesia) or *Gymnema sylvestre.*

The indication of treatment consists of taking a 300 mg capsule of placebo or *Gymnema sylvestre* with the first bite of breakfast and dinner.

Possible risks and inconveniences

Related to clinical evaluation procedures

Weight intake, size, blood pressure, waist and body composition analysis are noninvasive clinical studies that do not cause pain, discomfort or risk.

Related to laboratory procedures

You may experience some pain during the blood sample intake as a result of the puncture and a bruise may form at the site.

Related to drug treatment

Mild discomforts may occur mainly of gastrointestinal type such as stool softening or intestinal inflammation in the first few days.

Benefits of participating in the study

You will not receive payment for your participation in this study, nor will this study involve expense for you.

You will benefit from a metabolic assessment that includes measuring your sugar levels, body fat, blood pressure, body measurements, as well as receiving professional guidance on what steps you should take to reduce your risk of complications by our research team. At the end of the study you will be given nutritional follow-up and multidisciplinary support to prevent disease progression, individually.

Appointments, procedures and medication will have no cost to you.

The results of this study will contribute to the advancement in scientific knowledge of new therapeutic indications of *Gymnema sylvestre.*

Results or new information on treatment alternatives. During the course of this study, we will inform you of any new findings (whether good or bad) that are important to the decision to participate or withdraw from this study. If we provide you with new information, we will ask you again for the signature on your consent to be able to participate in this study again.

Participation or withdrawal: Your participation in the study is completely voluntary. You may decide not to enter or discontinue your participation at any time during the study without penalty or loss of profit. The researcher may also suspend your participation without your consent if you need additional treatment, do not follow the instructions, or suspect that the medicine is harmful to your health.

For the purposes of this investigation we will only use the information you have provided to us from the time you agreed to participate until the time you let us know that you no longer wish to participate.

Privacy and confidentiality. The information you provide to us may be used to identify you (a) (such as your name, phone number and address) but will be stored confidentially and separately as well as your questionnaire responses and clinical test results, to ensure your privacy.

The team of researchers, people involved in your health care, and your regular doctor will know that you participate in this study. However, no one else will have access to the information you provide to us during your participation in this study, unless you so desire. We will only provide your information if necessary to protect your rights or well-being (for example, if you experience physical harm or if you need emergency care), or

if required by law. When the results of this study are published or presented at conferences, no information will be given that could reveal your identity, it will be protected and hidden. To protect your identity, we will assign you a key that we will use to identify your data and use that key instead of its name in our databases.

Contact staff for questions and clarifications about the study. If you have questions or want to talk to someone about this research study you can contact from 8:00 to 16:00 hours, Monday through Friday with medical staff.

INFORMED CONSENT STATEMENT

Patient:
I have been clearly explained what the STUDY **EFFECT OF ADMINISTRATION OF *GYMNEMA SYLVESTRE* ON GLYCEMIC CONTROL, INSULIN SECRETION AND INSULIN SENSITIVITY IN PATIENTS WITH IMPAIRED GLUCOSE TOLERANCE** consists of. I have also read (or someone has read me) the letter content with consent. I have been given the opportunity to ask questions and all my questions have been answered with clear language my doubts. I have been given a copy of this document.

By signing this consent, I acknowledge that I have been informed about the methods of study and administration of the supplement, the inconveniences/benefits and adverse phenomena that may occur due to procedures and medications.
I understand that I am free to withdraw from the studio at any time, without losing any benefit or suffering any penalty. I freely and unreservedly consent to volunteer in this study.
Date:____/____/___

Patient's Name:

_____
_____
_____
_____

Signature:

_____

Address: Street:_____ No._____

Col: _____Cd: _____ Tel (home): _____ Tel (mobile):_____

RESEARCH PROJECT MANAGER: I have explained in detail what the research study consists of to the participant and I have answered all your questions. I believe that you understood the information described in this document and freely consent to participate in this research study. Date:____/____/___

Name: _____ Signature

_____

_____
_____

WITNESS 1. You certify that the participant signed this informed consent form in my presence, on a voluntary basis. Date: ____/____/____

Name: _____ Kinship: _____

Signature:

_____

WITNESS 2. You certify that the participant signed this informed consent form in my presence, on a voluntary basis. Date: ___/____/_____

Name: _____ Kinship: _____

Signature:

_____