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BIOAVALABILITY OF ANTIOXIDANT COMPUNDS AND EFFECT ON MARKERS OF INFLAMMATION BY INGESTION OF A HIBISCUS SABDARIFFA BEVERAGE WITH GLICEMIC AND INSULINEMIC RESPONSE

Study Protocol

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INTRODUCTION

There are numerous plant foods that are a source of bioactive compounds, which can induce an anti-inflammatory effect on various pathways of inflammatory processes in the body as it may be useful in decreasing markers of inflammation expressed by COVID-19 infectious disease and conditions such as obesity and its comorbidities. Given the above, Hibiscus sabdariffa hibiscus possesses advantages as a potential adjuvant in the management of COVID-19, as studies on the phytochemical properties of H. sabdariffa show that it has several health benefits, and could be used as a potent material for the therapeutic treatment of various diseases. Due to its high content of bioactive compounds, these can exert antioxidant, anti-inflammatory and anticarcinogenic effects, as well as help control blood glucose levels, prevent cardiovascular disease and obesity. In addition, it is a traditional component of the Mexican diet, of common consumption, easy incorporation in the diet, versatility in preparation and national production.

Therefore, to evaluate the bioavailability of bioactive compounds present in a beverage developed from the TECNM/ITESO collaboration, as well as the glycemic and insulinemic response produced by its consumption; besides establishing the effect on some inflammation markers that may be activated as a consequence of the SARS-COV-2 virus infection. This will help to increase knowledge about potential treatment/prevention schemes, avoid the development of severe manifestations of the disease, as well as boost the production and market of a national product.

BACKGROUND INFORMATION

GENERAL CHARACTERISTICS Hibiscus sabdariffa.

Hibiscus sabdariffa L., also known as roselle, karkadé, rosa de jamaica, among other names, is an herbaceous medicinal plant, which in Mexico is better known as jamaica. It is an annual cultivated shrub that belongs to the Malvaceae family (Duarte-Valenzuela et al., 2016), and grows to a maximum height of 3 m, with tri- or pentalobed leaves about 15 cm long, alternating on the stem. Its flowers measure 8 to 10 cm in diameter, are red at the base and pale at the ends, while the calyx is deep red, fleshy and rich in malic acid (Secretaría de Agricultura y Desarrollo Rural, 2021). In Mexico, hibiscus calyxes are used in the preparation of a refreshing drink widely consumed by the population, which gives it both economic and cultural importance (Duarte-Valenzuela et al., 2016), however, according to national production is insufficient and at least 50% of the hibiscus consumed is imported.

In Latin America it is used as a food and source of dietary fiber (Da-Costa-Rocha et al., 2014). Despite being native to Asia, it is also cultivated in many areas, including Central America and Africa. This species is usually cultivated for its fibers and calyxes, and includes three different genotypes: green, red which is the most commonly used type) and dark red (Andzi Barhé & Feuya Tchouya, 2016). Hibiscus calyxes have been recognized as a source of dietary fiber (DF) with antioxidant activity (Sayago-Ayerdi & Goñi, 2010) as well as anti-hypertensive, anti-hypercholesterolemic and anticarcinogenic effects (Lin et al., 2012).

The calyx is commercially important in the food industry for the production of foods and beverages such as tea, juices, jams, jellies and syrups (Borrás-Linares, Fernández-Arroyo, et al., 2015). H. sabdariffa herbal tea is commonly used as the sole ingredient for infusions. In many countries, the leaves are also consumed as a green leafy vegetable (Zhen et al., 2016). In general, there are several reports available on the consumption of the dried or fresh calyxes, seeds and leaves of H. sabdariffa used in the preparation of herbal medicines, beverages and fermented drinks or even cooked or raw (Da-Costa-Rocha et al., 2014). Hibiscus (Hibiscus sabdariffa L.) is an important crop for the tropical areas of Mexico. The area planted in Mexico is 20,061.31 ha, from which 7,538 tons of dried calyxes are obtained (SIAP, 2019), an amount that supplies less than 50% of the national demand; the deficit is imported from countries in Africa and Asia. In Mexico, the main hibiscus flower producing states are: Guerrero, Michoacán, Oaxaca, Puebla and Nayarit (SIAP 2019).

The state of Nayarit is among the top 5 producers nationwide (SIAP, 2019), with a production of 177.60 tons per hectare. Jala, is the municipality that occupies the second place in the whole country with a work in 60 hectares harvested, whose commercialization is propitiated mainly in the neighboring municipalities of Ahuacatlán, Ixtlán del Río, and cities such as Guadalajara and Monterrey. Although hibiscus production is of greater importance in the northern part of Nayarit, specifically in the municipalities of Huajicori, Rosamorada, Ruiz and Acaponeta, the incorporation of its production in the south and center such as La Yesca, Amatlán de Cañas, Ahuacatlán, Santiago Ixcuintla, Tepic and Jala, have made the latter the best positioned with its variety (SIAP, 2019; Cardona; 2020).

FUNCTIONAL PROPERTIES OF COMPOUNDS PRESENT IN *Hibiscus sabdariffa.*

H. sabdariffa is an important source of CF (Patel, 2014). Many studies have shown that H. sabdariffa calyxes contain bioactive compounds (BCs) that are generally believed to be the active components responsible for the therapeutic effects in such extracts, such as organic acids, anthocyanins, flavonoids, and phenolic acids (Sayago-Ayerdi et al., 2021; Ojulari et al., 2019), Table 2.1 presents some of the major FCs that make up the extracts of hibiscus calyxes.

In H. sabdariffa calyxes, their CBs are minority food components, considered non-nutrients, partially bioavailable in the body and have been shown to have various positive effects on consumer health. CF and carotenoids exert their main biological action through mechanisms of antioxidation and free radical sequestration, while the main effect of phytosterols is through the inhibition of intestinal cholesterol absorption (Sayago-Ayerdi & Goñi, 2010).

On the other hand, the biological activities of phenolic compounds in health promotion and disease prevention depend on intake, absorption, transport to target organs and metabolism. Despite claims that the health-promoting benefits of consuming H. sabdariffa extracts are related to the polyphenol content, there have been studies on the pharmacokinetics of H. sabdariffa anthocyanins in humans (Frank et al., 2005). Anthocyanins are a group of phenolic compounds found in a wide variety of flowers and fruits that exhibit a reddish-purple color and have been used as natural alternatives for the replacement of synthetic coloring additives in the food industry. The calyx of H. sabdariffa has several anthocyanins Table 2.1., these manage to competitively inhibit the angiotensin converting enzyme (ACE) such is the example of the inhibition of this enzyme in the lung of a rabbit, in a study with H. sabdariffa being this enzyme the main catalyst for the conversion of angiotensin I to angiotensin II (vasoconstrictor) leading to an increase in blood pressure (BP) (Ojeda et al., 2010). This mechanism of action is supported by

human clinical trials that linked the hypotensive activity of H. sabdariffa extract to ACE inhibition and serum sodium lowering (Beltrán-Debón et al., 2015).

Table 2.1. Flavonoid, anthocyanin, organic acids, phenolic acids and hydroxycinnamic acids content of calyxes of *H. sabdariffa*.

	Bioactives	Content
Organic acids	-Hydroxycitric acid, hibiscus acid, hibiscus acid dimethylester, 3-deoxy-D-lixoheptulosaric acid (DHA), hydroxyethyl ester of hibiscus acid, trimethylhydroxycitric acid	4000±1000 (mg/100 g)
Fenolic acids	- Ellagic acid, ferulic acid, P-coumaric acid, 3-O-caffeoylquinic acid, chlorogenic acid, neochlorogenic acid, methyl digallate, methyl chlorogenate, coumaroylquinic acid, dihydroferulic acid 4-o-glucuronide, 5-O-caffeoylshikimic acid ethyl chlorogenate, gallic acid, protocatechuic acid glycoside, pyrogallol, 3,4-dihydroxybenzoic acid, 3, 4-dihydroxyphenylacetic acid, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid, methyl gallate, 3,4-dihydroxyphenylpropionic acid, 4-hydroxyphenylacetic acid, syringic acid, 3-methoxy-4-hydroxyphenylacetic acid, 5-(3 ', 4' -dihydroxyphenyl)-γ-valerolactone, 4-hydroxyphenylpropionic acid, 3-hydroxyphenylacetic acid, 3-methoxy-4-hydroxyphenylpropionic acid, 3-hydroxyphenylpropionic acid.	2400±300-1000±400 (mg/100 g)
Hydroxycinamic acids	-3-caffeoylquinic acid, coumaroylquinic acid, 5-caffeoylquinic acid, 4-caffeoylquinic acid, methyl chlorogenate, 2-O-trans-caffeoyl-hydroxycitric acid, caffeic acid, isomer of caffeoylquinic acid, coumaroylquinic acid, caffeoyl-methylquinic acid, coumaric acid, caffeoylshikimic acid, feruloylquinic acid, ferulic acid	

Flavonoids	-Quercetin, Luteolin, kaempferol, Myricetin, Epigallocatechin, Glycosides, Hibiscitrin (hibiscetin-3-glucoside), Gossypetin, Kaempherol-3-sambubioside (Leucoside), Myricetin-3-sambubioside, Quercetin-3-sambubioside Quercetin-3-O-glucoside, (Isoquercitrin) Quercetin-3-O-galactoside, Kaempherol-3-O-ruthinoside, Methyl-epigallocatechin Kaempferol-3-glucoside, Kaempherol 3-O-(6″-O-acetyl) glucoside, Syringetin-3-O-glucoside Kaempherol-3-p-coumaroylglucoside. .	419±2 - 2260±70 (mg/100 g)
Antocianidins	- Delphinidin, hibiscentin cyanidin, hibiscin, delphinidin-3-glucoside, cyanidin-3-glucoside, delphinidin-3-sambubioside cyanidin-3-sambubioside.	115 ± 259 (mg/100 g)

Source (Borrás-Linares, Herranz-López, et al., 2015; di Lorenzo et al., 2021; Jabeur et al., 2019; Pimentel-Moral et al., 2018; Widowati et al., 2017)

Therefore, the biological properties of the antioxidants found in hibiscus depend on their bioaccessibility and bioavailability.

This is why in vivo studies of FC bioavailability are becoming more common and necessary, as this allows us to show beyond the detailed characterization of the compounds present in the various extracts. In the case of hibiscus, there are studies that have shown, both in hypercholesterolemic animals and in animals fed a high-fat diet, how the intake of the compounds present influences the decrease in markers of oxidative stress and in expression of satiety hormones (Moyano et al., 2016; Sayago-Ayerdi et al., 2014), On the other hand, it has been until the characterization of hibiscus calyx extracts from improved varieties and the in vitro predigestion of these calyxes, that it has been possible to establish a wide percentage of compounds present in Hibiscus capable of being bioaccessible (Sayago-Ayerdi et al., 2021). In this analysis, 49 compounds were obtained, of which five are organic acids such as Hibiscus, which is found in high concentration in the characterization but, after in vitro digestion, less than half remains, indicating that it may be bioavailable; Something similar happens with hydroxycitric acid, but this bioaccessibility is even more marked in the case of flavonoids such as quercertin-3-sambubioside or kaempferol-3-glucosides, which are not even

detected after in vitro digestion. Likewise, anthocyanins such as delphinidin-3-sambubioside contained in hibiscus are almost totally bioaccessible after digestion (Sayago-Ayerdi et al., 2021). This indicates the potential bioaccessibility of the phenolic compounds present in hibiscus calyxes that are the product of decoction.

BIOAVAILABILITY OF BIOACTIVE COMPOUNDS BY CLINICAL TRIALS

In vitro models provide a first and valuable insight into the transformations of bioactive compounds (BCs) during their gastric processing before reaching the target tissues. Human and animal models have been used to study the bioavailability and metabolism of BCs by in vivo assays. Each model has its advantages and disadvantages, some are the established ethical aspects, the Declaration of Helsinki (Osuna et al., 2016) must be taken into account, in addition to the possible ethical requirements of each institution and country. For human models, the informed consent of the volunteers is required (Fernández-Ochoa et al., 2022). The first step metabolism consists of the bioactive compound crossing the intestinal barrier before reaching the systemic circulation, at this point it is crucial to take into account whether it is an oral intake, whether the CB is free from the food matrix, encapsulated or in emulsion because it has to be able to cross the epithelial barrier. The second step is bioavailability, many researches focus on assessing the bioavailability of CBs, this means the proportion of an administered CB that is able to reach the systemic circulation and is a function of the opposite processes of absorption and clearance and can be calculated by considering the area under the curve (ABC) of the initial concentration of the CB between the amount found in the ABC at the intravenous level. The bioavailability of CBs, mainly phenolic CF compounds, is highly variable and it has been reported, for example, that flavonoids are very poorly absorbed glycosides that require the presence of β-glucosidases enzymes found in the small intestine before being absorbed as aglycones (Ávila-Román et al., 2021).

The main objective of bioavailability studies is to determine the formation of active metabolites from CB after absorption. It is important to evaluate whether metabolism has an effect on their biological activities, rather than the initial CB. Once absorbed in the liver, CBs are subjected to undergo different bioconversion reactions, from which they go on to produce metabolites according to two phases, phase I and II. Within these phases, conjugation reactions such as methylation, sulfation and glucuronidation take place, which facilitate their elimination via the biliary and urinary tract. Methylation reactions are catalyzed by catechol-methyltransferase with S-adenosyl-L-methionine as the methyl donor (Lee et al. 2002). Sulfation is catalyzed by sulfotransferases where 3'-phosphoadenosine-5'-

phosphosulfate is the sulfate group donor (Falany, 1997). CF themselves and their derived metabolites are transported to the liver by portal circulation and can be transferred back to the intestine through the enterohepatic cycle (Figure 2.1.) (Ávila-Román et al., 2021).

The bioavailability of BCs, mainly FCs, can be evaluated by assessing their metabolites in plasma and urine samples after ingestion of them in pure form, injected or as nutraceuticals. It can be assessed indirectly by the antioxidant capacity of plasma after consumption. They can also be evaluated by a single dose by acute assays, where in these assays the concentration of phenols increases in plasma samples transiently, and the capacity of the organism to take up these compounds from the food matrix is reflected. However, it is highly recommended to evaluate the entire diet of volunteers to whom such assay will be conducted and the use of repeated doses (Pandareesh et al., 2015).

The bioavailability and metabolism of phenolic compounds is a key point that needs to be investigated for a better understanding of the biological mechanisms of action, enabling the development of better applications based on these compounds. In recent years there has been an increase in this type of studies, which have succeeded in identifying new bioavailable metabolites in different biological matrices. Phase II metabolism, as well as colon metabolism, is the most prominent of these advances. All these advances have been made possible by the continuous progress of analytical and metabolomic approaches.

. IMMUNOMODULATORY RESPONSE OF BIOACTIVE COMPOUNDS

Inflammation is the response of an organism's immune system to damage caused to its cells and vascularized tissues by bacterial pathogens and by any other biological, chemical, physical or mechanical aggressor (García Barreno, 2008). Inflammation constitutes a non-specific response of the immune system to causative agents of diverse nature, which determine physiological changes that include increased blood flow and vascular permeability with the release of mediators derived from arachidonic acid, modified amino acids and cytokines, which amplify the process, while anti-inflammatory molecules (IL10, IL4, TGFβ, IL13, GC) are regulators of these events. Cytokines are a diverse group of proteins that include monokines, lymphokines, interleukins, interferons, colony-stimulating factors, and chemokines. They are small non-antibody proteins that are synthesized "as needed" during the immune response, usually produced as precursor forms by activated cells that mediate the inflammatory process (Brzustewicz & Bryl, 2015).

Proinflammatory cytokines are referred to as those that promote inflammation, with IL-1, IL-6 and TNF-α being prototypical. They act as endogenous pyrogens, induce the synthesis of secondary mediators and proinflammatory cytokines by macrophages and mesenchymal cells, stimulate the production of acute phase proteins and attract inflammatory cells. The net effect of an inflammatory response is determined by the balance between pro- and anti-inflammatory cytokines (IL-4, IL-10 or IL-13). Activated macrophages produce, in response to pathogens and other damaging stimuli, tumor necrosis factor (TNF), a cytokine with a relative molecular mass of 17 kDa that behaves as a necessary and sufficient mediator of local and widespread inflammation. TNF amplifies and prolongs the inflammatory response by activating other cells which, in response, release cytokines such as interlucin 1 (IL-1) and high mobility group protein 1 and amphoterin (HMGB1), and other mediators such as eicosanoids, nitric oxide and reactive oxygen species, which enhance inflammation and cause tissue injury (Garcia Barreno, 2008). Although it is true that the inflammatory process is essential for the survival of organisms, given the enormous number of existing environmental pathogens, this reaction capacity can often be exacerbated, generating morbidity and mortality due to the generation of a series of diseases. Therefore, it is important to maintain a balance between inflammation and anti-inflammation, where food is a key factor that can contribute to this (Caballero-Gutiérrez & Gonzáles, 2016). Phytochemicals present in food, in their original form or when metabolized, can form active metabolites and behave as intracellular messengers activating or inhibiting the expression of genes involved in inflammatory processes. One of the diets that meets this characteristic is the Mediterranean diet, rich in vegetables, legumes, fresh and dried fruits, cereals, low in saturated fats, constant in wine, olive oil and fish, which promotes the reduction of cardiovascular events, cancer and chronic diseases. The families of the main phytochemicals with anti-inflammatory properties are carotenoids, phenolic compounds, alkaloids, nitrogenous compounds and organosulfur compounds (Caballero-Gutiérrez & Gonzáles, 2016).

In 2019, the World Health Organization announced the existence of the infectious disease called COVID-19, caused by the SARS-CoV-2 virus, after an outbreak in Wuhan, China; subsequently, COVID-19 pandemic was declared on March 11, 2020 (López Pérez et al., 2020). SARS-CoV-2 is the causative virus of COVID-19 disease, which causes a predominantly respiratory syndrome and manifests with a broad spectrum ranging from moderate to severe clinical complications and even death. The pathogenesis of SARS-CoV-2 is complicated and multifactorial and involves both viral and host factors. Currently, nutritional deficiencies have been identified as being related to increased host susceptibility, which places the host in a situation where the pathogen can cause greater damage and progress to more severe levels of disease. Deficiencies of some nutrients are related to the exacerbated response of tissue-resident innate immune cells, which activates phagocytic function and signals triggering the inflammatory cascade. Likewise, malnutrition at the cellular level negatively affects several aspects of adaptive

immunity, including cytokine production, as well as antibody- and cell-mediated immunity of the immune system (Kunnumakkara et al., 2021).

The mechanism of SARS-CoV-2 infection begins when the virus enters the human body and binds to the angiotensin-converting enzyme 2 (ACE2) protein receptor. This group of proteins is widely expressed in the respiratory system, mainly in type II alveoli, oral mucosa, nasal epithelium and other organs. In addition to the respiratory tract, it can (adhere, infiltrate, bind, infect) other organs such as the cornea, heart, kidney, among others. Similarly, the virus binds to the transmembrane serine type II protease (TMPRSS2). A wide expression of this TMPRSS2 protein is reported in the nasal cavity, lungs, colon, gall bladder, kidney, prostate, pancreas, heart, among others and that is why it is susceptible to the virus (Sungnak et al., 2020). The cytokine cascade is positively correlated with the severity of infection, with the magnitude and severity of damage and with the risk of death. On the other hand, elevated levels of same cytokines such as IL-2R, IL-6 and IL-10 have been identified in subjects with COVID-19. In addition, in severe and advanced cases, a gradual reduction in absolute CD4+T, CD8+T, and B-cell counts has been found. A retrospective analysis in patients with COVID-19 and pneumonia demonstrated increased expression in serum samples of IL-6, interleukins, IL-10, IL-2, and interferon, as well as a decrease in CD3+ and CD4+, NK, and CD8+ (Kunnumakkara et al., 2021).

Certain benefits are more noticeable in cultures such as Asian cultures where the consumption of spices and various foods rich in phenolic compounds (PC) is very common in the diet. There are studies that attribute to these compounds the inhibition of infection by preventing the entry of the virus into the organism, such as the case of luteolin and quercertin in cultures of Vero E6 cells with an EC50 of 10μM and 83μM, respectively, where it was found that luteolin presented a high affinity for the protein present in SARS-CoV-S (Zhou et al., 2020). Likewise, another assay with resveratrol, which is found in grape skins, showed that it can modulate the severity of COVID-19 through regulating ACE2 expression and function (Horne & Vohl, 2020). The search for various CF-rich plants is a good alternative as an adjuvant to COVID-19 infection. However, it has the disadvantage that the extracts are not fully characterized to be able to identify the potential that these foods possess. The biological activity of FCs depends on their bioavailability and metabolic rate, as well as their digestive accessibility, which is determined by their release from the food matrix and their transepithelial passage efficiency. Many phenolic compounds can also be dose-dependent and can be recovered in urine in various metabolites (Mateos et al., 2016). This is why hibiscus extracts have ample percentage of bioaccessible compounds after digestion and this indicates that there may be a correlation between immune response and severity of progression with COVID-19 disease. There are numerous fruits, species and plants, as well as isolated bioactive compounds, which are the target of various pathways of

inflammatory processes, and which induce an anti-inflammatory effect in several chronic diseases, such as obesity, dyslipidemias and diabetes mellitus.

HYPOGLYCEMIC EFFECT OF HIBISCUS

One of the physiological mechanisms benefited by the consumption of hibiscus is the hypoglycemic effect that it is capable of producing by the action of its nutritional and functional components, positioning hibiscus as a beverage capable of reducing the incidence and maintaining glycemic control in individuals with type 2 diabetes mellitus (DM2), since it has been stated in various studies that low consumption of fiber and antioxidants, accompanied by high consumption of sugars, are the main causes that lead to its development (Mayasari et al., 2018), together with physical inactivity, excess body weight and genetic inheritance (Uyaguari-Matute et al., 2021).

Of course, Hibiscus sabdariffa L. has been catalogued as a functional food with a great hypoglycemic potential, being this granted by its abundant content of bioactive compounds (Villanueva-Carvajal et al., 2013), mainly organic acids, anthocyanins, flavonoids and polyphenols (Bule et al., 2020).

Among the wide variety of studies focused on the therapeutic properties of hibiscus are in vivo assays. Janson et al., (2021) focused on the glycemic response of obese rats when fed a high-fat diet accompanied by an aqueous extract of hibiscus in two proportions (250 and 500 mg/kg body weight) for 8 weeks, the result of which was a decrease in fasting blood glucose values, serum insulin and the value of the homeostatic model for evaluating insulin resistance (HOMA-IR). They also observed that postprandial glucose levels after 2 h were significantly reduced, in addition to showing a lower area under the curve than that of the control group. Thus, they claim that hibiscus is able to improve insulin sensitivity, which leads to increased glucose uptake in muscle and adipose tissue, thus reducing insulin resistance. A study with similar conditions (experimental model and high-fat diet) showed similarly favorable results, since the dose used of Hibiscus sabdariffa (HS), and the mixture of HS with agave fructans, decreased glucose concentration due to the increase in glucagon-like peptide type 1 (GLP-1) values after supplementation (Moyano et al., 2016).

Among the clinical studies conducted, those where the population is represented by diabetic individuals stand out, where hibiscus tea was administered (4 g. of hibiscus distributed in 4 intakes) was administered to elderly women with hypertension and diabetes (following their pharmacological treatment without modifications) which showed as a result the reduction of fasting blood glucose levels, which is attributable to the reduction of cortisol, it should be noted that this

hormone plays an important role in the homeostatic control of blood glucose by activating gluconeogenesis (Yusni & Meutia, 2020). On the other hand, Sarbini et al., (2019) presented contradictory results to the aforementioned, since the values of blood glucose, fasting insulin, and HOMA-IR, showed no significant differences between the placebo group and the HS group (administration for 8 weeks of 50 mg of pure extract in 2 capsules), although this is attributed to the consumption of antihypertensive drugs and failure in the effective dose. For this reason, it is preferable to perform clinical studies in apparently healthy people, in order to have a clearer answer about the hypoglycemic effect of hibiscus, without the intervention of pharmacological factors involved.

Similarly, Mayasari et al., (2018) focused their research on prediabetic women reporting the decrease in fasting blood glucose levels, after administration of Roselle-Stevia tea bags (5 g and 125 mg, respectively) twice daily for 14 days. Therefore, they assure that the amount provided is the effective dose for the reduction of fasting and two-hour postprandial glucose values in prediabetic individuals.

Likewise, several authors suggest that the antiglycemic effect provided by the existing compounds in hibiscus, may follow several mechanisms including inhibition of α-amylase and α-glucosidase enzymes, decrease of advanced glycation end products in plasma and reduction of oxidative stress caused by hyperglycemia (Bule et al., 2020), it is also able to increase insulin secretion and improve insulin resistance in diabetic individuals (Sarbini et al., 2019).

The aforementioned biological activity is dependent on the bioaccessibility and bioavailability of bioactive compounds, given that a considerable amount of them are bound to dietary fiber, as mentioned by Sáyago-Ayerdi & Goñi (2010) in their research when they named this interaction as "antioxidant dietary fiber", generating a favorable added value for human health when released during gastrointestinal digestion (Moyano et al., 2016). Villanueva-Carvajal et al., (2013) stated that the breakdown of the food matrix (calyxes of Hibiscus sabdariffa) facilitates the exposure of the functional groups of phenolic compounds allowing to exert the antioxidant effect, which they hypothesize may be generated by the high concentration of organic acids, such as hydroxycitric acid and hybisic acid, the above was confirmed after a simulation of gastrointestinal digestion in vitro.

One of the mechanisms by which hibiscus induces a hypoglycemic effect is through the inhibition of the aforementioned enzymes, which are related to carbohydrate digestion. As background we have the study conducted by Gondokesumo et al, (2017) where they point out that at a concentration of 37.5 µM of a methanolic extract of Hibiscus sabdariffa is able to inhibit the action of the enzyme α-glucosidase (IC50: 15.81 ± 0.78 µg/mL) while the mean inhibitory activity of the enzyme α-amylase was 18.09 ± 0.28 µg/mL at a concentration of 34.29 µM, this effect is attributed to the high concentrations of flavonoids in

hibiscus. Another previous study (Alegbe et al., 2019) points out that the aqueous extract of hibiscus administered to diabetic mice at 100, 200 and 400 mg/kg for 14 days inhibited α-amylase and α-glucosidase enzymes considering an IC50 of 490.79 and 475.31 µg / ml, respectively; this fact was reflected in the decrease of blood glucose values. They also performed an isolation of protocatechinic and gallic acids with ethyl acetate, proving that these compounds exert an inhibitory activity of the enzymes in question, besides being able to reduce blood glucose values and oxidative stress. Another relevant aspect is the capacity that these compounds showed for the regeneration of the islets of Langerhans of the pancreas, which coincides with that published by Adeyemi & Adewole (2019) who report that the methanolic extract of Hibiscus sabdariffa L. which they fed to rats with type I diabetes induced β-cell proliferation, as well as pancreatic islet volume, area and diameter, which translated into increased insulin secretion and enhanced glucose homeostatic control. Added to this study, the hibiscus extract reflected an improvement in the maintenance of body weight of the rats, even after the treatment was withdrawn, contrary to the control modulated with insulin protamine zinc, which continued with a reduction in weight when withdrawn.

DIGESTION AND ABSORPTION OF CARBOHYDRATES.

The hypoglycemic effect that hibiscus is capable of producing is related to the digestion and absorption of carbohydrates. These two mechanisms regulate the decomposition of food into small particles capable of crossing the intestinal lumen and reaching the portal system for subsequent distribution in the human body, contributing to a myriad of energy-demanding reactions. In this sense, nutrients are obtained from food through a series of processes in the gastrointestinal tract, where carbohydrates stand out as the major source of energy (Nadia et al., 2021).

Carbohydrates are hydrolyzed by enzymes to produce monosaccharides or disaccharides, which are absorbed in the small intestinal epithelium through passive mechanisms, as in the case of fructose, and active mechanisms, for the absorption of glucose and galactose (de Koning et al., 2020).

Carbohydrates can be ingested in the form of complex structures, such as polysaccharides containing very long carbohydrate chains (starch), as well as simpler structures, such as disaccharides (lactose, sucrose, maltose and trehalose) and monosaccharides (glucose, galactose and fructose) (McQuilken, 2021).

The digestion of this macronutrient is achieved in two stages, the first corresponds to the intraluminal phase, which is mediated by salivary and pancreatic amylase, and the second, by the mucosal phase where an interaction with the apical surface

of the enterocyte occurs (de Koning et al., 2020). Regarding the luminal phase, this begins in the mouth where carbohydrates begin to be assimilated by the reaction carried out by the enzyme α-amylase, or also known as ptyalin, which is produced by the salivary glands, in order to hydrolyze the α (1,4) bonds of linear glucose polymers, obtaining as a product smaller oligomers, such as maltose, maltotriose and α-limit dextrins (Sanders, 2016). It should be emphasized that this enzyme leaves intact α-bonds (1,6), α-terminal bonds (1,4) and also those that are close to branch points. In addition, α-amylase is usually inactivated by the modification of gastric juices on the pH of the stomach, so that carbohydrate digestion is incomplete, continuing until it reaches the small intestine.

As the pH of the chyme is neutralized in the small intestine by the action of bicarbonate, pancreatic α-amylase begins to be released to continue the chemical digestion of carbohydrates.

The products of the previous reactions carried out by the salivary and pancreatic α-amylase enzymes are again hydrolyzed on the apical surface of the enterocytes, although this time the enzyme complexes responsible are glucoamylase, saccharase-isomaltase and glucosidases. Glucoamylase goes on to hydrolyze the α (1,4) bonds at the non-reducing end of maltotriose and α-limiting dextrins, resulting in glucose and isomaltose monomers. The latter is not able to be hydrolyzed by this enzyme due to its inability to break α (1,6) bonds, giving rise to the saccharase-isomaltase enzyme, which is divided into 2 subunits, one of which is saccharase-maltase, responsible for catalyzing the digestion of sucrose and maltose, obtaining as product the monosaccharides that compose it; and the second subunit isomaltase-maltase, in charge of digesting isomaltose and maltose. And finally, there are the enzymes α and β Glucosidase, the first catalyzes the hydrolysis of terminal α-linked D-glucose residues (1,4) next to the non-reducing ends, thus releasing β-glucose. It should be noted that this enzyme is capable of hydrolyzing α (1,6) linkages, thus marking the end of the digestion of the macronutrient of interest. On the other hand, the enzyme β-Glucosidase is responsible for catalyzing the hydrolysis of the β (1,4) linkage of lactose, to obtain glucose and galactose, as well as breaking down glycolipids, obtaining a monosaccharide, such as glucose or galactose, and ceramide (Sanders, 2016).

Regulation of plasma glucose levels.

As a consequence of food consumption, blood glucose levels rise in response to carbohydrate consumption, which is influenced by the type of carbohydrate, the elimination of glucose from the bloodstream and the interaction with other food components (Sanders, 2016), so control systems are required to detect these changes in order to maintain blood glucose within normal values. Some of these

processes is the secretion of insulin and glucagon, these mechanisms are synchronized which allows maintaining stable blood glucose levels during the interprandial period (Pinés Corrales et al., 2020).

Insulin is a polypeptide of 51 amino acids distributed in 2 chains, which are linked by different disulfide bridges (5.8KDa) (Gómez Ayala, 2008). It is synthesized by the β cells of the Islets of Langerhans in the pancreas. Insulin action is mediated by intracellular signaling cascades, mainly by the entry of glucose into the β-cells of the pancreas through GLUT4 transporters (Sanders, 2016), which leads to increased metabolism causing elevation of the ATP/ADP complex, and consequently inhibition of the K+-ATP channel causing depolarization of the cell leading to an influx of Ca2+ and insulin secretion. Once released into the bloodstream, insulin binds to its receptor, which is composed of an extracellular α-subunit, which receives insulin, and a transmembrane β-subunit, which translates the signal through tyrosine kinase. As a consequence of the binding of insulin to its receptor, the signaling cascade is activated, managing to carry out its function by counteracting the action of hormones that cause hyperglycemia, and the regulation of glucose metabolism (Bonilla-Ocampo, 2014). In the presence of hypoglycemia, there are several response mechanisms: the first is the decrease in insulin secretion, the second physiological defense is the secretion of glucagon and the third is the increase in epinephrine secretion (Nares, M., Gonzalez et al., 2018).

Glucagon is a polypeptide made up of 29 amino acids, which is synthesized and secreted by the α cells of the Islets of Langerhans in the pancreas. Its release responds to plasma glucose levels lower than physiological concentrations by stimulating glycogenolysis, i.e. the hydrolysis of glycogen into glucose. Therefore, it is evident that glucagon and insulin have antagonistic effects.

There are extrinsic and intrinsic factors that modify the postprandial glycemic response, and therefore the glycemic index of foods, summarized in the physical form of the food (potato), the preparation and cooking used (raw and cooked carrot), the maturity of the fruits (banana), the production and manufacturing process of the products, the macronutrient mix (joint consumption of proteins and fats), the acidity of the food, as well as the consumption of fiber (Dávila, 2016).

The concept of the glycemic index has been expanded over the years, giving rise to mathematical products such as the glycemic load (GL), which is defined as the quantity and quality of carbohydrates consumed and estimates the total glycemic effect of the diet, for its obtaining it is necessary to multiply the amount of CHO contained in the food ingested by the GI value (GL = GI x available carbohydrates).

The increase to the glycemic load is associated with an unfavorable physiological response, mainly for people living with diabetes, since blood glucose and insulin values rise sharply. Foods with GL ≤10 are considered low GL and foods with GL ≥ 20 are considered high GL.

OBJECTIVES

General objective

To evaluate the in vivo bioavailability of bioactive compounds from a H. sabdariffa beverage and their immunomodulatory potential against moderate inflammation caused by SARS-CoV2 as well as enzymatic inhibition of α-amylase and α-glucosidase enzymes in vitro.

Specific objectives

1. To perform HPLC-DAD-MS characterization of a Hibiscus sabdariffa hibiscus-based beverage.

2. To perform an acute single-blind clinical trial, administering a hibiscus drink to normal-weight adults.

3. To evaluate the bioavailability of organic acids and phenolic compounds in urine and plasma samples present in the H. sabdariffa beverage.

4. To evaluate the immunomodulatory potential of the metabolites identified as bioavailable in plasma samples.

5. To evaluate the enzymatic inhibition of α-amylase and α-glucosidase exerted by the digested, placebo and treatment beverages.

6. To evaluate the in vivo GR and IR of the placebo beverage and treatment beverage.

. GENERAL METHODOLOGY

First Stage

Obtaining hibiscus-based beverage and placebo.

The hibiscus-based beverages (H.sabdariffa) and placebo were obtained from the reformulated company NUTRIYÉ SAPI de CV, located in the city of Guadalajara, Jalisco, Mexico.

Participants.

Twelve normo-weight volunteer participants without any medical condition were included. The invitation to participate was made through ITESO's communications office, as well as through announcements in social networks. They were selected according to the following criteria:

Within the inclusion criteria to be considered are those mentioned in Table 4.2.

Table 4.2. Non-inclusion and inclusion criteria for volunteer selection.

Inclusion criteria		**Non-inclusion criteria**	
Normolipidemia	Total cholesterol (plasma) < 200 mg/L-1	Hypertension	Hyperthyroidism
	Triglycerides < 150 mg/L-1	Diabetes	In diet
	Fasting glucose (plasma) < 100 mg/L-1	Smokers	Pregnant or lactating women
Normoglycemia	18.5 – 24.9	Cardiovascular disease	In medical treatment / supplements
BMI [kg/m2]	18 – 40	Secondary	Hepatic insufficiency

		hyperlipidemia	
Abdominal circumference [cm].	Men<90 Women < 80	Renal insufficiency	Infection / inflammatory disease (6 weeks prior to study)
Fat percentage [%] [cm	Men<19 Women < 33		
Systolic pressure [mmHg].	< 140		
Diastolic pressure [mmHg].	< 90		
Alcohol	Last consumption one week and a half		

The study adhered to the ethical principles for research on human subjects of the Declaration of Helsinki and was submitted to the ITESO Research Ethics Committee.

Acute Trial Clinical Intervention

An acute single-blind trial was conducted. Participants consumed a hibiscus-based drink (H. sabdariffa), or a 60ml placebo drink, and then received a Western-type diet characterized by excessive consumption of red meat, simple carbohydrates, ultra-processed products, colorants, saturated fats, refined sugars and very little intake of fiber and vitamins. After a washout period of more than 6 days, the participants underwent a second intervention with a different product from the first one (beverage to be evaluated or placebo, as appropriate), and the samples were repeated according to the following scheme

The protocol was previously explained to the volunteers and all those who give their written informed consent will be candidates to participate (Annex 1). On the day of the intervention, participants will arrive at 7 a.m., Mexico Central Time, with an overnight fast of 10-12h. They were requested a rest period of 30 min prior to blood pressure taking at the time of admission, in addition to the fact that they had to be seated, upright and with their feet on the floor during the process; the measurement was performed using a manometer on the left arm, elevated at the level of the heart (Gómez-León Mandujano et al., 2016).

Two types of sampling were performed:

(a) Urine.

For determination of phenolic compounds and organic acids in urine samples, a basal sample (T=0) was taken in a collection bottle. Once the hibiscus-based drink or placebo was ingested, samples were collected at the following times: 3h, 6h, 12h and 24h.

b) Blood

For the studies of inflammation levels (IL-6 and TNF-a), and for the bioavailability of phenolic compounds and organic acids, blood samples were taken with the help of collecting tubes by venous puncture. Eight samples were taken, the first on arrival of the patients at basal time (T=B), then at 30 min (T=2), 1h (T=3), 1:30h (T=4), 2h (T=5), 3h (T=6), 4h (T=7) and 6h (T=8) after having ingested the hibiscus-based drink or the placebo.

In both interventions, patients received a Western diet (breakfast and lunch) characterized by excessive consumption of red meat, simple carbohydrates, ultra-processed products, dyes, saturated fats, refined sugars and very little intake of fiber and vitamins, adjusted to their physical characteristics, and supervised by a team of specialist nutritionists. The participants remained at the trial site without any type of physical exercise during the course of the study and in a relaxed environment with selected recreational activities; they also had a water fountain within their reach from which they could drink indefinitely and a team of medical services that was located at the trial site until its completion.

Sample handling.

For biochemical tests, plasma samples were separated by centrifugation at 3000 rpm for 10 minutes at 4 °C and divided into aliquots and stored at -20 °C until further analysis. On the other hand, urine samples were divided into aliquots and frozen at -20 °C until further analysis.

Extraction of phenolic metabolites and organic acids from biological samples.

A liquid-liquid extraction and protein precipitation with acetonitrile and methanol was used to isolate metabolites from plasma following the procedure described by (Day et al., 2001; Gomez-Juaristi et al., 2018). with some modifications. A thawed plasma sample of 400 µl was acidified with 10 µl of 50% (v/v) aqueous formic acid. After shaking the aqueous mixture, it was added dropwise to 900 µl of cold acetonitrile containing 10% (w/v) ascorbic acid and shaken three times for 20 s before centrifugation at 14000 rpm for 10 min at 4 °C. The supernatant was decanted and the pellet was again extracted with 900 µl of methanol. After centrifugation (14000 rpm, 10 min 4 °C), the two supernatants were combined and dried under a stream of nitrogen at 30 °C. The dried samples were resuspended in 150 µl of aqueous formic acid (0.1% v/v) containing 10% acetonitrile acidified with 0.1% formic acid and centrifuged at 4°C for 15 min at 14000 rpm. The final supernatant was collected, filtered (0.45 µm pore size, cellulose acetate membrane filters, millipore) and 30 µL was analyzed by HPLC-DAD-MS. Urine samples were diluted with an equivalent volume of HPLC-grade water and centrifuged at 14000 rpm (10 min, 4 °C). The supernatants were filtered (cellulose acetate membrane filters with a pore size of 0.45 µm, millipore) and a 5 uL aliquot was directly injected into the HPLC-DAD-MS apparatus.

Characterization and quantification of phenolic compounds and organic acids from H. sabdariffa hibiscus-based beverage and biological samples by HPLC-DAD-MS.

The phenolic fraction and organic acids of the H. sabdariffa hibiscus-based beverage and the placebo beverage provided by Nutriyé were characterized. Where an aliquot of each beverage was filtered through a 0.45 µm millipore pore size filter and separately injected under gradient conditions into an HPLC high-performance liquid chromatograph coupled to an Agilent 1260 series mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) equipped with a UV-Vis diode array detector (DAD).

Metabolite identification in plasma and urine samples was carried out in the same system performed by the following methodology according to Mateos et al., (2016), using 30 µl of plasma and 5 µl of urine were injected separately under isocratic conditions in a high-performance liquid chromatograph (HPLC-DAD-MS) coupled to a mass spectrometer (Agilent 1260). Water (Phase A) and acetonitrile (Phase B) acidified with 0.1% formic acid at a flow rate of 0.4 ml/min were used as mobile phase. The phase was initially programmed with 95% of solvent A and then 5% of B. The elution program was increased to 10% of solvent B in 10 min and 60% of solvent B in 5 min, which was maintained for 2 min. The initial conditions (5% of solvent B) were recovered 1 min and maintained for 2 min. The conditions for the mass chromatograph were as follows. Identification of CF and organic acids was carried out using an Agilent 1260 series HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a UV-Vis diode array detector (DAD). Samples were injected onto a Poroshell 120 EC-C18 column (4.6 mm × 150 mm, particle size 2.7 µm) (Agilent Technologies). Detection will be performed in a specific nm range. For MS analysis, an Agilent Quadrupole LC / MS 6120 equipped with an electrospray ionization interface in negative ionization mode will be used with specific conditions of: drying gas flow; nebulizer pressure; gas drying temperature; and capillary voltage. Data analysis was performed using OpenLab CDS ChemStation Edition software (Agilent Technologies, Santa Clara, CA, USA). Characterization was based on retention time and mass spectrometry data. Compounds were first detected using a single MS scan in the specific range (m/z), followed by a targeted search based on peaks showing major signals in the UV-Vis chromatograms. Due to the lack of standards for phase II metabolites, tentative quantification of calibration curves corresponding to phenolic compounds and precursor organic acids was performed. The urine results will be normalized with the volume excreted in each interval studied.

Enzyme inhibition.

In vitro gastrointestinal digestion of the beverages.

Samples will be subjected to an in vitro digestion model adapted from the methodology by Blancas-Benitez et al., (2018). First, the samples will be subjected to an enzymatic hydrolysis process with pepsin at a temperature of 37°C for 1 h (step 1); the phenolic compounds (FC) released in the supernatant at this stage are considered as the FC present in the gastric fraction (GasF). Subsequently, the samples are hydrolyzed with pancreatin 5 mg/ml, P-1750, Sigma Aldrich) and α-amylase (120 mg/ml, A-6255, Sigma Aldrich) (step 2). The CF released at this stage of digestion is considered the CF present in the intestinal fraction (IntF).

α-amylase activity.

The products obtained from the various stages of sample digestion will be used to determine the α-amylase enzyme activity (U/mL) using the kinetic method proposed in the specifications of the amylase activity assay kit (Sigma-Aldrich #MAK009), which consists of taking an aliquot of 50 µL of the various samples and depositing them in a 96-well plate, then mixed with 100 µL of master solution, previously prepared with 50 µL of amylase assay buffer and 50 µL of α-amylase substrate mixture, then incubated at 25°C and at minute 3, the absorbance at 405 nm is measured to obtain the initial time (T-initial) using the Synergy HT microplate reader (Biotek Instruments, Inc. , USA), readings will be taken every 5 min until the values of the samples approach that obtained at the highest concentration of the standard (20 nM/well) giving rise to the T-final. A calibration curve will be performed with Nitrophenol at concentrations of 4, 8, 12, 16 and 20 nM/well. One enzyme unit is the amount of amylase that cleaves ethylidene-pNP-G7 to generate 1 mM p-Nitrophenol per min. The enzyme activity will be expressed as percentage inhibition (I%) and determined with the equation (Eq 6.1):

$$I\% = \frac{(B \times Factor\ de\ dilución)}{Tiempo\ de\ reacción \times V} \qquad \text{Ec 4.1}$$

Where:

B refers to the amount of Nitrophenol generated, reaction time is the T-final minus T-initial in minutes and V denotes the volume of sample added to the well (µL).

α-glucosidase activity.

The inhibition of α-glucosidase of the beverages will be evaluated using the products of the different phases of the in vitro digestion and will follow the methodology described in the specifications of the α-glucosidase kit (Sigma-Aldrich #MAK123) where first 220 µL of deionized water will be placed in a well of a reading plate and in another well 20 µL of deionized water will be placed and 200 µL of calibrator will be added to one of them. From the samples previously prepared in 50 mM phosphate buffer (pH 7. 0) 20 µL will be taken and placed in separate wells and 200 µL of master solution will be transferred to each of them, the solution will be previously prepared with 200 µL of ρ-nitrophenyl-α-D-glucopyranoside (NPG) buffer and 8 µL of NPG substrate, then incubated at 37° C and absorbance will be measured at 405 nm using Synergy HT microplate reader

(Biotek Instruments, Inc., USA), the initial measurement (T-initial) will be read and after 20 minutes the final absorbance (T-final) will be taken.

The enzyme activity will be expressed in units per liter (unt/L) and calculated with the equation (Eq 6.2) where one enzyme unit is the amount of α-glucosidase that catalyzes the hydrolysis of 1 mM ρ-nitrophenyl-α-D-glucopyranoside per minute at pH 7.0.

$$\text{unt/L}) = \frac{(Abs\ final - Abs\ inicial)}{Abs\ del\ calibrador - Abs\ del\ agua} \times 250\ \text{unt/L} \qquad \text{Ec 4.2}$$

Where:

Abs of the calibrator is the value for the calibrator at 20 minutes, Abs of the water represents the value of the water at 20 minutes and unt are units.

Evaluation of glycemic response.

To obtain the biological sample, a lancet will be used to puncture the thumb of the volunteer's finger and the sample will be collected on highly sensitive test strips for capillary glucose measurement of the OneTouch Ultra® System.

The portable system used for pre- and postprandial glucose measurement allows the monitoring of capillary glycemic response under experimental conditions for healthy subjects. With the use of this equipment, measurements will be obtained from t0 (preprandial), and at 15, 30, 45, 60, 90 and 120 min (postprandial). The results will be expressed in mg/dL.

Insulin analysis.

Participants will be required to hold a hand warmer (~40 °C) for 5 min before each capillary blood sampling, to stimulate peripheral blood flow.

Blood samples (approx. 250 µL, 4-5 drops) will be drawn through finger pricks (OneTouch Ultra® lancets). Measurements over time will be similar to the glycemic response assessment technique. Samples will be placed in BD Microtainer SST tubes and kept at room temperature for 30 min. Samples will then be centrifuged

(5000 rpm, 25°C) for 10 min to obtain serum and kept in a freezer at -20 degrees Celsius until further analysis (Hossain et al., 2021).

Serum insulin concentrations will be analyzed using a two-site solid-phase enzyme immunoassay kit (Human Insulin ELISA Kit, Millipore, EZHI-14K), where the direct sandwich technique is used with two antibody solutions targeting insulin epitopes (Figure 6. 2) in such a way that the plate will be coated with a monoclonal antibody (capture) and then the sample will be added, so that the analyte of interest (insulin) will bind with the capture antibody and subsequently a horseradish peroxidase-labeled detection secondary antibody will be added, this antibody will bind to the antigens that will be in turn bound to the capture antibody, consequently will be added the substrate 3,3',5,5'- tetramethylbenzidine in buffer (TMB), forming a bluish coloration dependent on the insulin concentration, the reaction will be terminated by the addition of stop solution (HCl) obtaining as colored product (yellow) after acidification, which is quantifiable at 450 nm in a microplate reader (BioTek®, Synergy HT, Winooski, Vermont, USA). The results will be expressed in µU/L.

Statistical Analysis.

Statistical analysis: first stage

The normality of the data distribution will be evaluated by means of the Shapiro-Wilk test; if so, a Student's t-test will be used, or, on the contrary, a nonparametric Mann-Whitney test will be used to evaluate the statistical differences between the variables. The level of statistical significance will be a value of ($p<0.05$). Statistical analysis will be performed with the STATISTICA program, version 10 (Statsoft.1984-2011 Inc., Tulsa, OK, USA).

Statistical analysis: second stage

Each sample will be considered as an experimental unit. Statistical analysis will be performed with the STATISTICA program, version 10 (Statsoft. 1984-2011 Inc., Tulsa, OK, USA). Differences in the response variables in the samples will be evaluated through a repeated-means ANOVA analysis. The level of statistical significance will be a value of ($p< 0.05$).

Calculation of the area under the curve.

The glycemic and insulinemic values obtained experimentally will be recorded in a database. The area under the curve (AUC) will be calculated according to the equation proposed by Wolover and Jenkins (1986) (Eq.4.7.):

$$AUC = \left(\left(A + B + \frac{D}{2}\right) * 15\right) + \left(\left(\frac{D}{2} + \frac{E}{2}\right) * 30\right) + \left(\left(\frac{E^2 * 30}{2} * (E + F)\right)\right) \quad \text{Ec 4.7.}$$

Where A, B, C, D, E, F; represent the increase in glucose and insulin above baseline and 15 and 30 represent the time interval between blood samples. Any area below the fasting level will be discarded.

EXPECTED RESULTS

This research aims to identify which metabolites are bioavailable in plasma and urine samples after ingestion of a hibiscus drink and to identify inflammation markers in the same normal-weight volunteers, as a potential adjuvant in the management of inflammation caused by COVID-19 disease, and generate a hypoglycemic effect by reducing the levels of glucose and serum insulin, consequent to the generation of possible modifications on the physiological mechanisms, including the inhibition of the activity of the α-amylase and α-glucosidase enzymes by the action of the bioactive compounds, as well as by the interaction with the rest of the ingredients that compose the beverage of interest.

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