NATIONAL POLYTECHNIC INSTITUTE

School of Medicine

SECTION OF GRADUATE STUDIES AND RESEARCH

"Gene modulation of NLRP3, IL-1β and TNF-α in peripheral blood of patients with exogenous obesity treated with Berberine."

Principal Clinical Research

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ABSTRACT

Background: In the treatment of exogenous obesity more than 800 traditional plants are used, such is the case of *Berberine* a phytopharmaceutical. This phytodrug has been shown to be anti-obesogenic in the Asian population, however its effect on our population is unknown. **Objective:** To determine the effects of *Berberine* on the gene modulation of NLRP3, IL-1β and TNF-α in peripheral blood of patients with exogenous obesity. 120 patients were studied, treated with: hypocaloric nutritional plan, hypocaloric nutritional plan and moderate aerobic exercise, hypocaloric nutritional plan, moderate aerobic exercise plus *Berberine* and finally a normo-weight group without treatment. Anthropometric measurements, biochemical tests, clinical and gene expression data of NLRP3, IL-1β and TNF-α were obtained. The anthropometric measurements, the treatment with *Berberina* obtained, with the exception of ICC, statistically significant results, while in the biochemical results the groups 2 and 3 showed similar results, those treated with Berberina only the VLDL

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1. INTRODUCCIÓN

Obesity is a systemic, progressive, chronic disease of multifactorial etiology, currently the most important and growing public health problem[1]. 1 This pathological condition has been present since remote antiquity, persists and is further increased by genetic and environmental factors, and is currently a pandemic with close links to the main causes of morbidity and mortality[2].

The process of globalization has brought about changes that have modified customs and habits, but above all, our lifestyle. This has led to a considerable increase in chronic non-communicable diseases, such as obesity[3].

According to the Clinical Practice Guideline 'Prevention, diagnosis and treatment of overweight and exogenous obesity', as well as NOM-008-SSA3-2017, for the comprehensive treatment of overweight and obesity, a personalized nutritional plan and physical activity/exercise is the initial basis in the management of this pathology, since lifestyle modification is the basis of treatment. The second option for treatment would be pharmacotherapy and ultimately bariatric surgery, depending on the Body Mass Index (BMI) of each patient[4,5].

Despite the advances in the treatment of obesity that Mexico has made in health in recent years, this continues to be a major problem to be solved in the country. This pathology is due to inadequate eating habits and sedentary lifestyles, which implies increasing the efforts of the population/authorities to reduce its prevalence. Therefore, the study proposes the implementation of a nutritional plan, moderate aerobic physical activity, as well as the use of phytopharmaceuticals as an alternative coadjuvant therapy for the management of obesity, from a clinical, biochemical and molecular point of view.

2. THEORETICAL FRAMEWORK

2.1. Obesity

""Chronic low-grade systemic inflammatory process due to abnormal secretion of cytokines and other inflammatory factors. "[6]

2.2. Definition

Obesity is defined, according to the World Health Organization (WHO), as an abnormal or excessive accumulation of fat that can be detrimental to health, although this concept has changed over time, from excessive accumulation of body fat, greater than or equal to 20% of body weight, greater energy intake, compared to that used, causing an increase in body weight/adipose tissue or as an unfavorable health condition, caused by a positive energy balance maintained over time, characterized by an excessive increase in body fat deposits and body weight [7, 8, 9.]

According to the World Health Organization (WHO), obesity is defined as abnormal or excessive accumulation of fat that can be harmful to health. This concept has changed over time, from excessive accumulation of body fat, greater than or equal to 20% of body weight, greater energy intake, compared to that used, causing an increase in body weight/adipose tissue or as an unfavorable health condition, caused by a positive energy balance maintained over time, which is characterized by an excessive increase in body fat stores and body weight [7, 8, 9]. It is not understood how the concept of obesity has changed. I suggest just leaving the WHO and the most recent or complete. Separate the ideas or definitions with a period.

While NOM 174-SSA1-1998 specifies "a disease characterized by the excess of adipose tissue in the body", on the other hand, the Official Standard for the Treatment of Obesity in Mexico 2010 defines it as "a disease characterized by the excess of adipose tissue in the body, determined with a BMI equal or greater than 30 kg/m^2 and 25 kg/m^2 in subjects with height below average height"[10], determined with a BMI equal to or greater

than 30 kg/m^2 and 25 kg/m^2 in subjects with height below the average height "[10] , and NOM 008-SSA3-2010, for the comprehensive treatment of overweight and obesity, defines it as 'abnormal and excessive accumulation of body fat'. Both, overweight and obesity, are accompanied by metabolic alterations that increase the risk of developing comorbidities such as arterial hypertension, type 2 diabetes, cardiovascular and cerebrovascular diseases, as well as breast, endometrial, colon and prostate neoplasms, among others[11]. Whatever the definition, there are 3 fundamental components that coincide: excess body fat, higher caloric intake with lower energy expenditure and weight gain.

2.3. Epidemiology of Obesity

Obesity has been recognized as a disease by the WHO in 1979 and is considered the greatest public health problem; since 1960 the prevalence of obesity has been increasing, and our country's transition from infectious diseases and malnutrition to having the first place in childhood obesity and the second place in adult obesity in the world, associating this pathology with other chronic non-communicable diseases[12,13].

In 1998, obesity was classified by the WHO as an epidemic, due to the fact that globally, there were more than 1 billion overweight adults and 300 million of these were obese. This epidemic is not restricted to developed societies, in fact, its increase is more rapid in developing countries, such as Mexico where it faces a double challenge together with its malnutrition problems[14].

The WHO reported in 2016, that the prevalence (1975 to 2016) of overweight/obesity worldwide increased to almost double with more than 1900 million adults aged 18 years and older, diagnosed as overweight and of these, more than 650 million are obese. About 39% of adults are overweight (39% men and 40% women); overall, 13% of the world's adult population are obese (11% men and 15% women), in both categories the prevalence is higher in women[15].

The highest prevalence of overweight and obesity was recorded in the adult population in the American continent (overweight: 62% in both sexes; obesity: 26%)[12] , with the United States of America (USA) in first place, with the highest obesity rate in the world, and Mexico in second place with 32%[16].

In the specific case of Mexico, data from the OMENT (Mexican Observatory of Noncommunicable Diseases) show that the epidemiological situation in the country has changed in recent decades[17], since malnutrition and infectious diseases were the public health problems[18]. Currently we are a nation with greater obesity in adults, by 2015 a report by Obesity Update referred that Mexico had the fastest increase in the world in overweight/obesity, for that year Mexico ranked second in adult obesity with 30.7% and it was projected that by 2030 it will increase to 39%[17].

In 2016 ENSANUT reported that the prevalence of overweight and obesity in women increased with respect to 2012, not only in adults but also in girls and adolescents, where the greatest increase was in rural areas. In the case of the combined prevalence of overweight/obesity in adults over 20 years of age, the data indicate that seven out of 10 adults suffer from this disease (72.5%), this percentage increased vs. 71.2% in 2012. The case of adult women was 75.6%, the increase was higher in rural areas (8.4%) vs. urban areas (1.6%). In men, the combined prevalence was 69.4%, with an increase of 10.5% vs. 201219,[20].

When comparing the abdominal phenotype of obesity in adults >20 years of age was 76.6% with a higher prevalence in the female sex with 87.7% compared to 65.4% in men; the highest proportion of this disease is concentrated in groups from 40 to 79 years of age. In this category, the only significant percentage difference with the ENSANUT 2012 was 4.9 points in the case of the female sex. In other words, 72% of adults continue to be overweight or obese [19]. Therefore, there is concern that generations born in recent decades, because of diseases and health damage caused by obesity, may have lower longevity than previous generations[13].

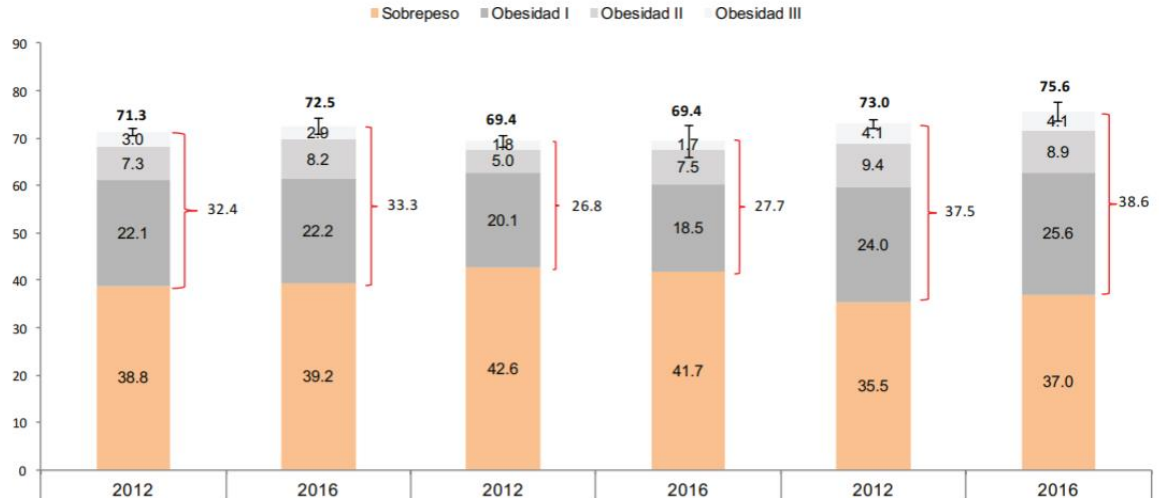

Figure 1: Comparison of the results of Prevalence of overweight and obesity in adult population in the ENSANUT, 2016.

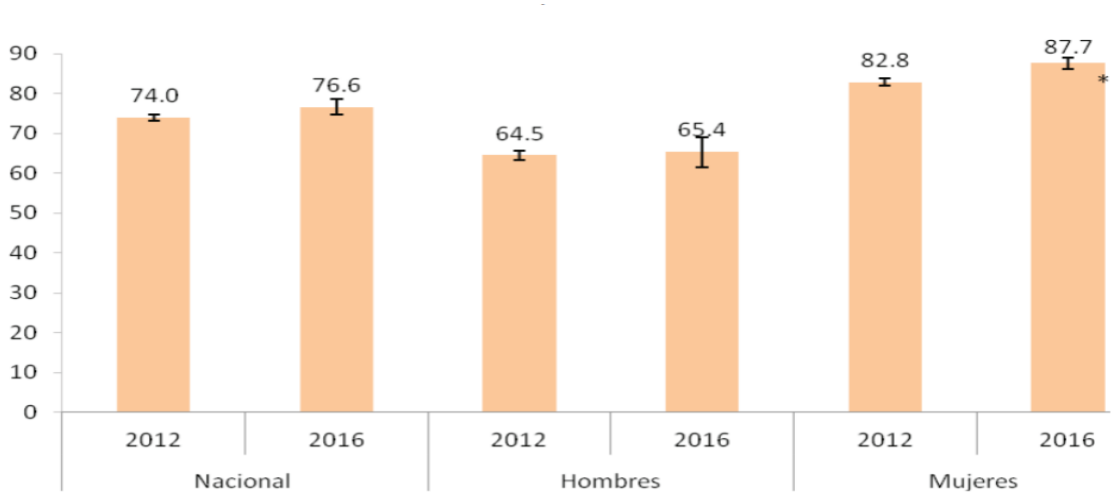

Figure 2: Comparison of the results of Trend of abdominal obesity in adult population in the ENSANUT, 2016.

2.4. Diagnosis and clinical classification

The diagnosis of overweight and obesity should be obtained by anthropometry (weight, height, waist and hip, % body and visceral fat), blood biochemistry, medical clinical history, as well as dietary clinical history[21,22].

The diagnosis of obesity is obtained by classifying the body mass index (BMI), i.e. a person's weight in kilograms divided by the square of the height in meters squared. A person with a BMI equal to or greater than 25 is considered overweight and with a BMI equal to or greater than 30 is considered obese[7].

The Mexican Official Standard 008 SSA3-2016, for the comprehensive treatment of overweight and obesity, makes the modification for the Mexican population according to short stature which, classifies it as a measurement less than 1.50 meters in women and less than 1.60 meters in men[10].

Table 1. Classification of overweight and obesity according to BMI.

Classification	**BMI (Kg/m2)**	**Classification**	**BMI (Kg/m2) Low height**
Normal weight	18.50-24.99		
Overweight	> 25 y <29.9	Overweight	≥ 23 y < 24.9
Obesity		Obesity	≥ 25
Grade 1	30 a 34.99		
Grade 2	35 a 39.9		
Grade 3	≥ 40		

. Table1. The classification of the degree of obesity using the body mass index as a criterion, distinguishing the following degrees referred to in Table 1: Being obesity as BMI equal to or greater than 30, while with short stature that classifies it as a measurement less than 1.50 meters in women and less than 1.60 meters in men referred to in Table 1, according to the Mexican Official Standard 008 SSA3-2016. Source: WHO and NOM 008.

The clinical classification of obesity is derived in two: exogenous and endogenous[23,24,25].

Exogenous: this obesity results from an imbalance between calories consumed and calories expended due to a lack of physical activity, i.e., excessive eating and sedentary lifestyle; exogenous obesity is currently considered the most prevalent not only in Mexico, but in the world[18,26].

Endogenous: caused by metabolic alterations, also called intrinsic or secondary[18,26,27,28].

Body fat distribution is divided into the following types:

Android (abdominovisceral or visceroportal), there is predominance of adipose tissue in the abdominal area, this type is more frequent in males and is strongly related to cardiovascular risk. Gynecoid (femoro gluteal), predominates in the lower abdomen, hips and buttocks and is more common in women[18,29,30]. The interest in the genetics associated with the obesity phenotype is due to the variability between individuals, where fat deposition is controlled by gene expression.

(http://reduvirtualcbs.xoc.uam.mx/obesidad/imagenes/m3_imag1.gif)

2.5. Etiology

Obesity is a multifactorial pathology. Obesity-related habits can be divided into two very important fields: genetic and environmental [31,32,33,34].

The most influential factor in obesity is the environmental factor, where poor nutrition is fundamental due to the imbalance of energy intake and lack of physical activity. Nowadays it is known that there is a direct relationship with energy intake, since currently unhealthy eating patterns such as the consumption of sugary drinks or industrialized foods, where Mexico has the highest per capita consumption of industrialized beverages,

which are the main causes of malnutrition, and although there have been public policies focused on the reduction of this type of products[35], in addition to the intervention in nutritional labeling, tax increase on foods high in sugars, to mention a few[36, 37], so far they have not been able to reduce in a potential way the national obesity prevalence, but in addition this prevalence has been increasing alarmingly.

There are other possible causes for the development of overweight and obesity that are not modifiable, such as age, genetics, race, ethnicity and sex[38]. While there are other modifiable causes such as sociocultural: family environment, economic income, educational level, marital status; biological: number of pregnancies (women); behavioral: smoking, alcoholism, depression, anxiety or some other psychopathologies; and those related to physical activity: daily activities, exercise or sport[39,40].

Among the genetic causes, genes related to the control of energy intake and use have been characterized and studied, in addition to their polymorphisms[40,41,42,43].

In addition, the current lifestyle with insufficient sleep, high stress, excessive use of electronic devices, among others, are the main causes of weight imbalance in Mexicans[44].

2.6. Comorbidities associated with obesity.

Obesity is considered the main risk factor for the development of other comorbidities, among which are[45,46]:

Type 2 diabetes: like obesity, diabetes is more prevalent in women and is more prevalent in urban areas than in rural areas[47,48,48,49,50]. Obesity is related to the causality of type 2 diabetes, with 90% of the prevalence attributed to it, due to the lipotoxicity inherent to prolonged obesity; thus, almost 10% of the Mexican population lives with diabetes, although it is considered that a similar number is undiagnosed. The complications of this disease are the leading cause of mortality in Mexico: every year, 98,000 people die from diabetes worldwide[48,49,50,51,52].

Arterial hypertension (AHT): it is fully recognized that obesity is a cardiovascular risk factor independent of the patient's blood pressure; android obesity is the highest risk factor, not only for AHT but also for increasing cardiovascular risk factors and, once again, the female sex has a higher prevalence [53,54,55,56,57].

Dyslipidemias: an adult with obesity is up to four times more likely to be diagnosed with dyslipidemias, compared to an adult with a healthy weight. In Mexico, the measurements recorded in the last 30 years show a trend towards an increase in overweight and obesity[42,58].

2.7. Pathophysiology of obesity

Obesity, according to its origin, can be classified in two: exogenous and endogenous. Endogenous obesity is related to endocrinopathies, such as: polycystic ovary syndrome, hypothyroidism, hyperinsulinism secondary to pancreatic tumor, Cushing's syndrome, hypogonadism, among others, this represents 5 to 10% (of what population?)[59, 60] .Obesity of exogenous origin is related to epigenetic factors induced by: abuse of high-fat food intake, processed foods, diets rich in refined carbohydrates, sugar-sweetened beverages and alcohol, which promote weight gain. In addition to inappropriate physical activity (sedentary lifestyle).

3. Nutritional plan

Nutritional management of obesity aims to reduce weight by 5% to 10%; this percentage is related to improved health due to a reduction in the comorbidities of this pathology; these benefits are greater with greater weight reduction in dyslipidemia, hyperglycemia and hypertension, and a reduction of at least 10% in weight is required to observe clinical changes, as well as a reduction in mortality of up to 15%. For a correct evaluation of the nutritional plan, individual anthropometric, biochemical, clinical and dietary data should be taken and a specific nutritional plan should be prepared according to the needs and conditions of each patient[62]. For this it is necessary to determine the

basal and total energy expenditure; with the evaluation the nutritional diagnosis is made and the degree of obesity of the patient is known and with this the nutritional plan is elaborated.

3.1 Energy intake

Energy intake is a homeostatic process of body functioning and balances the amount of energy that is stored as fat and its catabolism63; this regulation resides in the hypothalamus where the regulation of body composition is integrated with energy intake and expenditure[64].

Energy expenditure is the energy used and consists of four components: basal metabolic rate, endogenous thermogenesis, physical activity and resting energy expenditure[65].

The basal metabolic rate (BMR) of the individual is the energy expended at complete physical and psychological rest, for the correct functioning of all organs and for cellular respiration, and accounts for 60%-70% of an individual's total energy expenditure. On the other hand, thermogenesis is 10% of total expenditure in sedentary individuals and increases in athletes, reaching 50% of total expenditure in elite athletes[66, 67].

3.2 Energy expenditure

Basal energy expenditure (BEE) is calculated with different formulas, the Harris-Benedict formula being the most used, determined as follows:

$$\text{TMB Woman} = 655 + (9.6 * P) + (1.8 * A) - (4.7 * E)$$

$$\text{TMB Men} = 66 + (13.7 * P) + (5 * A) - (6.8 * E)$$

The total energy expenditure (TEE) is then estimated by multiplying the GEB by the individual's physical activity. The GET indicates how much is required to cover energy needs, the result is complemented with 24-hour recall and weekly food frequency, validated by ENSANUT [65].

The calorie prescription that induces a negative balance in obese patients is from: Kcal/day= GET or GEB-caloric deficit, this deficit is from 50 to 1000 Kcal to decrease 500-1,000 g per week[66].

3.3 Macronutrient distribution

CARBOHYDRATES: is the main base of the diet, they are 50% of the total caloric value, they are composed of (glucose): carbons (6), hydrogens (12) and oxygens (6), carbohydrates are divided into three groups:

Monosaccharides, disaccharides and polysaccharides, the first two are considered simple and polysaccharides, complex; of the total carbohydrates consumed it is recommended that 90% be complex [67-69].

FATS: constitute up to 30% of the total caloric value of the diet, and their composition is carbon, hydrogen and oxygen; the term fats refers to fats and oils in food and are a mixture of fatty acids, which are divided into three:

Saturated, monounsaturated and polyunsaturated, and it is recommended that 7%-9% calories be saturated fatty acids, monounsaturated 15%-18%, and polyunsaturated 7%-9%: (omega 6 ratio of 10:1 and omega 3 of 5:1), and although it is promoted to ingest mono and polyunsaturated fatty acids for their protective effect, currently the intake of fats is higher than recommended with an obesogenic effect [69,70].

PROTEINS: like carbohydrates and fats, proteins have carbon, hydrogen, oxygen and nitrogen and are determined by the biological value of the protein, which can be of low (vegetable origin) or high biological value (animal origin), the importance of this macronutrient lies in its functions of growth, cellular repair, to mention a few, and its recommended consumption is 20% of the total value[69].

3.4 Hypocaloric diet

The hypocaloric diet is the nutritional plan with energy restriction, to generate a negative energy balance and reduce body weight. It is recommended in overweight or obese adults, where the intake of macronutrients, vitamins and minerals is adequate; if the intake is less than 1200 Kcal, it is supplemented to ensure proper nutrition[71].

4. Exercise

The effect of exercise on obesity goes beyond decreasing weight, as it improves anthropometry, biochemistry and clinical, and improves mental health, so it is important to increase physical activity and exercise for greater energy consumption and, therefore, use the body's energy reserve. When implementing exercise should consider the characteristics of the individual to be programmed, be pleasant and consistent with their lifestyle so that they adapt appropriately.

Studies in different populations where physical activity, exercise and nutritional changes were combined obtained good results in the treatment of overweight/obesity[72].

4.1 Moderate aerobic exercise

Moderate aerobic exercise 30 minutes a day, at least 3 times a week increases lipolysis up to 3 times, since fatty acids are released and used as a source of energy and the risk of diseases such as hypertension, diabetes and cancer is reduced, insulin sensitivity increases, triglycerides decrease and cardiac output increases[73, 74].

5. Adipose tissue

Adipose tissue is very important in obesity[67]; white adipose tissue (unilocular) stores triglycerides in a single compartment[68]. It is distributed in subcutaneous adipose tissue

and visceral adipose tissue[69]; adipocytes generate and receive information from the environment and are involved in the chronic inflammation of obesity[76].

In the pro-inflammatory state associated with obesity, the expansion of adipose tissue plays a determining role: adipose tissue increases the production of adipocytokines that mediate pro- and anti-inflammatory and immunological reactions, triggering pathophysiological processes related to inflammation, such as microvascular complications, where pro-inflammatory adipokines are elevated and anti-inflammatory ones are decreased[75,77]. In other words, although individuals are genetically programmed, interaction with environmental factors modulates gene expression.

Cellular components of adipose tissue

Adipose tissue is composed of different cell lines: endothelial, mesenchymal, vascular, fibroblasts, leukocytes and macrophages, the latter being part of the immune system and derived from monocytes formed in the bone marrow[77].

In obesity, inflammatory and metabolic pathways are associated with an increase in the number of resident macrophages in the adipose tissue[78] and can be M1 (classic or pro-inflammatory) or M2 (alternative or anti-inflammatory)[79]. It is not only important how many macrophages there are in adipose tissue, but also their function; thus, M2 macrophages have greater expression of anti-inflammatory genes in lean subjects, whereas in obese subjects there are more M1 macrophages with greater release of pro-inflammatory adipocytokines[77].

Adipose tissue and inflammation

Exogenous obesity is producing an accumulation of free fatty acids resulting from lipogenesis in the form of triglycerides to adipocytes. This accumulation and differentiation of mesenchymal stem cells cause hypertrophy and hyperplasia, inflaming the AT, which in turn causes activation of the first line of immune defense with specialized cells such as:

macrophages and dendritic cells; which recognize endogenous and exogenous ligands, this recognition is performed by Toll-like receptors (TLR's) that activate signaling pathways culminating in the translocation of nuclear transcription factor kappa B (NF-kB) which plays a fundamental role in inflammation. Because the secretion of inflammatory cytokines by activated leukocytes, macrophages, and other tissues are mainly regulated by this signaling pathway, this pathway activates inflammasome proteins which is a mediator of inflammation, that is, inflammation is promoted by TLR's through the production of proinflammatory cytokines and this condition causes an increase in the cellular compartments of the subcutaneous adipose tissue, increasing macrophages. Such infiltration by macrophages derived from circulating monocytes have the function of tissue repair and homeostasis, although due to inflammation such macrophages differentiate under the pressure of the tissue microenvironment into M1 macrophages that secrete a large number of inflammatory mediators, that is, the synthesis and secretion of proinflammatory cytokines are mainly by the macrophage, however, cytokines of the IL-1β family are exceptional. Unlike the rest of the cytokines, these, too, are dependent on NLRP3 inflammasome formation, although these cytokines differ in their initial activation, they converge and coincide with the same inflammatory genes, to summarize; the interaction between proinflammatory pathways (NF-kB) and cellular response to genomic damage, coexist in that microenvironment of the obese AT, where, the deregulated activation of the NLRP3 inflammasome contributes in pathological processes, being the inflammation characteristic of obesity one of the crucial risk factors since plasma levels of certain cytokines such as: NLRP3, IL-1β and TNFα are elevated in obese subjects and are related to their body fat and body weight. On the other hand, total body weight loss has been associated with a decrease in these cytokines[90, 91, 92, 93, 94, 95].

NLRP3

The best characterized inflammasome to date is NLRP3, its gene located on chromosome 1q44, has nine exons; it codes for the 1034 aa cryopyrin protein, the expression of this protein is highly regulated and is affected by pro-inflammatory processes and local environmental factors. During its activation conformational changes are induced to recruit the adaptor protein ACS, and this interacts with procaspase-1 in its inactive form, through

the CARD domain, this domain is present in both proteins. Subsequently, activated caspase-1 is responsible for the maturation of pro.IL1β, to obtain the biologically active form of IL-1β90, [91, 92, 93, 94, 95].

Tumor necrosis factor-alpha (TNF-α):

Obesity-associated inflammation alters the functionality of AT, releasing free fatty acids into the circulation, resulting from TNF-α-favored lipolysis, initiating inflammatory signaling. TNF-α is an adipose tissue protein that is elevated in obesity and contributes to insulin resistance. Its gene is located on chromosome 6p21.3; this gene has a promoter region, four exons and three introns. This cytokine is synthesized by macrophages, T lymphocytes, endothelial cells among other cells, a membrane-bound precursor that exerts an inflammatory biological role locally and depending on the interaction with other cells, and another soluble one that exerts its inflammatory functions at a distance from the cells that synthesize it, the membrane-bound form gives rise to the soluble form of this cytosine (REVIEW HOW TNF IS SYNTHETIZED), the latter is made up of 233 aa and has a molecular weight of 26KDa while TNF-α is made up of 157 aa and has a molecular weight of 17 Kda; Finally, the active form of TNF-α consists of a 51 KDa homotrimer, which can dissociate at low concentrations, thus losing its biological activity[96, 97].

IL-1β:

Like TNF-α, IL-1β initiates inflammatory signaling through its receptors. IL-1β is linked in the pathogenesis of DT2, as it promotes insulin resistance and induces apoptosis and alters the function of β-cells of the islets of Langerhans of the pancreas because of inflammation. In addition, IL-1β promotes the synthesis of TNF-α, which decreases insulin signaling. Saturated fatty acids released into the bloodstream cause macrophages to decrease AMPK (Adenosine Monophosphate-Activated Protein Kinase)[98].

Specifically, IL-1β synthesis is given in the mRNA coding for pro-IL-1β and is subsequently translated by polysomes in the cytosol until activated by IL-1β converting enzyme (ICE). This molecule can be translocated to facilitate its localization at the cell membrane, the converting enzyme is translated in the endoplasmic reticulum as an

inactive precursor, remains in the cytosol and requires two internal anchors (also by ICE) to form an enzymatically active heterodimer. Then, two heterodimers form a tetramer in association with two pro-IL-1 β molecules and anchoring occurs. After anchoring the 17kD IL-1 β is secreted into the extracellular milieu through a membrane channel. IL-1 β (amino acids 1-116) can be found both inside and outside the cell. A small amount of pro-IL-1 β can be transported into intact cells, presumably through the same channel. In the absence of ICE, pro-IL-1ß can be activated by extracellular proteases.

In the specific case of obesity, fatty acid secretion tips the balance towards increased recruitment of M1, secreting pro-inflammatory factors, including TNF-α and IL-1β, among others. This accumulation of M1 macrophages in adipose tissue is the most important source of IL-1β and caspase-1 through the formation of the NLRP inflammasome3 [98, 99, 100].

5. Treatment

According to the Clinical Practice Guide 'Prevention, diagnosis and treatment of overweight and exogenous obesity', the initial evaluation identifies individuals with overweight/obesity, as well as those at risk of suffering from them, who can benefit from weight loss. In the study of obesity, anthropometry, blood biochemistry, clinical and diet, as well as economic, are evaluated to have a detailed overview of the individual and how to make a comprehensive intervention[5].

The Mexican Official Standard NOM-008-SSA3-2017 for the comprehensive treatment of overweight and obesity refers to the set of actions based on the complete and individualized study of the patient with overweight or obesity, and includes medical, nutritional, psychological, rehabilitation, physical activity and, where appropriate, surgical, aimed at changing the lifestyle, reducing health risks, comorbidities and improving the quality of life of the patient[4]. In addition, to treat an overweight/obese individual, the following must be available: Clinical scale with stadiometer and equipment to treat overweight/obese individuals, anthropometric tape, and those established by applicable regulations.

Because the diagnosis of obesity by BMI can be imprecise, other variables in addition to the weight and height of the individual must be taken into account; thus, the Edmonton Obesity Staging System (EOSS) identifies comorbidities and functional limitation in obesity, to have precise therapeutic objectives in individuals with a BMI greater than or equal to 30.[101]

The consensus of the Spanish Society for the study of Obesity 2016 for the prevention, diagnosis and treatment of obesity, the treatment guidelines according to the BMI of the obese individual as another form of intervention in their treatment [102].Currently, Mexico does not have effective treatments against obesity. This is evident since ENSANUT MC 2016 reported an increase compared to ENSANUT 2012, placing Mexico in second place worldwide in adult obesity and first place in childhood obesity, indicating that strategies to reduce this pathology have not been successful; therefore, it is necessary to adapt comprehensive and multidisciplinary treatments that contribute to the intervention in this pathology.

The objective of using drugs is to reduce complications caused by obesity and increase quality of life; thus, the greatest advantage in reducing risk factors is obtained by losing 5-10% of weight in 6 months and attenuating health risks, especially cardiometabolic ones. This indicates that drugs for overweight/obesity are prescribed when there is evidence of non-response to diet therapy, physical activity and exercise, and that in 3-6 months there has been no loss of >5% of weight in individuals with a BMI of 30 kg/m2 with/without comorbidities or in those with a BMI greater than 27 with one or more comorbidities, a drug is used when the aforementioned criteria are met[106].

There are various drugs used for weight loss such as orlistat, and recently the European Medicines Agency approved two new drugs: liraglutide and the combination of bupropion with naltrexone (2015), with side effects: nausea-vomiting, with liraglutide: headaches, dry mouth, nausea and dizziness, with bupropion/naltrexone: CNS alterations in the case of anorexigenics due to its interaction with catecholamines, which is why the implementation of alternative treatments with fewer adverse effects is required[102, 106, 107].

Use of phytopharmaceuticals

The General Health Law in article 224 defines phytopharmaceuticals or herbal medicine as:
"Products made with plant material or a derivative thereof, whose main ingredient is the aerial or underground part of a plant (extracts and tinctures), as well as juices, resins, fatty and essential oils, presented in pharmaceutical form, whose therapeutic efficacy and safety has been scientifically confirmed in national or international literature"[108]. Currently, plants are used in infusion and in capsules, syrups or extracts[109]. While the regulation of health supplies defines:

"Herbal medicines, in addition to containing plant material, may add excipients and additives in their formulation. Those that are associated with isolated and chemically defined active ingredients, or those proposed as injectables, are not considered herbal medicines. In the formulation of a herbal medicine, narcotic or psychotropic substances of synthetic origin, or mixtures with allopathic medicines, procaine, ephedrine, yohimbine, chaparral, germanium, animal or human hormones or other substances that contain hormonal or antihormonal activity or any other that represents a risk to health may not be included"[110]. In this way, the quality of a phytopharmaceutical and its use as an effective medicine for a specific disease can be guaranteed. It must also be supported by medical research that endorses its results[111].

The difference between a phytopharmaceutical and a chemical drug is that the former contains as an active ingredient a plant preparation instead of a synthesized chemical substance[112, 113].

Berberine

Berberine is an alkaloid of Rhizoma coptidis, Cortex pellodendri and Hydrastis canadensis, and is used as an over-the-counter medicine in China to treat infectious

diarrhea, inflammation, diabetes, non-alcoholic fatty liver disease, dyslipidemia, cardiovascular diseases, etc.[114, 115, 116].

Berberine has activity against bacteria, viruses, fungi, protozoa, helminths and chlamydia, with in vitro and clinical phase results. Berberine is antidiarrheal by inhibiting peristalsis and, probably, by its intestinal antisecretory action. Different clinical studies show that Berberine is effective in treating acute diarrhea caused by enterotoxins from E. coli, Vibrio cholerae, among others. It is also more effective than ranitidine for duodenal ulcers. In the cardiovascular system it has positive inotropic, negative chronotropic, anti-arrhythmic and vasodilatory action. The decrease in platelet aggregation and thromboxane B2 could be involved in its anti-ischemic effect; it also has cytostatic activity in vivo and in vitro, stimulates the secretion of bile and bilirubin, improves the symptoms of chronic cholecystitis and normalizes tyramine concentration in patients with liver cirrhosis[110, 117, 118].

It also has anti-tumor effects in many types of cancer, since it suppresses proliferation by inducing apoptosis, inhibiting metastasis, and has very low or no toxicity in healthy tissue and is protective in the liver against chemically induced hepatotoxicity[111,112,113,117,118,119].

Berberine and its interaction with obesity and inflammation

The main component of Rhizoma coptidis is Berberine, it is biologically active and is an over-the-counter drug with multiple properties, among which anti-inflammation stands out, reducing IL-1β, TNF-α, iNOS, ICAM-1, IL-6 and the activation of NF-κB in vivo. Berberine reduces inflammation via TLR4, since it inhibits the activation of SRC and cell mobility by TLR in macrophages stimulated with LPS; in addition, it inhibits the expression of TNF-α, IL-6, TLR 2, TLR 4 and TLR 9 in early-phase sepsis in an animal model[114,120].

The suppression of inflammation by Berberine is a complex phenomenon, the NF-κB pathway plays an important role, since TLR-NF-κB mediate inflammation and could be targeted by Berberine to induce anti-inflammatory activity. Inflammatory stimuli include

TNF-α, in the NF-κB pathway; these factors could be inhibited by Berberine, which would negatively regulate the NF-κB pathway with a decrease in pro-inflammatory cytokines by macrophages and suppression of inflammation[121, 122, 123]. In addition, Berberine and metformin promote glucose metabolism by stimulating glycolysis, which is probably due to the inhibition of the mitochondrial respiratory chain complex, independent of AMPK activation, meaning that this phytopharmaceutical has a hypoglycemic effect[124]. Berberine protects the heart from ischemia/reperfusion injury, since it reduced myocardial injury by decreasing myocardial autophagy and apoptosis via AMPK/mTOR in animals[125].

Berberine interactions with inhibitory and activating genes of inflammation.

In silico Berberine shows a strong binding with TLR4 receptors and other anti- and pro-inflammatory genes, coinciding with results reported in vitro and experimental model.

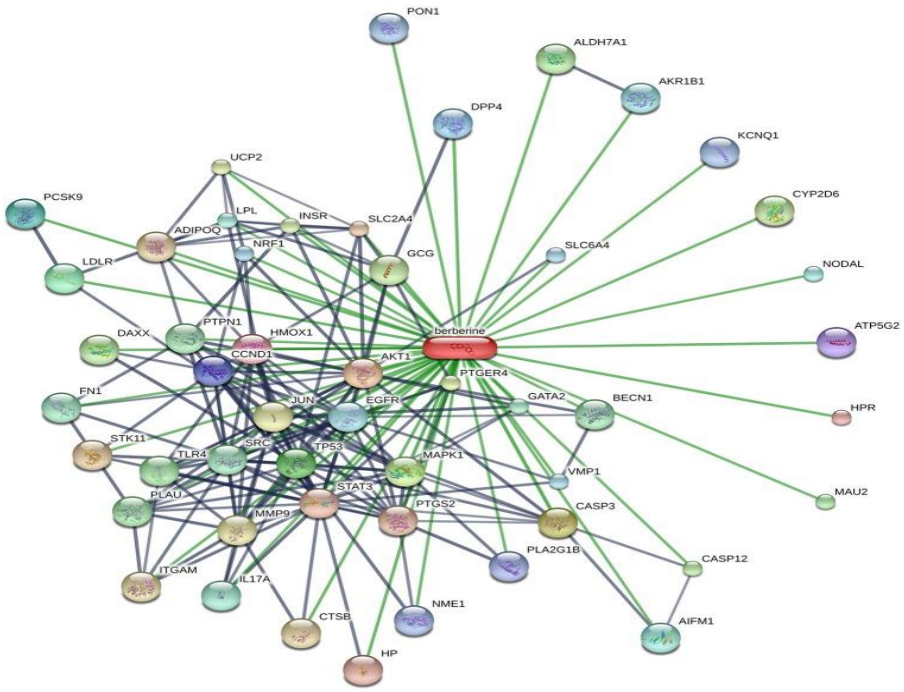

Figure 10. Image taken from the reference: Di Pierro Francesco, the at. Clinical role of a fixed combination of standardized Berberis aristata and Silybum marianum extracts in diabetic and hypercholesterolemic patients intolerant to statins. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy 2015:8. 89-96

6. Justification

The inflammatory process in chronic non-communicable diseases, such as obesity, alters the patient's health, and also promotes tissue damage and insulin resistance, which in turn induce type 2 diabetes or hypertension, to name a few, so the immunomodulatory effect of Berberine may be a viable alternative to reduce this process in exogenous obesity.

7. Problem statement

Obesity is highly prevalent in our country and is associated with chronic inflammation and high morbidity and mortality. Despite scientific advances in its study, the mechanisms that lead to this state are not clear. In obese individuals, prone to comorbidities, the use of additional intervention is justified, and although there are various treatments, the development of new products or combinations of them is increasingly important. Animal studies indicate that Berberine inhibits the production of pro-inflammatory cytokines (IL-1β and TNF-α) although they do not report on NLRP3.

The clinical/molecular evaluation of Berberine in obese individuals will determine whether the phytopharmaceutical has therapeutic potential.

8. Research Question

What is the effect of Berberine on the gene expression of IL-1β, TNF-α and NLRP3, in peripheral blood cells in individuals with exogenous obesity?

9. Objectives

General objective:

To determine the effects of Berberine on gene expression of cytokines IL-1β, TNF-α and NLRP3 in individuals with exogenous obesity.

Specific Objectives:

1. To form groups diagnosed with exogenous obesity.

2. To evaluate anthropometric parameters

3. To evaluate biochemical parameters

4. To quantify the expression (mRNA) of NLRP3, IL-1β and TNF-α

5. To establish the differences in gene expression between individuals with exogenous obesity and control subjects.

10. Hypothesis

Berberine inhibits gene expression of NLRP3, IL-1β and TNF-α in individuals with exogenous obesity.

11. Material and methods

Type of study:

An experimental, analytical, longitudinal, prospective study of comparative groups was carried out.

Study subjects

Ninety patients with type I and II obesity will be selected, as well as 30 control subjects (healthy adults), who agreed to be donors of a peripheral blood sample, 2 tubes (1: biochemistry, 2: gene expression) channeled to the Obesity Program unit (Comprehensive Medical Service, ESM, IPN), with the following selection criteria.

12.1. Selection criteria

Inclusion

Human peripheral blood obtained from adult donors with the following characteristics:

- Patients with BMI ≥ 30kg/m2
- With risk factors for type 2 diabetes (history of diabetic parents or siblings, sedentary habits)
- Fasting blood glucose ≤ 126 mg/dL or glycosylated hemoglobin < 6.5%
- Controlled arterial hypertension
- >18 years of age
- Accept and sign the informed consent

No inclusion

- Hypersensitivity to the components of the formula
- History of hypoglycemic episodes
- History of obesity of endogenous origin
- History of serious psychological, psychiatric, eating or neurological disorders

- History of hyperthyroidism, heart disease, diabetes, liver or kidney failure, glaucoma, epilepsy
- Pregnancy or lactation

Exclusion criteria

- Pregnancy
- Diabetics
- Presenting any serious adverse effect
- Patients who decide to withdraw from the protocol

Elimination

- Samples contaminated during processing
- Lysed blood samples
- Coagulated blood simple.

Variable Description

Independent Variable

Berberine

Category: quantitative.

Measurement scale: ratio.

Measurement unit: 500 mg with excipient q.s.

Operational definition: the tested dose of Berberine was 500 mg, given to patients for 3 months.

Dependent

Gene expression of IL-1β, TNF-α and NLRP3.

Category: quantitative.

Measurement scale: discrete, continuous.

Measurement unit: arbitrary units.

Operational definition: Cytokines were determined with commercial qPCR equipment/reagents, and their arbitrary units/relative expression were determined by a specific standard curve for each one.

Observation method: open.

Sampling method: non-probabilistic sampling of consecutive comparative groups that met the selection criteria, until reaching the sample size that constituted the study groups (patients with exogenous obesity).

Operational description of the study: Berberine has been provided by the Medix laboratory, in 500 mg capsules, to treat patients for 3 months.

Study groups

The individuals will be distributed into four groups:

• Group 1. Individuals with exogenous obesity: nutritional plan intervention for lifestyle change.
• Group 2. Individuals with exogenous obesity: nutritional plan intervention, moderate controlled aerobic exercise for lifestyle change.
• Group 3. Individuals with exogenous obesity: nutritional plan intervention, moderate aerobic exercise and Berberine phytopharmaceutical. With biweekly sessions for 3 months.
• Group 4. Normal-weight individuals without intervention.

Blood samples were taken after informed consent from the participants, they were informed of the intervention and the group to which they belonged, they were also taken at the end of the study.

Procedure

In the selection, obtaining information, anthropometry and blood biochemistry, the subjects of the study will be asked for authorization and their participation will be requested after a detailed explanation of the risks and benefits of the protocol, obtaining signed informed consent.

Obtaining the samples

The universe of the study will be individuals from 19 to 55 years of age with exogenous obesity; they will be recruited in the Obesity Projects area of the Postgraduate Studies and Research section of the Higher School of Medicine of the National Polytechnic Institute, referred by the Comprehensive Medical Service.

Each participant will be asked in writing to enter the study, each one was identified, and a clinical history was prepared with anthropometric measurements: weight, percentage of body fat, percentage of visceral fat, height, BMI. Peripheral blood will also be taken for blood biochemistry and messenger RNA extraction and qPCR tests to determine gene expression of NLRP3, IL-1β and TNF-α.

Biochemical tests.

Peripheral blood samples will be evaluated by an external laboratory.
Blood samples will be collected at the beginning and three months after treatment. Participants were called at 7 am, after fasting for at least 8 hours, and the sample was collected in Vacutainer tubes (BD, USA), added with ethylenediaminetetraacetic acid (EDTA) to prevent coagulation. 4 mL of blood was taken from everyone; from there, 1 mL of Trizol (Sigma-Aldrich, USA) was added to 2 2 mL tubes with 100 µL per tube. The blood was vortexed for 30 s and stored at -70 °C until processing. The rest of the blood was stored at -20 °C.

Anthropometric evaluation

The somatometry of each participant will be determined by wearing light clothing and without shoes: weight, height, % body fat, % visceral fat.

Height

It will be defined as the distance between the vertex and the support plane.

• Instrument: Stadiometer (Seca mod. 208).

• The measurement will be expressed in cm with a precision of 1 mm.

The subject stood up straight, with the heels together and supported on the back stop and so that the inner edge of the feet formed an angle of 60 degrees. The buttocks and the upper part of the back were in contact with the vertical table of the stadiometer. The subject was instructed to breathe deeply without getting up and maintaining the position of the head. The horizontal platform of the stadiometer was slowly lowered until it contacted the head of the subject, applying gentle pressure to minimize the effect of the hair and placing the head in the Frankfort plane.

Weight, % body fat and % visceral fat

• Instrument: Body composition analyzer (InBody770).

• The measurement will be expressed in Kg, with a precision of 0.1 kg.

It will be determined with the body analyzer, programmed with the required parameters (age, sex, height, among others). The subject stood in the center of the body analyzer platform, distributing the weight equally on both legs, without the body being in contact with anything around it, and placed his feet and hands on the equipment electrodes with his arms extended on both sides of the body, until the record was displayed on the screen and monitor.

Body Mass Index

The body mass index (BMI), also known as the Quetelet index, is an index of adiposity and obesity, as it is directly related to the percentage of body fat. The body mass index

was calculated by dividing the weight in kilograms by the square of the height in meters. This index has the advantage that its components (weight and height) can be easily measured. The nutritional status of participants was classified based on international anthropometric indicators, according to the WHO.

Nutritional Plan

There is no unanimity regarding the "ideal diet" because everyone has different anthropometry, blood biochemistry, clinical features, habits, economy, customs, pathologies, family/social interactions, which make standardization impossible. Therefore, based on the initial assessment, the following were prescribed:

"Classic" low-calorie diet: Recommended in most guides and consensus, it establishes a deficit of 500 to 1,000 calories per day, and a balanced distribution of nutrients: 45-55% carbohydrates, 15-25% protein, 25-35% total fat, and 20-40 g of fiber 1, [41].

"Low-calorie" Mediterranean diet: This is the model supported by SEEDO because it represents this balanced and healthy approach, with a low intake of saturated and trans fatty acids and added sugars, and a high consumption of vegetable fiber and monounsaturated fatty acids. Its health benefits, including mortality, are clearly established in the literature.

High-protein diet: Based on the satiating effect and less loss of lean mass. The intake is increased to >1.2 g/kg/day, with a minimum intake of 90 g daily.

Nutritional Management

The nutritional management plan in this project will be aimed at improving the lifestyle and quality of life of patients, as well as reducing their body weight. This intervention will be done according to the nutritional management for obesity developed by nutritionists from INCMNSZ.

The goals of the nutritional plan were to establish healthy eating habits with a 10-15% reduction in body weight during treatment.

Nutritional Consultations

The consultations will be biweekly to evaluate body weight, anthropometry and adherence to the nutritional regimen, in addition to monitoring the modification of eating habits.

Clinical-nutritional history

In this clinical history, the lifestyle of the patients will be evaluate, the activities from the time they will wake up until bedtime will be questioned, their physical activities, allergies or intolerances, eating habits such as: meal times, tastes, preferences, quantities, type of food preparation, economic amount allocated, places of food, if they ate with electronic distractions, among others, to know the needs of each participant and adequately prescribe the food plan.

Food frequency

A food frequency survey from ENSANUT 2012 will be applied to learn about eating habits and determine the consumption of energy, important macro and micronutrients. The same survey analyzes and reports the results when capturing the data. With the evaluation of the diet and the anthropometric, biochemical, clinical, dietary and economic indicators, a nutritional diagnosis and the degree of obesity of each patient were established.

Energy expenditure

After the diagnosis, the prescription of the individualized food plan was prepared. The basal energy expenditure (BEE) was calculated with the Harris Benedict formula, and the current body weight.

While the total energy expenditure (TEE) was estimated from the physical activity of everyone by multiplying the BEE by the physical activity factor, according to the category of each one.

Energy prescription

A negative balance was induced to decrease 1kg of body weight per week, reducing from 500 to 1,000 Kcal per day. The caloric deficit was determined from the TEE for their current weight, the daily energy intake of the participants where the number of calories was calculated having the total energy expenditure minus 20% to obtain homogeneity in all participants.

Dietary distribution and composition.

The nutritional distribution with 50% carbohydrates was recommended, 10% simple carbohydrates, from fruit consumption, while the distribution with 30% fats was recommended, 10% saturated fats, and finally the distribution with 20% protein was half of animal origin and half of vegetable origin. The meal plans were adapted according to the requirements of each participant.

Each meal plan contained sample menus and an additional food exchange brochure, which contained the food groups and their equivalents, depending on whether the participants' weight decreased or the number of portions of each food group was maintained, as well as the calories of each nutritional plan.

The number of mealtimes was distributed in 5: 3 main meals (breakfast, lunch and dinner) and two additional snacks (morning and afternoon).

The lists of the Mexican System of Equivalents and the Institutional Program for the Prevention and Control of Diabetes Mellitus, IMSS, were used to prepare a list of portions

of the most consumed/common foods that were organized into food groups. The objective was to facilitate the participants' practice of new eating habits.

Anthropometric Evaluation

It allowed us to identify alterations in the nutritional status of each participant, as well as the associated risks; the anthropometric variables that were evaluated at the beginning of the program, every 15 days and at the end allowed us to assess the changes in the participants beyond weight loss, granting nutritional plans according to the specific needs for their anthropometric, biochemical, clinical, dietary, economic assessment and according to their family history.

Phytopharmaceutical

Therapeutic indications

A bottle of Berberine of 500 mg with excipient cbp of 102 tablets each was given, as well as a new bottle at each consultation and the participants handed over the empty bottles or with leftover pills. The way they should ingest them was 3 tablets: 3 times a day before each meal.

Treatment adherence log

The patients will give a treatment adherence log to indicate the day and time they took the tablets, as well as if they did not take them.

Adverse effects log

The patients will give a log in which they should indicate if they had any adverse events, to report them.

Controlled Aerobic Exercise

Controlled aerobic exercise on a treadmill, supervised by a sports physician, the frequency with which participants carried out their sessions was 3 times a week for 30 minutes each, at 60-70% of maximum heart rate. Due to the sedentary lifestyle of the patients, coupled

with obesity and its complications, an ‘app’ was installed on the participants’ cell phones (with the exception of two: one did not have the device and one could not read or write, so they were provided with a semi-automated pedometer so that all participants had the same intervention), all were asked if they exercised, which was denied and their usual physical activity was minimal; in the initial phase of the protocol, their usual physical activity was determined (using the ‘app’), which they were instructed to increase, with a goal of 10,000 steps or 150 min per week.

Gene expression

Extraction of total RNA

Blood samples will be lysed, and the material was extracted with TRIzol®Reagent, a mixture of guanidine isocyanate and phenol-chloroform, following the manufacturer's instructions. Once isolated, the total RNA will be suspended in RNase-free water to prevent its degradation and stored at -80°C. 140 µL of peripheral blood were used.

Extraction technique

Sample lysis will be carried out by adding one milliliter of TRIzol reagent and allowing it to incubate for 10 minutes.

1) Separation phase. Phenol-chloroform (0.2 mL per sample) was added, and the mixture was then centrifuged. Three phases were separated: a lower organic phase with the proteins, an interface with the DNA, and a transparent upper aqueous phase with the RNA. This fraction was transferred to a new tube to precipitate the RNA.

2) RNA precipitation by adding 0.5 mL of isopropyl alcohol. Centrifugation at 12,000g for 10 min at 4□C occurs, and the precipitated RNA forms a pellet or adheres to the walls and base of the tube.

3) RNA washing. This is done with 1 mL of 75% alcohol and centrifugation at 7500g for 5 minutes. The pellet is dried by vacuum centrifugation (spin vac pump) until the alcohol evaporates.

4) Re-suspension of total RNA. The samples are dissolved in RNAse-free water, treated with 0.1% diethylpyrocarbonate (DEPC) (nuclease inhibitor). The integrity of the tRNA is checked on an agarose gel. Quantification of total extracted RNA

Sample concentration and purity will be measured by spectrophotometry (NanoDrop2000™, Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). Samples with a purity index (at 260/280nm) of 1.80-2.0 were considered adequate, indicating the absence of contamination.

Complementary DNA synthesis by reverse transcription

cDNA amplification will be performed using the "Transcriptor First Strand cDNA" kit for Rt-PCR from Roche (Roche Diagnostics GmbH, Mannheim, Germany), according to the manufacturer's instructions. 500 µg of the cDNA sample was used in 20 µl volume. For the reaction, the following was done: The cDNA was synthesized from the tRNA of the samples and with the Invitrogen Roche kit (CA USA), in the following reaction mixture:

1) H2O mili Q - 12.5 µL (the volume varies)
2) Buffer 10x MMLV – 2.5 µL
3) 'Random primer' - 0.5 µL
4) DTT 0.1 M—2.5 µL
5) dNTPs mix –1 µL
6) MMLV RT

The volume of the mixture is adjusted to 25 µL and the final product after the reaction in the conventional thermocycler (MasterCycler, Eppendorf) for 1 hour and stored at -20ºC.

Real-time polymerase chain reaction, qRT-PCR

Procedure to determine the relative mRNA expression of genes of interest, using probes from the human transcriptome library (Roche, Library), LightCycler nano thermal cycler and TaqMan reaction mix (Roche Diagnostics). Oligosequences (sense and antisense) were

designed with ProbeFinder software version 2.45 (http://www.universalprobelibrary.com). The reaction mix was prepared according to the manufacturer's protocol with 5 µl of cDNA of the appropriate dilution, in 10 µl. The assay included a standard curve of 5 serial dilutions for each gene, and samples were normalized with the endogenous control β-actin. Each sample was performed in triplicate and data were analyzed with LightCycler nano software.

The thermocycler was programmed with the following scheme: preincubation for one cycle of 10 minutes at 95°C; the reaction for 45 consecutive cycles (denaturation at 95°C for 10 seconds, annealing at 60°C for 30 seconds and extension at 72°C for 1 second) and the final incubation at 40°C for 30 seconds.

Table 5. Oligonucleotide sequences used to determine gene expression.

Gen	Proteins	Sequences	Concentration
NLRP3	Proteína 3 que contien dominios NACHT, LRR y PY	F: 5´ GCA AGA CTT TGA CAA CAT GC3´ R: 3´CAC CTG TTG TGC AAT CTG AAG5´	10nM
TNF-α	Factor de necrosis tumoral alf	F: 5´ CAGCCTCTTCTCCTTCCTGAT3´ R: 3´GCCAGAGGGCTGATTAGAGA5´	10nM
IL-1β	Interleucina 1 beta	F: 5´ TTG GGT AAT TTT TGG GAT CTT AC3 R: 3´CTG TCC TGC GTG TTG AAA GA5´	10nM
B-actina	Gen constitutivo	F: 5´ AGC CAC ATC GCT CAG ACA C3´ R: 3´GCC CAA TAC GAC CAA ATC C5´	10nM

Statistical analysis

The values of each determination are expressed as the mean ± SE. The differences between the groups were calculated with ANOVA. Differences between the groups will be determined by using Student's T test. In all tests, a value less than or equal to 0.05 was considered significant.

Ethical aspects

The study will be of lower than minimum risk, since no blood was obtained from the participants in quantities greater than the limit established by the Regulations for Research on Human Beings of the General Health Law. The project was evaluated,

approved and accepted by the Research and Ethics Committee of the Higher School of Medicine of the National Polytechnic Institute.

The study was carried out in accordance with the ethical principles:

- Declaration of Helsinki of the WMA - Ethical principles for medical research involving human beings[126].
- Written and signed informed consent as stipulated in article 21 of the General Health Regulations on Health Research[127].
- As stipulated in article 17 of the General Health Regulations on Health Research, this project is classified as minimal risk[127].
- The protocol was carried out in compliance with the recommendations established in NOM-087-ECOL-SSA1-2002, which addresses matters related to Environmental Protection and Environmental Health; Biological-infectious Hazardous Waste[128].

Material Resources

The project will develop in the Molecular Biology Laboratory and the Obesity Project, which have the infrastructure for the protocol. The material, consumables and reagents were provided by the responsible researchers, while the Berberine was donated by Medix laboratories.

Financial Resources

The financial support was provided by the researchers' own resources and by the institution. As for the phytopharmaceutical, it was provided by Medix Laboratories.

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