Research Project

Randomized Controlled Trial Evaluating the Effect of Rosmarinus Officinalis Extract on Metabolic, Hepatic, Renal, Inflammatory, and Oxidative Stress Biomarkers in Patients With Type 2 Diabetes.

Responsible

Dr. Blanca Patricia Lazalde Ramos

Contributors

M. Internist . Judisett Mirabal Rodríguez

Dr. Sol María Quirarte Báez

Dr. Marisol Galván Valencia

QFB. Diana Jazmín Soriano Gálvez

M. Internist . Sandy Fidel Rodríguez Reyes

Folio: CEI/ISSSTE/2025/043

03/09/2025

INDEX

LIST OF ABBREVIATIONS

CVD: Cardiovascular Disease

DM 2: Type 2 Diabetes Mellitus

RI: Insulin resistance ()

Carnosic Acid

Rosmarinic Acid

AGL: Free Fatty Acids

TNF- α : Tumor necrosis factor alpha

IL-6: Interleukin 6

OBD: Obesity

Tg : triglycerides

Col: cholesterol

MAD: Malondialdehyde

SOD: Superoxide dismutase

GSH: Reduced Glutathione

TABLE OF CONTENTS

LIST OF FIGURES

SUMMARY

Type 2 diabetes mellitus is a prevalent chronic condition characterized by elevated blood glucose levels, primarily due to insulin resistance in the tissues. Despite conventional treatments, many patients do not achieve adequate control and therefore turn to the use of medicinal plants, including *Rosmarinus. Rosemary (Pseudomonas officinalis L.*). Rosemary is a medicinal plant known for its antioxidant, anti-inflammatory, and antidiabetic properties. Preliminary studies suggest that rosemary extract may improve metabolic parameters in diabetic patients. However, scientific evidence regarding its clinical efficacy and safety in this context is very limited, making it crucial to investigate new therapies that can complement or improve existing treatments. To evaluate the effects of rosemary on clinical metabolic parameters, a randomized controlled clinical trial will be conducted using a methanolic extract of rosemary in patients with this condition. Patients diagnosed with type 2 diabetes mellitus, over 18 years of age, who agree to participate by signing an informed consent form will be included. Patients with serious illnesses such as cancer, renal or hepatic insufficiency, a history of rosemary allergy, and pregnant or breastfeeding women will be excluded. A medical history will be taken from each patient, and their glycemic and lipid profiles, atherosclerotic indices, oxidative stress, and inflammation will be evaluated. Statistical analysis will be performed using SPSS version 23. Depending on the normality of the data, the t-test will be used for parametric data, and the Mann-Whitney U test for non-parametric data. For unpaired data, the t-test will be used for parametric data, and for paired data, the Wilcoxon signed-rank test for non-parametric data. Repeated measures ANOVA will be used for data with more than three means. This project could open new avenues of research into the use of medicinal plants for the treatment of chronic diseases, promoting a holistic and natural approach in modern medicine.

KEYWORDS

Rosemary, Type 2 Diabetes Mellitus, Cardiovascular Disease

1.INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease characterized by elevated blood glucose levels that, over time, leads to serious damage to the heart, blood vessels, eyes, kidneys, and nerves. The most common type is type 2 diabetes, generally in adults, which occurs when the body becomes resistant to insulin or does not produce enough insulin (Valer Pelarda Angela 2020). In the last three decades, the prevalence of type 2 diabetes has increased dramatically in countries of all income levels. Diabetes is a disease with a high prevalence worldwide; an estimated 415 million adults have diabetes. Globally, the number of people with diabetes mellitus has quadrupled in the last three decades, making it the ninth leading cause of death. The prevalence of DM has increased dramatically in the last three decades (Figure 1). It is estimated that by 2035 the global prevalence will affect 592 million people (10.1% of the population) (Martín-Peláez S 2020).

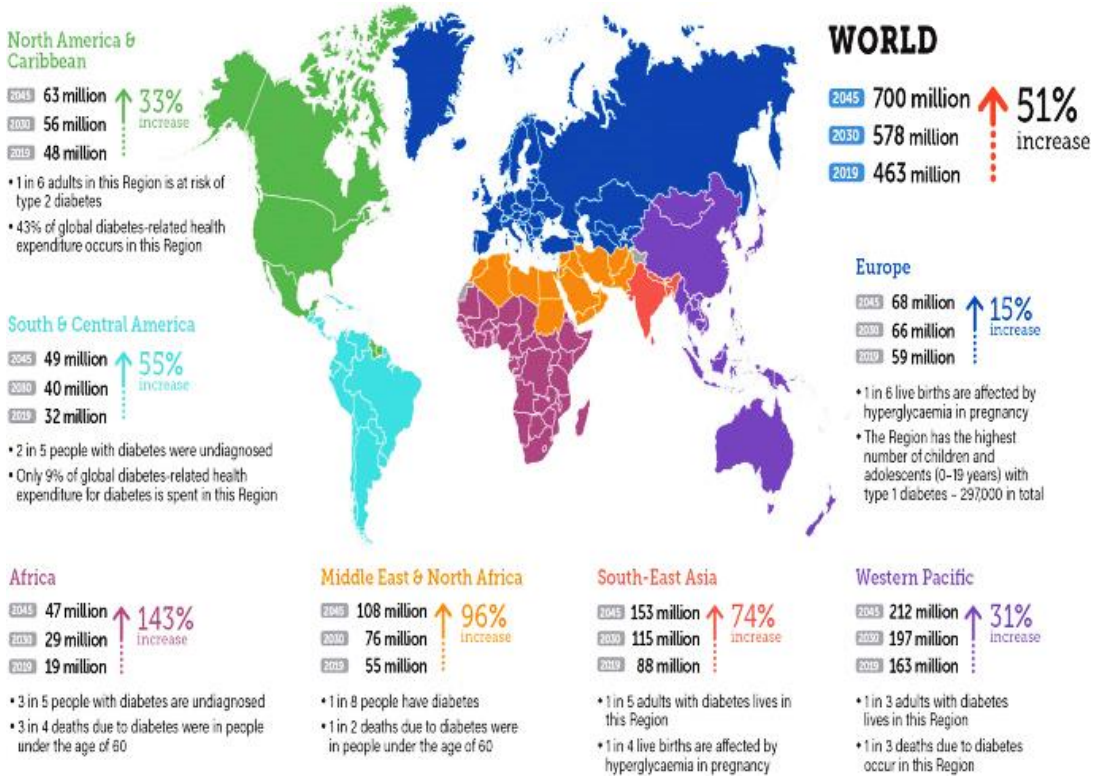


Figure 1 Global prevalence of diabetes mellitus in adults aged 20 to 79 years (2019, 2030 and 2045)

In 2019, it was estimated that, in Latin America, there were 31.6 million people with diabetes (Since January 2020) and according to the WHO, it was the sixth leading cause of death (244,084 deaths) (Pan American Health Organization 2021).

In Mexico, the prevalence of diabetes in 2018 was 16.8%, making it the second leading cause of death and the leading cause of disability in the country. In the 2022 National Health and Nutrition Survey (ENSANUT), the prevalence of both diagnosed and undiagnosed diabetes was 18.3%, or approximately 14.6 million people. From 2006 to 2022, the prevalence of diabetes gradually increased by 3.9%, while the prevalence of undiagnosed diabetes decreased by 1.3%, indicating that the detection of diabetes has increased, consequently reducing the risk of complications from the disease. (ENSANUT 2022)

The costs associated with the treatment and management of diabetes complications represent a significant financial burden for both patients and healthcare services. The Mexican Health Foundation (Funsalud) estimates that this cost is equivalent to at least 2.25% of the gross domestic product (GDP), primarily due to the cost of complications. Insulin therapies can be 7.5 times more expensive than with oral medications.

According to the report by Pérez-Lozano et al. (2023), the annual cost per medication for monotherapy is $3,720.76 MXN. Metformin generated an average patient cost of $6,729.95 MXN, while glibenclamide generated an average patient cost of $3,402.93 MXN, making it 190% cheaper than metformin. Neither alternative resulted in any hospitalization or emergency room admission costs. For biotherapy, the annual cost of the medication was $118,561.79 MXN, while the cost of medical consultations was $327,414.00 MXN and the cost of hospitalization was $243,756.00 MXN for this condition. On average, a patient without subsidy from any health institution would have to pay $50,756.92 MN per year for metformin/ dapagliflozin therapy , $97,200.01 MN for metformin/ sitagliptin , $318,795.16 MN for metformin/NPH insulin, and $26,744.84 MN for pioglitazone / lispro- protamine insulin (Pérez-Lozano et al., 2023) ,pharmacoeconomic analysis report , the patient would spend more than 50% of their salary on treatment with oral hypoglycemic agents, and if insulins are included, the cost becomes prohibitive. This leads to poor adherence to treatment and, consequently, uncontrolled disease, increasing the likelihood of developing complications and other comorbidities. Therefore, health policies must create cost-effective strategies that benefit both patients and healthcare services.

Diabetes, due to its magnitude, severity, and economic implications, represents the main public health problem among nutritional and metabolic diseases, as it is associated with multiple acute and chronic complications. Despite the numerous current therapies for controlling type 2 diabetes mellitus, it remains among the leading causes of mortality and morbidity worldwide. The foregoing allows us to conclude that this disease currently constitutes a global health problem, with a significant gap in its therapeutic approach and deficiencies in preventing its fatal outcome. Therefore, its high prevalence and incidence, coupled with its impact on mortality—its most feared complications being microvascular and macrovascular diseases —demands an urgent solution. For this reason, exploring new, affordable, accessible, and safe therapeutic options is of vital importance today. It is precisely this aspect that this research aims to investigate .

2. THEORETICAL FRAMEWORK

2.1. Type 2 Diabetes Mellitus

Type 2 Diabetes Mellitus (T2DM) is a chronic, degenerative, and incurable but controllable disease characterized by elevated blood glucose levels resulting from impaired glucose, lipid, and carbohydrate metabolism, generally due to peripheral tissue resistance to insulin or pancreatic beta cell dysfunction. This leads to symptoms and signs secondary to these alterations, as well as acute and chronic complications that can result in the patient's death. (American Diabetes Association 2023. Ruano Imbaquingo 2023)

2.1.1. Metabolic effects of DM2

Regarding the metabolic effects of type 2 diabetes (T2D), insulin resistance stands out, in which the body's cells (especially muscle, liver, and adipose tissue) respond less efficiently to insulin (Figure 1). This leads to decreased glucose uptake and increased hepatic glucose production. As a result, blood glucose levels remain elevated. Insulin resistance causes increased insulin release from the pancreas, which can initially compensate for hyperglycemia. However, over time, the pancreatic beta cells become exhausted and cannot produce enough insulin, leading to elevated blood glucose levels (Sussman, J., 2023). This results in alterations in blood lipids, such as hypertriglyceridemia and decreased HDL cholesterol. This contributes to an increased risk of cardiovascular disease, a key factor in morbidity and mortality in people with T2D (Zhang, 2022).

Insulin resistance also promotes fat storage in the liver, leading to non-alcoholic fatty liver disease (NAFLD). This condition exacerbates insulin resistance and is associated with an increased risk of progressing to cirrhosis or liver cancer. Furthermore, because glucose cannot be utilized, the liver in diabetic patients increases glucose production, even when blood glucose levels are high. This contributes to persistent hyperglycemia. As a result, chronic hyperglycemia can damage the kidneys, potentially leading to progressive kidney failure (diabetic nephropathy). This complication is associated with impaired glomerular filtration and may require dialysis in advanced stages. It has

been observed that type 2 diabetes is linked to a state of chronic low-grade inflammation. (KimD.2021).

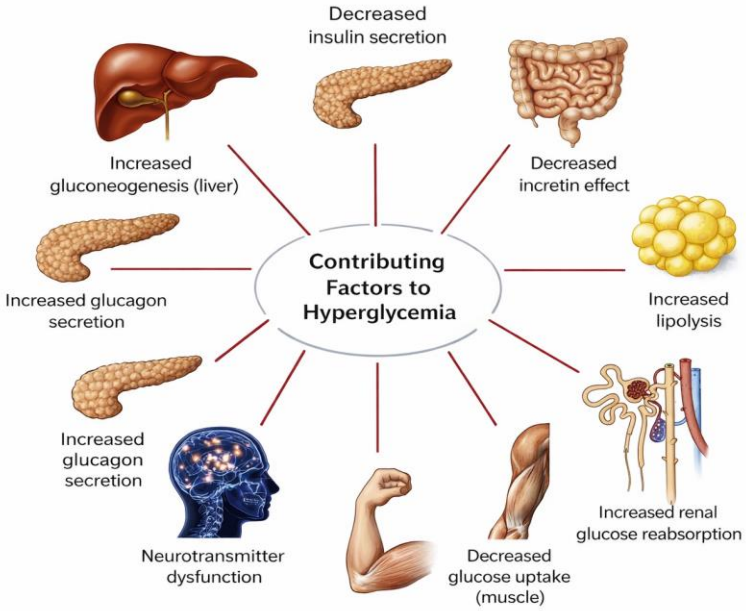


Figure 2Summary Scheme of the Pathophysiology of Type 2 Diabetes Mellitus (Taken from - Jerez Fernández 2022)

Insulin resistance and dyslipidemia promote the release of inflammatory mediators, such as cytokines, which contributes to the progression of micro- and macrovascular complications . Furthermore, in type 2 diabetes (T2D), protein metabolism is also altered. Despite hyperglycemia, patients may experience muscle wasting due to impaired protein synthesis and breakdown, which can exacerbate insulin resistance. Metabolic alterations, such as dyslipidemia and hypertension associated with T2D, increase the risk of cardiovascular diseases, including coronary artery disease, myocardial infarction, and stroke. In addition, recent research suggests that the gut microbiome plays a crucial role in T2D, as alterations in gut flora can influence insulin resistance and inflammation. (Hunter, L. 2020).

2.1.4 Risk factors for DM2

There are risk factors for developing the disease, which can be divided into modifiable factors, when we refer to those in which, after an intervention, a reduction in the risk of developing DM2 is obtained, and non-modifiable risk factors, where it is not possible to intervene to obtain a modification of the risk (Paladines Blacio Arlet Jocelyn 2024).

In general, the most important factors found in the diabetic population are:

Advanced age : The risk increases with age, especially after 45 years, and even more so when it exceeds 60 years of age.

Obesity or overweight : Abdominal obesity is the most important characteristic of this factor, as it contributes to insulin resistance.

Sedentary lifestyle : Lack of physical activity reduces the effectiveness of insulin and increases the risk of developing type 2 diabetes. At least 30 minutes of physical activity should be performed 3 times a week to reduce this risk.

Family history of diabetes : Having close relatives with type 2 diabetes significantly increases the risk of developing the disease due to genetic factors.

Race or ethnicity: Individuals from certain ethnic groups, such as African Americans, Hispanics, Native Americans, and Asians, have a higher risk of developing type 2 diabetes.

History of gestational diabetes : Women who have had diabetes during pregnancy have a higher risk of developing type 2 diabetes later in life; this risk increases if they had a macrofetus or a child with malnutrition or placental abnormalities.

Metabolic syndrome : Metabolic syndrome, which includes hypertension, dyslipidemia, abdominal obesity, and elevated blood glucose levels, thus increasing the risk of diabetes.

Hyperlipidemia : High levels of triglycerides or LDL cholesterol and low levels of HDL cholesterol are factors that favor the development of type 2 diabetes, as well as its complications.

High blood pressure: Hypertension is closely related to type 2 diabetes and also increases cardiovascular risk.

Pre-diabetes : People with blood glucose levels higher than normal, but not high enough to be diagnosed as diabetic (impaired fasting glucose or glucose intolerance) have a higher risk of developing type 2 diabetes.

As can be seen, type 2 diabetes mellitus is characterized by a combination of genetic, environmental, and metabolic factors. Symptoms usually develop gradually, so the disease is often not detected until complications arise. Identifying risk factors is crucial for implementing prevention strategies,

such as lifestyle changes, diet, and exercise. All these factors, along with biological susceptibility, socioeconomic status, education, access to healthcare, and self-care, influence the development of diabetes worldwide. Currently, several tools exist to help identify patients at risk of developing type 2 diabetes, and a widely used method is the FINDRISC scale , which assesses the risk of developing type 2 diabetes within 10 years. A family history of diabetes is one of the main risk factors considered in this scale (Paladines Blacio Arlet Jocelyn 2024, Correia JC 2022).

2.1.2. Association of DM2 with obesity

The close relationship, coexistence, and pathophysiological similarities between type 2 diabetes (T2D) and obesity (OBD) led to the creation and study of the complex term "diabesity," considered the new disease of the 21st century due to its global epidemiological reach. OBD is recognized as the determining factor in this pandemic because adipose tissue has a limited storage capacity and, to avoid its collapse, expresses an insulin-resistant phenotype . If this damage is permanent and the patient does not treat their OBD, it can trigger T2D, which, in turn, causes serious damage to liver tissue, such as hepatic steatosis; insulin resistance develops in skeletal muscle; and cardiovascular damage occurs, such as hypertension, endothelial dysfunction, and atherosclerosis. (Delgado García AF, 2016. Mera-Richard Flores, 2021. Jerez Fernández, 2022)

2.1.3. Main symptoms and signs of DM2 :

Type 2 diabetes is a multisystemic disease that affects multiple systems and organs; its main *symptoms* include:

- *Polyuria:* Increased frequency and amount of urination. Elevated blood glucose levels cause the kidneys to filter excess glucose, which draws water into the urine and increases the urge to urinate. This intensifies when blood glucose levels exceed 14 mmol/L.
- Polydipsia: Excessive thirst. Water loss due to polyuria causes dehydration, which generates a constant feeling of thirst.
- Polyphagia: Excessive hunger. Although blood glucose levels are elevated, cells cannot use glucose efficiently due to insulin resistance, leading to feelings of hunger.

- *Fatigue:* Insulin resistance prevents glucose from entering cells to be used as energy, causing general tiredness.
- Blurred vision: High glucose levels can affect the blood vessels in the eyes, causing temporary blurred vision, which if it persists could lead to permanent blindness or cataracts.
- *Frequent infections:* People with type 2 diabetes have a higher risk of infections due to decreased immunity, especially urinary tract or skin infections.
- *Slow wound healing* : Hyperglycemia affects the body's ability to heal wounds and injuries.
- Unexplained weight loss: Although they experience excessive hunger, some people with type 2 diabetes may lose weight, especially if their diabetes is not properly controlled. (Paladines Blacio Arlet Jocelyn 2024)

Among its clinical signs we can find:

- *Hyperglycemia* : High blood glucose levels, which is the main sign of the disease.
- *Abdominal obesity* : People with type 2 diabetes often have excess fat in the abdominal area, which increases the risk of the disease.
- *High blood pressure* (hypertension): This is common in people with type 2 diabetes, which contributes to a higher cardiovascular risk.
- *Dyslipidemia:* Alterations in blood lipid levels, such as increased triglycerides and LDL cholesterol, and low HDL levels.
- *Acanthosis nigricans:* A clinical sign characterized by dark, velvety patches on the skin, usually in areas such as the neck, armpits, or groin. It is a sign of insulin resistance. (Correia JC 2022, Paladines Blacio Arlet Jocelyn 2023)

Once we know what DM2 consists of, as well as its most important pathophysiological aspects, its risk factors and the clinical diagnosis, it is necessary to know what the diagnostic criteria are to establish or rule out the presence of the disease.

2.1.4 . Diagnostic criteria for DM2

The screening and diagnostic criteria according to the ADA 2023 are shown in Table 1. The ADA considers that for the definitive diagnosis of DM2 in the absence of symptoms of DM2, 2 abnormal results in the screening tests are required.

Diagnostic Criteria for Prediabetes and Diabetes

Diagnostic Test	Prediabetes	Diabetes
Glycated Hemoglobin (HbA1c)	5.4–6.4% (39–47 mmol/mol)	≥6.5% (≥48 mmol/mol)
Oral Glucose Tolerance Test (OGTT, 2-hour plasma glucose after 75 g glucose load)	140–199 mg/dL (7.8–11.0 mmol/L)	≥200 mg/dL (≥11.1 mmol/L)
Fasting Plasma Glucose (FPG)	100–125 mg/dL (5.6–6.9 mmol/L)	≥126 mg/dL (≥7.0 mmol/L)
Random Plasma Glucose	—	≥200 mg/dL (≥11.1 mmol/L) with classic symptoms of diabetes

(ADA 2023)

2.1.5 . Complications of DM2

Complications of diabetes mellitus can appear around 5 to 10 years after diagnosis, even if the patient is receiving adequate treatment. These complications accelerate with persistent poor control. Complications can be acute (diabetic ketoacidosis, hyperosmolar coma, hypoglycemia) (Regüeiferos Montoya JC 2024) and chronic (Revueltas Agüero M 2022), where we can see:

- *Eye disease:* This is due to changes in fluid levels, tissue swelling, and damage to the blood vessels of the eyes. Diabetic retinopathy and cataracts are prominent examples (Faselis C 2020).
- *Foot problems* : Caused by nerve damage and reduced blood flow to the feet. Ulcers are a notable example, including diabetic foot ulcers (Arias-Rodríguez 2023, Jiménez-Castillo GA 2023).
- *Gum disease and other dental problems* : A high amount of blood sugar in saliva helps harmful bacteria grow in the mouth. The bacteria mix with food to form a soft, sticky layer called plaque. Plaque also comes from eating foods that contain sugars or starches. Some types of plaque cause gum disease and bad breath. Others guys cause cavities (Fonseca Escobar D 2021)

- *Heart disease and stroke :* Caused by damage to the blood vessels and nerves that control the heart and blood vessels (Revueltas Agüero M 2022)
- *Kidney disease* : This occurs due to damage to the blood vessels of the kidneys. Many people with diabetes develop high blood pressure . This can also damage their kidneys. The most common are Chronic Kidney Disease and Diabetic Nephropathy. (Regüeiferos Montoya JC 2024. Batista Téllez 2024)
- *Nerve problems (diabetic neuropathy)* : Caused by damage to the nerves and small blood vessels that carry oxygen and nutrients to the nerves (Jiménez-Castillo GA 2023)
- *bladder problems* : Occur due to nerve damage and reduced blood flow to the genitals and bladder (Neurogenic bladder, erectile dysfunction) (Jiménez-Castillo GA 2023)
- *Skin conditions* : Some are caused by changes in small blood vessels and reduced circulation. People with diabetes are also more likely to have infections, including those affecting the skin (acanthosis nigricans , recurrent skin infections, delayed wound healing, vitiligo) (Arias-Rodríguez 2021, Jiménez-Castillo GA 2023).

2.1.6. Treatment

When type 2 diabetes is correctly diagnosed, it is necessary to implement a feasible treatment plan to prevent the onset of complications, which can be fatal. Comprehensive diabetes mellitus management is reported to rest on four fundamental pillars: diabetes education, diet, physical exercise, and medication. (Díaz-Piñera A 2024)

These pillars are developed as follows:

Lifestyle Modification

- Healthy diet: Dietary modification is essential. A balanced diet, low in refined carbohydrates, is recommended, with a focus on foods rich in fiber, healthy fats, and lean protein. Calorie reduction is essential for overweight or obese patients.
- Regular physical exercise: Physical activity improves insulin sensitivity and contributes to weight management. At least 150 minutes of moderate physical activity per week, such as walking, swimming, or cycling, is recommended.

- Weight loss: Weight loss, especially in overweight individuals, significantly improves glycemic control and may even induce remission of diabetes in its early stages.

Drug treatment focuses on controlling blood glucose levels, reducing insulin resistance, and increasing insulin release. It also aims to prevent or reduce complications. The main drug classes include:

- Metformin : It is the first-line drug in the treatment of type 2 diabetes. It helps reduce glucose production in the liver and improves insulin sensitivity.
- SGLT-2 inhibitors : Drugs such as empagliflozin and dapagliflozin help eliminate glucose through urine and have shown cardiovascular benefits.
- GLP-1 agonists : Drugs such as liraglutide and semaglutide that increase glucose-dependent insulin secretion and reduce food intake.
- Insulin : In advanced stages of the disease, when insulin production is insufficient, insulin injections may be needed.

During treatment, regular monitoring of blood glucose levels is essential for adjusting the treatment and maintaining adequate control. Patients may need to measure their glucose several times a day, especially if they are taking insulin or other medications that affect glucose levels. (Díaz-Piñera A 2024)

Another important part of treatment is the prevention and/or management of complications. Furthermore, ongoing patient education is essential for understanding the disease, learning to manage it, and making informed decisions about their treatment. Psychosocial support is also crucial, as many patients may face emotional or psychological difficulties due to the diagnosis and chronic management of the disease.

The treatment of diabetes mellitus (DM) is far from sufficient to prevent its complications, always taking into account the individual needs of each patient. This highlights the need for complementary treatments that are, above all, effective, affordable, economical, and safe. The use of medicinal plants in the treatment of various diseases has increased in modern medicine, and in particular, there has been a clear rise in interest in rosemary in recent years.

2.2. *Rosmarinus officinalis L.* (Rosemary)

Rosemary is a fragrant, evergreen, green-leaved plant with bluish-white flowers. Native to the Mediterranean, North and South Africa, and Western Asia, it grows in many parts of the world (in dry or moderately moist soils), reaching a height of 1 to 2 meters. In Mexico, it is produced throughout the country and is used as a medicinal plant in the states of Guerrero, Hidalgo, Jalisco, Michoacán, Mexico State, Morelos, Oaxaca, Puebla, Sonora, Tlaxcala, and Veracruz. Rosemary does not tolerate anaerobic or very wet soils, but it does tolerate soils of medium salinity. (Flores-Villa, Emmanuel 2020)

2.2.1. Pharmacological Uses of Rosemary

Rosemary essential oil and leaf extracts have been described as having antimicrobial, anti-nephrotoxic , anti-inflammatory (Sousa Borges Raphaelle, 2019) , antitumor (Kallimani 2022) , anti-hepatotoxic , anthelmintic, anticancer, antifungal, insecticidal, antidiabetic, antimutagenic (Gonçalves Catarina, 2022) , anti-toxigenic , cytotoxic (Tremêa 2024), analgesic, herbicidal, hypoglycemic, hypolipidemic (Liu X. Y, 2024 and Kallimanis 2022), antiarthritic, antidepressant, antiobesity , and neuroprotective properties. antihyperglycemics , antihyperlipidemics , antidiarrheals, seed germination inhibitors, inhibitory effect against HIV, (Ahmed 2020) Vasorelaxants , antivirals, antithrombotics (Santos Rodríguez Ana Paula, 2024) , hypouricemics , proapoptotics , anti-infectives , spasmolytics, among others (Flores-Villa, Emmanuel, 2020). It has also been found to improve the production of nerve growth factor (Li, T, 2024). Rosemary extract increases neuronal glucose uptake independently of insulin signaling and activates AMPK in neurons, which may make it essential in the treatment of dementias such as Alzheimer's (Baron DC, 2021) .

2.2.2. Main secondary metabolites of Rosemary

The medicinal effects of plants are associated with their secondary metabolites; 25 phenolic compounds have been identified, mainly flavonoids and flavones, 13 terpenes including phenolic terpenes and a diterpenolactone , two glycosides corresponding to 6-O-caffeoyl-β-D-fructofuranosyl- (2→1)-α-D- glucopyranoside and primulaverine , an aromatic compound identified as phenanthrenone , and a growth regulator, 12-hydroxyjasmonic acid (Pérez et al., 2020). Since

carnosic acid , rosmarinic acid , and carnosol are the main bioactive ingredients of rosemary extract, which possess multiple biological activities, such as the inhibition of digestive enzyme activity, inhibition of lipid absorption, antioxidant properties (Liu, X 2024), anti-inflammatory properties, and antiglycation properties , this suggests a potential therapeutic effect on diabetes.

2.2.3. Hypoglycemic mechanisms of rosemary

The hypoglycemic effect of rosemary has been attributed to its activation of peroxisome proliferator-associated receptor gamma (PPARγ), which increases the production of enzymes involved in primary metabolism, leading to reduced blood levels of fatty acids and glucose. Rosemary, specifically Carnosol , has been shown to act as an agonist of this receptor, which in turn is an agonist of oral hypoglycemic medications, primarily biguanides and sulfonylureas. This justifies its use as an adjunct to conventional treatment (Hassani 2016). Rosemary also exhibits anti-inflammatory and antitumor effects (Agatonovic-Kustrin 2021). The phenolic diterpenes in the methanolic extract of rosemary suppress the cyclic adenosine monophosphate (cAMP) response of gluconeogenic promoter genes (Yun et al., 2013).

Similarly, Vijayan, M., & Surya, K. (2017) reported that rosemary potentially increases hepatic glycolysis and fatty acid oxidation by activating AMPK and PPAR pathways. These components have the ability to suppress the cAMP response of the PEPCK-C gene and glucose 6-phosphatase, which contributes to its hypoglycemic activity (Ahmed, H 2020, Correa, VG2024). By suppressing the cAMP response, key transcription factors in hepatic carbohydrate metabolism are activated, such as the bifunctional enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFK-2/FBPase2) and the carbohydrate response element-binding protein (ChREBP). Rosmarinic acid improved insulin resistance by decreasing IRS1 phosphorylation in skeletal muscle of insulin-resistant rats (Jayanthy et al., 2017).

Another study demonstrated that carnosic acid inhibited SREBP activity and prevented de novo lipid synthesis. In primary hepatocytes, carnosic acid reduced the nuclear abundance of steroid regulatory element-binding proteins (SREBPs) and downregulated genes such as stearoyl - coenzyme A desaturase (SCD), fatty acid synthase (FAS), and 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), thereby inhibiting de novo fatty acid and cholesterol biosynthesis.

Furthermore, carnosic acid alleviated insulin resistance, hyperlipidemia, and fatty liver by restricting the expression of SREBP target genes in the liver (Akinmoladun , Afolabi, et al. (2020)). Carnosic acid has been shown in primary hepatocytes to reduce the nuclear abundance of SREBPs and downregulate target genes such as stearoyl -coenzyme desaturase (SCD), fatty acid synthase (FAS), and de novo lipid synthase.

2.2.4. Background of the use of Rosemary for the treatment of diabetes mellitus.

Labban et al. (2014) evaluated the effect of rosemary leaves at doses of 2, 5, and 10 g per day on glucose levels, lipid profile, and lipid peroxidation in 48 individuals over a period of 4 weeks. The results showed a decrease in glucose, total cholesterol, triglyceride, LDL-c, and malondialdehyde levels , and an increase in HDL-c, vitamin C, and B carotene levels. Therefore, it was described that rosemary not only has an effect on hyperglycemia but also on dyslipidemia and lipid peroxidation, in a dose-dependent manner (Labban et al., 2014).

Subsequently, the effect of rosemary on glucose levels and lipid profile in patients with type 2 diabetes (T2D) was reported. The author selected 45 individuals with T2D and glucose levels between 160 and 300 mg/dL, who also had elevated lipid profiles, and 15 non-diabetic individuals. All participants took 3 g of rosemary leaves daily for one month. The author reported a 31–35% decrease in cholesterol, triglyceride, and LDL levels, a 21% reduction in glucose levels, and a 22% increase in HDL lipoproteins (Al Jamal et al., 2014). However, the intervention was for a short period, which may be due to the large volume of the plant consumed, as the whole plant, rather than an extract, was used.

In 2019, a study was conducted evaluating the impact of rosemary supplementation in type 2 diabetic patients, finding that patients who consumed rosemary showed a significant reduction in blood glucose levels and improved their lipid profiles compared to the placebo group. Nawaz, Waseem, et al. (2019). The proposed mechanisms for these effects include improved insulin sensitivity and rosemary's antioxidant activity. Based on these properties, other pharmacological research has demonstrated that rosemary extract and its phenolic components, especially carnosic acid, rosmarinic acid , and carnosol , could significantly improve diabetes mellitus by regulating glucose metabolism, lipid metabolism, and promoting anti-inflammatory and antioxidant activity,

thus showing extremely high research value. This is further supported by the study conducted by Bao , T (2020), which identified the main rosemary compounds and their metabolites associated

with antidiabetic mechanisms, while also suggesting the need for clinical trials to expand the scientific evidence. In 2020, Jiang , L., and colleagues evaluated the effect of rosemary in the treatment of type 2 diabetes, based on insulin resistance and the fundamental action of rosmarinic acid . They demonstrated that rosmarinic acid was able to produce better regulation of GLUT4, thus favoring not only more efficient glucose transport but also better glucose utilization by tissues. (Jiang , L., et al. (2020).

Ameer, OZ, et al. (2021) conducted a study evaluating more than 10 medicinal plants, including rosemary, for use in treating type 2 diabetes. The results showed that rosemary had the best antidiabetic properties, followed by turmeric and moringa.

Hypertension is one of the diseases most frequently associated with diabetic patients; in fact, it is more common to find a patient with both hypertension and diabetes than with a diagnosis of type 2 diabetes alone. Another study related to this topic was conducted to evaluate the effect of a standardized ethyl acetate extract based on its rosmarinic acid content , as well as the effect of different doses of rosmarinic acid in mice with chronic hypertension induced by fatty acids (FAIs). This involved the chronic administration of FAIs (12 weeks) to mice at 0.2 µg /kg i.p. The study found a significant difference in systolic and diastolic blood pressure (SBP and DBP) values ($p < 0.001$; $F = 1.91$) between the affected group and the healthy group, as well as between the values of the other treatments, including telmisartan (10 mg/kg), Oba- EtOAc (25 mg/kg), Ose- EtOAc (25 mg/kg), and all doses of rosmarinic acid (0.39 to 6.24 mg/kg). Furthermore, a statistically significant difference was also found after evaluating blood glucose levels (Alegría Herrera E. 2019). This study coincides with the one conducted by Prasannarong in 2019, which confirmed the hypotensive effect of rosemary. In that study, acute treatment with rosmarinic acid decreased blood pressure by an average of 46–64 mmHg , while chronic treatment decreased blood pressure by an average of 33–58 mmHg . Intake at 40 mg/kg reduced plasma glucose levels. Chronic treatment with rosmarinic acid at 10, 20, and 40 mg/kg prevented ANG II-induced hyperglycemia.

Our research group evaluated the effect of rosemary tea intake in patients with type 2 diabetes over a 90-day period, reporting a statistically significant decrease in anthropometric parameters such as body mass index and waist-to-hip ratio, as well as in glycated hemoglobin percentages, insulin resistance, pancreatic β-cell function, and malondialdehyde levels . Therefore, the use of a rosemary extract was suggested to reduce the dosage and duration of treatment and thus achieve a more promising therapeutic effect. Consequently, rosemary tea may be a promising option for patients with drug-resistant type 2 diabetes (Quirarte-Báez et al., 2019). Subsequently, a study was conducted in HIV patients undergoing antiretroviral therapy with ATRIPLA, to whom complementary therapy was added. We used a methanolic extract (400 mg/day) and an aqueous extract of rosemary (4 g/L per day) for 4 months to evaluate genomic instability using the buccal mucosa micronucleus assay. The groups that received complementary therapy with the rosemary extracts showed a decrease in genomic instability, which was most evident in those who ingested the methanolic extract (Lazalde-Ramos et al., 2020).

Although the active ingredients in rosemary are known to be non-toxic, they can cause adverse reactions at high doses. The most common are allergies or contact dermatitis, seizures, and at higher doses, an increase in the number of micronucleated cells and chromosomal aberrations. While overdose from rosemary infusions is very rare, it could cause abdominal spasms, gastroenteritis, vomiting, kidney irritation, and uterine bleeding. Ghasemzadeh Rahbardar 2024.

2.2.5 Toxicological studies of rosemary

In recent years, rosemary (*Rosmarinus) Rosemary (Officinalis*) has been the subject of various toxicological investigations, evaluating its potential beneficial and adverse effects. This research focused on identifying possible adverse reactions to prevent harm to patients. A 2020 study evaluated the effects of rosemary extracts in mice, both in the acute and subchronic phases. The results indicated that, at low doses, rosemary does not present significant toxic effects, but at high doses (above 1000 mg/kg) signs of liver and kidney toxicity were observed, although not reaching lethal levels. This study highlights the importance of controlled dosage and the evaluation of long-term effects (El-Abhar, 2020).

Regarding its effect on the cardiovascular system, research published in 2021 indicated that rosemary can have beneficial effects on blood circulation, but it can also present risks if consumed in excess. It was observed that some of its active components, such as rosmarinic acid and eucalyptus, can induce a slight decrease in blood pressure, but they can also cause adverse reactions in individuals with pre-existing conditions, such as hypertension. (Badria, FA, & Fouad, MA (2021).

On the other hand, in 2022, a dermal toxicity analysis of rosemary essential oil showed that, at high concentrations, it can cause irritation or dermatitis in people with sensitive skin. The studies recommended proper dilution of the essential oil before topical application. (Kollipara, MP, & Reddy, S. (2022)

A study reported in 2023 explored the potential genotoxic effects of rosemary using in vitro tests. It was observed that rosemary extracts did not exhibit significant mutagenic activity in the cells tested, suggesting that rosemary does not have a direct carcinogenic effect. However, caution is advised regarding its excessive or prolonged use due to its high concentration of phenolic compounds (Marzocco , S., 2023). Regarding interactions with other medications, there are reports of interactions between the active compounds in rosemary and certain anticoagulant and antihypertensive drugs. Therefore, rosemary could potentiate the effects of these medications, increasing the risk of bleeding or hypotension (Almeida, PC, & Costa, JM, 2023).

3. PROBLEM STATEMENT

Type 2 diabetes mellitus is a complex, systemic health condition and one of the most common conditions affecting adults seeking primary care. It is a significant risk factor for all age groups, races, and sexes, and is considered an essential adult chronic disease with a devastating socioeconomic impact on healthcare systems worldwide. This is because it is incurable, with mostly disabling sequelae that increase the risk of cardiovascular, renal, and neurological diseases, which are among the leading causes of death globally. Despite available treatments, many patients do not adequately control associated risk factors such as dyslipidemia and hypertension, and the number of complications and premature deaths from this cause has not been minimized, making it a global health problem . This data may be useful for future studies and for the development of evidence-based clinical guidelines. Furthermore, if rosemary capsules prove effective, they could be integrated into public health programs for diabetes management, especially in communities with limited access to conventional medications. Rosemary is a medicinal plant with antioxidant and anti-inflammatory properties that could have beneficial effects in improving these risk factors and reducing complications. However, scientific evidence regarding its efficacy and safety in the clinical treatment of diabetes is limited, as most studies have been preclinical. Given this, the author poses the following question: Does the methanolic extract of rosemary, as an adjunct to conventional treatment of type 2 diabetes, protect the patient's vascular health at a dose of 500mg daily for 6 months by improving the metabolic, clinical, and inflammatory profile without affecting renal or hepatic function ?

4. JUSTIFICATION

Rosemary is a medicinal plant known for its antioxidant, anti-inflammatory, and antidiabetic properties. It is widely available and has very low toxicity. Preliminary studies suggest that rosemary extract may improve metabolic parameters in patients with type 2 diabetes. However, scientific evidence regarding its efficacy and safety in this context is very limited, making it crucial to investigate new therapies that can complement or improve existing treatments. Furthermore, it is vital to consider the socioeconomic impact of this disease, which represents a significant burden on healthcare systems due to its long-term complications. Effective and accessible interventions such as the use of rosemary could reduce the costs associated with the treatment and management of this condition. Hence, the need to determine whether rosemary can be a viable therapeutic option by establishing the safety of rosemary capsules in this specific population. Based on in vivo and in vitro evidence demonstrating that rosemary has an agonist effect on oral hypoglycemic medications, primarily due to its active metabolite carnosol , and that after stabilizing metabolic, inflammatory, and oxidative stress parameters, it can delay and/or prevent the onset of damage and complications in diabetic patients, this justifies focusing on newly diagnosed patients. Once meta-inflammation is established in diabetic patients, leading to complications, the damage is irreversible. This project could open new avenues of research into the use of medicinal plants for the treatment of chronic diseases, promoting a holistic and natural approach in modern medicine. Conducting this research can provide significant empirical data on the efficacy and safety of rosemary in diabetes management.

5. HYPOTHESIS

H1: The administration of methanolic extract capsules of Rosemary as an adjunct to treatment for a period of 6 months, stabilizes metabolic, clinical, inflammatory parameters, oxidative stress and DNA damage, decreasing cardiovascular involvement in the newly diagnosed diabetic patient.

$H_{0:}$ The administration of methanolic extract capsules of Rosemary as an adjunct to treatment for a period of 6 months does not stabilize the metabolic, clinical, inflammatory parameters, oxidative stress and DNA damage, and therefore does not decrease the cardiovascular impact in the newly diagnosed diabetic patient.

6. OBJECTIVES

6.1 GENERAL

- To demonstrate that the methanolic extract of Rosemary (Rosmarinus officinalis) administered as an adjunct to the conventional treatment of type 2 diabetes mellitus improves clinical, metabolic, inflammatory and oxidative parameters without affecting the renal and hepatic profile, thus reducing cardiovascular involvement.

6.2 SPECIFIC

1- Obtain the methanolic extract of rosemary

2- Encapsulate, package and label the methanolic extract of rosemary and cellulose as a placebo in accordance with current Mexican official standards

3- To determine the effect of complementary therapy with methanolic extract of rosemary on the glycemic profile, lipid profile, oxidative stress, DNA damage and inflammatory parameters.

4- To determine the clinical effect of complementary therapy with rosemary in patients with type 2 diabetes through exhaustive physical examination at different times

5- To evaluate the safety of ingesting the methanolic extract of Rosemary at a dose of 500 mg using the adverse effects questionnaire and the analysis of the hepatic and renal profile

6- To compare the effects of complementary rosemary therapy on clinical, biochemical, oxidative stress and inflammation parameters in relation to the control group (patients who continued only with their established pharmacological treatment and placebo).

7. METHODOLOGY

7.1. Type and design of the study

A longitudinal study will be conducted, allowing for clinical, biochemical, and healthcare follow-up over the 6 months of intervention with the methanolic rosemary extract in patients recently diagnosed with type 2 diabetes. In another group, cellulose will be used as a placebo, encapsulated with the same characteristics and in accordance with Mexican regulations.

This is a mixed, experimental, randomized controlled clinical trial (RCT) where biomarkers associated with the metabolic, oxidative and inflammatory profile of the patients included in the study will be quantified using colorimetric enzyme assays and enzyme-linked immunosorbent assays (ELISA).

7.2. Stages of the study

The study will be divided into three main stages:

STAGE I. *Preparation of the methanolic extract of Rosmarinus officinalis*

The rosemary leaves are dried at room temperature, then mechanically pulverized into a fine powder, which is passed through a sieve for particles between 355 and 255 nm in size (mesh No. 60). Once the dry powder is obtained, the extract is prepared with 50g of dried rosemary powder and 300ml of methanol. It is covered with aluminum foil and left to stir for at least 2 days. Then it is refluxed for 3 hours at 70°C. After this, the macerate is filtered, trying to remove as much leaf powder as possible, and 15g of activated carbon is added to the extract to remove chlorophyll. It is left protected from light and stirred for 2 days. After this time, the activated carbon is filtered out and the mixture is subjected to rotary evaporation at 80°C to separate the methanol from the solution. Once only the concentrated extract is obtained, cold triple-distilled water is added in a 1:3 ratio. It is filtered, and the resulting extract is dried in an oven at 37°C for 2 days. Once dry, it is stored protected from sunlight and then, following the necessary sanitary measures, it is encapsulated in size 1 capsules.

***STAGE II** . Patient Recruitment*

In this stage, the recruitment of patients recently diagnosed with DM2 will be carried out under ADA-2024 guidelines and selected according to the established criteria.

Board 1Selection criteria

Inclusion criteria	***Exclusion criteria***	***Elimination criteria***
1.Confirmed diagnosis of DM2 2. Older adults in an age range above 18 years. 3. Informed Consent Signature (Annex 1) 4. Clinical stability or absence of acute complications associated with diabetes or another disease at the time of the study. 5. Absence of other associated diseases except for Obesity and/or High Blood Pressure 6. Glomerular filtration rate greater than or equal to 60ml/min/1.73m2sc according to the EKD-EPI 2021 formula	1. Allergy or hypersensitivity to any of the components of Rosemary. 2. Pregnant or breastfeeding women. 3. Patients taking other supplements or medications that may interfere with the study results. (antioxidants) 5. Patients who are participating in another clinical trial simultaneously.	1. Patients who experience any adverse reaction to the phytopharmaceutical during the study. 2. Patients who decide to withdraw from the study

Patient recruitment will be conducted through information sessions. Invitations will also be extended through doctor-patient information sessions.

Patients who meet the inclusion criteria will be asked to sign the informed consent letter (Annex 1) and will have their medical history taken and initial anthropometric and biological measurements taken for the determination of the established initial biochemical parameters.

***STAGE III.** Intervention and follow-up of patients*

Sample size: A study will be conducted with a minimum of 35 patients.

Study Groups: Participants will be randomly selected and divided into two groups: an intervention group (receiving methanolic rosemary extract capsules in addition to conventional treatment at a

dose of 500 mg per day for 6 months) and a placebo group (receiving 500 mg of cellulose) in addition to conventional treatment. Patients will be assigned to these groups to ensure the feasibility and speed of the research, given the dynamic nature of this population, and to minimize patient attrition. Randomized block sampling will be used.

Study period : October 2025 to May 2026.

Patient follow-up

Patient follow-up will be conducted monthly for six months. A WhatsApp group will be created for participants to share their experiences with the intervention, report any discomfort, and facilitate closer communication.

They will also be sent a reminder call before their appointment to avoid losing track of the patient.

Patients will attend their assigned healthcare facilities monthly, where they will be monitored through clinical, anthropometric, and biochemical evaluations to establish scientific evidence of the efficacy and safety of consuming an extract of *R. officinalis* L. in the treatment of patients with type 2 diabetes mellitus in a controlled clinical trial. The field treatment with the patients will be explained in detail later.

Laboratory tests corresponding to the complete blood count (Hemoglobin (Hb), Hematocrit (Hct), Erythrocyte Sedimentation Rate (ESR)), white blood cell count (WBC), glucose profile, renal profile, lipid profile, and liver profile will be evaluated at the clinic to which you belong. The inflammatory and oxidative stress profiles will be evaluated at the UAZ laboratories by our research group using the following techniques:

INFLAMMATION : IL-6, TNF-alpha, and CRP. This will be performed using a competitive, indirect sandwich ELISA. *Sample collection* : A serum sample is drawn from the patient.

1. *ELISA plate preparation* : In a microtiter plate , the wells are coated with specific antibodies against IL-6, TNF alpha, or PCR.
2. *Sample addition* : The sample is added to the well and incubated to allow IL-6, TFN alpha or PCR of the sample to bind to the antibody.
3. *Washing:* The cups are washed to remove any unbound substance.
4. *Addition of secondary antibody:* A secondary antibody conjugated to an enzyme (e.g., peroxidase) is added that binds to the antigen-antibody complex.

5. *Second wash and centrifugation* : This is done to remove complexes that were not bound to the secondary antibody

6. *Substrate reaction:* A substrate is added that reacts with the conjugated enzyme, producing a color change.
7. *Spectrophotometric reading:* Absorbance is measured at a specific wavelength (e.g., 450 nm). The color intensity is proportional to the amount of IL-6 present in the sample.
8. *Calculation:* An IL-6 standard curve is used to calculate its concentration.

OXIDATIVE PROFILE: malondialdehyde (MDA), GSH, SOD
MDA is a product of lipid peroxidation and can be measured by an assay based on its reaction with malondialdehyde in the presence of thiobarbituric acid (TBA). This assay is known as the TBA assay using the Yagi technique (1998).
Procedure: Place 0.3 ml of the sample (serum or plasma) in a tube, and add 2 ml of 1/12N sulfuric acid, 0.3 ml of 10% phosphotungstic acid , and 1 ml of 0.6% TBA. Mix by vortexing . Place the mixture in a water bath at 95°C for 60 minutes. After this time, allow the tubes to cool and add 1.3 ml of n-butanol. Mix by slow shaking for 5 minutes and then centrifuge for 10 minutes at 3,000 rpm. The organic phase is read at a wavelength of 534 nm, using n-butanol as a blank in quartz cells. Lipid peroxide levels are calculated and expressed in terms of MDA; the results obtained are incorporated into a calibration curve made with 1,1,3,3 tetramethoxypropane , under the same conditions as the sample, in triplicate.

In the case of SOD and GSH, specific kits will be used for their measurement by sandwich ELISA , competitive and indirect, and the manufacturer's instructions will be followed.

7.3. Operationalization of variables

Variables to be quantified

1. ***Medical record***

A medical history will be compiled to gather information from the patient's relevant clinical practice, which will include family history of type 2 diabetes, non-pathological and pathological personal history, gynecological and obstetric history (if applicable), diabetes history, physical examination data and pharmacological treatment (appendix 2)

2. ***Variables obtained from the interrogation and physical examination.***

   Age and sex will be evaluated among the main general variables, while among the categorical variables we will evaluate those obtained from the patient's interview and

physical examination, such as the presence or absence of diabetic retinopathy, superficial and deep reflexes, peripheral pulses, and subcutaneous cellular tissue.

Table 2Operationalization of the variables obtained from the interview and physical examination

Profile evaluated	**Variable**	**Variable type**	**Frequency**	**Description**
General variables	Age	continuous quantitative	Initial	According to chronological age at the start of the study
	Sex	Categorical dichotomous	Initial	According to sex
Other categorical variables	Diabetic Retinopathy	Categorical dichotomous	Initial and 6 months	It will be measured through physical examination with an ophthalmoscope and classified into Presence of retinopathy Absence of Retinopathy
	Reflectivity	Categorical dichotomous	Initial and monthly	It will be measured through physical examination and with the use of a reflex hammer for deep tendon reflexes, and will be classified into Reflexes preserved Hypoesthesia
	Peripheral pulses	Categorical Polytomous	Initial and monthly	It will be measured through physical examination, and it should be noted that the absence of a pedal pulse does not necessarily indicate disease, as it can be absent in up to 10% of the population. It will be classified as follows: Present and normal Present and diminished in a member Present and diminished in both members
	TCS (Subcutaneous cellular tissue)	Categorical dichotomous	Initial and monthly	It will be measured through physical examination, since early detection of progressive kidney

				damage in diabetic patients is vital. It will be classified as Infiltrate Not infiltrated

3. Anthropometric measurements

Weight, height, body mass index (BMI), waist circumference, hip circumference, and waist-to-hip ratio (WHR) will be determined at baseline and every month during the 6 months of intervention.

Table 3Operation of anthropometric variables

Profile evaluated	**Variable**	**Variable type**	**Frequency**	**Description**
Anthropometric Measurements	Body weight	continuous quantitative	Initial and monthly	Patients are weighed using a mechanical medical scale.
	Size	continuous quantitative	Initial and monthly	The length is measured from the feet to the crown using a measuring tape.
	Body mass index	continuous quantitative	Initial and monthly	It will be measured through the calculation of a specific formula. Weight/Height $^{2.}$ Where underweight (BMI less than 18.5), normal weight (BMI from 18.5 to 24.9), overweight (BMI from 25 to 29.9) and Obesity (BMI greater than 30) are considered.
	Waist measurement	continuous quantitative	Initial and monthly	The waist circumference will be obtained by measuring at the height of the last floating rib (approximately two fingers above the navel) using a measuring tape
	Hip measurement	continuous quantitative	Initial and monthly	The maximum hip circumference will be obtained at the level of the buttocks using a measuring tape.
	Waist-to-hip ratio (WHR)	continuous quantitative	Initial and monthly	It will be obtained by the ratio of the waist and hip circumference, with >102 cm considered at risk in men and 88 cm in women.

4. *Biochemical Assessments*

Glycemic profile

The glycemic profile will be determined by measuring glucose levels, glycosylated hemoglobin, and insulin resistance using the triglyceride/glucose and triglyceride/HDL-C ratios.

Table 4Operation of the variables of the glycemic profile

Profile evaluated	Variable	Guy	Frequency	Description
Glycemic profile	Glucose	continuous quantitative	Initial and monthly	A reference enzymatic method will be used employing hexokinase (HK), which catalyzes the phosphorylation of glucose by ATP to form glucose-6-phosphate and ADP. The reaction continues with the use of a second enzyme, glucose-6-phosphate dehydrogenase (G6PDH), which catalyzes the oxidation of glucose-6-phosphate by $NADP^+$ and glucose to form NADPH. The concentration of NADPH formed is directly proportional to the glucose concentration.
	HbA1c	continuous quantitative	Initial and 6 months	immunodetection method will be used . In this method, the detection antibody in the buffer binds to the antigen in the sample, forming antigen-antibody complexes. These complexes then migrate to the nitrocellulose matrix where they are captured by the other antibody immobilized on the test strip. The more antigen in the sample that forms the stronger the antigen-antibody complex, the greater the fluorescence signal intensity in the detection antibody. The result will be expressed as a percentage of glycosylated hemoglobin in whole blood.
	Insulin resistance	continuous quantitative	Initial and 6 months	The TG/HDL-c ratio and the TG / glucose ratio will be used as one of the markers of insulin resistance along with the TG/ Glu ratio , because HDL-c has the opposite effect to LDL-c, that is, it collects cholesterol from peripheral tissues and takes it to the liver to be excreted, generating glucose utilization at the muscle level and consequently improving insulin function

Lipid profile

Poor glycemic control adversely affects lipid and lipoprotein metabolism; therefore, the lipid profile will be evaluated in these patients to assess the effect of complementary therapy on glycemic control and, consequently, the lipid profile.

The concentration of cholesterol, triglycerides, HDL-C, LDL-C, and VLDL-C will be quantified.

Table 5Operationalization of lipid profile variables

Evaluated Profile	Variable	Variable Type	Frequency	Description
Lipid profile	Triglycerides	continuous quantitative	Initial and monthly	The increase in fatty acids in patients will be measured using a colorimetric enzyme assay on blood samples, as described by the provider.
	Cholesterol	continuous quantitative	Initial and monthly	Cholesterol concentration is determined by a colorimetric enzymatic assay on a blood sample according to the supplier's specifications
	HDL Cholesterol	continuous quantitative	Initial and monthly	The concentration of c-HDL in blood samples is determined by a precipitation assay and a colorimetric enzymatic assay according to the supplier's specifications.
	LDL Cholesterol	continuous quantitative	Initial and monthly	The concentration of c-LDL in blood samples is quantified using a colorimetric enzyme assay according to the supplier's specifications.
	VLDL Cholesterol	continuous quantitative	Initial and monthly	The concentration of c-VLDL is calculated by dividing the triglyceride level by five, using mg/ dL as the unit .

Kidney function tests

Chronic hyperglycemia secondary to diabetes is the etiopathogenic axis of dysfunction in various types of kidney cells that leads to progressive renal failure; therefore, the renal profile of patients will be evaluated before complementary therapy and renal function will be monitored during complementary therapy.

The tests will evaluate levels of urea, blood urea nitrogen (BUN), albumin, and creatinine.

Table 6Operationalization of the renal profile variables

Profile evaluated	Variable	Variable Type	Frequency	Description
Renal profile	Urea	continuous quantitative	Initial and monthly	The concentration of urea in the blood samples will be determined by a colorimetric enzymatic assay according to the supplier's specifications.
	BUN	continuous quantitative	Initial and monthly	The concentration of urea nitrogen in the blood samples will be determined by a colorimetric enzymatic assay according to the supplier's specifications.
	Albumin	continuous quantitative	Initial and monthly	The concentration of albumin in the blood samples will be determined by a colorimetric enzymatic assay according to the supplier's specifications.
	Creatinine	continuous quantitative	Initial and monthly	Creatinine concentration in blood samples will be determined by a colorimetric enzyme assay according to the supplier's specifications.

Liver function tests

Liver involvement is very common in diabetes mellitus. In type 2 diabetes, patients frequently present with hepatic steatosis and, occasionally, non-alcoholic steatohepatitis. Therefore, it is important to evaluate liver function in these patients.

The liver function markers will include the quantification of the enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

Table 7Operationalization of the liver profile variables

Evaluated Profile	Variable	Variable type	Frequency	Description
Liver profile	ALT	Continuous quantitative variable	Initial and monthly	The catalytic activity of ALT will be determined by an enzymatic assay by measuring the decrease in NADH in the medium. Sample quantification will be performed according to the supplier's specifications.
	AST	Continuous quantitative variable	Initial and monthly	The catalytic activity of AST will be determined by an enzymatic assay by means of the decrease of NADH in the medium. The quantification of the sample is carried out following the supplier's specifications.

The studies that include blood count, renal and hepatic profile, glucose, and lipid profile will be performed at the IMSS.

Oxidative stress

It has been widely demonstrated that hyperglycemia, both intracellular and extracellular, generates increased production of free radicals (FR), thereby increasing oxidative stress damage and producing structural and functional alterations at various levels of biological organization, gene expression, and specific cell signaling pathways. A greater increase in oxidative stress has been observed in diabetics with poor metabolic control and complications compared to those without chronic complications and with optimal disease control. Therefore, one of the goals of complementary therapy with *R. officinalis* is to reduce oxidative damage in these patients.

Among the markers of oxidative stress, oxidative damage at the level of total lipids is measured by quantifying malondialdehyde (MDA), and damage at the level of proteins is measured by advanced protein oxidation products (PAOPs).

Table 8Operationalization of oxidative stress variables

Evaluated Profile	Variable	Variable type	Frequency	Description
Oxidative stress	MDA	continuous quantitative	Initial and monthly	The concentration of MDA in serum will be determined using a colorimetric method in liver, kidney, and pancreas samples. A 10% homogenate is prepared from the tissues in KCl (1.15%), and the blood serum in H_2SO_4 (12 $_N$). The addition of TBA (0.3%) creates colored compounds that are measured photometrically at a wavelength of 534 nm. The calibration curve of MDA of known concentration allows for the determination of MDA levels in the samples (Uchiyama & Mihara, 1978).
	GSH	continuous quantitative	Initial and 6 months	Blood, urine, or tissue samples are collected and must be stored correctly to preserve the stability of the biomarkers. The concentration of **reduced glutathione (GSH)** is measured by a colorimetric assay with dinitrobenzene sulfonate (DNBS), where the intensity of the color is related to the amount of GSH.
	SOD	continuous quantitative	Initial and 6 months	Blood, urine, or tissue samples are collected and must be stored correctly to preserve the stability of the biomarkers. Superoxide **dismutase**

				(SOD) activity is measured by a colorimetric assay that evaluates the inhibition of nitrobluetetrazolium reduction, using a spectrophotometer.

Inflammation Assessment

Type 2 diabetes mellitus (T2DM) is characterized by a state of chronic inflammation where immune cell activation and pro-inflammatory cytokines play a role in the development, progression, and pathogenesis of its complications. Therefore, the effect of complementary therapy on the inflammatory state in patients with T2DM will be evaluated to determine whether complementary therapy with **R. officinalis** will have an effect on the progression and pathogenesis of diabetic complications associated with inflammation.

C-reactive protein, interleukin 6, and tumor necrosis factor alpha (TNF-α) will be determined.

Table 9Operationalization of the variables of the inflammatory profile

Profile evaluated	Variable	Guy	Frequency	Description
Inflammation	C-reactive protein	continuous quantitative	Initial and monthly	dL is determined after agglutination occurs , and subsequently, a series of dilutions are performed to determine the relevant concentration.
	IL-6	continuous quantitative	Initial and 6 months	A sandwich enzyme immunoassay will be used for the quantitative measurement of human IL-6 in serum. The plate is coated with an IL-6-specific antibody. The sample and standards are added to the wells, and the IL-6 present in each sample binds to the wells via the immobilized antibody. After incubation, the wells are washed and then incubated with biotinylated anti-IL-6 antibody , which binds to the captured IL-6 present in each well. After incubation, the unbound biotinylated detection antibody is removed by washing, an HRP- streptavidin conjugate is added to the wells, and the microtiter plate is incubated . After incubation and washing, TMB substrate solution is used

				to visualize the enzymatic reaction of HRP catalysis, producing a blue product that turns yellow after the addition of an acidic stop solution. The density of yellow is proportional to the amount of IL-6 captured in each well.
	TNF-α	continuous quantitative	Initial and 6 months	Quantitative measurement of TNF-α will be performed using a sandwich enzyme immunoassay in serum. The microtiter plate of the kit is coated with a TNF-α-specific antibody. The corresponding standards or samples from the microtiter plate containing a biotin-conjugated antibody preparation specific for TNF-α are added to the wells. Horseradish peroxidase-conjugated avidin (HRP) is then added to each well of the microplate and incubated. After adding the TMB substrate solution, only the wells containing TNF-α, the biotin-conjugated antibody, and the enzyme-conjugated avidin will exhibit a color change. The enzyme-substrate reaction is terminated by adding a sulfuric acid solution, and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 10 nm.

Cardiovascular Evaluation

Cardiovascular involvement will be assessed by measuring Troponin T levels , given that this troponin has been most strongly associated with asymptomatic cardiovascular damage in type 2 diabetic patients.

Profile to be evaluated	**Variable**	**guy**	**Frequency**	**Description**
Cardiovascular	Troponin T	Continuous quantitative	Initial and 6 months	Troponin T measurement will be performed using the enzyme immunoassay technique with the use of a kit,

	and following the manufacturer's instructions.

7.4. Clinical fieldwork

Patients will be recruited at the assigned research clinic, with prior authorization from the Director, with the goal of obtaining at least 30 volunteers. These volunteers will be randomly assigned to two groups: one for the experimental treatment (Rosemary Capsules) and another for the control treatment. Consultations for patients enrolled in the study will take place at the Research Clinic on Wednesdays from 11:00 AM to 5:00 PM (to cover both regular medical appointment times). If a participant is unable to attend during these hours, they will be provided with a telephone number to contact the researcher and schedule an appointment at their convenience.

First date

Once patients have been referred to our clinic following the inclusion and exclusion criteria, a comprehensive interview will begin, where we will assess the patient's personal and family medical history, lifestyle, reported symptoms, and medications they take for diabetes.

A physical examination will then be performed, respecting the patient's modesty, but thorough in search of important signs in the diabetic patient.

The next step will be the anthropometric measurements, in which weight will be taken using an OMRON HBF-514C digital scale with a reading accuracy of 0.1 kg (0.220 lb) and a weighing capacity of 149.69 kg (330 lb). Without shoes or heavy clothing, the participant should stand in the center of the scale, placing their feet in the designated footrests and remaining still during the measurement. The weight will be recorded when the numbers on the digital scale stabilize, along with the time the measurements are taken. Height will be measured using a SECA wall-mounted mechanical stadiometer , which has a measuring range of 0 to 220 cm. The participant, barefoot, will stand with their heels together, legs straight, and shoulders relaxed. Their heels, hips, shoulder blades, and the back of their head should be in contact with the vertical surface on which the stadiometer is mounted . The head should be positioned in the Frankfort horizontal plane, which is represented by a line between the lowest point of the eye socket and the tragus (the cartilaginous prominence in front of the opening of the external auditory canal). Just before the measurement is taken, the individual should inhale deeply, hold their breath, and maintain an erect posture. The hair will then

be compressed, and the measurement taken. Any hair ornaments that could interfere with the measurement should be removed.

Using the weight and height data obtained, BMI will be calculated (using the formula Height / Weight²) based on the WHO tables (2007) to stratify each participant into the appropriate BMI category according to the data obtained (underweight, normal weight, overweight, obese). Participants will be given a request form for laboratory tests, and the appointment will be scheduled by the Researcher (Master's student) within a maximum of 10 days. The samples will be collected and processed at the IMSS facilities by laboratory personnel, with prior authorization. The results

will be entered into the CILAB system, which is accessible only to medical personnel with a password.

The data is recorded on the Clinical form (Annex 2)

At this first appointment, each patient will be informed about the study, its objectives, and, of course, any possible adverse reactions they might experience with the use of the herbal medicine. Informed consent will also be obtained (Appendix 1). A follow-up appointment will be scheduled for 14 days later.

Second appointment (day 0)

Laboratory results will be collected and reported in the corresponding section of Appendix 3. Blood pressure, anthropometry, and a buccal mucosal sample will be taken. If patients meet the criteria, they will be admitted to the research study. They will be assigned the initial treatment with a consecutive number corresponding to the same number as their questionnaire. Subsequently, the questionnaire, the clinical record, and the questionnaire on possible adverse effects (Appendix 4) will be completed. This questionnaire will record any symptoms present before the start of treatment that are not attributable to the treatment itself, and patients will be asked at each visit about possible side effects, covering all organ systems (tolerability).

They will be randomly assigned the treatment to follow (first allocation), giving verbal and written instructions: (Annex 5)

1) Regarding taking the medication, schedules and dosage.

2) In the case of amenorrhea in women of childbearing age, they will be instructed to immediately discontinue the medication and consult with the responsible physician.

3) Procedure to follow in case of severe side effects (immediately stop treatment and seek medical advice).

4) Be alert for the presence of side effects.

5) Attend each of the following appointments on time.

A follow-up appointment will be scheduled in 7 days.

Third appointment (30 days):

Following the fourth week of treatment, a physical examination, blood pressure measurement, anthropometric measurements, and oral swab will be taken. The follow-up questionnaire and questionnaire on potential adverse effects will be completed, gathering information in the specific sections of the respective questionnaire. Any questions will be answered. A second supply of the assigned treatment will be provided, along with a further explanation of how to identify warning signs. An open appointment will be scheduled for emergency care if needed, and patients will be monitored for any side effects. During this appointment, the effectiveness, tolerability, and adherence to the treatment will be evaluated (by counting ingested doses). Subsequent appointments will be scheduled every 30 days.

Appointments at 2, 3, 4, 5 and 6 months of intervention

Patients will be comprehensively re-evaluated, with a thorough physical examination, the presence or absence of adverse effects will be recorded, oral samples will be taken every month, and biochemical studies corresponding to the month of evaluation will be indicated.

At the final appointment, each patient will be informed of the test results and their progress with the complementary treatment. Any questions will be addressed, and the form will be completed with the information gathered at this appointment.

We must bear in mind that weekly consultations will be held to recruit new patients, as well as educational talks about self-care for diabetic patients, questions will be answered, and capillary

blood glucose tests will be performed sporadically and randomly using reagent strips and glucometers.

7.5. Statistical Analysis

A database will be created in the EXCEL 2019 program with the results of the questionnaires, proceeding to the validation of the data and subsequent statistical analysis.

Sample size calculation: The calculated sample size for this study is N = 30 participants. This value was obtained using G Power software, considering a 95% confidence level, 80% statistical power, and a large effect size (assessed based on the glucose variable with an expected decrease of 180 mg/dL). This sample size is adequate to detect significant differences in the variables of interest,

ensuring the validity and reliability of the results. Block random sampling will be used to ensure that both groups are adequately represented, following the aforementioned inclusion and exclusion criteria. The sample will be representative of the target population.

Descriptive statistics: Qualitative variables will be reported with absolute frequencies, relative frequencies and cumulative relative frequencies, in addition to measures of significance of proportions; quantitative variables will be reported with measures of central tendency and dispersion as appropriate (parametric variables will be evaluated with means and standard deviation and non-parametric variables with medians and interquartile ranges). This normality analysis will be performed with a Shapiro test.

Intergroup Comparison Analysis

To compare the variables between the intervention group and the control group, the following statistical tests will be used depending on the type of variable:

- Quantitative variables: The independent samples t-test will be used if the variables follow a normal distribution. If the variables do not follow a normal distribution, the Mann-Whitney U test will be applied. This analysis will be performed initially, that is, when collecting clinical and laboratory data before the ingestion of the extract, in order to establish our initial comparisons between the control group and the case group. Once we have the data from the second laboratory measurements, as well as the monthly clinical observations, we will establish the comparisons using paired data, employing t-tests for parametric variables and

- Wilcoxon signed-rank tests for non-parametric variables. rank test. In the case of variables where we have 3 or more measurements, a repeated measures ANOVA will be performed if they are parametric, and if they are not, a Friedman test will be performed.
- Categorical variables: The chi-square test will be used to compare the proportions between the two groups. If the expected frequencies are less than 5, Fisher's exact test will be used. For repeated measures categorical variables, McNemar's test will be performed.

For the reliability index, a p-value of 0.05 will be used to reject null hypotheses and evaluate whether or not there are significant differences after using the extract. All data will be analyzed using SPSS version 23.

7.6. Writing and acceptance of the protocol

The protocol will be written by the internist Judisett Mirabal Rodríguez using Microsoft Word software, to be submitted for consideration to the National Research Commission of the IMSS and to the Research Ethics Committee of the Autonomous University of Zacatecas Francisco García Salinas.

7.7. Conflicts of interest

There are no conflicts of interest.

7.8. Ethical considerations

This research project will be conducted in accordance with the guidelines established in the Regulations of the General Health Law on Health Research, Title Two, Ethical Aspects of Research in Human Beings, Chapter II, Article 65, Pharmacological Research. Pharmacological research is defined as activities for which there is no prior experience in the country, that have not been registered by the Ministry of Health, and therefore are not commercially distributed, as well as registered and approved medications when their use is investigated with modalities, indications, doses, or routes of administration different from those established, including their use in combinations. According to Article 66, this is considered a Phase II clinical pharmacology drug research study. Furthermore, we will follow the guidelines of Article 67, where our research group has conducted preclinical studies in different species (dog, rat, and mouse). We are also awaiting any other guidelines that the aforementioned Regulations may require.

As part of the ethical considerations, an information session will be held with participants, and the project's objectives and scope, as well as its potential health implications and adverse effects, will be provided in writing. If individuals agree to participate in the project, they will be asked to provide their informed consent, which they must sign (See Annexes).

Informed consent will be obtained in accordance with the Nuremberg Code, which clarifies the basic principles governing the ethical conduct of research, such as the capacity to give consent, absence of coercion, and understanding of the risks and benefits involved. The Code also establishes the fundamental ethical principles of respect for persons, justice, and beneficence. Participants will not receive any payment for their participation in the study, their personal data will be handled confidentially, and the results will be used solely for research purposes.

The protocol will be registered at the Autonomous University of Zacatecas and submitted to the ethics and research committees of IMSS and UAZ

Therefore, it is considered that the procedures to be carried out comply with the ethical standards and the Regulations of the General Health Law on Health Research, adhering to the recommendations for biomedical research in human beings, dictated in the Declaration of Helsinki in 1964 and revised in Tokyo in 1975, respecting the principles contained in the Nuremberg Code, the Belmont Report and the United States Code of Federal Regulations (Common Rule); prior authorization by the Ethics Committee of the National Coordination of Research of the IMSS.

Regarding ISO Standards, we mention that our protocol complies with ISO 19609 (Parts 1, 2 and 3) which governs matters related to botanical products used as medicines:

- Part 1: General quality requirements.
- Part 2: Identification and testing.
- Part 3: Pollutants and waste.

Also with ISO 9001 on quality management systems: being applicable to laboratories or companies that produce the extract, it guarantees traceability, control and continuous improvement.

Furthermore, we mention those ISO standards that regulate clinical studies involving human intervention:

- ISO 14155:2020 On clinical trials of medical devices in humans – Good clinical practice (GCP): Widely used as an ethical and technical basis for any RCT.
- ISO 20916:2019 Relevant in our case since laboratory tests are used to measure the response of patients (such as glucose levels or HbA1c) and it governs the protocols for these tests.
- ISO/IEC 27001 Information Security Management: This is of vital importance in our environment for the security of sensitive patient data and requires confidentiality and protection of clinical information.

  For conducting laboratory studies and techniques such as ELISA, our protocol will follow the following ISO standards to maintain the quality of the results of these studies: ISO 15189:2022 on particular requirements for quality and competence applicable directly to medical laboratories that process human samples; ISO 13485:2016 Quality management systems for medical devices; in this case, we will use a kit for performing ELISA, ensuring that the kit manufacturer complies with good practices for in vitro diagnostic devices.

7.9 . Human, physical and financial resources of the study

Human resources

The execution of this project relies on intra-institutional and inter-institutional collaboration networks.

Within the Autonomous University of Zacatecas there is a collaboration network between the Academic Groups, Experimental Models for Diagnosis and Therapeutics (CA-UAZ-235) and Pharmacology in Molecular Biomedicine (CA-UAZ-175)

Equipment

The Laboratory of Pharmacology in Molecular Biomedicine and Neuroscience at the Autonomous University of Zacatecas has the equipment for obtaining the methanolic extract of *R. officinalis.* (rotary evaporators, water baths, irons, scales, -20°C refrigerator, mill, among others) as well as an ultra-freezing unit (-70°C) for the preservation of plasma samples until processing

The acquisition of a semi-automatic encapsulator is necessary, which will be used to encapsulate the methanolic extract of *R. officinalis*

This research project aims to provide information on the benefits of complementary therapy with *R. officinalis* as an antidiabetic treatment, which will be reflected in the reduction of treatment costs and comorbidities

7.10. Biosafety aspects

The study will be conducted in accordance with institutional biosafety regulations, taking into account the Official Mexican Standard that establishes the characteristics, identification procedures, classification, and list of hazardous waste (NOM-052-SEMARNAT-2005). All liquid or solid Biological Infectious Hazardous Waste (RPBI) generated in the laboratory will be decontaminated by laboratory personnel before collection and disposal by qualified personnel, in accordance with the procedures of NOM-087-ECOL-SSA1-2002 (Environmental Protection, Environmental Health,

Biological-Infectious Hazardous Waste, Classification and Handling Specifications). The handling of biological waste will take into account that all material in contact with human samples (tubes, pipettes, ELISA plates) must be inactivated (e.g., with hypochlorite) and then disposed of as infectious biological waste according to local regulations.

The waste will be delivered to the person in charge of the warehouse in the Health Sciences area, who is responsible for delivering it to the contracted collection company for its final disposal in accordance with the provisions of the official Mexican standard NOM-087-ECOL-SSA1-2002

Furthermore, the laboratories at the Medical Units are equipped with the necessary equipment for sample evaluation, storage, and processing, all in accordance with the institution's regulations. Safety measures such as the use of gloves, gowns, and face masks will be in place to prevent contact and aspiration during handling. Appropriate aseptic techniques will also be followed for the preparation and encapsulation of the methanolic extract. The students involved have received prior training on working with the material and the proper technique for preparing the rosemary methanolic extract.

Regarding biosafety requirements directly related to patients, an Informed Consent form will be signed, which includes an explanation of the risks, even the minimal ones associated with the supplement. Medical monitoring will be carried out, including tracking for possible adverse effects of the rosemary extract (for example, interactions with hypoglycemic medications). A thorough pre-treatment evaluation for allergies or possible adverse reactions to rosemary compounds will be

performed through a comprehensive interview. We will also ensure that the encapsulated product is free of microbiological contaminants (microbiological standards for ingestible products).

A follow-up of possible adverse reactions to complementary therapy reported by patients will also be carried out (Annex 4).

Regarding international biosafety standards, our study will be guided by ISO 10993, which establishes the biological evaluation of medical devices and is widely used to assess biocompatibility, toxicity, and dermal or systemic sensitization. Furthermore, the collection, storage, and analysis of human biological samples (blood, serum) will be conducted in accordance with ISO 20387:2018, respecting ethical consent, sample traceability, and storage and processing conditions.

9. EXPECTED RESULTS AND IMPACTS.

Among the expected results of this project, we hope to generate scientific knowledge. We will have a validated method for the simultaneous quantification of rosmarinic acid , carnosol , and carnosic acid . Likewise, we will obtain the metabolic and inflammatory profile of the methanolic extract of *Rosmarinus. The study will also examine the bioavailability of R. officinalis* secondary metabolites (rosmarinic acid , carnosol , and carnosic acid) in patients with type 2 diabetes mellitus. Undergraduate and postgraduate students will be trained. Researchers will participate in national and international forums and publish their findings in indexed journals. Meetings will be held at healthcare centers to disseminate the results to the public.

By carrying out the intervention and clinical, biochemical, and healthcare follow-up of patients with type 2 diabetes included in the study, implementing support, counseling, and continuing education strategies to ensure their continued participation, the aim is to improve the health of these patients by contributing to the treatment of metabolic and inflammatory alterations present in patients with type 2 diabetes through the administration of a standardized extract of *R. officinalis,* thus having a positive impact on the quality and life expectancy of the patients.

Type 2 diabetes (T2D) has a direct economic impact on healthcare spending. Diabetic patients die prematurely or live with the disease daily, experiencing significant economic consequences that represent a social cost. This economic burden affects a country's economic and human development, the capacity of its workforce, and conditions of equity and poverty. This project aims to reduce the economic burden of T2D by positively impacting the quality of life and life expectancy of patients with the disease. This will be reflected in both direct economic costs (outpatient care and treatment of complications) and indirect costs (premature death, absenteeism, work disability, invalidity, and presenteeism).

10 . BIBLIOGRAPHIC REFERENCES

1. Valer Pelarda A. **Literature Review on Type 2 Diabetes Mellitus.** Electronic Journal of PortalesMedicos.com. 2020;15(14):738. Available from: https://www.revista-portalesmedicos.com/revista-medica/revision-bibliografica-sobre-la-diabetes-mellitus-tipo-2/
2. Escobar Trinidad AJ, Arredondo López A. **Review and analysis of the effectiveness of the multidisciplinary model for diabetes care.** Mexico; 2019. Available from: http://www.scielo.org.mx/pdf/hs/v18n3/2007-7459-hs-18-03-261.pdf
3. Martín-Peláez S, Fitó M, Castañer O. **Mediterranean diet effects on type 2 diabetes prevention, disease progression, and related mechanisms: A review.** Nutrients. 2020;12(8):1–15.
4. Wu Y, He X, Zhou J, Wang Y, Yu L, Li X, et al. **Impact of healthy lifestyle on the risk of type 2 diabetes mellitus in southwest China: A prospective cohort study.** J Diabetes Investig. 2022;13(12):2091–2100.
5. Li Y, Teng D, Shi X, Qin G, Qin Y, Quan H, et al. **Prevalence of diabetes recorded in mainland China using 2018 diagnostic criteria from the American Diabetes Association: A national cross-sectional study.** BMJ. 2020;369.
6. Avilés-Santa ML, Monroig-Rivera A, Soto-Soto A, Lindberg NM. **Current state of diabetes mellitus prevalence, awareness, treatment, and control in Latin America: Challenges and innovative solutions.** Curr Diab Rep. 2020;20(11).
7. Elsevier. **COVID-19 Resource Centre.** 2020. Available from: https://www.elsevier.com/connect/coronavirus-information-center
8. Pan American Health Organization (PAHO). **National overview of diabetes prevention and control in the Americas.** 2021;1–9.
9. National Institute of Statistics and Censuses (INEC). **Vital Statistics: Statistical Registry of Live Births and Deaths 2018.** 2018. Available from: https://www.ecuadorencifras.gob.ec/documentos/webinec/Poblacion_y_Demografia/Nacimientos_Defunciones/2018/Principales_resultados_nac_y_def_2018.pdf
10. National Institute of Public Health (INSP). **Prevalence of prediabetes and diabetes in Mexico: ENSANUT 2022.** Updated November 14, 2023. Available from: https://www.insp.mx/avisos/prevalencia-de-prediabetes-y-diabetes-en-mexico-ensanut-2022
11. **Ruano Imbaquingo, D.E., Ruano Imbaquingo, H.J., Yépez Salazar, D.A., Herrería Rodríguez, M.A., Falcón León, K.D., & López Hoyos, E.J. (2023).** Current treatment of type 2 diabetes mellitus.

Ciencia Latina Multidisciplinary Scientific Journal, 7(2), 379–395. https://doi.org/10.37811/cl_rcm.v7i2.5300

12. **American Diabetes Association. (2023).** Standards of Medical Care in Diabetes—2024**.** ***Diabetes Care*, 46(Suppl 1), S1–S291.**
13. **Delgado García, A.F., Valdés Rodríguez, Y.C., & Marcel, E.A. (2016).** Visceral obesity: A predictor of type 2 diabetes mellitus and cardiovascular disease**.** ***Revista Latinoamericana de Patología Clínica y Medicina de Laboratorio*, 63(2), 67–75. Available from:** https://www.medigraphic.com/pdfs/patol/pt-2016/pt162b.pdf
14. **Mera-Richard Flores, R., Colamarco-Delgado, D.C., Rivadeneira-Mendoza, Y., & Fernández-Bowen, M. (2021).** General aspects of diabesity: Pathophysiology and treatment**.** ***Cuban Journal of Endocrinology*, 32(1). Retrieved October 5, 2024, from** http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S1561-29532021000100010
15. **Jerez Fernández, C.I., Medina Pereira, Y.A., Ortiz Chang, A.S., González Olmedo, S.I., & Aguirre Gaete, M.C. (2022).** Pathophysiology and clinical alterations of type 2 diabetes mellitus: A literature review**.** ***NOVA Journal*, 20(38).** https://doi.org/10.22490/24629448.6184
16. **Correia, J.C., Waqas, A., Huat, T.S., Gariani, K., Jornayvaz, F.R., Golay, A., et al. (2022).** Effectiveness of therapeutic patient education interventions in obesity and diabetes: A systematic review and meta-analysis of randomized controlled trials**.** ***Nutrients*, 14(18).**
17. **Paladines Blacio, A.J. (2023).** Individual risk assessment for developing type 2 diabetes mellitus**.** ***Electronic Journal PortalesMedicos.com*, 18(5), 213.**
18. **Regüeiferos Montoya, J.C., Suarez Rivero, B., Toledo Fernández, S.O., Rosell Suárez, A., Garzón Argudín, J.J., & Pérez Montes de Oca, E. (2024).** Complications in type 2 diabetic patients with comorbidities**.** ***Archives of Hospital Universitario "General Calixto García"*, 12(2), e02401220. Available from:** http://revcalixto.sld.cu/index.php/ahcg/article/view/1220
19. **Revueltas Agüero, M., & Molina Esquivel, E. (2022).** Diabetes mellitus as a cardiovascular risk factor**.** ***Archivo Médico Camagüey*, 26, 8715. Available from:** http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S102502552022000100050
20. **Faselis, C., Katsimardou, A., Imprialos, K., Deligkaris, P., Kallistratos, M., & Dimitriadis, K. (2020).** Microvascular complications of type 2 diabetes mellitus**.** ***Current Vascular Pharmacology*, 18, 117–124.**

21. Arias-Rodríguez, F.D., Jiménez-Valdiviezo, M.A., del Cisne-Ríos-Criollo, K., Murillo-Araujo, G.P., Toapanta-Allauca, D.S., Rubio-Laverde, K.A., Barreno-Yandún, Y.P., Moposita-Alvarado, M.M., & Trejo-Pincay, M.B. (2023). **Diabetic foot: Update on diagnosis and treatment. Literature review**. *Angiología*, 75(4), 242–258. https://doi.org/10.20960/angiologia.00474
22. Fonseca Escobar, D., Parada Fernández, F., Carvajal Guzmán, M., Sepúlveda Verdugo, C., & Cortés Vásquez, S. (2021). **Dental management of the diabetic patient: A narrative review**. *Revista de la Asociación Odontológica Argentina*, 109(1), 64–72. https://doi.org/10.52979/raoa.1119
23. Batista Téllez, D., Estrada Hernández, J., & Morell Pérez, L. (2024). **Chronic kidney disease and progression factors in patients with type 2 diabetes mellitus**. *Revista Información Científica*, 103, e4611. https://doi.org/10.5281/zenodo.10999895
24. Bravo, L.E., & Anaya-Escamilla, A. (2023). **Diabetic neuropathy: A narrative review of pathophysiology, diagnosis, and treatment**. *Acta Médica Peruana*, 40(3), 243–251. https://doi.org/10.35663/amp.2023.403.2731
25. Díaz-Piñera, A., Rodríguez-Salvá, A., Achiong-Estupiñán, F., Cardona-Garbey, D., Maldonado-Cantillo, G., Londoño-Agudelo, E., & Vander Stuyft, P. (2024). **Therapeutic adherence of patients with type 2 diabetes mellitus in four health areas of the country**. *Finlay Journal*, 14(1). Available from: https://revfinlay.sld.cu/index.php/finlay/article/view/1367
26. Tremêa, G.T.F., Kleibert, K.R.U., Krause, L.S., Fell, A.P.W., Scapini, A.R., Marschall, K.W., Baiotto, C.S., da Silva, M.H.T., da Silva, J.A.G., & Colet, C.F. (2024). **Aesthetic radiofrequency associated with Rosmarinus officinalis supplementation is safe and reduces oxidative stress in women: A randomized, double-blind clinical trial**. *Journal of Evidence-Based Integrative Medicine*, 29, 2515690X241246293. https://doi.org/10.1177/2515690X241246293
27. Flores-Villa, E., Sáenz-Galindo, A., Castañeda-Facio, A.O., & Narro-Céspedes, R.I. (2020). **Rosemary (Rosmarinus officinalis L.): Its origin, importance, and general aspects of its secondary metabolites**. *TIP: Specialized Journal in Chemical-Biological Sciences*, 23, e20200266. https://doi.org/10.22201/fesz.23958723e.2020.0.266
28. Huang, M., Wang, H., Xu, X., Lu, X., Song, X., & Zhou, G. (2020). **Effects of nanoemulsion-based edible coatings with a composite mixture of rosemary extract and ε-poly-L-lysine on the shelf life of ready-to-eat carbonado chicken**. *Food Hydrocolloids*, 102, 1–9. https://doi.org/10.1016/j.foodhyd.2019.105576
29. Hendel, N., Sarri, D., Sarri, M., Napoli, E., Palumbo Piccionello, A., & Ruberto, G. (2024). **Phytochemical analysis and antioxidant and antifungal activities of powders, methanol extracts, and essential oils from Rosmarinus officinalis L. and Thymus ciliatus Desf. Benth.** *International Journal of Molecular Sciences*, 25(14), 7989. https://doi.org/10.3390/ijms25147989
30. Li, T., Wang, W., Guo, Q., Li, J., Tang, T., Wang, Y., Liu, D., Yang, K., Li, J., Deng, K., Wang, F., Li, H., Wu, Z., Guo, J., Guo, D., Shi, Y., Zou, J., Sun, J., Zhang, X., & Yang, M. (2024). **Rosemary (Rosmarinus officinalis L.) hydrosol based on serotonergic synapse modulation for insomnia**. *Journal of*

Ethnopharmacology, 318(Pt B), 116984. https://doi.org/10.1016/j.jep.2023.116984Garros Ferreira L., Barbosa Evora PL, Kise Capellini V., et al. Effect of Rosmarinic acid on the arterial blood pressure in normotensive and hypertensive rats: role of ACE. Phytomedicine [Internet]. 2017 [Accessed 18 Sep 2024] ; Available from: https://doi.org/10.1016/j.phymed.2017.02.006

31. Prasannarong M., Saengsirisuwan V., Surapongchai J., et al. Rosmarinic acid improves hypertension and skeletal muscle glucose transport in angiotensin II-treated rats. BMC Complementary and Alternative Medicine [Internet]. 2019 [Accessed 18 Sep 2024] ; 19:165. Available at: https://doi.org/10.1186/s12906-019-2579-4
32. Zhang, Y., & Li, X. (2022). "Impact of Type 2 Diabetes on Lipid Metabolism and Cardiovascular Risk." Frontiers in Endocrinology, 13, 657-664.
33. Hassani, F.V., Shirani, K., & Hosseinzadeh, H. (2016). Rosemary (Rosmarinus officinalis) as a potential therapeutic plant in metabolic syndrome: a review. Naunyn-Schmiedeberg's archives of pharmacology, 389(9), 931–949. https://doi.org/10.1007/s00210-016-1256-0
34. Borges RS, Ortiz BLS, Pereira ACM, Keita H, Carvalho JCT. Rosmarinus officinalis essential oil: A review of its phytochemistry, anti-inflammatory activity, and mechanisms of action involved. J Ethnopharmacol . 2019 Jan 30; 229:29-45. doi : 10.1016/j.jep.2018.09.038. PMID: 30287195.
35. Quirarte-Báez SM, Zamora-Pérez AL, Reyes-Estrada CA, Gutiérrez-Hernández R, Sosa-Macías M, Galaviz-Hernández C, Manriquez GGG, Lazalde-Ramos BP. A shortened treatment with rosemary tea (Rosmarinus officinalis) instead of glucose in patients with type 2 diabetes mellitus (T2D). J Popul Ther Clin Pharmacol . 2019 Dec 3;26(4): e18-e28. doi : 10.15586/ jptcp.v26i4.634 . PMID: 31909576.
36. Bao, T.Q., Li, Y., Qu, C., Zheng, Z.G., Yang, H., & Li, P. (2020). Antidiabetic Effects and Mechanisms of Rosemary (Rosmarinus officinalis L.) and its Phenolic Components. The American journal of Chinese medicine, 48(6), 1353–1368. https://doi.org/10.1142/S0192415X20500664
37. Ahmed, H.M., & Babakir -Mina, M. (2020). Investigation of rosemary herbal extracts (Rosmarinus officinalis) and their potential effects on immunity. Phytotherapy research : PTR, 34(8), 1829–1837. https://doi.org/10.1002/ptr.6648
38. Lazalde-Ramos, BP, Zamora-Pérez, AL, Ortega-Guerrero, AI, Quintero-Fraire, SZ, Palacios-Lara, O., Quirarte-Báez, SM, Galaviz-Hernández, C., Sosa-Macías, M., Ortiz-García, YM, & Morales-Velázquez, G. (2020). Genomic Instability Decreases in HIV Patients by Complementary Therapy

with Rosmarinus officinalis Extracts. Journal of Medicinal Food, 23(10), 1070–1076. https://doi.org/10.1089/jmf.2019.0199

39. Jayanthy G, Roshana Devi V, Ilango K, Subramanian SP. Rosmarinic Acid Mediates Mitochondrial Biogenesis in Insulin Resistant Skeletal Muscle Through Activation of AMPK. J Cell Biochem . 2017 Jul;118(7):1839-1848. doi :10.1002/jcb.25869
40. Labban L, Mustafa US, Ibrahim YM. The effects of Rosemary (Rosmarinus officinalis) leaves powder on glucose level, lipid profile and lipid peroxidation. Inte J Clin Med 2014;5:207 –304.
41. . Jiang, L., et al. (2020). " Rosmarinic acid in rosemary: A potential remedy for improving insulin resistance in type 2 diabetes." *Pharmacological Research* . (Accessed September 20, 2024)
42. Ameer, OZ, et al. (2021). "A review of medicinal plants used for diabetes management: Focus on rosemary." *Journal of Medicinal Plants Studies. (Accessed September 20, 2024)*
43. Baron, DC, Marko, DM, Tsiani, E., & MacPherson, REK (2021). Rosemary extract increases neuronal cell glucose uptake and activates AMPK. Applied physiology, nutrition, and metabolism
44. = Physiologie appliquee , nutrition et metabolisme , 46(2), 141–147. https://doi.org/10.1139/apnm-2020-0014
45. Agatonovic-Kustrin , S., Balyklova , K.S., Gegechkori , V., & Morton, D.W. (2021). HPTLC and ATR/FTIR Characterization of Antioxidants in Different Rosemary Extracts. Molecules (Basel, Switzerland), 26(19), 6064. https://doi.org/10.3390/molecules26196064
46. Tu Z, Moss-Pierce T, Ford P, Jiang TA. Rosemary (Rosmarinus officinalis L.) extract regulates glucose and lipid metabolism by activating AMPK and PPAR pathways in HepG2 cells. J Agric Food Chem. 2013 Mar 20;61(11):2803-10. doi :10.1021/jf400298c.
47. Gonçalves C, Fernandes D, Silva I, Mateus V. Potential Anti-Inflammatory Effect of Rosmarinus officinalis in Preclinical In Vivo Models of Inflammation. Molecules. 2022 Jan 18;27(3):609. doi :10.3390/molecules27030609. PMID: 35163873; PMCID: PMC8840442.
48. Kallimanis , P., Chinou, I., Panagiotopoulou, A., Soshilov, AA, He, G., Denison, MS, & Magiatis , P. (2022). Rosmarinus officinalis L. Leaf Extracts and Their Metabolites Inhibit the Aryl Hydrocarbon Receptor (AhR) Activation In Vitro and in Human Keratinocytes: Potential Impact on Inflammatory Skin Diseases and Skin Cancer. Molecules (Basel, Switzerland), 27(8), 2499. https://doi.org/10.3390/molecules27082499
49. Santos Rodríguez AP, Faria E Souza BS, Alves Barros AS, de Oliveira Carvalho H, Lobato Duarte J, Leticia Elizandra Boettger M, Barbosa R, Maciel Ferreira A, Maciel Ferreira I, Fernandes CP, Cesar

Matias Pereira A, Tavares Carvalho JC. The effects of Rosmarinus officinalis L. essential oil and its nanoemulsion on dyslipidemic Wistar rats. J Appl Biomed. 2020 Dec;18(4):126-135. doi : 10.32725/jab.2020.016. Epub 2020 Nov 6. PMID: 34907765.

50. Zhang, L., & Lu, J. (2024). Rosemary (Rosmarinus officinalis L.) polyphenols and inflammatory bowel diseases: Major phytochemicals, functional properties, and health effects. Fitoterapia, 177, 106074. https://doi.org/10.1016/j.fitote.2024.106074
51. Ghasemzadeh Rahbardar, M., & Hosseinzadeh, H. (2024). Toxicity and safety of rosemary (Rosmarinus officinalis): a comprehensive review. Naunyn-Schmiedeberg's archives of pharmacology, 10.1007/s00210-024-03336-9. Advance online publication. https://doi.org/10.1007/s00210-024-03336-9
52. Akinmoladun , Afolabi, et al. (2020). "Rosmarinus officinalis L. (rosemary) as a potent natural source for managing hyperlipidemia: A review of its molecular mechanisms". *Environmental Toxicology and Pharmacology* .
53. González Madariaga1 Yisel, Boffill Cárdenas María de los Ángeles. Metabolic syndrome in basic research: Its potential treatment from a plant species. Medicent Electrón 2024;28: e4057 ISSN 1029-3043
54. Liu, XY, Wang, WZ, Yao, SP, Li, XY, Han, RM, Zhang, D., Zhao, Z., Wang, Y., & Zhang, JP (2024). Antioxidation Activity Enhancement by Intramolecular Hydrogen Bond and Non-Browning Mechanism of Active Ingredients in Rosemary: Carnosic Acid and Carnosol. The journal of physical chemistry. B, 128(31), 7627–7638. https://doi.org/10.1021/acs.jpcb.4c02949
55. Vijayan, M., & Surya, K. (2017). "The role of herbal remedies in managing diabetes: Focus on rosemary." *Journal of Ethnopharmacology .* Consulted September 20, 2024
56. Alegría Herrera E., Herrera Ruiz M., Román Ramos R., et al. Effect of Ocimum basilicum , Ocimum selloi , and Rosmarinic Acid on Cerebral Vascular Damage in a Chronic Hypertension Model. Biol Pharm Bull. (2019) [Internet]. 2019 [cited 2024 Sep 27]; 2019;42(2):201-211. Available from: https://www.ncbi.nlm.nih.gov/pubmed/30713252
57. Nawaz, Waseem, et al. (2019). "Effects of Rosmarinus officinalis (rosemary) on glucose and lipid levels in patients with type 2 diabetes: A randomized clinical trial." *Journal of Ethnopharmacology*
58. Pérez-Mendoza MB, Llorens-Escobar L, Vanegas-Espinoza PE, Cifuentes A, Ibáñez E, Villar-Martínez AAD. Chemical characterization of leaves and calli extracts of Rosmarinus officinalis by UHPLC-MS. Electrophoresis. 2020 Oct;41(20):1776-1783. doi :10.1002/elps.201900152.

59. Al Jamal A. Effect of Rosemary (Rosmarinus officinalis) on lipid profiles and blood glucose in human diabetic patients (type-2). Afr J Biochem Res 2014;8:147 –50
60. El-Abhar, H.S., & El-Masry, T. (2020). Acute and sub-chronic toxicity evaluation of Rosmarinus officinalis L. in mice. Toxicology Reports, 7, 287-296. doi :10.1016/j.toxrep.2020.04.001
61. Badria , FA, & Fouad, MA (2021). Cardiovascular effects of Rosmarinus officinalis: Beneficial or harmful Phytomedicine, 82, 153436. doi : 10.1016/j.phymed.2020.153436
62. Kollipara, M.P., & Reddy, S. (2022). Dermatological safety of essential oils from Rosmarinus officinalis. Journal of Dermatological Science, 106(1), 65-72. doi : 10.1016/j.jdermsci.2021.09.004
63. Marzocco, S., & Greco, F. (2023). Genotoxicity and carcinogenicity testing of Rosmarinus officinalis L. extracts in vitro. Environmental Toxicology and Pharmacology, 81, 103538. doi : 10.1016/j.etap.2022.103538
64. Almeida, PC, & Costa, JM (2023). Interactions of Rosmarinus officinalis with pharmaceutical drugs: A review of potential risks and benefits. Phytotherapy Research, 37(7), 2228-2237. doi:10.1002/ptr.7714
65. Sussman, J., & Xie, H. (2023). "Metabolic Effects of Type 2 Diabetes: Beyond Glucose Control." Journal of Clinical Endocrinology & Metabolism, 108(6), 1072-1085
66. Kim, D., & Lee, Y. (2021). "Insulin Resistance and Liver Metabolism in Type 2 Diabetes." Diabetes Research and Clinical Practice, 176, 108737.
67. Hunter, L.J., & Bailey, C.J. (2020). "Chronic Inflammation and Insulin Resistance in Type 2 Diabetes." Diabetes & Metabolism Journal , 44(4), 485-494.
68. effectiveness of type 2 diabetes mellitus treatment in Mexico of treatment of type 2 diabetes mellitus in Mexico]. Rev Med Inst Mex Seguro Soc. 2023 Mar 1;61(2):172-180. Spanish.

1. ANNEXES

Annex 1. Informed Consent

Informed consent form for participation in research protocols

Study name :	Evaluation of the clinical metabolic effects of the methanolic extract of Rosemary (Rosmarinus officinalis L.) in patients with Type 2 Diabetes Mellitus . An interventional study
Sponsor external :	Not applicable .
Place and date :	October - November 2025
Registration number institutional :	CEI/ISSSTE/2025/043
Justification and objective of the study:	Type 2 diabetes mellitus is among the top nine causes of death in Mexico. The primary goal of treating type 2 diabetes is to normalize blood glucose levels to prevent or avoid associated complications, regardless of the medications used. However, this is sometimes insufficient to stop these complications, which is why patients turn to medicinal plants. Natural products made from plants remain an important part of traditional medicine, and there has been a recent increase in interest in their use. One of the plants used worldwide for treating various ailments, as well as in food preparation, is *rosemary (Rosmarinus). Rosemary officinalis* contains substances that can be used to treat various diseases, such as type 2 diabetes, and also has hypoglycemic and hypotensive effects. This study will be conducted to determine the antidiabetic activity of a phytomedicine based on methanolic extract of rosemary.
Procedures :	Participants will be randomly assigned to two groups: one receiving only conventional treatment and the other receiving complementary therapy with 1.5g of methanolic rosemary extract for 6 months. An initial medical history will be taken, and blood samples will be collected before starting the rosemary complementary treatment and again at 3 and 6 months of treatment. A 10 ml blood sample will be taken on each occasion.

Possible risks and inconveniences :	The treatment generally does not cause discomfort. If any side effects occur, they should be mild, although a hypersensitivity reaction (a minor or major allergic reaction), vomiting, or diarrhea may develop. If you experience any discomfort, you should stop the treatment and go to your Family Medicine Unit or, if necessary, to the Emergency Room for medical attention.
Potential benefits you will receive by participating in the study:	You will not receive any payment for your participation. The benefits lie in the improvement of clinical and laboratory parameters, as well as offering another therapeutic alternative for your condition.
Information on results and treatment alternatives:	Your progress will be explained to you in a simple and clear way, and if any complications arise, you will receive the necessary medical support. Referrals to other specialists will be provided if needed.
Participation or withdrawal :	The decision to participate in the study is voluntary, and if at any time you decide to leave the study, you may or may not inform us of your reason, which will not be judged negatively and your decision will be respected at all times.
Privacy and confidentiality :	At all times, the information obtained will be treated with respect and handled with the utmost discretion and secrecy by the research team. Furthermore , you will not be identified in any potential [unclear/unclear]. publications .

Declaration of consent:

After having read and having all my doubts about this study explained to me:

I do☐t agree to participate in the study.

I ag☐ to participate and give my authorization for laboratory tests to be taken only for this study.

If you have any questions or require clarification regarding the study, you may contact Dr. Judisett Mirabal Rodríguez, Internist, at 8180269497 or judisettm@gmail.com ; Dr. Sol María Quirarte Báez, at 4929490914 or sol.quirarte@imss.gob.mx ; or Dr. Blanca Patricia Lazalde Ramos, at 84921702977 or lazalderamos@uaz.edu.mx .

_____	_____
Patient 's name and signature	Name and signature of the person obtaining consent
_____	_____
Witness 1	Witness 2
Name, address, relationship and signature	Name, address, relationship and signature

Annex 2 . Initial clinical record

Evaluation of the clinical metabolic effects of the methanolic extract of rosemary (Rosmarinus officinalis L.) in patients with type 2 diabetes mellitus. An interventional study

Instructions:

The purpose of this clinical record is to identify the patients participating in the study.

Please fill in each and every one of the following points in pencil.

1) GENERAL DATA:

Patient's name: _____

Membership Number: _____ Age: _____

Affiliated clinic: _____

Home: _____

Telephone: _____ Telephone 2: _____

Work address: _____

Date: _____

Folio Number: _____

Consultation number: _____

Assigned treatment: _____

Sex:

Female _____ Male_____

Schooling:

1) Primary: Complete____ Truncated____
2) Secondary: Complete____ Truncated____
3) Preparatory: Complete____ Truncated____
4) Degree: Complete____ Truncated____
5) Specialty: Complete____ Truncated____
6) Mastery: Complete____ Truncated____
7) Doctorate: Complete____ Truncated____

Occupation:

3) PERSONAL BACKGROUND

- Gynecological and obstetrical (if female):

Are you currently pregnant? Yeah____ No____

Are you currently breastfeeding? Yeah____ No____

- Past medical history

Do you suffer from any serious illness? No____ If Yes, Please Specify _____

Heart disease? No____ Yeah____

Nephropathy? No____ Yeah____

Liver disease? No____ Yeah____

Cancer? No____ Yeah____

Diabetes mellitus? No____ If ____ Specify type _____

Do you suffer from other illnesses? No____ If Yes, Please Specify _____

Do you suffer from any mental illness? No____ If Yes, Please Specify _____

Do you smoke? No____ Yeah____ Cigarettes per day_____

Do you drink alcohol? No____ Yeah____ Type and frequency_____

Do you use or have you used prohibited substances? No____ Yeah____ Type and frequency_____

Examination with an ophthalmoscope:

Is the patient on a Diabetes Diet? (YES / NO) if YES, previusly: YES / currently NO ( )

Toma antidiabetic drugs taken (YES / NO) Current Treatment: 1) _____ 2) _____

Insulin administered (YES / NO) Type and Dosage 1) _____ 2) _____

Has attended any educational session? (YES / NO)

Capillary blood glucose monitorization performed? (YES / NO) Latest Result ( )

Fundus examination (YES / NO) (A) (M) (D) _____

PHYSICAL EXAMINATION DATA

Weight _____ Kg. _____ Talla _____ Mts. BMI _____ Cms.

Pressure antel in Brazo NO dominante 1) _____ 2) _____) _____ FC _____

Head (eyes, ears, throat, mouth) 1) _____

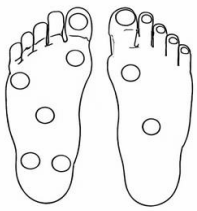
Oral cavity _____

Neck _____ Presente _____ Diminished _____ Absent

Neck _____

Extremities _____

Pedal Pulses	(YES / NO)	(I III / MID)	
Deot pedis	Present _____	Diminished _____	(MII / MID)
Vibration percection	Present _____	Diminished _____	Absent _____
Achilles reflex	Present _____	Ausente _____	(MII / MID)
Fungal infection	Present _____	Ausente _____	(MII / MID)
Keratotic lesions	Present _____	Ausente _____	(MII / MID)
Charcot's foot	Present _____	Ausente _____	(MII / MID)



Diabetic neuropathy signs Other findings: _____

LABS: Gluc _____ Urea _____ Creat _____ HDL _____ LDL _____ LDL _____

Trigl ____ HbA1C _____ EGO _____ Ácid Uric _____

Electrocardiogram ( ) Normal (abnormal

Annex 3. Laboratory sheet

IDENTIFICATION SHEET

Name:_ _____ Folio:_ _____

Number : _____ Date:_ _____

Age._ _____ Sex:_ _____ FUM:_ _____ MPF._ _____

Initial Weight:_ _____ Size:_ _____ BMI:_ _____

3 months Weight:_ _____ Size:_ _____ BMI:_ _____

6 months Weight:_ _____ Size:_ _____ BMI:_ _____

Parameter	Initial	3 months	6 months
Hb			
Hto			
Erythrocyte			
Leukogram			
AST			
ALT			
Tg			
Cholesterol			
Fasting glucosa			
Postprandial glucosa			
Glycosylated Hemoglobin			
Creatinine			
Urea			
Uric Acid			
Final Degree Project		---	
MDA		---	
SOD		---	
IL-6		---	
Triglyceride/glucose index		---	
Triglyceride/HDL-C ratio		---	

Annex 4. Questionnaire about effects adverse

General Patient Information			
Age:_ _____ Sex._ _____			
Have you previously used rosemary extract or related products?	No:_ _____	Yeah:_ _____	Please specify:_ ____ _____
Are you currently taking any medication or supplements?	No:_ _____	Yeah:_ _____	Please specify:_ ____ _____
General Symptoms			
Have you experienced any general discomfort after starting treatment with rosemary extract?	No:_ _____	Yeah:_ _____	Please specify:_ _____ _____
Have you noticed excessive fatigue or unusual drowsiness?		No:_ _____	Yeah:_ _____
Have you experienced fever or chills?		No:_ _____	Yeah:_ _____
Digestive Effects			
Have you had stomach problems, such as nausea, vomiting, or abdominal discomfort?	No:_ _____	Yeah:_ _____	Intensity:_ _____ _____
Have you experienced diarrhea or constipation?		No:_ _____	Yeah:_ _____
Have you had any digestive problems, such as heartburn or acid reflux?		No:_ _____	Yeah:_ _____
Skin Effects			
Have you noticed any skin reactions, such as rashes, redness, or itching?	No:_ _____	Yeah:_ _____	Area:_ _____ _____
Have you experienced any allergic reaction, such as swelling of the face, lips, or tongue?		No:_ _____	Yeah:_ _____
Respiratory Effects			
Have you had breathing difficulties or a feeling of shortness of breath?		No:_ _____	Yeah:_ _____
Have you experienced persistent coughing or wheezing?		No:_ _____	Yeah:_ _____
Effects on the Nervous System			
Have you noticed frequent or severe headaches?		No:_ _____	Yeah:_ _____
Have you experienced dizziness or vertigo?		No:_ _____	Yeah:_ _____
Have you had trouble concentrating or felt confused?		No:_ _____	Yeah:_ _____
Cardiovascular Effects			
Have you noticed any changes in your heart rate, such as palpitations or irregular heartbeats?		No:_ _____	Yeah:_ _____
Have you experienced high or low blood pressure after taking the extract?		No:_ _____	Yeah:_ _____
Other Effects : _____			
Have you had any adverse reactions that were not mentioned in the previous questions?	No:_ ____	Yeah:_ _____	Describe it:_ ___ _____

Have you noticed any changes in your emotional state, such as anxiety, depression, or irritability?	No:_ ____	Yeah:_ _____	Please specify: ____ _____
Frequency and Duration of Symptoms			
How long after starting treatment with rosemary extract did you notice adverse effects?	At the moment:_ _____ After 1-2 days:_ _____ After 1 week or more:_ _____		
How often do you experience the adverse effects mentioned?	Diary._ _____ Weekly:_ _____ Occasionally:_ ____ Only once :_ _____		
Have the symptoms improved, worsened, or remained the same since treatment began?	Improved:_ _____ Worsened:_ _____ Equal:_ _____		
Action Taken			
Have you stopped using rosemary extract due to adverse effects?	No:_ ____	Yeah:_ _____	
Have you consulted a healthcare professional about the adverse effects?	No:_ ____	Yeah:_ _____	
Have you received any treatment to alleviate the adverse effects?	No:_ ____	Yeah:_ _____ Which._ _____	

Annex 5. Patient Instructions (Format for the proper administration of the assigned treatment)

Aimed at patients with type 2 diabetes who voluntarily begin the study to receive treatment with methanolic extract of rosemary

Patient's name: _____

Membership number: _____

Date: _____

Please read the following instructions carefully before starting your prescribed treatment:

1) Do not take any other medication, natural product or remedy for your illness.
2) Your treatment lasts 6 months from today, concluding on the next day: _____.
3) You must not discontinue treatment unless instructed to do so by the responsible physician.
4) Please take the medication at the indicated time.
5) The treatment is taken three times a day, morning, afternoon and evening.
6) Make sure that the medication is always taken at the same time.
7) During treatment you can eat your normal diet and do physical activity.
8) During treatment you can do all your usual tasks.
9) At each appointment, you will be given the necessary amount of medication to complete your treatment until your next appointment.
10) Please attend an evaluation with the responsible physician on the following dates and times:

Appointment 1: Date: _____; Time: _____.
Appointment 2: Date: _____; Time: _____.
Appointment 3: Date: _____; Time: _____.
Appointment 4: Date: _____; Time: _____.
Appointment 5: Date: _____; Time: _____.
Appointment 6: Date: _____; Time: _____.
Appointment 7: Date: _____; Time: _____.

If you have any questions, you can visit the research office from 11:00 AM to 5:00 PM, Monday through Friday, or call 8180269497. You can also email judisettm@gmail.com

Annex 6. MN Questionnaire

Data general			
No. of record:			
Name:			
Date birthdate :			**Age:**
Sex:	**M**	**F**	**Weight and size:**

In case of be women
Last date of menstruation :
Duration of the period menstrual:

Place birthdate :
Home:

Schooling:
Company where he /she works:
Guy of job that performs :
Years working in bliss company:
Jobs Previous:

Background of depression familiar	Yeah	No
Background of diabetes	Yeah	No
Background of diseases cardiovascular	Yeah	No
Background of arthritis rheumatoid	Yeah	No
Background of lupus erythematous systemic	Yeah	No

Background of cancer	Yeah	No	

Background of disorders food				Yeah	No
Consumption tobacco :	Yeah	No	Guy:		
Exposure to the tobacco (passive either asset):					
Age of start (exposure either consumption):					
Time of consume tobacco:					
consumption :					
Packs consumed to the anus :					
Consumption of drugs:	Yeah	No	Which is it:		
Frequency of consumption:					
Consumption of alcohol:	Yeah	No	Guy:		
Frequency of consumption:					
Age to start:	Amount of alcohol (weekly):				
Consumption of vitamins I accessories food:				Yeah	No
Guy: Vitamin (to) (b) (c) (and) (Acid) pholic) Others:					
Yeah remember the brand write bullet					
Consumption of medications:	Yea h	No			
Specify (time, guy and dose):					

Employment of contraceptives hormonal:	Yeah	No	Guy:

Contact with solvents, fertilizers, gasoline:	Yeah	No
Please specify:		