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CARACTERIZACIÓN FISICOQUÍMICA Y EVALUACIÓN DEL EFECTO ANTIOBESIDAD DE LAS PLANTAS COMESTIBLES: *Malva parviflora Y Myrtillocactus geometrizans*

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i. Abreviaturas

ABTS: 2,2´-azino-bis-(3-etilbenzotiazolin-6-sulfónico]

DPPH: 1,1 difenil-2-picrilhidrazil

CAM Terapias complementarias y alternativas

OMS: Organización de la Salud

FAO: Organización de las Naciones Unidas para la Alimentación y la agricultura

IMC: Índice de masa corporal

OECD: Organización para la Cooperación y el Desarrollo Económicos

POMC: Proopiomelanocortina

NPY: Neuropéptido Y

AgRP: Péptido relacionado con la proteína Agout)

MSH: α−melanocitos

TSH: Hormona estimulante de la tiroides

CRH: Hormona liberadora de corticotropina

MCH: Hormona concentradora de melanina

UGT: glucuronosiltransferasas

SULT: sulfotransferasas

NOM: Norma Mexicana

DM2: Diabetes tipo 2

DM1: Diabetes tipo 1

MC3/4R: receptores de melanocortina 3 y 4

CART: Transcripción relacionada con la cocaína y las anfetaminas

FDA: Alimentos y administración de drogas

FD: Fibra dietética

COFEPRIS: Comisión Federal para la protección contra riesgos sanitarios

SNC: Sistema Nervioso Central

SAHOS: Síndrome de apnea-hipopnea obstructiva

COMT: Catecol O- metiltransferasa

C/EBPá: receptor

PPARα: Proliferador de peroxisomas alfa

ERK: signal-regulated kinase

JNK: cinasas aminoterminales c-Jun

AMPK: Proteína kinasa activada

CBG: ®- glucosidasa citosólica

UDP: Glucoronosiltran

I. Resumen

México se caracteriza por tener una gran variedad de plantas de origen silvestre que son comestibles. La *malva parviflora* L., se caracteriza por ser una planta herbácea y perenne, está conformada de hojas de pecíolo largo, reniformes y onduladas y cuenta con florescencias color lila claro. Y por otra parte se encuentran las inflorescencias comestibles conocidas como flor de garambullo, las cuales se caracterizan por ser de color blanco/rosa, que se desarrolla en la parte superior de las cactáceas conocidas como garambullo (*Myrtillocactus geometrizans)*. Por lo tanto, el objetivo de este trabajo fue determinar las características nutrimentales, antioxidantes y su efecto anti-obesidad *in vitro* e *in vivo* de las plantas *Malva parviflora y Myrtillocactus geometrizans,* para su utilización en la alimentación o como nutracéutico.

Las características generales sobre la malva y la flor de garambullo fueron presentadas en una revisión bibliográfica sobre las plantas de estudio (*Malva parviflora y Myrtillocactus geometrizans*). En otro documento se revisó la importancia de la presencia de compuestos bioactivos presentes en las plantas comestibles y su efecto sobre la obesidad.

Para conocer la influencia por el cocinado en las plantas se realizó un análisis proximal, determinación de fibra dietética, compuestos fenólicos y capacidad antioxidante (DPPH• ABTS•+ y FRAP), así como propiedades fisicoquímicas (color, CRA, CRA, CH, IRG y MEB) de las muestras liofilizadas de las plantas. De acuerdo con los resultados las plantas presentaron una importante composición nutricional relacionado a las proteínas, fibra dietética y contenido antioxidante. Sin embargo, tras el tratamiento térmico, este provocó cambios en los compuestos solubles (carbohidratos y compuestos fenólicos totales), capacidad antioxidante, propiedades fisicoquímicas. Además, se determinó una alta retención de compuestos fenólicos y vitamina con la matriz del alimento, contribuyendo con una alta capacidad antioxidante (DPPH, ABTS y FRAP).

Asimismo, la administración de un extracto o liofilizado de la malva mantiene efectos positivos sobre la obesidad como es la disminución de peso, mayor tránsito intestinal. En los estudios histológicos se encontró que el tejido hepático no presentó daños, mientras que la hipertrofia presentada en las células adiposas fue revertida por la administración del extracto y liofilizado de la malva. Es importante mencionar que se requieren de más estudios preclínicos que ayuden a afirmar el efecto que mantiene la malva en la obesidad. Además de realizar estudios preclínicos con la utilización de flor de garambullo

II. Abstract

Mexico is characterized by having a wide variety of plants of wild origin that are edible. The *Malva parviflora L.*, is characterized by being an herbaceous and perennial plant, is formed of leaves of long petiole, reniform and wavy and has light lilac florescence. And on the other hand, there are the edible inflorescences known as garambullo flower, which are characterized by being white/pink, which develops on top of the cacti known as garambullo (*Myrtillocactus geometrizans*). Therefore, the aim of this work was to determine the nutritional characteristics, antioxidants and their anti-obesity effect *in vitro* and *in vivo* of the plants Malva parviflora, Myrtillocactus geometrizans, for use in food or as nutraceutical.

The general characteristics of mallow and garambullo flower were presented in a literature review on study plants (*Malva parviflora* and *Myrtillocactus geometrizans*). Another paper reviewed the importance of the presence of bioactive compounds present in edible plants and their effect on obesity.

To determine the influence of cooking on plants, a proximal analysis, determination of dietary fiber, phenolic compounds and antioxidant capacity (DPPH• ABTS•+ and FRAP) was performed, as well as physicochemical properties (color, CRA, CRA, CH, IRG and MEB) of freeze-dried plant samples. According to the results, the plants presented an important nutritional composition related to proteins, dietary fiber and antioxidant content. However, after heat treatment, this caused changes in soluble compounds (carbohydrates and total phenolic compounds), antioxidant capacity, physico-chemical properties. In addition, a high retention of phenolic compounds and vitamin with the food matrix was determined, contributing with a high antioxidant capacity (DPPH, ABTS and FRAP).

Also, the administration of an extract or freeze-dried mallow maintains positive effects on obesity such as weight loss, greater intestinal transit. Histological studies found that liver tissue did not present damage, while hypertrophy presented in adipose cells was reversed by the administration of the extract and freeze-dried mallow. It is important to mention that more preclinical studies are needed to help affirm the effect that mallow maintains on obesity. In addition to preclinical studies with the use of garambullo flowers.

III. Introducción

Dentro de la diversidad botánica de México, se tienen plantas que normalmente crecen de manera silvestre en los alrededores de sembradíos o como hierbas, siendo consideradas plagas. Sin embargo, muchas de estas plantas han sido consideradas como comestibles y adoptadas como parte de la alimentación de las poblaciones, que además contribuyen a efectos benéficos en pro de la salud, considerándose con potencial alimentario importante. Ejemplos de estas plantas son la malva (*Malva parviflora*) y la flor de garambullo (*Myrtillocactus geometrizas*). La malva pertenece a la familia de las malváceas (25-30 especies), la *Malva parviflora* comúnmente llama malva, malva de quesitos, malva de campo, malva de castilla, malva alta, malva rosa, malva real, y en inglés mallow. Por su parte las malvas se distinguen por su aporte en fibra dietética, proteínas, hidratos de carbono, ácidos grasos (oleico, linolénico), además de presentar en su composición mucílagos, minerales (Fe, Zn, F, Mg, K, N y Mn). Además, en esta planta se han identificado fitoquímicos (flavonoides, ácidos fenólicos, carotenoides, terpenos). Por otra parte, la flor de garambullo, es una inflorescencia que crece de una cactácea llamada garambullo, esta flor es poco estudiada, sin embargo, se ha encontrado que presentan nutrientes como hidratos de carbono, proteínas, lípidos, fibra dietética y minerales (N, F, Ca, Mg, Fe, Cu, Zn y Mn). Además de la composición general de la flor está constituida de compuestos bioactivos (ácidos fenólicos, flavonoides, terpenoides, taninos y saponina).

La evidencia científica con estudios preclínicos con malva ha demostrado beneficios como: antioxidantes, antiinflamatorios, anticancerígenos, antimicrobiano y antidiabético. Mientras que *Myrtillocactus geometrizans* (cactus) solo se ha demostrado tener efecto anticancerígeno y antiinflamatorio.

Por tal motivo, la ejecución de la investigación se sitúa en obtener información acerca del potencial uso en estudios *in vitro* e *in vivo*, permitiendo la revalorización de las plantas silvestres.

IV. Justificación

La obesidad es la enfermedad nutricional más frecuente y un problema creciente de salud pública a nivel mundial como nacional. Además, se considera como un precursor de enfermedades subyacentes que reducen la esperanza de vida entre la población.

Las estimaciones mundiales realizadas marcan que el 39 y 40 % en hombres y mujeres respectivamente, mostraron sobrepeso y del 11 al 15 % presentaron obesidad recalcando que la población con sobrepeso desarrollará obesidad. Mientras que en la niñez 340 millones de niños y adolescentes tienen sobrepeso y obesidad. México cuenta con el 73 % de su población con sobrepeso y el 34 % padece obesidad mórbida. La proyección estimada para el 2030 reporta que el 39 % de la población será obesa. Por otra parte, la obesidad infantil ha ido incrementó del 7.5 al 15 % durante el 2016. Todo esto debido a diversos factores entre los que se encuentra la transición alimentaria que encamina a una alimentación hipercalórica alta en azúcares, grasas saturadas, con alimentos industrializados, alimentos de origen animal, con un escaso o incluso nulo consumo de vegetales convencionales o plantas silvestres incluidas en la alimentación mexicana, lo que conlleva hacia un camino seguro al desarrollo de obesidad entre la población.

Los tratamientos en la obesidad son de tipo quirúrgico y farmacológicos que benefician la regulación del peso y eventos. Sin embargo, son costosos y han mostrado efectos secundarios graves.

La realización de investigación básica y aplicada en estos productos, se orienta en obtener información sobre su potencial de utilización ante estudios *in vitro* e *in vivo*, permitiendo la revalorización de las plantas silvestres.

V. Objetivo general

Determinar las características nutrimentales, antioxidantes *in vitro* y efecto anti-obesidad *in vivo* de las plantas *Malva parviflora y Myrtillocactus geometrizans,* para su utilización en la alimentación o como nutracéutico

VI. Objetivos específicos

1. Analizar información sobre las plantas de estudio (*Malva parviflora, Myrtillocactus geometrizans*), para la construcción de una compilación de antecedente generales de las plantas de estudio

2. Analizar y recabar información actualizada acerca de los compuestos bioactivos presentes en plantas comestible y su efecto en la obesidad

3. Evaluar la influencia del cocinado en las plantas:

a) Análisis proximal, fibra dietética
b) Compuestos fenólicos y capacidad antioxidante (DPPH$^{\bullet}$ ABTS$^{\bullet+}$ y FRAP)
c) Propiedades fisicoquímicas (color, CRA, CRA, CH, IRG y MEB) de las muestras liofilizadas de las plantas

4. Evaluar en el efecto térmico del extracto del liofilizado de las plantas:

a) Compuestos fenólicos totales y actividad antioxidante
b) Identificación y cuantificación de compuestos fenólicos y ácido ascórbico

5. Evaluar en un modelo obesogénico en ratas Wistar, el efecto de la administración del liofilizado de la hoja de malva y extracto hidroalcohólico de hoja *Malva parviflora* sobre parámetros

a) Antropométrico (peso, longitud, IMC e índice de Lee),
b) Índice glucémico
c) Estrés oxidativo
d) Tránsito intestinal,
e) Histopatología del hígado y tejido adiposo

VII. Diseño metodológico

El diseño metodológico se describe para fines de la investigación considerando los 5 objetivos planteados en este trabajo (figura 1).

El primer objetivo y segundo objetivo del presente trabajo fue llevar a cabo el análisis de información sobre las características generales y antecedentes de las plantas de estudio *Malva parviflora, Myrtillocactus geometrizan.* Y en segundo término sobre los compuestos bioactivos presentes en plantas comestibles y su efecto fisiológico en el padecimiento de la obesidad. Como objetivo 3 se evaluó la influencia del cocinado en las plantas. Las muestras crudas y cocidas de las plantas de estudio se liofilizaron, y homogeneizaron (500 mm). Se almacenaron en bolsas herméticas resguardadas de la luz y a una temperatura de -30°C, hasta su análisis. Las determinaciones químicas posteriores realizadas fueron el análisis proximal y fibra dietética, compuestos fenólicos y capacidad antioxidantes ((DPPH• ABTS•+ y FRAP), además de las propiedades fisicoquímicas (color, CRA, CRA, CH, IRG y MEB) de las muestras.

Para el cumplimiento del objetivo 4 se evaluó el efecto térmico del extracto del liofilizado de las plantas sobre la retención de compuestos fenólicos totales y actividad antioxidante e identificación y cuantificación de compuestos fenólicos y ácido ascórbico.

El objetivo 5 fue realizado con un análisis preclínico con la muestra de malva (liofilizado y extracto hidroalcohólico), durante esta fase se determinó la DL_{50}, parámetros antropométricos, índice glicémico, estrés oxidativo, transito gastrointestinal e histopatología de hígado y tejido adiposo.

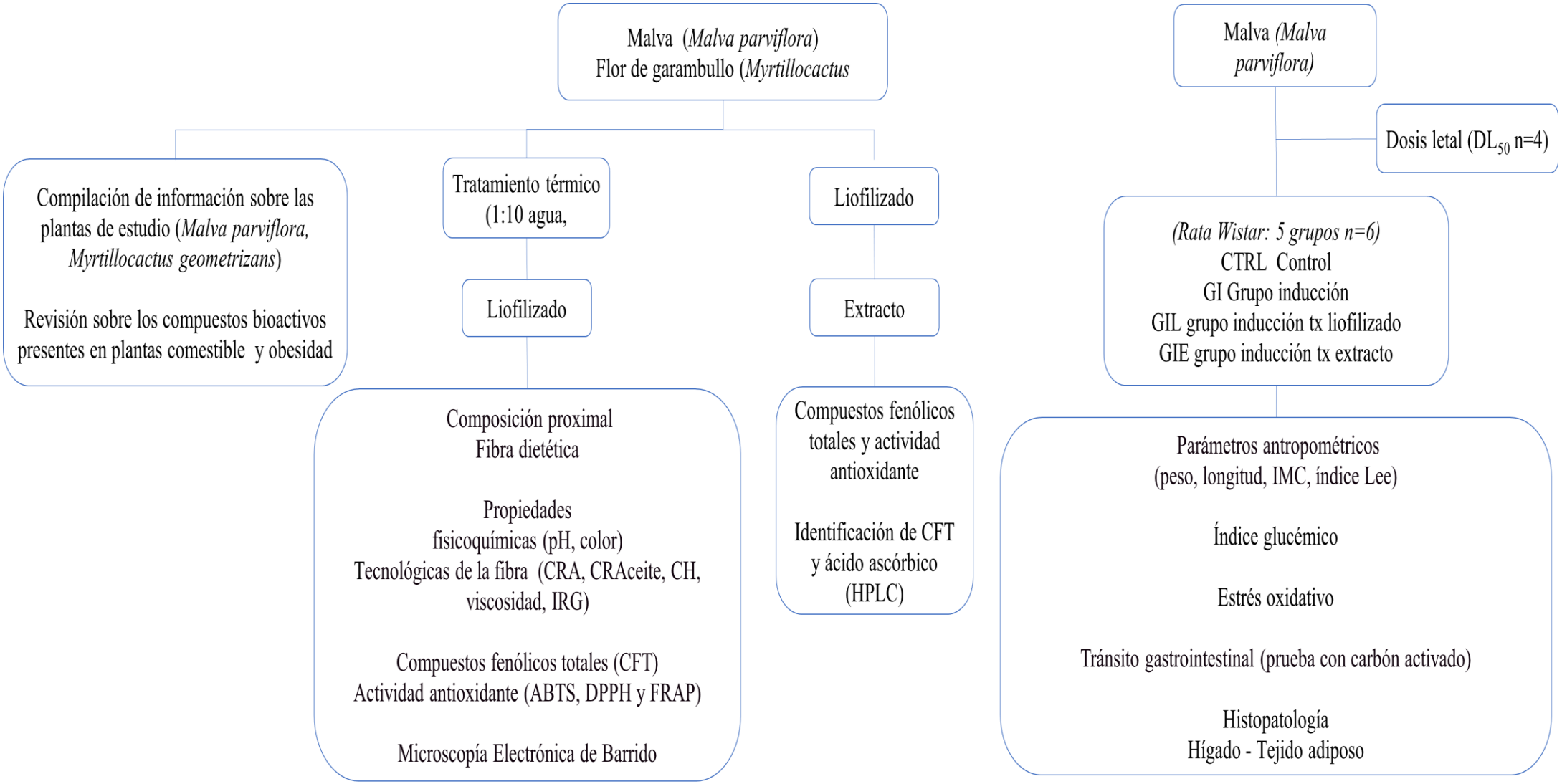

Figura 1. Diseño Metodológico

Para la presentación de resultados la información obtenida de esta investigación se dividió en capítulos acorde con los objetivos los cuales se abordará capítulos I, II, III, IV, V, descrita en forma de artículos, lo que permitirá presentar la información ya publicada se presenta en el idioma correspondiente, los resultados restantes quedan pendientes a su publicación.

El capítulo I (Antecedentes generales) describe las características etnobotánicas, etnofarmacología, acciones farmacológicas, compuestos bioactivos presentes, efectos antiinflamatorios, anticancerígenos, antidiabéticos de ambas plantas. En el mismo capítulo se desarrolla información relacionada a la obesidad como su descripción, fisiopatología, su relación con el desarrollo de otras enfermedades, tratamientos alternativos con compuestos bioactivos, sus mecanismos de acción frente a la obesidad, así como estudios preclínicos y clínicos de los efectos de los compuestos bioactivos.

En el capítulo II se reporta información relacionada al análisis proximal y fibra, la determinación de compuestos fenólicos y capacidades antioxidantes determinada por los mecanismos establecidos de actividad antioxidante (DPPH•, $ABTS^{\bullet+}$ y FRAP), así como sus propiedades fisicoquímicas (color, CRA, CRA, CH, IRG y MEB).

El capítulo III desarrolla la información sobre el impacto que presenta el tratamiento térmico sobre la retención de CFT y su actividad antioxidante, así como los compuestos presentes en la malva y la flor de garambullo.

Por último, el capítulo IV, refiere información de los resultados recabados del efecto del tratamiento con malva (liofilizado y extracto) en un modelo obesogénico.

CAPÍTULO I

ANTECEDENTES GENERALES

Características botánicas, nutricionales y su efecto etnomedicinal de malva (*Malva parviflora*) y flor de garambullo (*Myrtillocactus geometrizans*).

Resumen

Dentro de la extensión territorial mexicana, se ha mostrado una gran biodiversidad vegetal entre árboles, flores y plantas, pero que además de embellecer al país, se les han implementado una variedad de usos como de ornato, plaguicidas, medicinales y comestibles. Esta última, se centra dentro del rol alimentario uno de los más importantes, especialmente entre las poblaciones rurales e indígenas, quienes usualmente las consumen como parte de su dieta, además de contribuir con efectos en pro de la salud. Debido a la composición que presentan tanto las flores como plantas, de tal manera que en este artículo la información se centra entre las características botánicas, así como, de su composición nutricional, además de referir los efectos del consumo de flores y plantas sobre algunas patologías.

Introducción

México se ha distinguido por tener una gran diversidad biológica de plantas, principalmente durante la época de lluvia, alrededor de sembradíos, o como hierbas no deseables consideradas a veces como enemigas del cultivo. El uso de plantas comestibles sigue siendo una práctica vigente, observándose en muchos mercados tradicionales. Por otra parte, su rol alimentario es uno de los más importantes, especialmente entre las poblaciones rurales e indígenas, quienes usualmente las consumen como parte de su dieta, además de contribuir con efectos en pro de la salud, lo que hace necesario considerarlas como potencial alimentario valioso (Kibar & Kibar, 2017; Pinela, Carvalho, & Ferreira, 2017; Cilia, Aradillas, & Díaz-Barriga, 2015).

En el estado de Hidalgo; se han reportaron 611 especies de plantas, de las cuales, 461 son de uso medicinal, 236 son consideradas comestibles, 149 las utilizan de ornato y 73 tienen función plaguicida (Pérez Escandón, Villavicencio Nieto, & Ramírez Aguirre, 2003).

La producción de plantas comestibles silvestres es de forma natural y espontánea en la naturaleza y no son sembradas por el hombre. Se distinguen dos tipos de plantas silvestres: Las arvenses las cuales crecen entre campos cultivados o ambientes antropogénicos y las rudelares que se desarrollan entre el entorno de la habitación humana o sobre las vías de comunicación (Diccionario de términos botánicos, 2009). Las plantas

silvestres han coexistido con el hombre durante siglos y normalmente son consumidas en crudo como ensaladas o cocidas en recetas tradicionales (Ortiz-Quijano, 2007).

Malva parviflora L: Malva

La *Malva*, pertenece a la familia de las Malváceas, comprende un grupo numeroso de especies tropicales y templadas, existen entre 25-30 especies diferentes tales como *Malva sylvestris, Malva neglecta Wallr., Malva verticillata L., Malva nicaensis All y Malva parviflora L*. por mencionar algunas (Sharifi-Rad 2021), esta última conocida popularmente con los nombres de malva, malva de quesitos, malva de campo, malva de castilla, malva alta, malva rosa, malva real, y en inglés mallow (Abdalla *et al*., 2016, Kahramanoglu, 2020, Zambrana, P. N. Y., *et al*., 2020). Son nativas de Asia, África, Europa y adventicias de América (Rashed 2017). En las zonas de México se distribuye como maleza ruderal y arvense en los estados de Aguascalientes, Baja California Norte, Baja California Sur, Chiapas, Chihuahua, Coahuila, Colima, Ciudad de México, Durango, Guanajuato, Guerrero, Hidalgo, Jalisco, Michoacán, Morelos Nuevo León, Oaxaca, Puebla, Querétaro, San Luis Potosí, Sinaloa, Sonora, Tamaulipas, Tlaxcala, Veracruz y Zacatecas. Dentro del estado de Hidalgo se producen en los municipios de Actopan, el Arenal, Atotonilco el Grande, Epazoyucan, Huasca, Mineral del chico, Mineral del Monte, Omitlán, Pachuca, Santiago de Anaya, Singuilucan, Tepeapulco, Tolcayuca, Zapotlán y Zempoala.

La *malva parviflora* L., se caracteriza por ser una planta herbácea y perenne, que mide de 8 a 45 cm de altura, los tallos son ascendentes, sin embargo, no se mantienen perpendiculares debido a que carecen de fuerza para mantenerse erguidos, cuenta con ramificaciones de color verde en su totalidad raramente, pero con coloraciones purpura oscuro en la parte inferior del tallo. Las hojas que forman parte de esta planta se caracterizan por tener un peciolo largo, reniformes y ondulado, las láminas de las hojas miden aproximadamente 6 a 7 cm de ancho y 4.5 cm de largo. Además, cuenta con inflorescencias de entre 3 o 4 en axilas que miden 0.5 cm de diámetro de color lila claro, escasamente solitarias (CONABIO) (Figura 1).

Figura 1. Malva. (*Malva parviflora)*

Composición nutrimental

Las malvas han formado parte de la alimentación por su sabor y facilidad de consumo y cuenta con un alto valor nutricional, debido a su aporte de fibra dietética (36.47 g/100g bs), proteínas (34.94 g/100g bs) e hidratos de carbono (48.05 g/100g bs), entre los cuales se encuentran caracterizados por un contenido importante de mucílagos entre los que se encuentran ácido glucurónico, ácido galacturónico, ramnosa, galactosa, fructosa, glucosa, sacarosa y trehalosa, ácido urónico, arabinosa, manosa, xilosa, fucosa y rafinosa. También se han encontrado glucuronosil-xilotriosa considerada como componentes con efectos terapéuticos (Sandoval-Gallegos, *et al*., 2021; Gasparetto, J., *et al*., 2012). La planta también contiene ácidos grasos (1.84 g/100g bs) como el oleico, linolénico de importancia nutricional, entre otros (cáprico, láurico, mirístico, palmítico, palmitoleico, esteárico, linoleico, araquidónico, behénico, erúcico, lignocérico). Otros componentes importantes son las vitaminas A, B1, B2 y C, así como, minerales (hierro, zinc, fósforo, magnesio, fósforo, potasio, nitrógeno y manganeso. Además de su composición nutricional principal, también se han encontrado fitoquímicos como flavonoides, ácidos fenólicos, carotenoides y esteroles terpenos (Chiclana F. C 2009, Abdel-Ghani 2013; Rasheed; 2017, Sandoval-Gallegos, *et al*., 2021).

Compuestos bioactivos presentes en la Malva

El interés por encontrar nuevos compuestos bioactivos con efectos positivos a la salud ha ido creciendo día con día. En este sentido, y de acuerdo con la práctica de la medicina tradicional se han encontrado una gran diversidad de plantas con efectos beneficiosos. Tal es el caso de la *malva parviflora que* ha sido utilizada desde las hojas, ramas, tallos, hasta la raíz, o incluso toda la planta por la población en diferentes partes del mundo

(Bolivia, Ecuador, Colombia, Perú, Etiopía, Pakistán, entre otros) a través de infusiones, pasta, cataplasmas, emolientes con la finalidad de mejorar el estado de salud, para aliviar diversos padecimientos como infecciones gastrointestinales, problemas renales, fiebre, en la cicatrización de heridas, tos o carcinomas (Zambrana, N. Y., *et al*., 2020).

Dentro de la familia de las malvas se ha encontrado como parte de su composición a las coumarinas, polifenoles, flavonoides, terpenos, taninos, antocianinas, β-sitosterol, alcaloides, quinonas, entre otros. (J. Pothier 2000, Sharifi-Rad-2019). Estos compuestos identificados y muchas veces aislados, suelen ser los responsables de los efectos antiinflamatorios, antioxidantes, antimicrobianos, entre otros como se enlista en la Tabla 1.

Tabla: 1 Compuestos bioactivos identificados y sus efectos en diferentes especies de malvas

Especie	Compuestos bioactivos identificados	Efecto	Autor
Malva sylvestris	Terpenoides, monoterpenos, diterpenos, sesquiterpenos y norterpenos, malvona A (2-metil-3-metoxi-5,6-dihidroxi-1,4-naftoquinona fenoles derivados (Ácido 4-hidroxibenzoico, ácido 4-metoxibenzoico, ácido 4-hidroxi-3-metoxibenzoico, ácido 2-hidroxibenzoico, ácido ferúlico y tirosol. Sulfito oxidasa Coumarinas Esteroides campesterol, estigmasterol y γ-sitosterol	Antiinflamatoria Antioxidante Anticomplementaria Anticancerígena Integridad del tejido cutáneo Antiulcerogénica Antimicrobiano Responsable de la reacción final en la degradación oxidativa de los aminoácidos que contienen azufre	Qawasmeh, A., *et al*., 2020; Gasparetto, J., et al., 2012
Malva neglecta Wall	Alkaloides Taninos Saponinas Ácido hidroxicinámico Flavonoides, Flavonoles Proantocianidinas Antocianinas Ácidos orgánicos.	Antimicrobiano Antioxidante Anti-inflamatoria Anti-ulcerogénico, hepatoprotector Anti-urolitiasis Inhibidora de las enzimas anticolinesterasa, convertidora de angiotensina, α-amilasa, α-glucosidasa y lipasa pancreática. Antiurolítica	Al-Snafi, A. E. 2020 Khalid, S. *et al.*, 2018
Malva verticillata	benzil-〈-D-galactopiranósido (–)-secoisolariciresinol-9′-O-®-D glucopiranósido Éster metílico del ácido trans- ferúlico	Antidiabético Cardioprotector Antiobesidad	Ko, J.-H., *et al*., 2018 Ibrahim, D., *et al*., 2020
Malva rotundifolia	Alcaloides Glicósidos Saponinas Taninos Terpenoides Flavonoides Compuestos fenólicos	Antioxidante	Ganaie, A.A., *et a*l., 2017

Malva parviflora	Ácidos fenólicos: ácido gentísico, rosmarínico, vanílico, Ferúlico, sinápico, *p*-coumárico, cinámico y Flavonoides: Apigenina-7-glucósido, catequina, luteolina, naringenina, apigenina, kaempferol, Ácido oleanólico, Tilirosida Escopoletina	Antioxidante Analgésica antiinflamatoria Antiartrítica Antibacterial, Antidiabético, Antifungico Hepatoprotector, Neuroprotector, Antiulcerogénica	Abd El-Salam, 2019, Ajeet Singh, 2017, Martínez-Hernández 2020 Shale TL, 2005 Afolayan AJ,2010 Bouriche H, 2011 Pérez-Gutiérrez RM., 2012 Aslam M, 2014 Dugani, A, 2016 Lagunas-Herrera, 2019

Efectos etnomedicinales de la malva

Martínez-Hernández (2020), reportó que el extracto de diclorometano y fracciones Mpf4 (diclorometano:metanol) y MpFhy (precipitado acetona:metanol) de la malva, disminuyen la inflamación como el dolor articular, además refirió que Mpfhy a 1.0 mg /kg incrementó la IL4 e IL10 y disminuyó IL-17, IL1β- y TNF-α. Dichos efectos, se deben a la composición química de la *Malva parviflora*, en específico por los esteroles como de otras moléculas como los ácidos grasos. Por otra parte, Medrano-Jiménez, (2019) mostró que el extracto de *Malva parviflora,* disminuye eficazmente la astrogliosis, así como la presencia de péptidos Ab insolubles en el hipocampo y la alteración del aprendizaje espacial.

Otro efecto que ha sido atribuido a los compuestos de las plantas es su efecto antimicrobiano. Tal es el caso del compuesto extraído β-sitoesterol con hexano, metanol y agua de *malva parviflora* en un estudio realizado por Medihin Ododo *et al*., (2016) quien mostró que el extracto de cloroformo mantuvo una actividad antibacteriana contra *Staphylococcus* y *Escherichia coli*, por otra parte, el extracto etanólico mostró actividad contra *Staphylococcus aureus*. Por su parte, Al-Otibi *et al*., (2021) reportó que las nanopartículas de planta biosintetizadas y el extracto de hoja de *Malva parviflora* mitigan activamente a los hongos como *Helminthosporium rostratum*, *Fusarium oxysporum* y *Alternaria alternata.* Además, se ha reportado que la *Malva parviflora* posee un potencial importante antioxidante, ya que el extracto a base de alcohol demostró ser un excelente restaurador de la memoria defectuosa de ratones, inyectados con amiloides β, además de reducir los niveles reducidos de cerebro, se restauraron enzimas antioxidantes como la glutatión peroxidasa, glutatión reductasa, la catalasa y superóxido dismutasa. Al mismo tiempo los niveles de peroxidasa lipídica disminuyeron significativamente (Muhammad Aslam, 2014). Por su parte, Pérez-Gutiérrez, (2012) reportó la administración del extracto de hoja de *Malva parviflora* de hexano en dosis de 100, 200 y 400 mg/kg durante 28 días en un modelo de diabetes inducido por estreptozotocina en ratas, hallando un restablecimiento casi a valores normales sobre los parámetros bioquímicos entre ellos los niveles de glucosa en sangre, las enzimas hepáticas, sustancias reactivas del ácido tiobarbitúrico, la hemoglobina glucosilada, los niveles de insulina. En dicho estudio se refirió que el compuesto principal está presente en las hojas de

la *Malva parviflora,* el derivado del ácido oleanólico 2α, 3β, 23α, 29α tetrahidroxi olean-12 (13) -en-28-oico 1, mantuvo efectos positivos en los ratones con diabetes inducida por estreptozotocina en dosis de 20 mg/kg durante 4 semanas. Como consecuencia a la administración de este derivado mostraron que tienen actividades hipolipidemicas e hipoglucémicas, antiinflamatorias, mejorando además la resistencia a la insulina y las enzimas hepáticas en ratones diabéticos por estreptozotocina.

Myrtillocactus geometrizans: Flor de garambullo

Como parte de la alimentación de la población mexicana también se encuentra una gran variedad de flores ampliamente distribuidas dentro del territorio mexicano, de Norte a Sur, encontrando 100 especies nativas que representan al menos 49 géneros en 25 familias de plantas, siendo las Agavaceae, leguminosae, Arecaceae, Cactaceae y Curcubitaceae con una diversidad de especies (Mapes, 2016). El garambullo es una cactácea de nombre científico *Myrtillocactus geometrizans*, única especie que crece en zonas áridas. En ella se desarrolla una inflorescencia conocida como flor de garambullo, crece en la parte superior de la cactácea arborescente, erecta, de 2-7 m de altura. Las flores de garambullo se encuentran previstas en las areolas, miden 1.8 cm de longitud y tienen una corola blanca. En México se distribuyen en gran parte del territorio nacional (Hidalgo, Querétaro, Guanajuato, Michoacán, Jalisco, San Luis Potosí, Zacatecas, Tamaulipas, Nuevo León, Estado de México, Puebla, Veracruz, Guerrero y Oaxaca) (Guzmán *et al.*, 2003; Sanjuan-Trejo, 2021).

Figura 2. Flor de garambullo (*Myrtillocactus geometrizans)*

Composición nutrimental

La flor de garambullo es fuente de nutrientes como hidratos de carbono (68.13-70.75 mg/ 100 g bs), proteínas (12.53-13.25 mg/ 100 g bs), lípidos (1.69-2.30 mg/ 100 g bs), y fibra dietética (42.98 g/100 g bs). Además, de la composición general, la flor contiene componentes fitoquímicos tales como ácidos fenólicos, flavonoides, terpenoides, taninos, saponinas (Solís R David, 2017; Pinedo-Espinoza., *et al.*, 2020; Sandoval-Gallegos, *et al*., 2021).

Tabla 2. Contenido de minerales en la flor de garambullo

Minerales	g/100g bs
Nitrógeno	2
Fósforo	0.26
Potasio	2.90
Calcio	0.21
Magnesio	0.18
Hierro	65,80
Cobre	6.20
Zinc	37.70
Manganeso	40.90
Boro	53.07

(Solís R David, 2017; Pinedo-Espinoza., *et al.*, 2020; Sandoval-Gallegos, et al., 2021)

Compuestos bioactivos

De los componentes químicos que se encuentran en la flor de garambullo identificados por Pinedo-Espinoza fueron del grupo de los ácidos fenólicos y flavonoides. En la tabla 3 se describen los compuestos identificados de la flor de garambullo, así como el posible efecto biológico de la flor de garambullo.

Tabla 3. Efectos biológicos sobre las plantas de estudio

Especie	Compuestos bioactivos identificados	Efecto	Autor
Myrtillocactus geometrizans (Cactus)	Peniocerol Macdougalina	Anticancerígeno Antiinflamatorio	Harlev Eli; et al., 2013 Salazar 2011
Myrtillocactus geometrizans (flor)	Ácido clorogénico, siringico, vanilinico, p-hidroxibenzoico, cafeico, ferúlico, p- cumárico, rutina, florizidina floretina, naringenina, myrecitina, apigenina, galangina.	No se han reportado efectos de los compuestos	Pinedo-Espinoza *et al.*, 2021

Efectos etnomedicinales de flores

Por otra parte, se encuentran otro tipo de cactáceas en la cuales también se desarrollan inflorescencias y se ha encontrado efectos biológicos tales como anti analgésicos, antiinflamatorio y anti genotóxico de las flores de *Opuntia microdasys Lem.Pfeiff* (Hassiba Chahdoura, 2017); actividad antiinflamatoria de las flores de *Opuntia delli* (Sharma, C. *et al*., 2015), actividad anticancerígeno, diurético, efecto antiepiléptico de las flores *Opuntia ficus indica* (Kamble, S., *et al*., 2017; Galati, E., *et al*., 2002), o como el encontrado en las flores de *Opuntia dejecta*, las cuales se ha inhibido el estrés oxidativo hepático (Zouaoui, 2021).

Tabla 4. Compuestos bioactivos identificados y sus efectos en diferentes flores comestibles

Flores	Compuestos bioactivos identificados	Efecto	Autor
Antigonon leptopus *Tagetes erecta* *Cosmos sulphureus* *Bougainvillea hybrida* *Cassia siamea* *Clitorea ternatea* *Malvaviscus arboreus* *Ixora chinensis* *Leucaena leucocephala* *Nelumbo nucifera* *Plumeria obtusa L.* *Telosma minor*	Ácido gálico, protocatequiico, p-hidroxibenzoico, clorogénico, vanílico, cafeico, siríngico, *p*-cumárico, ferúlico, sinápico. Rutina, miricetina, querecetina, apigenina, Kaempferol	Actividad antioxidante celular supresión de la proliferación de células cancerosas Inhibición de las enzimas α-glucosidasa y lipasa	Kaisoon,2012
Cylindropuntia rosea *Opuntia oligacantha var Ulapa* *Opuntia matudae var* *Cuaresmeño Rosado* *Echinocereus cinerascens*	4-o-glucósido de ácido cafeico 3-O-(-malonil-glucósido)-7-o-glucósido de quercetina 3-O-xilosil-glucurónido de quercetina 3-O-glucósido de quercetina	Antioxidante antiinflamatorio antimicrobiano anticáncer agentes antidiabéticos	Pesamiento-Niño, 2021

	3-O-(600-acetil-glucósido) de Peonidina 4-O-glucósido de Ácido protocatequiico 4-O-glucósido de Ácido ferúlico Sinensetina 3-O-glucósido 7-O-ramnósido de Isorhamnetina 3-(3,4-dihidroxifenil)-2-hidroxipropanoato Derivado del ácido elágico 3,5-O-diglucósido de malvidina (+)-catequina (-)-Epicatequina 3-O-glucosil-ramnosil-glucósido de quercetina 3-O-xilosil-glucurónido de quercetina 6,8-di-C-glucósido apigenina 3,7-dimetilquercetina	antiviral antidepresivo antimicrobiano cardioprotector actividad antimutagénica	
Dahlia mignon *Rose* *Calendula officinalis L.* *Centaurea cyanus L*	Tocoferol Ácidos grasos poliinsaturados	Antioxidante antiinflamatorio anticáncer anti-obesidad hipoglucémico neuro protectores, protector hepático y gastroprotector	Pires, 2017
Sambucus nigra L. *Arnica L.* *Matricaria L.* *Centaurea cyanus L.* *Bellis perennis L.* *Calendula officinalis L.* *Acacia Mill.* *Anthyllis vulneraria L.* *Lavandula L.* *Lamium album L.* *Malvae arboreae L.* *Tilia cordata Mill.* *Malva L.* *Primula L.* *Crataegus L.* *Verbascum L.*	Carotenoides Triterpenoides Ácidos fenólicos Procianidina polímeros	Actividad antienvejecimiento, propiedades antihiperglucemiante	Nowicka, 2019

Conclusión

Esta revisión destaca la importancia que representan la malva y la flor de garambullo los cuales pueden ser considerados como alimentos funcionales y plantas medicinales, permitiendo esto, que se han apreciadas y revalorizadas. Sin embargo, es importante que se realice una investigación toxicológica, así como la relación con la composición y sus efectos sobre algunas patologías, con la finalidad de conocer mejor los beneficios de estos vegetales en beneficio de la salud humana.

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Review

Role of Bioactive Compounds in Obesity: Metabolic Mechanism Focused on Inflammation

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Abstract: Obesity is a disease characterized by an inflammatory process in the adipose tissue due to diverse infiltrated immune cells, an increased secretion of proinflammatory molecules, and a decreased secretion of anti-inflammatory molecules. On the other hand, obesity increases the risk of several diseases, such as cardiovascular diseases, diabetes, and cancer. Their treatment is based on nutritional and pharmacological strategies. However, natural products are currently implemented as complementary and alternative medicine (CAM). Polyphenols and fiber are naturally compounds with potential action to reduce inflammation through several pathways and play an important role in the prevention and treatment of obesity, as well as in other non-communicable diseases. Hence, this review focuses on the recent evidence of the molecular mechanisms of polyphenols and dietary fiber, from Scopus, Science Direct, and PubMed, among others, by using key words and based on recent in vitro and in vivo studies.

Keywords: obesity; inflammation; bioactive compounds

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1. Introduction

Obesity has been cataloged as a slow-motion disaster [1], so exhaustive research to find efficient alternatives against obesity has been the subject of continuous debate. In accordance with the WHO, worldwide obesity has nearly tripled since 1975 (OMS. Global Health Observatory [GHO] data, 2019), and has been considered as a public health problem. Mexico is one of the countries with the highest prevalence of obesity. In addition, obesity is defined as abnormal or excessive fat accumulation that increases the risk of developing a secondary disease. Adipose tissue was previously considered as a static tissue (reservoir for energy). Studies have referred to adipose tissue as a dynamic tissue (metabolically active organ) [2–4]. The morphophysiological change of adipose tissue during obesity induces a chronic low-grade inflammatory state, also referred to as parainflammation (intermediate state between basal and inflammatory) or metainflammation (metabolically triggered inflammation) [5–7]. On the other hand, visceral adipose tissue could have a local immune response [8], and it is linked with the stimulation and release of detrimental cytokines and chemokines implicated in metabolic disorders [9]. In addition, the inflammation associated with obesity could be triggering other comorbid conditions, such as diabetes, cardiovascular disease, and cancer, among others [2].

Some researchers have demonstrated that the consumption of plant-based foods could decline the inflammation state in obesity due to their content of bioactive compounds. Bioactive compounds are found in a minor amount in these food items and they have been reported to be effective in the treatment of obesity [10–14]. Among the compounds reported with beneficial effects are flavonoids, polyphenols, betalains, and fiber, which have been studied as factors with probable effects on specific pathways (PPARα, cyclooxygenase-2, glucose transporter (GLUT4), SIRT1, and PGC1-α) conferring anti-inflammatory activities, with remarkable implications for health and disease [15–17] Therefore, this review aims to provide a comprehensive overview of recent studies about the possible role and effect of specific bioactive compounds on weight management and obesity consequences. Several investigations have been focused on the research for natural alternatives that, in agreement with the work, tend to be promising treatments against obesity progression.

2. Physiopathology of Obesity

Energy self-regulation is a complex system that includes coordinated neurogastrointestinal and endocrine pathways to maintain adequate metabolism and the use of nutrients. Peripheral or afferent systems generate signals exerted on adipocytes (secrete leptin: modulate satiety [18], the pancreas (secretes insulin: regulates the body's energy supply, cell growth, and metabolism [19]), the stomach (secretes ghrelin: stimulates appetite [20]), and the ileum and colon (secrete peptide YY: appetite regulation [21]). These signals are generated and processed by the arcuate nucleus of the hypothalamus and create new signals that are subsequently emitted by catabolic and anabolic-type neurons. Finally, the efferent system, constituted by hypothalamic neurons, is controlled by the arcuate nucleus and is, therefore, responsible for the effect on food inhibition or intake [22]. Therefore, the effect of food consumption and a lack of caloric expenditure cause obesity through the development of the preadipocyte to a mature state. This event occurs through transcription factors, such as peroxisome proliferator-activated receptor γ (PPARγ), and other transcription factors, including CCAAT/enhancer-binding proteins (C/EBPs, AP-1), signal transducers and activators of transcription (STATs), and Kruppel-like factor (KLF), that promote preadipocyte differentiation into mature adipocytes (adipogenesis) [23]. This mature state is characterized by presenting a low-grade chronic inflammation state caused by the accumulation of proinflammatory macrophages. Immune cells, such as eosinophil, neutrophil, treg cells, and killer T cells, are also responsible for the secretion of inflammatory cytokines, as well as proteins, such as galectin-3, and exosomes [24,25]. On the other hand, the presence of obesity also triggers the activation of nuclear factor erythroid-2-related factor 2 (Nrf2), whose function is characterized by the maintenance of redox and metabolic homeostasis, through regulating the antioxidant endogenous response and decreased inflammatory stress [26,27].

2.1. Inflammation in Adipose Tissue

Under normal conditions, adipose tissue regulates essential biological processes through the autocrine, paracrine, and endocrine pathways [28–30]. When obesity occurs, an inflammatory process originates, which is known as a low–grade chronic inflammation response of prolonged time [31], and is the result of increasing fat tissue (hypertrophy related to an increase in the size of adipocytes and an overproduction of pro-inflammatory mediators by exogenous or endogenous stimuli, Figure 1) due to excess nutrient consumption [32].

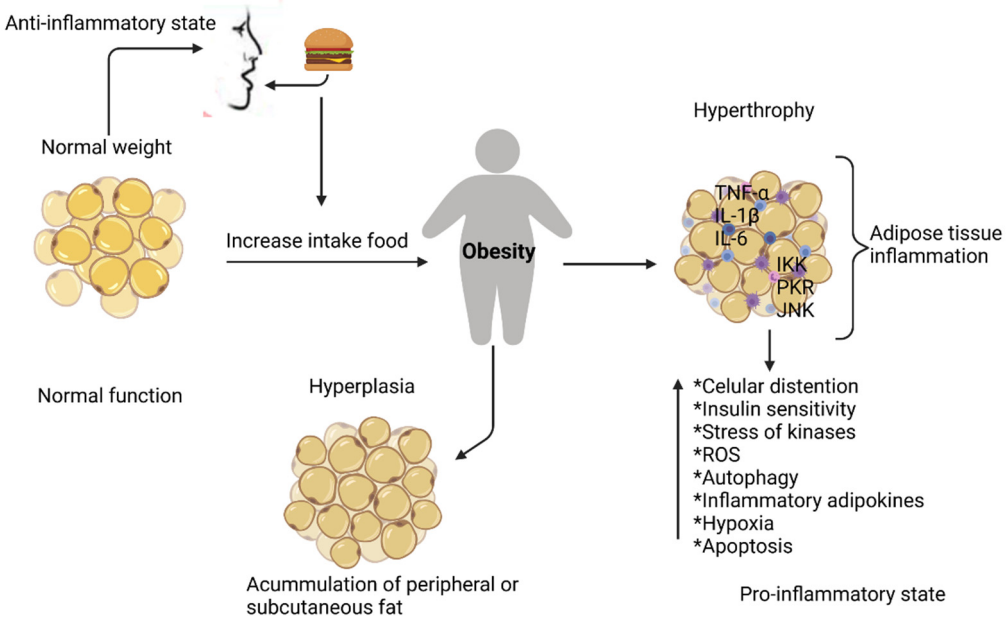

Figure 1. Adipose tissue inflammation. Excessive growth of adipose tissue in obesity induces the production of pro-inflammatory cytokines that activate protein kinase pathways, at the same time stimulating macrophage infiltration and a change in the phenotype of M2-type macrophages to proinflammatory M1, leading to an inflammatory state with consequences locally and systemically. Tumor necrosis factor-alpha (TNF-α), interleukin-1b (IL-1β), interleukin-6 (IL-6), N-terminal c-JUN (JNK), nuclear factor-kappa kinase inhibitor β (IKK), protein kinase R (PKR). Created with BioRender.com.

On the other hand, inflammation of adipose tissue is also described as a body's natural or biological reaction against pathogens and harmful stimuli caused by toxic compounds, damaged cells, and metabolic factors [33]. There are two types of inflammation: acute (short time), characterized by edema and the migration of leukocytes; and chronic inflammation (long time), differentiated by a constant secretion of proinflammatory molecules by lymphocytes and macrophages on blood vessels and connective tissue [33–36]. This inflammatory response depends on the origin of the initial stimulus, the location of adipose tissue in the body, as well as the mechanism to counteract it. Existing factors that trigger inflammation, such as (1) cell surface pattern-recognition receptors that detect different detrimental stimuli; (2) the activation of several inflammatory pathways, such as the mitogen-activated protein kinase (MAPK), nuclear factor kappa-B (NF-κB), and Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathways; (3) the delivery of inflammatory markers as transcription factors: NF-κB, STAT 3, and inflammatory cytokines (TNF-α, IL-1, IL-6, IL-8), pro-inflammatory enzymes as metallopeptidase-9 (MMP-9), cyclooxygenase (COX-2), vascular endothelial growth factor (VEGF), cell adhesion molecules (CAM), such as VCAM-1 and ICAM-1, etc., and (5) the immune response by hypoxia-induced factor (HIF) [33,37,38]. Therefore, factors such as the production of inflammatory intermediaries and dysregulating inflammatory pathways cause the chronic triggering of collateral injury that then impairs tissue homeostasis, developing several chronic diseases related to low-grade inflammation (LGI), such as atherosclerosis, type-2 diabetes, gout, and multiple neurodegenerative diseases, that negatively affect people's health and life expectancy [17,39–42].

Many studies report that, during this inflammatory process, there is excessive segregation of inflammatory factors known as adipokines, bioactive molecules responsible for the origin of inflammation and insulin resistance associated with obesity [43], segregated by adipocytes that include TNF-α, IL-6, IFN-γ, plasminogen activator inhibitor (PAI-1), monocyte chemoattractant protein-1 (MCP1), IL-1β, IL-8, IL-10, IL-15, leukemia inhibitory factor (LIF), hepatocyte growth factor (HGF), apolipoprotein amyloid A3 seric (SAA3),

macrophage migration inhibitory factor (MIF), potent inflammatory modulators, such as leptin, adiponectin, resistin, and C-reactive protein (CRP), and these maintain both negative and positive effects, such as the maintenance of oxidative stress, changes in autophagy patterns, tissue necrosis, etc. (Table 1).

It has been observed that obesity is related to metabolic pathways, food intake, and energy expenditure (Figure 2), which leads to the alteration of various inflammatory pathways, such as Janus-N-terminal kinase system/signal transducer and transcription activators (JNK/STAT), IκB-kinase β, and protein kinase C (PKC) [44,45], Besides an increased infiltration of cells into adipose tissue [46–49], due to the systemic circulation of inflammatory factors that stimulate the endothelial cells. Thus, an inflammatory state is triggered by the relationship between adiposity and metabolic pathways, macrophages, adipocytes, and other factors [7,28,50]. All of these alterations trigger the development of other diseases.

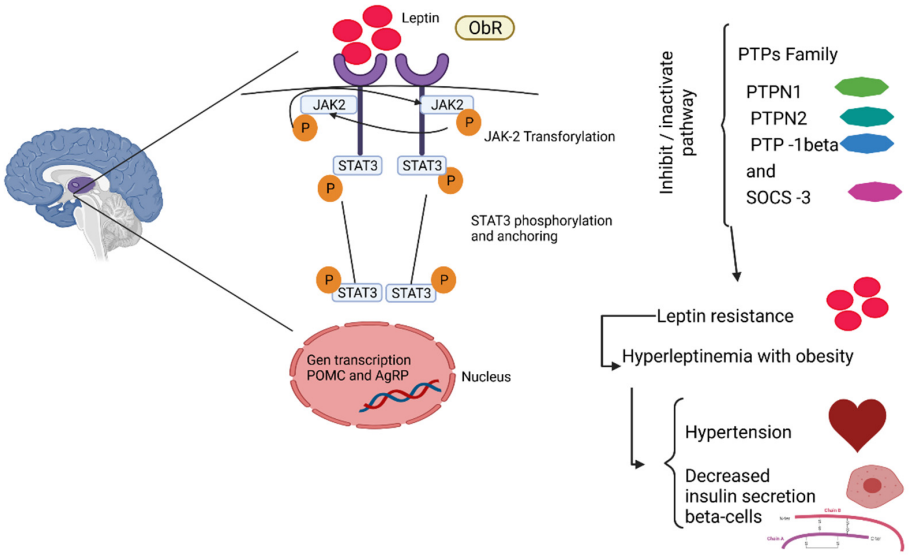

Figure 2. Leptin pathway. Leptin binds to the ObR receptor and JAk-2 transphosphorylation occurs, translocating the phosphate groups, giving rise to the anchoring and phosphorylation of STAT3. These STA3 travel to the nucleus, where the transcription of target genes, such as POMC (decreases hyperphagia) and AgRP (increases food intake), takes place. This pathway can be inactivated by the interaction of tyrosine-protein phosphatase 3 (PTP3) and suppressor of cytokine signaling 3 (SOCS3), causing resistance to leptin, resulting in hyperleptinemia, which leads to cardiovascular problems, such as hypertension, as well as causing a decrease in insulin secretion in β cells. Created with BioRender.com.

Table 1. Adipokines' effect on obesity.

Adipokines	Segregation Molecules	Effect	Author
CRP	Increases the expression of vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion (ICAM-1), and E-selectin in vascular endothelial cells Increases the secretion of monocyte chemoattractant protein-1 (MCP-1)	Participates in the coronary and aortic atherosclerosis that leads to cardiac events	[51]
TNF-α	Decrease of nitric oxide (NO) Increase of endothelin1 (ET-1), angiotensin II (ATII), oxidized low-density lipoproteins (oxLDL), ICAM-1, VCAM-1, MCP-1, CD40/CD40L, and leukocyte adhesion	Increases foam cell formation Increases smooth muscle cell (SMC) proliferation and migration Expansion of the injury area Increases platelet adhesion Increases leptin concentration	[52]

Table 1. *Cont.*

Adipokines	Segregation Molecules	Effect	Author
IL-6	Increases the concentration of free fatty acids (FFAs), C-reactive protein (CRP), and nitric oxide (NO)	Induces insulin resistance Decreases hepatic insulin clearance, insulin-dependent hepatic glycogen synthesis, glucose uptake in adipose cells	[53,54]
IL-1β		Inhibition of the insulin-transduction pathway Inhibition of β-cell function Destruction of β-cell mass Induces the transcriptional activation of inflammatory genes	[55,56]
MCP1		Strongly implicated in adipose tissue macrophage (ATM) recruitment, adipose expansion and remodeling, and angiogenesis	[30]
IFN-γ	Cytokine secretion	Induces attraction of monocytes towards the activation of M1-type macrophages originating from proinflammatory cytokine secretion	[57]
PAI-1	Increases the proliferation and migration of smooth muscle cells (SMCs)	Increases foam cell formation Increases platelet adhesion (thrombosis) Inhibition of the residual plasminogen activator	[58]
Resistin	Increases endoteline-1 (ET-1), angiotensine (ATII), oxLDL, intracellular adhesion (ICAM-1), VCAM-1, MCP-1, CD40/CD40L, leukocyte adhesion, and VSMC Stimulates the synthesis and secretion of cytokines in adipocytes and endothelial cells	Decreases NO release Increases in foam cell formation Increases in proliferation and migration of SMC and the expansion of injury area Increases in platelet adhesion and, as a consequence, thrombosis	[59,60]
Visfatin	Induces ICAM-1, VCAM-1, E-selectin, IL-8, IL-6, MCP-1, fibroblast growth factor-2(FGF-2), and metalloproteinase MMP-2/-9 production Increases the release of ROS (reactive oxygen species)		[60,61]
Vaspin		Overexpressed in the obesity state Induces phosphatidylinositol 3-kinase/ Protein kinase (PI3K/AKT) activation, increases both glucose transporter type-4 (GLUT4) expression and translocation, and promotes insulin-stimulated glucose	[62]
Angiotensinogen	Stimulates ICAM, VCAM-1, MCP-1, and factors stimulant of colonies of macrophages M-CSF production.	Decreases NO bioavailability Decreases vasorelaxation mechanisms and increases platelet adhesion to the vascular wall	[44,63]
Leptin	Increases VCAM-1	In hyperleptinemia, the inflammatory process increases Increases oxidative stress Improves vasorelaxation Increases vascular permeability	[52,64]

2.2. Obesity and Its Comorbidities

The development of obesity and body mass index (BMI) are concomitant with several chronic diseases, such as type-2 diabetes, cardiometabolic diseases (including hypertension, dyslipidemia, and cardiovascular disease), cancer, non-alcoholic fatty liver disease, among other less prevalent diseases (Table 2) [65–67].

2.2.1. Type-2 Diabetes

Since the 1990s, observational studies in humans described that plasma biomarkers of inflammation (CRP and IL.6) are higher in type-2 diabetic patients [68]. Studies in vitro showed that TNF-α could impair insulin signaling in 3T3-L1 adipocytes, leading to the reduced expression of insulin receptor substrate-1 (IRS-1) and Glut4 [69]. In the early 2000s, it was reported that obesity increases low-grade inflammation by maintaining the IKK/NFκB, JNK1/AP1, and PKC pathways [70,71], and this correlates with serum inflammatory markers in type-2 diabetic patients [72,73].

Kahn and collages described immune cell infiltration as concomitant with cytokine secretion by adipose tissue, leading to insulin resistance by retinol-binding protein 4 (RBP4) [74]. They also that found an upregulation of the fatty acid synthesis pathway in the adipose by carbohydrate response element-binding protein (ChREBP), a transcription factor that regulates lipogenesis and glycolysis, leads to GLUT4 overexpression [75].

Chronic obesity progression also induces an inflammatory process in the pancreas caused by the increased flux of no esterified or free fatty acids (FFA) [74] and the subsequent penetration of macrophages to increase cytokine infiltration, including TNF-a, IL-6, and MCP-1 [76], leading to β-cell dysfunction [77]. Likewise, an increase in the glucose and fatty acid levels activates the inflammasome complex in the pancreas, promoting the release of proinflammatory cytokines, such as IL-1β, CRP, IL-6, TNF-α, MCP-1, IL-8, and PAI-1, considered as pro-inflammatory markers in diabetes [43,77–81].

As summarized by Ortega and collages [82], obesity increases chronic inflammation and cytokine production, which affects insulin-dependent tissues and beta cells; peripheral tissues are impaired by the lipotoxicity exerted by ectopic lipid stores in obese subjects, and the increased secretion of a set of autocrine and paracrine products by adiponectin downregulation finally produces the loss of insulin sensitivity concomitant with impaired insulin production in type-2 diabetic patients.

2.2.2. Cardiovascular Disease

Cardiovascular disease (CVD) is one of the first causes of mortality in several countries. Hyperlipidemia, T2D, and hypertension are common pathologies that increase the risk of CVD, and inflammation is a key mechanism for the progression and complications of CVD [83].

Factors that are considered key to the development of endothelial dysfunction are plaque formation and plaque instability, which constitute the main mechanism of vascular damage in atherosclerotic disease. In people with obesity, there is an activation of the systemic inflammation unchained from the accumulation of macrophages in adipose tissue that at the same time stimulate the secretion of pro-inflammatory proteins, mainly TNF-α, IL-6 and C-reactive protein (CRP), leptin, adipocyte fatty acid-binding protein, and several novel adipokines, such as chemerin resistin, visfatin, and vaspin. These inflammatory mediators are responsible for the induction of CVD, such as plaque formation [84], endothelial dysfunction [85], and cardiac dysfunction [86].

In a two-decade prospective follow-up study, the cardiometabolic profile (HOMA-IR, hs-CRP, and serum HDL) was more adverse in recent-onset obesity and persistent obesity youths (23 years old) compared to never obese participants. However, participants who had obesity in early childhood or preadolescence but transitioned to a non-obesity status had similar characteristics to those who were never obese [87]. Therefore, the reduction of weight gain triggers inflammation and cardiometabolic consequences. In a clinical study in

adults, the use of an anti-interleukin (IL)-1β antibody in patients with hs-CRP > 2 mg/L decreased the IL-6 and CRP levels associated with decreased cardiovascular events [88].

2.2.3. Cancer

According to different authors, inflammation linked to obesity is considered a risk factor that improves the initiation and progression of various types of cancer [89]. The relationship between obesity and cancer due to alterations such as insulin metabolism, insulin-like growth factor-1 (IGF-1) axis, sex steroids hormones, adipokines, and chronic low-grade inflammation, has been investigated, which contribute to the adverse effects of obesity in cancer development and progression [90,91].

Kolb [92] described that excess nutrients lead to the activation of different metabolic signaling pathways, cytokine release, hyperplasia, and hypertrophy of adipocytes, which, in turn, increased macrophages on white adipose tissue, triggering a low-grade inflammatory response on the organism, promoting a carcinogenic environment.

In addition, the presence of macrophages in obesity causes the infiltration of tumors, and increases the inflammatory tumor microenvironment caused by cytokines, prostaglandins, and angiogenic factors [93]. On the other hand, the obesogenic status also increases growth factor signaling and vascular perturbations, provoking microenvironment changes and inflammation, causing an increased risk of cancer or its progression [93].

2.2.4. Non-Alcoholic Fatty Liver Disease

Non-alcoholic fatty liver disease (NAFLD) is a very complex disorder and is the most common liver disorder related to T2D [94]. NAFLD is characterized by increased lipid accumulation and subsequent inflammatory response to progress to liver cirrhosis, fibrosis, or non-alcoholic steatohepatitis (NASH).

The liver is a metabolic organ that uses fat as fuel during starvation. The increase in ectopic fat and visceral adipose tissue leads to increased secretion of inflammatory markers, such as TNF-α, IL-6, CCL3, soluble intercellular adhesion molecule-1 (sICAM-1), and CRP [95,96].

It has been shown that, during NAFLD, hepatic stellate cells (HSCs) and Kupffer cells increase the secretion of TNF-α and promote the recruitment of immune cells, perpetuating the inflammatory process [97]. On the other hand, the cytokines produced from adipose tissue under obese conditions induce hepatic insulin resistance and fibrosis [98].

Table 2. Obesity and its relationship with other diseases.

Diseases	Description	Author
Dyslipidemia	This pathology is due to the consequence of lipolysis produced in the adipocyte, increasing the levels of free fatty acids and increasing the synthesis of hepatic triglycerides, which, in turn, leads to an increase in VLDL. On the other hand, the decline in HDL-c is due to the decrease of Apo A-I, CETP, and LCAT, which inhibits the expression of ABCA1, ABCG1, and SR-B1. The cytokines and adipokines are responsible for these alterations in the adipose tissue	[99]
Gallbladder disease	Gallstones originate from the accumulation of cholesterol monohydrate crystals precipitating in gallbladder bile. Therefore, an increase in weight stimulates the risk of gallstones.	[100]
Hyperuricemia	An alteration with increased serum uric acid level development to gout due to monosodium urate crystals depositing mainly in the joints. These conditions increase with obesity due to the production of urates.	[101]

Table 2. *Cont.*

Diseases	Description	Author
Osteoarthritis	Although the damage is not clear, it has been found that the dysregulation of adipokines (adiponectin, apelin, leptin, lipocalin-2, visfatin, chemerin, and resistin) and cartilage extracellular matrix degradation in the muscle–skeletal system exerts deleterious effects on the joint.	[102]
Hypothyroidism	Lower free irosin 4 and higher tirosin-stimulating Hormone levels are associated with fat accumulation. Modified thyroid function with normal feedback regulation may be the cause of alterations in energy expenditure with subsequent increases in BMI and weight	[103]

3. A Brief Overview of the Properties and Dietary Effects of Some Bioactive Compounds in Obesity

Due to the complicated pathophysiology of obesity, it is necessary to strengthen the consumption of a healthy diet based on vegetables to favor a decrease in obesity and its complications. These diets are characterized by the presence of vegetables, grains, or legumes containing single or mixed compounds with synergistic effects [104–107]. These are named bioactive compounds or phytochemicals [108,109], and are found in all plants as secondary metabolites. The concentration of bioactive compounds (such as fiber dietary, minerals, vitamins, fatty acids, proteins, some carbohydrates, and polyphenols) varies depending on the parts of the plant growth phase and the season [110].

3.1. Polyphenol Compounds

Close to 8000 polyphenol compounds have been identified in nature [111], with a great diversity of structures from simple molecules to polymers with high molecular weight [112]. Known as structures of aglycones, the number of aromatic rings depends on the structural elements; polyphenol compounds are classified as flavonoids, phenolic acids, lignans, stilbenes, alkylphenols, curcuminoids, furanocoumarins, phenolic terpenes, and others. Polyphenol compounds are the most abundant phytochemicals in fruit and vegetable-based diets [113,114], and are bioavailable after absorption by the intestine into the circulatory system, after the ingestion of food. However, its bioavailability is affected by various factors, such as food processing, the amount of food ingested, interactions with other molecules, or intestinal factors, which may depend on the pharmacokinetic profile (absorption, distribution, metabolism, and excretion: ADME) [115,116]. Despite this, recent studies on the natural bioactive compounds present in foods linked them with effects on cell functions in obesity, such as a decrease in the inflammatory response [117], inhibit adipogenesis and lipogenesis [118], induce apoptosis [119], regulation genes involved in adipogenesis, lipolysis, and fatty acid oxidation [120], and others.

On the other hand, the biological effect of phenolic compounds on obesity, these compounds maintain other functions, such as the regulation of insult oxidative, inflammation, and autophagy in diabetic nephropathy due to the action mechanism of ferulic acid, which is based on the regulation of the AGE, MAPK, and NF-kB pathways. Additionally, ferulic acid inhibits excessive ROS production, stimulates autophagy, and inhibits apoptotic cell death in a high-glucose environment on cultured NRK-52E cells [121].

Quercetin is effective for gut dysbiosis, improving with the administration of 0.2%, before antibiotic treatment in mice as it restores the diversity of the gut bacteria as well as intestinal barrier function [122].

Lignans have been found as a neural protector due to their inhibitory effect on NO production in LPS-activated microglia. Other compound derivative-lignans attenuate the production of NO and PGE2, as well as inhibit the expression of iNOS and COX-2 by suppressing I-kB-a degradation and the nuclear translocation of the p65 subunit of NF-kB [123].

3.1.1. Phenolic Acids

Phenolic acids confer health-promoting properties due to antioxidant functions by the reactivity of the phenol moiety (hydroxyl substituent on the aromatic ring) or electron donation and singlet oxygen quenching [124–126]. The study conducted by Aranaz [127] reported that phenolic acids have an inhibitory effect on adipogenesis in 3T3-L1 adipocytes at three different doses (10, 50, and 100 mM) and remained in the medium for 8 days. Additionally, this effect was accompanied by the down-regulation of Scd1 and Lpl, and PPARγ activation by phenolic acid [127]. Another study conducted by Hsu and Yen [128], concluded that 3T3-L1 adipocytes treated with rutin at doses of 0–250 μM for 12 and 24 h have an inhibitory effect on intracellular triglycerides and glycerol-3-phosphate dehydrogenase (GPDH) activity, which could be mediated by a decrease in the expression of adipogenic transcription factors PPAR-γ and C/EBPR, and leptin, as well as an increase in the expression of adiponectin [128]. In general, some studies on phenolic acids have demonstrated the inhibition of macrophage infiltration and inflammatory cytokine release, such as TNFα, MCP-1, and PAI-1 through NF-kB downregulation [129–131]. On the other hand, phenolic acids contribute to the increased secretion of anti-inflammatory adiponectin from adipocytes, avoid adipocyte differentiation, and regulate adverse lipid profiles [129]. There is a wide variety of compounds that maintain positive functions against obesity, as shown in Table 2.

3.1.2. Flavonoids

The biochemical activities of flavonoids and their metabolites depend on their chemical structure, which may vary with one or more hydroxyl substituents, including their derivatives. According to different studies, flavonoids have been related to the reduction of weight due to the loss of adipose tissue [132], β-oxidation stimulation [133], adipogenesis, and lipogenesis inhibition by decreasing the expression of LPL, SREBP1c, and PPARγ [134]. In addition, flavonoids (25–100 μM) decrease the mRNA expression of adipogenic transcription factors (*C/EBPá, PPAR*-α, and SREBP-1) on 3T3-L1 cells.

Additionally, it has been reported that flavonoids (quercetin to 10, 50, and 100 μM)) induce the apoptosis of mature adipose tissues through the modulation of extracellular signal-regulate kinase (ERK) $\frac{1}{2}$ and JNK on 3T3-L1 [135]. On the other hand, during the inflammatory response, flavonoids could inhibit the expression and secretion of pro-inflammatory cytokines [136]. Therefore, flavonoids have shown a positive effect on obesity and reduce its complications [137].

3.1.3. Betalains

Betalains are water-soluble and nitrogen-containing pigments, divided into betacyanins and betaxanthins [138]. Normally, they are widely used as colorants. Diverse studies have indicated that betanin has antioxidant, anti-inflammatory [139], hepatoprotective [140], anticancer [141], and anti-diabetes activities [142].

3.1.4. Carotenoids

Carotenoids have many effects on obesity, such as restricting the adipogenesis and hypertrophy of adipocytes [143]. According to Mounien et al. [144], carotenoids downregulate gene expression in adipocytes through NF-κB and MAPK, or via the transcription factors implicated in detoxification, such as aryl hydrocarbon receptor (AhR), nuclear factor erythroid-2-related factor 2 (NRF2), or Pregnane X receptor (PXR). In addition, they have an inhibitory effect on adipocyte differentiation, anti-adipogenic effects via the regulation of adipogenic transcription factors, such as C/EBPα (CCAAT/Enhancer-binding Protein α) and PPAR-γ (Peroxisome proliferator-Activated receptor), reducing LPS-mediated induction of TNF-α in macrophages via NF-κB and JNK, and attenuating macrophage infiltration [145], among others. The mechanisms of the action of specific bioactive compounds on animal models of obesity are summarized in Table 3.

Table 3. Molecular mechanisms of bioactive compounds on animal models of obesity.

Bioactive Compounds	In Vivo	Mechanisms of Action	Toxicity	Author
Phenol acids				
Caffeic acid	C57BL/6 mice with diet HFD	The mechanism focuses on an increase of the phosphorylation of AMP-activated protein kinase and decreasing acetyl carboxylase, a downstream target of AMP-activated-protein kinase (AMPK).	No maternal toxicity	[145]
Ellagic acid	High-fat diet-induced obesity SD rats.	Decreases the mRNA expression of Zfp423 and Aldh1a1 (responsibilities of WAT plasticity) and increases the mRNA expression of the brown adipocyte, as well as markers UCP1, PRDM16, Cidea, PGC1α, and Ppar-α; and beige markers, including CD137and TMEM26. It also elevates the expression of UPC1 in iWAT (specific protein of brown adipocyte).	No-observed-effect level 3011 mg/kg bw/day (males) No-observed-effect level 3254 mg/kg bw/day and 778 mg/kg (females) (rats)	[13,146]
Gallic acid	Mice (Swiss) model fed with high-fat diet	Induces an increase in SIRT1 and PGC1-α, might be responsible for thermogenesis activation under a high-fat diet.	Non-toxic >100 mg /L	[12,147]
p-Coumaric acid	Mouse model of high-fat diet-induced obesity	The mechanism of the action on obesity is mediated by the mTORC1-RPS6 pathway, regulating the Ucp1, HSL, and GUT-4 proteins	Low toxicity 2850 mg/kg bodyweight (mice)	[14,148]
Vanillic acid	High-fat diet (HFD)-induced obese mice and genetically obese db/db mice	The mechanism of action is due to the increase in the cellular NAD levels, and AMPK activates the NAD-dependent deacetylase SIRT1, which results in the deacetylation or activation of PGC1 and, therefore, a thermogenic effect.	1000 mg/kg b.w (rats)	[149,150]
Flavonoids				
Capsaicin	Mouse (Adult male WT and TRPV1−/− (B6.129X1Trpv1 tm1Jul/J) model of HFD-induced obesity.	Intracellular Ca2+ rises via TRPV1 channels stimulated by CAP, activating CaMKII/AMPK, which phosphorylates and activates SIRT-1. This causes the deacetylation of PPAR-γ and PRDM-16 and facilitates their interaction to promote the browning of WAT (white adipose tissue).	Oral LD50 118.8 mg/kg for males and 97.4 mg/kg for females (mice) Male rats—161.2 mg/kg, and female rats—148.1 mg/kg	[151,152]
Anthocyanins	Male C57BL/6J mice fed a modified AIN-93M control diet containing high fat/high cholesterol	Inhibition of IKKε expression in adipose tissue occurs. Prevents the action of macrophage infiltration by attenuating the action of IKKε in energy preservation.	No toxic effects of anthocyanins identified 20 mg/kg/d mice; >3 g/d guinea pigs and rats; >2.4% body weight in beagle dogs and 9 g/kg/d in rats, mice, and rabbits	[153,154]

Table 3. *Cont.*

Bioactive Compounds	In Vivo	Mechanisms of Action	Toxicity	Author
Pterostilbene	Zucker rats (fa/fa) model of genetic obesity	Present effect thermogenic and oxidative capacity of brown adipose tissue, due to increase of gene expression of Ucp1, peroxisome proliferator-activated receptor γ co-activator 1 α (Pgc-1α), carnitine palmitoyl transferase 1b (Cpt1b), nuclear respiratory factor 1 (Nfr1), and cyclooxygenase-2 (Cox2); PPARα, PGC-1α, p38 mitogen-activated protein kinase (p38 MAPK), UCP1 and glucose transporter (GLUT4); and enzyme activity of CPT 1b and citrate synthase (CS) were assessed in interscapular brown adipose tissue.	No significant toxic effects	[11,155]
Resveratrol	High-fat diet (HFD)-induced adipogenesis and inflammation in the epididymal fat tissues of mice C57BL/6J.	There are changes in the GalR1, GalR2, PKCd, and p-ERK protein expressions, with subsequent changes in the Cyc-D and E2F1 expressions, on galanin-mediated adipogenesis cascades in the epididymal adipose tissue. Decrease adipogenic transcription factors (PPARg2, C/EBPa, SREBP-1c, and LXR) and their target genes (FAS, LPL, aP2, and leptin) were suppressed. TLR4 uses MyD88-dependent and MyD88-independent pathways, whereas TLR2 signals only in the MyD88-dependent manner. The MyD88-dependent pathway uses TRAF6 and IRF5, leading to its nuclear translocation and cooperation with NF-kB. The MyD88-independent pathway uses TRIF in activating NF-kB in either a TRAF6-dependent or TRAF6-independent mechanism. TRIF associates with TBK1 and IKKi, which in turn leads p-IRF3. Resveratrol limits changes in the expression of TLR2, TLR4, and downstream molecules (MyD88, Tirap, TRIF, TRAF6, IRF5, p-IRF3, and NF-kB), along with the subsequent changes in the cytokines (TNFα, IFNα, IFNβ, and IL-6) implicated in the TLR2/4-mediated pro-inflammatory signaling cascades on adipose tissue	No toxic effect in humans	[10,156]

Table 3. *Cont.*

Bioactive Compounds	In Vivo	Mechanisms of Action	Toxicity	Author
Curcumin	Mice C57BL/6 fed a high fat diet	There is a suppression of acetyl CoA conversion to malonyl CoA. Lower levels of malonyl CoA increase CPT-1 expression, promoting fatty acid oxidation. The phosphorylated AMPK also suppresses the expression of GPAT-1, which results in reduced fatty acid esterification. The phosphorylated AMPK inhibits PPAR-γ and C/EBP-α transcription factors.	No toxicity from curcumin	[157,158]
Quercetin	Diet-induced obese (DIO) ICR mouse	Blocked protein levels of the key adipogenic factors C/EBPβ, C/EBPα, PPARγ, and FABP4, and the TG-synthesis enzymes lipin1, DGAT1, and LPAAT. Inhibited MAPK, ERK1/2, JNK, and p38MAPK, and MCP-1 and TNF-α in adipocytes and macrophages	285–3000 mg/kg toxicity present	[159,160]
Apigenin	High-fat diet (HFD)-induced obese C57BL/6 (C57) mice	Apigenin binds to non-phosphorylated STAT3, reduces STAT3 phosphorylation and transcriptional activity in visceral adipose tissue, and consequently reduces the expression of the STAT3 target gene cluster of differentiation 36 (CD36). The reduced CD36 expression in adipocytes reduces the expression of peroxisome proliferator-activated receptor-gamma (PPAR-γ) which is the critical nuclear factor in adipogenesis.	300 mg/kg (mice) No toxicity	[161,162]
Scutellarein	Mouse model of obesity induced by high-fat diet (HFD) feeding.	There is suppression of the expression of cytokine genes TNF-α, IL-6, IL-1β, ICAM-1, VCAM-1, and NF-κB.	Minimally toxic or non-toxic in rodents	[163,164]
Luteolin	C57BL/6J mice model of DIO (diet-induced obesity: high-fat diet)	It is modulated the TLR signaling pathway on pro-inflammatory response. There is a decrease in EMR1 and CCL7, which impacts adipose tissue, increases lipolysis and the TCA cycle, reduces the pro-inflammatory response, adipokine dysregulation, adipocyte macrophage infiltration and accumulation, fibrosis, pancreatic β cell dysfunction, hepatic lipotoxicity, insulin resistance, and chronic inflammation. Another mechanism of action is the interaction in the AMPK/PCG1α. Elevates the expressions of thermogenic genes and the activities of AMPK/PCG1a signaling molecules.	No adverse effect or toxicity	[165–167]

Table 3. *Cont.*

Bioactive Compounds	In Vivo	Mechanisms of Action	Toxicity	Author
Chlorogenic acid Caffeine	ICR mice with high-fat diet	Increases AMPK phosphorylation and p-AMPK up-regulates the expression of ATGL and HSL, promoting the hydrolysis of triglycerides and the release of FA. Elevates ACO expression by the activation of AMPK (accelerated β-oxidation). Down-regulation of LXR-α and increase in p-AMPK restrain the expression of SPEBP1c, thereby down-regulating the expression of SCD1 and FAS to inhibit lipid synthesis and regulate lipid metabolism.		[168]
Catechin, Picatechin, Procyanidins	High-fat diet-fed C57BL/6 mice	Activated AMPK-α also induces the expression of UCPs and PGC-1a, which are involved in energy expenditure and thermogenesis		[169]
Cyanidin-3 O galactoside	Mice (C57BL/6) model with high-fat diet-induced obesity	Related to adipogenesis-related transcription factors (C/EBPs, PPAR-γ, and SREBP-1c) and coactivators (PGC-1α), and the down-regulation of specific adipogenesis-related genes affected by these transcription factors.		[170,171]
Other compounds				
Betacyanins	High-fat diet (HFD)-induced obese mice	Reduces HFD-induced body weight gain, and ameliorates adipose tissue hypertrophy, hepatosteatosis, glucose intolerance, and insulin resistance. Increases the expression levels of lipid metabolism-related genes (AdipoR2, Cpt1a, Cpt1b, Acox1, PPAR-γ, Insig1, and Insig2) and FGF21-related genes (β-Klotho and FGFR1/2), and decreases the expression level of Fads2, Fas, and FGF21		[171]

3.2. Clinical Evidence

Clinical evidence regarding the effect of bioactive compounds to treat obesity and its comorbidities is limited compared with animal models of obesity, and clinical results are not conclusive. Although the evidence suggests that bioactive compounds are not effective for weight loss in humans, the anti-inflammatory response in the obesogenic state is still a field of research.

Clinical studies with a combination of bioactive compounds showed controversial results. In a pilot study, the effectiveness of dietary herbal supplements of rhubarb, ginger, astragalus, red sage, and turmeric was found to reduce food intake and cause weight loss in women with a 700 kcal/day diet. After 8 weeks, no changes in weight were observed [172]. The consumption of two cups of strawberry drinks daily by women with metabolic syndrome after 4 weeks reduced the levels of oxidized LDL without changes in CRP and adiponectin [173]. The acute consumption of 250 mL of Hibiscus sabdariffa calyces (HSC) extract, which is rich in polyphenols, for two weeks was proven in men with cardiovascular disease to increase the flow-mediated dilatation of the branchial artery. Although Gallic acid, 4-O-methylgallic acid, 3-O-methylgallic acid, and hippuric acid reached a maximum plasma concentration at 1 to 2 h post-consumption of the extract, changes in other clinical parameters and the CPR levels were not observable [174]. However, the chronic consumption of a combination of bioactive compounds (epigallocatechin gallate, capsaicin, piperine, and L-carnitine) for 8 weeks in overweight subjects showed diminished

HOMA-IR, leptin/adiponectin ratio, LDL, ghrelin, and CRP [175]. The evidence suggests that some combinations could reduce their protective effects in clinical trials.

In trials with a single compound, the protective effect seems to be more evident. In a double-blind, randomized trial controlled with a placebo, 22 subjects with T2D received 180 mg of ellagic acid per day for 8 weeks and 22 subjects with T2D received a placebo. At the end of the study, fasting plasma glucose, insulin, HOMAIR, and Fetuin A were reduced and serum sirtuin1 was increased by the treatment of ellagic acid [176].

In a pilot study with subjects with multiple sclerosis, the consumption of 800 mg epigallocatechin gallate and 60 mL of coconut oil decreased IL6 and the fat percentage [177]. In overweight women and those with obesity, the consumption of epigallocatechin-gallate and resveratrol (282 mg/d and 80 mg/d, respectively) for 12 weeks did not cause changes in adipocyte size and distribution, but caused changes in pathways related to adipogenesis (β-estradiol and Prolactin), the cell cycle and apoptosis were downregulated, as well as oxidative stress (nuclear factor and erythroid 2-like 2 (NRF2)) and inflammation (TNF-α) [178].

Resveratrol consumption (150 mg/day) has been proven in obese men in a randomized double-blind crossover study for 30 days. The effects of Resveratrol were: decreased intrahepatic lipid content, circulating glucose, triglycerides, HOMA index, systolic blood pressure, and inflammation markers (CRP). Although changes in the BMI were not observable, this trial suggests that resveratrol induced metabolic changes in obese humans mimicking calorie restriction [179].

Another factor to take into account is the possible interference with meals. For example, a daily intake of 25 mg of pure (-)-epicatechin (EPI) for two weeks does not reduce cardiometabolic risk factors in overweight and obese adults [180]. However, the consumption of a higher dose of 100 mg of EPI before meals for 4 weeks showed a significant reduction in the TG/HDL ratio and hsCRP [181].

Additionally, genetic factors could interfere with the observable effects. In a study (double-blind, placebo-controlled cross-over trial with 6-week treatment periods separated by a 5-week washout period) with ninety-three overweight or obese adults with metabolic syndrome, the effect of 150 mg of quercetin was evaluated. The consumption of quercetin reduced the systolic blood pressure and plasma-oxidized LDL without changes in serum TNF-alpha and CRP [182]. This could be explained by a genetic predisposition; Egert and colleagues reported that the reduction of TNF-alpha was dependent on the apolipoprotein E genotype [183]. Later, the Egert group studied the effect of the consumption of 162 mg/d quercetin in overweight-to-obese patients with pre- and stage-1 hypertension, without changes in systemic and adipose tissue inflammation after 6 weeks of treatment [184].

The use of curcumin in clinical trials has been widely evaluated. The effects of curcumin in a 6-month randomized, double-blind, and placebo-controlled clinical trial with subjects diagnosed with type-2 diabetes showed a positive effect on the reduction of the pulse wave velocity and leptin with increased levels of serum adiponectin [185]. Ganjali and collages conducted a randomized, crossover, and controlled trial in obese individuals who consumed 1 g of curcumin daily for 4 weeks. They observed a significant reduction in IL-1β, IL-4, and VEGF with curcumin consumption [186]. In a study on overweight girls who consumed 500 mg of curcumin per day for 10 weeks, a reduction in serum IL-6 and CRP was observed [187]. However, in another study, the consumption of 1 g of a phosphatidylcholine complex of curcumin in individuals with metabolic syndrome resulted in no changes in the BMI and clinical parameters [188]. Additionally, the use of resveratrol in combination with curcumin has no impact on the postprandial inflammatory markers of obese individuals in an acute intervention [189], and the use of curcumin alone or with fish oil in older overweight adults and those with obesity did not result in additional benefits to the fish oil alone, which improved dyslipidemia [190].

The use of a network platform has been useful to evaluate the synergistic mechanism of Sanghuang–Danshen (SD) phytochemicals in the homeostatic protection against high-fat-induced vascular dysfunction in healthy subjects. The acute consumption of 600 and 900 mg of SD phytochemicals had synergistic effects and fumaric acid, cryptotanshinone, and

ellagic acid would exert a synergistic influence on vascular health by regulating adhesion molecule production [191]. Therefore, the use of new bioinformatic tools could be useful for understanding the interactions of bioactive compounds and their potential effects.

3.3. Antioxidant Fiber Dietary

There is a diversity of plant and by-products that have been considered as potential sources of dietary fiber and bioactive compounds, in such a way that it has been called "antioxidant dietary fiber" (ADF) and is defined as a product with a content of natural antioxidants associated with the fiber matrix [192], which is characterized by the combined beneficial properties of both dietary fiber and antioxidants [193] and could be considered as a bioactive compound.

Dietary fiber maintains the functional integrity of the gastrointestinal tract, improves constipation, improves cardiovascular diseases and diabetes, and reduces the risk of developing cancer [169,193–196]. The action mechanisms that develop the fiber depend on the dietary fiber type. A diversity of studies on animals has demonstrated the effect of consuming dietary for obesity control, as well as to decrease gastric bloating and promote satiety, through the interaction between fiber (soluble and insoluble) and its contact with water to increase its viscosity, which will depend on different factors, such as the structure of the fiber, and the chemical composition, concentration, and molecular weight of the dietary fiber. Additionally, ADF decreases caloric intake, aids in weight reduction (fat), induces changes in body fat distribution, decreases the fatty tissues, and even inhibits glucose absorption, and high cholesterol and triglycerides levels [195,197–205].

Zou et al. [206] reported that insoluble fiber induces the expression of IL-22, which in involved in reducing the attack on the microbiota due to the building of the epithelium by the regeneration of crypts and the expression of antimicrobials that protect against a series of inflammatory processes, such as those produced by obesity [206]. The results obtained by Sanchez et al. showed that the intake of soluble fiber may enhance the pro-inflammatory state characterized in obesity [207], as fiber could protect against the oxidative stress characterized by this pathology. On the other hand, Ma. et al. [208] proved that soluble and insoluble fiber are related to low CRP concentrations, as fiber decreases lipid oxidation and, therefore, reduces inflammation. In addition, another study reported that fiber consumption decreases inflammatory markers due to reduced LPS production and improves gut permeability [209].

4. Conclusions

The modification of lifestyle is suggested as a treatment for obesity control, mainly including the consumption of natural foods, which could help to improve health due to the content of bioactive compounds, such as flavonoids, phenolic acids, or dietary fiber. In addition, other relevant aspects, such as the bioavailability, metabolic pathways, and action mechanics, of the resultant metabolites of bioactive food compounds are important aspects that reduce obesity and its related diseases. However, more research is required to justify the use, efficacy, and safety of foods with bioactive compounds. Additionally, clinical validation is necessary with finality to implement correct treatment strategies.

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Capítulo II

Efecto del hervido sobre la composición nutricional, antioxidantes y propiedades fisicoquímicas de las plantas comestibles (*Malva parviflora y Myrtillocactus geometrizans)*

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RESEARCH ARTICLE

Effect of boiling on nutritional, antioxidant and physicochemical properties of edible plants (*Malva parviflora* and *Myrtillocactus geometrizans*)

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ABSTRACT

The aim of the present research was to determine the effect of boiling on nutritional composition, total phenolic compounds, antioxidant capacity, physicochemical and morphological characteristics of two edible plants *Malva parviflora* (mallow leaf) and *Myrtillocactus geometrizans* (garambullo flower). The plants had an important nutritional composition as carbohydrates (48-70 %), dietary fiber (36-42 %) and protein (13 %), as well as total phenolic compounds (468-750 mg GAE/100 g db) with a high antioxidant capacity. However, boiling originated the decrease of soluble compounds, carbohydrates, total phenolic compounds, antioxidant capacity and physicochemical properties. Plants changed to dark colors and physicochemical properties were affected, except to water retention capacity, oil retention capacity and viscosity, which had the same values in mallow leaves (raw and boiled), but increased water retention capacity in garambullo flowers, it may be by changes in the morphology observed. Therefore, is to suggest the raw consumption or with minimal cooking of these plants to avoid changes caused by thermal treatment.

Keywords: *Plants; antioxidants; boiling; nutrimental composition; physicochemical properties*

INTRODUCTION

Mexico is distinguished for having a great regional biological diversity of plants. There are about of 25,000 to 30,000 species, which 7,461 are registered, and 2,168 are considered edible species (Mapes and Basurto, 2016), from example: *Erythrina americana, Yucca filifera, Chenopodium* spp., *Suaeda torreyana, Portulaca oleracea, Porophyllum* spp., *Agave salmiana*, *Amaranthus hybridus* L., *Malva parviflora, Myrtillocactus geometrizans,* among others. These plants have an important role in complementing population diet with great impact on health due to they provide dietary fiber, vitamins, minerals and antioxidant compounds (Cilia López et al., 2015; Kibar and Kibar, 2017; Pinela et al., 2017).

The structural matrix of plants mainly formed of dietary fiber (soluble and insoluble fiber) determine the physicochemical characteristics and therefore healthy properties after being consumed (Ahmed et al., 2011; Lattimer and Haub, 2010; Dhingra et al., 2012; Deepak and Sheweta, 2019). When the food is subjected to several thermal treatments (boiling, fraying, dry, blanched, among others), several changes are carried out with the disruption of the food matrix (composed mostly of dietary fiber). The rheological properties of the cooked vegetables are dependent on the cellular disruption and the ability of fibers to absorb and hold water (Waldron, Parker, and Smith, 2003; Vetter and Kunzek, 2002). The interaction of water with fibers such as pectin, β-glucan or gums with high molecular weight causes a high viscosity in the food, which may exhibit a positive action on the glycemic response, cholesterol and lipids (Guillon and Champ, 2000; Vetter and Kunzek, 2002). The free diffusion of water and low molecular weight compounds that could be in the matrix

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(Southon and Faulks, 2002) will influence the color, texture, flavor and nutritional value of cooked vegetables in different ways. The release of compounds of the food matrix (as sugar molecules by hydrolysis of complex glycosidic chains, fat, protein molecules, minerals and bioactive compounds) are leaching into the boiling water (Nafir et al., 1992; Traoré et al., 2017; Okibe et al., 2015; Ikanone and Oyekan 2014; D. T. and Crosby, 2016). In addition, other reactions could take place in the food during thermic treatment, as the formation of complexes between fiber and other released compounds of the food (Takeyama, Yokokawa and Tanimura, 1996), reactions of degradation (oxidation of vitamin C), diminution of antinutritional compounds (oxalic acid, phytic acid), and therefore, the increasing of the bioavailability of some nutrients by their transformation into more active molecular structures (Southon and Faulks, 2002).

There are limited studies on the effect of cooking on many edible wild leaves and flowers of high consumption in mexican gastronomy. Finding only research on changes of the cooked edible plants as *Chenopodium* spp., *Suaeda torreyana, Portulaca oleracea and Porophyllum* spp. (Arias-Rico et al., 2020), and reports forms of consumption of flowers such as squash blossoms, flowers as coral trees and yucca, mexican marigolds, dahlias, cactus flowers, among others (Mulík and Ozuna, 2020). Few studies cover the importance of the changes in the thermic treatment in edible plants as mallow leaves, while in garambullo flowers the research have been on phytochemicals and antioxidants properties (Abdalla and el-Aal, 2016; Singh, 2017; Pinedo-Espinoza et al., 2020). However, there is a lack of studies to be carried out that complement the information in both plants.

For this reason, the purpose of study was to determine the effect of boiling on nutritional composition, total phenolic compounds, antioxidant capacity, physicochemical and morphological characteristics of two edible plants *Malva parviflora* (mallow leaf) and *Myrtillocactus geometrizans* (garambullo flower).

MATERIALS AND METHODS

Plant materials and sample preparation

Fresh plants mallow leaves (*Malva parviflora*) and garambullo flowers (*Myrtillocactus geometrizans*) were purchased from the local market in Pachuca, Hidalgo, Mexico during the period January to June 2019. The general characteristics of the studied plants were described in Table 1. The samples were manually cleaned with distilled water and chopped. Then, the non-edible portions in both samples were discarded. An edible part of these plants (leaves and stems) was maintained in raw and another part was boiled. Boiling conditions were performed by preliminary experiments carried out for each vegetable, considering the minimum cooking time to reach similar softness, palatability, and taste according to the Mexican consumption habits. Each plant batch was divided into three parts to have at least three repetitions in the experiments. A total of 10 g of plant was chopped and boiled in a beaker with 100 mL of distilled water (relation 1:10 food/water) (Figure 1) to complete the cooking (around 90 seconds). The boiling water was drained off for 60 s. Raw and cooked plants were freeze-dried (Freeze dryer VWR26671-581 Labconco, USA), ground to 500 mm mesh, and stored at -20 ◦C in black bags for further analysis. All determinations of proximal analysis, antioxidant, and physicochemical properties were performed in lyophilized samples at least in triplicate. Results of processed samples have been corrected considering a factor that takes into account the soluble solids' loss due to changes of moisture after processing.

Chemical analysis

Proximate analysis

Samples were analyzed using AOAC methods (Latimer, 2012): moisture (method 925.09), protein (method

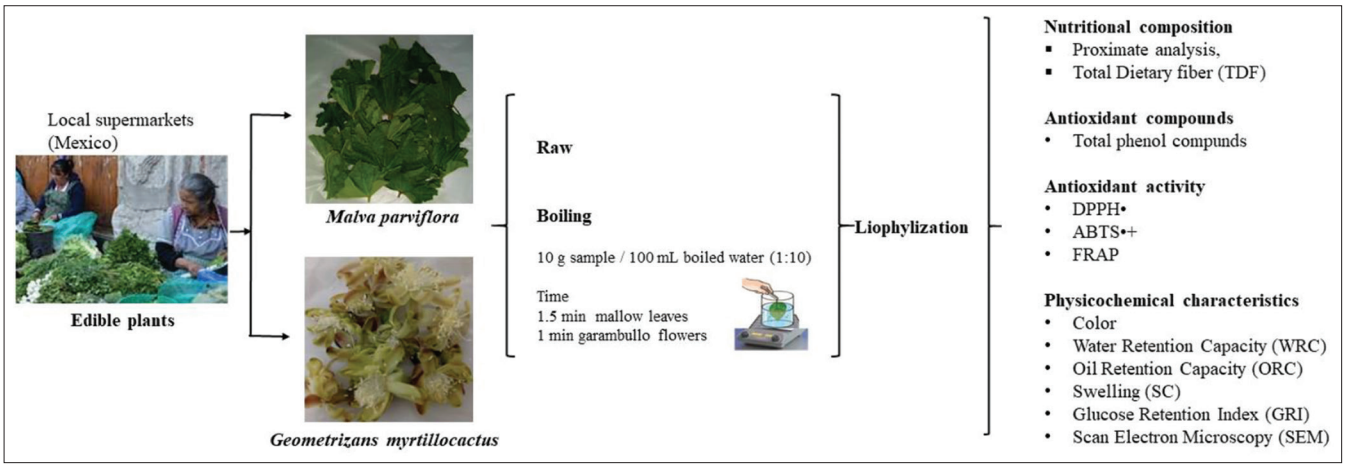

Figure 1. Graphical abstract methodology.

Table 1: Botanical characteristics and thermal treatment conditions of studied plants.

Plants photographs	Plants name	Edible parts
	*Scientific name: Malva parviflora L.* Common name: Spanish: Malva; English: Mallow Is an herbaceous plant, 50 to 70 cm high, reniform, wavy leaves; light lilac flowers; fruit of a squat appearance, blooms from July to September. It is found as ruderal and weeds (Villavicencio-Nieto and Pérez-Escandón, 2006).	Leaves and stems
	*Scientific name: Myrtillocactus geometrizans:* Common name Spanish: gflor de garambullo; English: garambullo flower Flower of Myrtillocactus geometrizans: The flower of garambullo grows at the top of the arborescent, erect cacti. The flowers of garambullo are planned in the areolas, have a white corolla (Muñoz Zurita, 2012).	flowers

950.48), fat (method 983.23) and ash (method 930.05) Finally, carbohydrates were calculated by difference of the proximate parameters. The results were expressed as grams per 100 grams of dried basis (g/100 g db) according with the following formula:

Total Carbohydrates=(100[moisture+protein+lipids+ash]

Total dietary fiber

Total dietary fiber (TDF), soluble (SDF) and insoluble (IDF) dietary fiber were analyzed according to AOAC (Latimer, 2012), with an enzymatic–gravimetric method using a Total Dietary Fiber Assay Kit (Sigma TDF-100A Kit, Sigma-Aldrich). All the results were expressed as grams per 100 grams of dried basis (g/100 g db).

Antioxidant capacity

Antioxidant compounds extraction

The extraction of antioxidants from dried plants was performed in two extraction cycles with aqueous-organic solvents with different polarities (Saura-Calixto et al., 2007). 250 mg of sample with 10 mL of methanol:water (50:50, v/v) was stirred during 1 h and centrifuged (3000 *g*, 15 min) (Allegra 25R ™, Beckman Coulter, CA, USA), then the supernatant was transferred to a volumetric flask of 25 mL. The pellet was re-extracted with 10 mL of acetone:water (70:30, v/v) and centrifuged again. Then, the combination of both supernatants was carried out and the flask was graduated to 25 mL using previously prepared solutions of methanol and acetone (50:50, v/v). The extract was used to determine total phenolic content and antioxidant capacity.

Total phenolic content was performed according to the Folin-Ciocalteu procedure (Montreau, 1972; Singleton et al., 1999). Gallic acid was used as a reference standard, and the results expressed as milligrams of gallic acid equivalents per 100 g of dry basis (mg GAE/100 g db).

The antioxidant activity was evaluated by radical scavenging assays: DPPH$^{\bullet}$ (Brand-Williams et al., 1995 and described by Morales and Jiménez-Pérez (2001) and ABTS$^{\bullet+}$. (Re et al., 1999 and described by Kuskoski et al., 2005). In both determinations Trolox was used as a standard and the results were expressed as micromol of trolox equivalents per 100 g of dried basis (μmol TE/100 g db).

FRAP ferric reducing antioxidant power was accomplished according to the methodological process described by Benzie and Strain, (1996) and referred methodology by Gulcin et al., (2003). Ferrous sulfate was used as standard and the result was expressed as micromoles of Fe (II) per 100 g of dried basis [μmol Fe (II)/100 g db].

Physicochemical analysis

Color

The color parameters were measured with a Minolta CR300 Japan colorimeter using a CIELab system on the basis of CIE *L* (luminosity)*, *a* (redness)* and *b* (yelloness)* values. Also, Hue angle [h°= tg-1(b/a)] and chroma [C= ($a^{*2} + b^{*2}$) ½)] parameters (Janin et al., 2001) were calculated.

Functional properties

The water retention (WRC) and swelling (SC) capacity were analyzed according to Robertson et al., (2000) methodology. The results of WRC were expressed as g/g, while SC was defined as mL/g db.

The oil retention capacity (ORC) was determined under the methodology of Garau et al., (2007). ORC was expressed as oil retained by 1 g of sample.

To measure the viscosity, a dispersion with distilled water (3 %) was obtained from each lyophilized sample using a Brookfield DV-E viscometer at temperatures of 27°C. The results were expressed as centipoise (cP).

The glucose retention index (GRI) was determined on the basis of the retardation of diffusion of glucose according to Goñi et al., (2002) and de Cortes Sánchez-Mata et al., (2002) methodology according the next formula:

$$GRI = 100 - \frac{(Glucose\ diffused\ bag\ with\ fiber)}{(Glucose\ diffused\ bag\ without\ fibre)} * 100$$

SEM (Scanning electron microscopy)

To determine the microstructure of plants, the scanning electron microscopy (SEM) was used. An amount of dried sample was placed on samples holders with Denton Vacuum LLC (Moorestown NJ, USA), at a pressure of 20 millitorr and a current of 20 mA for 4 minutes. The samples were covered with gold and were observed in a JEOL IT300 to x1000.

Statistical analysis

All the determinations were performed in triplicate and the results of the evaluated samples were expressed by mean ± standard error of the mean (SE). To determine the difference and the levels of statistical significance between raw and cooked of each plant a Student t test was used (95%) using the statistical package SPSS System for WINTM version 19.0.

RESULT AND DISCUSSION

Nutritional composition

Proximal analysis

These plants are used by populations into their traditional dishes, and usually are consumed boiled.

Table 2 shows the results of chemical composition of the studied food vegetable (mallow leaves and garambullo flowers). In general, the raw wild foods had a range of moisture between 94 – 97 % and characterized mainly by carbohydrates 48 - 70 %, followed by protein (13 - 34 %) and ashes (8 - 13 %). On the other hand, both plants had a low content of lipids of around 2 %, therefore these foods are considered as low-calorie foods (Pinedo-Espinoza, et al., 2020). These results were similar to other studies in jew´s mallow leaves and mallow leaves (*Malva parviflora*) (Abdalla and el-Aal, 2016) and garambullo flower (Pinedo-Espinoza et al., 2020).

The thermal treatment caused an increase ($p<0.05$) of 6 % (mallow leaves) and 15 % (garambullo flower) in carbohydrates and the content of proteins increased in mallow leaves (26 %) while garambullo flowers decreased (2.8 %) in comparison with raw samples. The increase in carbohydrate content could be attributed to hydroxylation of complex glucidic chains freeing sugar molecules, while that the high proteins content could be explained by the nature and form of proteins of the plants (Traoré et al., 2017). A significant loss of soluble compounds was observed in both studied samples, as in ethereal extract (around 23 - 55 %) and ashes (50 %), similar to other studies (Ahmed and Ali, 2013; Arias-Rico, 2020).

Total dietary fiber (TDF)

Wild plants are related to human nutrition and folk medicine because of its high content of dietary fiber (Koca et al., 2015). A content of TDF in raw mallow leaf was obtained (36.47 g/100 g db), composed mainly of IDF (31.32 g/100 g db) and the rest of SDF (5.15 g/100 g db) (Table 2). It was higher compared to a study on *Malva neglecta Wallr* leaves (Koca et al., 2015). The garambullo flowers had a content of 42.98 g/100 g db of TDF, with a high content of IDF (40.02 g/100 g db) in comparison with SDF (2.96 g/100 g db). Several authors established the content of crude fiber on garambullo flowers (6.92 g/100 g db) as well as in different edible flowers (*Aloe vera, Agave salmiana, Arbutus xalapensis, Cucurbita pepo, Erythrina americana, Erythrina caribaea, Euphorbia radians, Yucca filifera*) (12-17 g/100 g db) (López-Cervantes et al., 2018; Sotelo et al., 2007), however, this methodology underestimate value dietary fiber in the foods due to loss of fiber material chemical treatment produced (Dhingra et al., 2012).

The thermal processing showed a decrease ($p<0.05$) in TDF (12.2- 26.8 %) of the studied samples which could be attributed to partial degradation of cellulose and hemicellulose into simple carbohydrates during boiling (Zia-ur-Rehman et al., 2003). In comparison with the raw samples, the fraction of SDF increased (19 and 8 %), with an important decrease of IDF (35 and 13 %) in mallow leaf and garambullo flower, respectively. These changes could be caused by modification of the structure of fiber (Caprita et al., 2011) as depolymerization of the cell walls (Huang and Hsieh, 2019) or disruption of covalent and non-covalent bonds between polysaccharide chains and proteins moieties or glycosidic linkages on the dietary fiber components affected by heat treatment (Bader Ul Ain et al., 2019; Caprita et al., 2011; Margareta and Nyman, 2003; Yang et al., 2017) increasing the SDF content. The changes on the wall cell and therefore in the food matrix cause important changes of physicochemical properties of the vegetable foods (Fouad And Rehab, 2013; Miglio et al., 2008; Ramírez-Moreno et al., 2013).

Total phenolic compounds and antioxidant capacity

The antioxidant compounds as phenols are substances with a main function on the oxidation process, inhibiting free radicals, therefore, these bioactive compounds have important physiological effects (Farhan et al., 2012). Vasco et al., (2008) establishes that foods could be classified according to phenolic compounds in low content (< 100 mg GAE/100g db), medium (101-1000 mg GAE/100 db) and high content

Table 2: Effect of thermal treatment on nutritional composition of edible plants in Mexico (g/100 g db).

Parameter	Mallow leaf		Garambullo flower	
	Raw	Boiled[A]	Raw	Boiled[A]
Moisture	97.51 ± 0.29	98.14 ± 0.04	94.56 ± 0.86	94.16 ± 0.38
Carbohydrate	48.05 ± 0.73	51.96 ± 1.31*	70.75 ± 0.56	81.58 ± 0.54*
Protein	34.94 ± 0.62	44.05 ± 1.26*	13.25 ± 0.15	12.87 ± 0.06*
Etheral extract	1.84 ± 0.12	1.41 ± 0.59*	2.30 ± 0.53	1.02 ± 0.27*
Ash	12.82 ± 0.49	6.50 ± 0.20*	8.23 ± 0.57	4.35 ± 0.42*
Total dietary fiber	36.47 ± 0.01	26.69 ± 0.01*	42.98 ± 1.37	37.73 ± 0.15*
Dietary fiber fractions				
Soluble	5.15 ± 0.00	6.12 ± 0.00*	2.96 ± 0.00	3.20 ± 0.00*
Insoluble	31.32 ± 0.02	20.56 ± 0.01*	40.02 ± 1.37	34.53 ± 0.02*

Values are mean ± SEM (n=3) *The asterisk indicates a significant difference between raw and boiled of each plant. [A] Corrected value taking into account the soluble solid loss during cooking, [B] Carbohydrates were calculated substrate the values of moisture, protein, ethereal extract and ash.

(>1001 mg GAE/100g db). In this case, the studied plants presented a medium content of polyphenols, raw mallow leaf was of 468.43 mg GAE/100 g db, while raw garambullo flower showed a value of 750.89 mg GAE/100 g db (Table 3). In comparison with other studies in other mallow leaves the TPC content of mallow leaf was low due they found values of TPC between 789 - 1180 mg GAE/100g db (Abd El-Salam and Morsy, 2019). While TPC from garambullo flower was higher than the found by others studies (35 - 189 mg GAE/100 g db) (Solís-Ramírez and García-Vieyra, 2017; Pinedo-Espinoza et al., 2020). The variable polyphenols content between studies of these plants may be due to several factors such as the botanical characteristics, antioxidant composition, time and the used of different solvents for extraction (Abd El-Salam and Morsy, 2019; Farhan et al., 2012; Messaoudi et al., 2015; Mohammed et al., 2014; Petkova et al., 2019; Solís-Ramírez and García-Vieyra, 2017).

The efficacy of antioxidant compounds in samples were analyzed by DPPH$^{\bullet}$, ABTS$^{\bullet+}$ and FRAP methodologies. The values of antioxidant capacity of mallow leaves measured by DPPH$^{\bullet}$, ABTS$^{\bullet+}$ and FRAP were of 1,264.11 μmol TE/100 g db, 331.01 μmol TE/100 g db and 5.55 μmol Fe (II)/100 g db, respectively, while garambullo flowers showed higher values of 1,4004.90 μmol TE/100 g db, 2,344.94 μmol TE/100 g db and 95.72 μmol Fe (II)/100 g db, respectively (Table 3). The values of antioxidant activity measured by the three methodologies in mallow leaf were higher in ABTS$^{\bullet+}$ than other study of *Malva sylvestris* (ABTS$^{\bullet+}$ 34 μmol TE/100 g db) (Benso et al., 2016) and lower in *Malva parviflora* [FRAP 65,416 μM Fe (II)/100 g db] (Teixeira et al., 2016). The activity antioxidant by mallow leaf could possibly possess radical scavengers activity and therefore maintain a good antioxidant activity (Abd El-Salam and Morsy, 2019).

The values of DPPH$^{\bullet}$ inhibition shown by antioxidant values from garambullo flowers were found among the ranges reported for other edible flowers 2,300 - 67,500 μmol TE/100 g db (Pinedo-Espinoza et al., 2020)

Figure 2. (a-d) Samples of mallow leaves and garambullo flowers before and after of boiled.

and low in ABTS$^{\bullet+}$ in comparison with other study (24,523 - 180, 700 mmol/TE 100 g) (Hu et al., 2019). FRAP values of garambullo flowers here reported were found among the values of other studies (0.02 - 112.49 μmol Fe II/100g db) (Li et al., 2014; Roy et al., 2011). The antioxidant capacity in flowers of garambullo could be correlated with the content of phenolic compounds or other metabolites as phloridzin and rutin mainly, compounds considered as good antioxidants (Pinedo-Espinoza et al., 2020; Hu et al., 2019; Shi et al., 2019).

Furthermore, the cooking significantly affected the TPC, inducing a decrease of 22 % in mallow leaf and 10 % in garambullo flower, respect to TPC in raw samples (Table 3),

which are according with the loss of TPC in other studies of cooked vegetables as pepper, squash, green beans, peas, leek, broccoli and spinach (12-26 %) (Turkmen et al., 2005). These losses could be attributed to the diffusion of these soluble compounds into the boiling water or the degradation of these compounds during boiling (Gunathilake et al., 2018; Preti et al., 2017). The losses of bioactive compounds could be strongly related with the decrease of the antioxidant activity of the boiled samples, because mallow leaf and garambullo flower showed a decrease ($p<0.05$) around 30 % of antioxidant capacity measured as DPPH•, ABTS$^{\bullet+}$ and FRAP, with a strong affectation in garambullo flowers (around 30 % until 70 %).

Physical-chemical properties

Color

The parameters of color (*L**, *a**, *b**, h° and C) are shown in Table 4. In the fresh samples, the luminosity showed 31.37 and 44.53 values in mallow leaf and garambullo flower, respectively. The color presented by raw mallow leaf was found in the yellow-green quadrant (values of *a** -3.56 and *b** 14.73) which contributes with a fresh appearance characteristic of chlorophyll content, while the garambullo flower was observed in the yellow-red quadrant (values of *a** 1.13 and *b** 13.60). This color could be due to the presence of phenolic compounds, carotenoids or betalains. Hue showed values of 103.09 and 85.20, while in chroma had results of 15.31 and 13.64 in mallow leaves and garambullo flowers, respectively. The cooking in plants caused color changes (Figure 2). The luminosity increased slightly in both samples, while in the color parameters of *a** and *b**, mallow leaf tended to greener color (*a** -8.80 and *b** 14.50) and garambullo flowers tended to be redder color (*a** 3.76 and *b** 11.66) (Table 4). In addition, hue angle increased green tonality in mallow leaves and decreased the red tonality in garambullo flowers. The color saturation (chroma) was maintained ($p>0.05$) in both plants. The green color developed after boiling could not necessarily be attribute to the normal conversion of chlorophyll to pheophytin (Trivedi et al., 2018), but probably caused by other derivatives of chlorophyll (chlorophyllides) that change from non or less- colored precursor of green color to more visible green color (Turkmen et al., 2005) or this caused by the inactivation of enzymes to avoid changes of color (Mohamed et al., 2012). On the other hand, the deaeration of plants tissue substituting oxygen by water molecules of the boiling causes the loss of opacity of the plants to an increase in the intensity of green color (Turkmen et al., 2005). While that the changes of color to red tonalities in garambullo flowers could be due to the compounds present in the flowers such as carotenoids or betalains, since the caraotenoids may trans - cis transformated of double bonds and therefore leads a decrease of color intensity (Khoo et al., 2011; Schieber and Carle, 2005). After thermal treatment, the betalains could be have several changes as isomerization, deglycosylation, hydrolysis, dehydrogenation and decarboxylation (Rodriguez-Amaya, 2019) or the loss of these hydrosoluble compounds for leaching into cooking water (Sawicki and Wiczkowski, 2018).

Properties of dietary fiber (WRC, ORC, SC, viscosity and GRI)

The functional or technological properties of powder plants are determined by the soluble and insoluble fractions, which have an important impact on functionality and nutritional effects (Lecumberri et al., 2007). The studied samples were characterized with a high content of insoluble

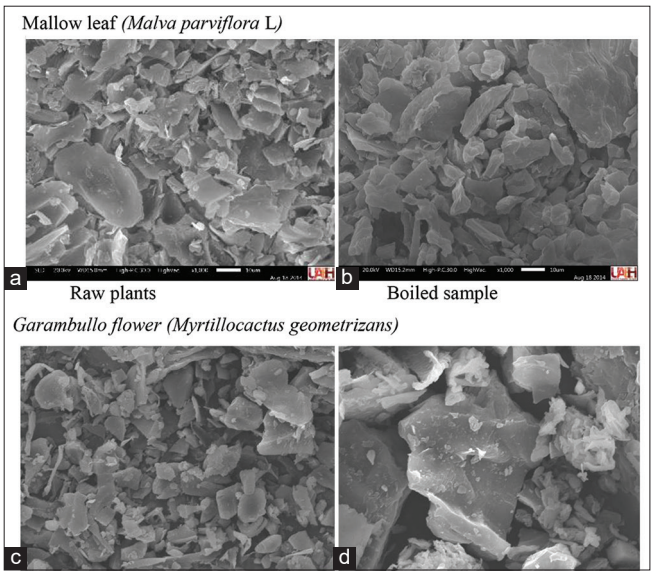

Figure 3. Scanning electron microscopy (SEM) of raw a) and c), and boiling b) and d) tissues of mallow leaf (*Malva parviflora* L.) and garambullo flower (*Myrtillocactus geometrizans*), respectively.

Table 3: Effect thermal treatment on total phenolic compounds and antioxidant activity of edible plants in Mexico (100 g db)

Parameter	Mallow leaf		Garambullo flower	
	Raw	Boiled	Raw	Boiled
TPC (mg GAE)	468.43 ± 4.18*	364.07 ± 4.21	750.89 ± 9.95*	672.53 ± 4.14
DPPH (µmol TE)	1264.11 ± 109.34*	947.77 ± 38.63	14004.90 ± 2694.74*	4192.81 ± 1292.63
ABTS $^{\bullet+}$ (µmol TE))	331.01± 4.45*	243.37 ± 3.37	2344.94 ± 9.73*	1638.95 ± 79.51
FRAP (µmol Fe (II)	5.55 ± 0.17*	3.98 ± 0.07	95.72 ± 0.94*	68.72 ± 0.72

Values are mean ± SEM (n=3) *The asterisk indicates a significant difference between raw and boiled of each sample. [A] Corrected value taking into account the soluble solid loss during cooking.

Table 4: Effect thermal treatment on physico-chemical characteristics of edible plants.

Parameter	Mallow leaf		Garambullo flower	
	Raw	Boiled[A]	Raw	Boiled[A]
Color				
L*	31.37 ±0.86	32.10±1.65*	44.53 ± 1.76	50.83 ± 1.84*
a*	-3.56 ± 2.81	-8.80 ± 0.81*	1.13 ± 0.25	3.76 ± 1.30*
b*	14.73 ± 0.64	14.50 ± 1.50	13.60 ± 0.60	11.66 ± 0.30*
h*	103.09 ± 10.18	121.32 ± 2.86*	85.20 ± 1.27	72.26 ± 5.55*
croma	15.31 ± 1.13	16.97 ± 1.50	13.64 ± 0.58	12.29 ± 0.61
WRC[a] (g/g db)	5.43 ± 0.44	5.31 ± 0.15	5.99 ± 0.15	7.79 ± 0.16*
ORC[b] (g/g db)	2.22 ± 0.38	2.15 ± 0.19	2.40 ± 0.29	1.92 ± 0.30
SC[c] (mL/g db)	2.46 ± 0.05	0.96 ± 0.11*	2.87 ± 0.12	2.40 ± 0.24*
Viscosity (mPa)	6.80 ± 0.17	7.10 ± 0.45	7.05 ± 0.47	7.42 ± 0.10
GRI[d] (mg/g)	4.45 ± 0.13	1.23 ± 0.14*	2.96 ± 0.46	2.38 ± 0.11*

Values are mean ± SEM (n=3) *The asterisk indicates a significant difference between raw and boiled of each plant. [a]WRC: Water Retention Capacity, [b]ORC: Oil Retention Capacity, [c]SC: Swelling Capacity, [d]GRI: Glucose Retention Index. [A] Corrected value taking into account the soluble solid loss during cooking

dietary fiber (85-93 % of total dietary fiber). The results of WRC, ORC, SC and viscosity in raw samples presented values behaved similarly (Table 4).

The values obtained of WRC (around 5 g/g db) and SC (around 2 mL/g db) (Table 2) were similar to commercial supplements of dietary fiber which are characterized as high content of insoluble fiber (WRC of 3.1 to 4.8 g/g db and 6 mL/g db of SC) (Goñi and Martin-Carrón, 1998) and fiber-rich cocoa products (WRC and SC of 4.76 g/g and 6.52 mL/g, respectively). The data of ORC (2 g/g db) were similarities with to the values reported for extracted pectins of agroindustrial residues as soy hull, passion fruit peel and orange pomace (among 2 - 4 g/g db) (de Moura et al., 2017). According to Benítez et al., (2017), particles with bulk density had a higher capacity to absorb or bind lipid components related with a greater surface area.

The results of viscosity in mallow leaf and garambullo flower (6.80-7.05 cP) had the same behavior that some edible plants (*Chenopodium nuttalliae safford*, *Suaeda Torreyana. Watson*, *Portulaca oleracea* L., *Chenopodium album* L. and *Porophyllum ruderale* with values of 2.56–7.68 cP) (Arias-Rico et al., 2020).

Glucose retention index

The GRI is considered an important *in vitro* index to assess the effect of fiber on delay in glucose absorption by gastrointestinal tract (López et al., 1996), mallow leaves showed a higher GRI than garambullo flowers (4.11 g/g db and 2.96 mg/g db, respectively); however, both were similar to obtained for fiber-rich cocoa products (4.40 mg/g db) (Lecumberri et al., 2007).

Several conditions such as thermal processing (boiling), by cross-links holding the cell wall, type and duration time, temperature and hydration grade, among others, could cause the solubilisation of pectic components, often accompanied by the swelling of cell wall (Holland et al., 2020; Waldron et al., 2003). These gradual changes originated from the disruption of the cell wall (cellulose) and the exposure to the environment of the protoplasmic structure, affected the hydration and oil properties (Paciulli et al., 2016; Ranganathan et al., 2016; Xu et al., 2015). The values of ORC and viscosity of both samples studied remained (p>0.05) after cooking, the maintenance of viscosity could be due to minimal increases of soluble components (SDF) after thermic treatment in our study. On the other hand, the SC decreased (p<0.05) in both samples (60 % in mallow leaves and 16 % in garambullo flowers) as well as GRI (between 16 to 19 %). These last could be due to the restructuring of the matrix towards a less compact plant structure after treatment (Margareta and Nyman, 2003; Raghavendra et al., 2006; Requena et al., 2016). The WRC remained in the mallow leaf, while an increase in garambullo flowers was observed. The increase of WRC may be due to hydrophilic matrix of IDF (do not form gelatinous matrix) in which also water is entrapped (Vázquez-Ovando et al., 2009). These changes physically modify the plant fiber, impacting on technological functionality and beneficial effects on health (Holland et al., 2020; Paciulli et al., 2016; Ranganathan et al., 2016).

Scanning Electron Microscopy (SEM)

The function of heat treatment is focused on modifying the sensory characteristics as well as ensuring safety foods. Nevertheless, this treatment could cause change on the matrix of plants. Figure 3 shows the morphology of the tissue of the plant studied raw and boiled. It can be observed in Figure 3a and Figure 3c tissue regular and smooth texture in raw mallow leaves and garambullo flowers, respectively. After boiled treatment there is more amorphous extracellular matter in both samples (mallow leaf and garambullo flower), irregular fragments and small clumps stiffer were observed (Figure 3b and Figure 3d). After cooking treatment the organization of plants is lost due to that the heating provoke alterations in the

microstructure of plants tissue, causing modification on texture by loss of turgor pressure and occluded air, thermal degradation of middle lamella pectins and others cell wall polysaccharides that constitute plant tissue (Gonzalez and Barrett, 2010; Llano et al., 2003), having to the end an impact on physicochemical properties of dietary fiber, nutrimental composition and sensory characteristics (Butz et al., 2002; Gonzalez and Barrett, 2010).

CONCLUSION

According to the results the plants presented an important nutritional composition related to protein, dietary fiber and antioxidant contents. However, the thermal treatment provoked changes on the soluble compounds, mainly carbohydrates and total phenolic compounds, affecting the nutritional composition and antioxidant capacity. Also, cause alterations in physicochemical properties as a consequence plants matrix altered. Short time thermal treatments led modifications as darker colors, hydration properties, water retention capacity in garambullo flowers, swelling and oil retention capacity in the other plant. On the other hand, it showed a decrease in glucose retention index. All the changes could be attributed to modifications in plant structures. In this sense, short thermal treatments are recommended to minimize changes of these plants or inclusive intake raw from these. Further research could be extended to other edible plants. Moreover, studies in plants should be redirected to conducting studies related to human health.

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Authors' contributions

Ramírez-Moreno E. conceived, designed the experiments, and analyzed the data, in addition to serving as director of the thesis of E.M.S.-G.; Sandoval-Gallegos E.M. performed the experiments and analyzed the data; Arias-Rico J.; Cruz-Cansino N. S.; Ramírez-Ojeda D. Zafra-Rojas Q.Y. contributed with reagents/materials/analysis tools and contributed to a valuable discussion; Hernández-Ávila J contributes with analysis and discussion of scanning electron microscopy.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

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Capítulo III

Effect of thermal treatment on the retention of total phenolic compounds and antioxidant activity of edible plants (Mallow and Garambullo flower)

Effect of thermal treatment on the retention of total phenolic compounds and antioxidant activity of edible plants (mallow and garambullo flower)

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Abstract

Mexico has a high variety of edible plant species, which are considered potential sources of nutrients, phytochemicals, organic or inorganic acids, etc., such is the case of mallow and garambullo flowers. Therefore the objective of this study was to evaluate the effect of thermal treatment on total phenolic compounds and antioxidant capacity of two edible plants in Mexico. The studied plants were characterized by a high amount of TPC and were characterized by several phenolics as ferulic, *p*-coumaric, caffeic acids, quercetin and apigenin. After thermal treatment the phenolic compounds of studied samples had a high retention (78 and 89 %) in the food matrix and therefore a high antioxidant capacity (around 70 %) measured asf DPPH, ABTS and FRAP respectively. On garambullo flowers these values present 28, 70, and 72 % retention of DPPH, ABTS and FRAP respectively. On the other hand, coumarin and ferulic acid in both samples was identified, while apigenin was found in the mallow and quercetin in the garambullo flower. However, research is needed to continue characterizing and investigating the effect with these plants

Key words: *bioactive compounds, phenolic acids, flavonoids, Malva parviflora, Myrtillocactus geometrizans*

Introduction

Several plant parts (leaves, flowers, pistils, stems) have been part of the population diet and are rich in bioactive compounds, therefore they have been investigated for their biological activities and potential health benefits. The plant leaves present nutrients as carbohydrates, dietary fiber and minerals (calcium, magnesium, potassium, iron, zinc and phosphorus) (Singh, G., et al., 2001, Onyeka et al., 2007). In addition, these leaves are a source of phytochemicals such as alkaloid, steroid, tannin, anthocyanin, carotenoids and flavonoids etc., (Duma, M. et al., 2014). On the other hand, edible flowers are characterized by nutritional and medicinal properties (de Lima Franzen, et al., 2019). Some studies reported that the flowers are rich in protein, fiber, minerals, phytochemicals (carotenoids, catechins, flavonoids and phenolic acids). With low ethereal extract, resulting in low caloric value, which maintain an important role on metabolic processes (de Lima Franzen, et al., 2019; Grzeszczuk, M., et al., 2018; Ohmiya, 2011; Chen D., et al., 2020; Han, A. R., et al., 2019; Kucekova, Z., et al. 2013).

The mallow leaves have been considered with a nutritional composition important (proteins, carbohydrates, fatty acids, dietary fiber, vitamins and minerals) (Sandoval-Gallegos, et al., 2021; Gasparetto, J., et al., 2012). Also phytochemicals content has been reported as total flavonoid compounds, phenols, tannins, saponins, alkaloids, resins and polyunsaturated acids (Table 2). Due to composition of the mallow, have been attributed with beneficial effects such as anti-inflammatory, anti-microbial, antioxidant, hypolipidemic and hypoglycemic (Martínez-Hernández, et al., 2020; Medrano-Jiménez, et al., 2019; Ododo et al., 2016; Pérez-Gutiérrez, 2012).

The garambullo flowers are a source of nutrients (proteins, carbohydrates, fatty acids, dietary fiber, vitamins and minerals) (Pinedo-Espinoza, et al., 2020; Sandoval-Gallegos, et al., 2021). In addition of the general composition, they contains phytochemical components such as phenolic acids, flavonoids, terpenoids, tannins and saponins (Solis R David, 2017; Pinedo-Espinoza, et al., 2020; Sandoval-Gallegos, et al., 2021) (Table 1) which is characteristic of edible flowers. In relation to the composition, it has been found that in other cacti flowers have anti-inflammatory, and anti-genotoxic, anticancer, diuretic and antiepileptic effects

(Chahdoura, 2017; Sharma et al., 2015; Kamble, S et al, 2017; Galati et al., 2002; Zouaoui et al., 2021).

Table 1. Proximal, phytochemical and fatty acids composition of mallow and garambullo flower (g/100 g db)

Proximal composition	Mallow	Garambullo flower
Carbohydrates	28.7 - 48.05	68.13 - 70.75
Proteins	22.9 - 34.94	12.53 - 13.25
Ethereal extract	1.84 - 2.5	1.69 - 2.30
Ash	12.82 - 18.2	8.23 - 10.73
Total dietary fiber	21.5 - 36.47	42.98
Phytochemicals		
Total Phenolic Compound	1.098 - 87 g GAE/100 g	
Gentisic acid	10.155	
Rosmarinic acid	5.785	
Sinapic acid	8.644	
Gallic acid		0.0089
Chlorogenic acid		0.0557
Syringic acid		0.0056
Vanillic acid	1.133	0.0054
p-hydroxybenzoic acid		0.0089
Caffeic acid		0.0009
Ferulic acid	0.399	0.0271
p-coumaric acid	37.186	0.0096
Cinnamic acid	12.607	
Total Flavonoid Compound	0.00083 – 0.00107 g CAE/100g	
Rutin		1.1365
Naringenin	55.733	
Phloridzin		2.0900
Phloretin		0.4770
Myricetin		0.0470

Apigenin	1.080
Galangin	
Catechin	3.578
Luteolin	27.166
Kaempferol	2.754
Chrysin	0.604
Fatty acids	Decanoic, Dodecanoic, Tetradecanoic, Hexadecanoic, cis-9-Hexadecanoic, Octadecanoic, cis-9-Octadecanoic, cis-9,12-Octadecadienoic, cis-9,12,15-Octadecatrienoic, Eicosanoic, all cis-5, 8, 11, 14 Eicosanoic, Docosanoic, cis-13-Docosanoic, Tetracosanoic

Authors: Ereifej, Khalil et al., 2015; Ekram A et al., 2019; Farhan, H et al., 2012a; Shadid, K. A. et al., 2021; Farhan, H., et al., 2012b; Pinedo- Espinoza et al., 2021, Solis 2019

The consumption of mallow is typically cooked like vegetables in broth or eaten fresh in salads (Dugani et al., 2016). While, garambullo flowers (cacti flowers) are prepared in dishes such as roasted on the griddle, stewed Mexican-style, stewed, then added to quesadillas, savory pancake with or without tomato broth, egg-battered, with or without tomato broth stewed Mexican-style, or as an additional ingredient of meat stews, after thermal treatment (Figueredo-Urbina, et al., 2022).

The study of foods is generally reported in raw matter, so it is important to describe the changes of the food as it was consumed. In addition, other uses of thermal treatment we can find the preservation of food by elimination of pathogenic and spoilage microorganisms, also helps denature or inactive enzymes and toxins of foods, providing quality to the food and a higher useful life (Dewan, Md et al., 2020). This information will be closed to have a complete nutritional composition and its properties. In general, after thermal treatment (boiling, blanched, fried, etc.,) of vegetable foods, several modifications of sensorial characteristics (smell, taste, texture) and nutritional characteristics could be carried out, through thermal treatments. These processes allow food to be edible and palatable, producing changes in texture, making plants softer, but causing the loss of freshness and firmness

(Callcott, E. et al., 2018). These effects are mainly due to the disruption of the structure of the cell wall, of the middle lamellae, as well as of the cellulose, which undergoes an increase in the degree of hydration causing its separation. This process cause gradually, the damage of the cell membrane, as well as the disorganization of the protoplasmic structure, in addition to the loss of cell turgor, which in the end develop alterations in the food matrix, leaving the fibers exposed to the medium, affecting the physicochemical properties and therefore its technological and health functionality (Callcott, E. et al., 2018).

Therefore, the objective of this study was to determine the effect of thermal treatment of phenolic compounds and antioxidant capacity of edible parts of plants: *Malva parviflora L* leaves and *Myrtillocactus geometrizans* flowers.

Materials and methods

Plant material

Plant material and preparation of the freeze-dried extract and hydroalcoholic extract: Fresh plants mallow leaves (*Malva parviflora*) and garambullo flowers (*Myrtillocactus geometrizans*) (Table 2) were purchased from a local market in Pachuca, Hidalgo, Mexico during the period January to June 2019. A total of 10 g of plant was chopped and boiled in a beaker with 100 mL of distilled water (relation 1:10 food/water) to complete the cooking (around 90 seconds). The boiling water was drained off for 60 s. Raw and cooked plants were freeze-dried (Freeze dryer VWR26671- 581 Labconco, USA), ground to 500 mm mesh, and stored at -20°C in black bags for further.

Table 2. Botanical characteristics and thermal treatment conditions of studied plants

Plant photographs	Plants name	Edible parts
	*Scientific name*: *Malva parviflora L.* Common name: Spanish: Malva; English: Mallow Is an herbaceous plant, 50 to 70 cm high, reniform, wavy leaves; light lilac flowers; fruit of a squat appearance, blooms from July to September. It is found as ruderal and weeds (Villavicencio-Nieto and Pérez-Escandón, 2006).	Leaves and stems
	*Scientific name*: *Myrtillocactus geometrizans*: Common name Spanish: flor de garambullo; English: garambullo flower Flower of Myrtillocactus geometrizans: The flower of garambullo grows at the top of the arborescent, erect cacti. The flowers of garambullo are planned in the areolas, have a white corolla (Muñoz Zurita, 2012).	flowers

Phenolic compound total and antioxidant activity

Total phenolic compounds

Total phenolic content was performed according to the Folin-Ciocalteu procedure (Montreau, 1972; Singleton et al., 1999). Gallic acid was used as a reference standard, and the results expressed as milligrams of gallic acid equivalents per 100 g of dry basis (mg GAE/100 g db)

Antioxidant activity

The antioxidant activity was evaluated by radical scavenging assays: DPPH• (Brand-Williams et al., 1995 and described by Morales and Jiménez-Pérez 2001) and ABTS●+. (Re et al., 1999 and described by Kuskoski et al., 2005). In both determinations Trolox was used as a standard and the results were expressed as micromol of Trolox equivalents per 100 g of dried basis (µmol TE/100 g db).

FRAP ferric reducing antioxidant power was accomplished according to the methodological process described by Benzie and Strain, (1996) and referred methodology by Gulcin et al., (2003). Ferrous sulfate was used as standard and the result was expressed as micromoles of Fe (II) per 100 g of dried basis [µmol Fe (II)/100 g db]

Identification and quantification of phenolic acid, flavonoids and vitamin C

Phenolic acids and flavonoids obtention

From identification and quantification of phenolic acids and flavonoids was made an alkaline hydrolysis extraction: 10 mg of lyophilized sample was weighed and homogenized in 2 mL NaOH (2 mol/L), incubated at 35° for 30 min. in the darkness. Then, pH was adjusted at 2 with HCl (3 mol). Subsequently, used 4 mL of diethyl ether and was transferred to a flask (3 times). Finality was samples roto evaporated at 40 °C (Pérez-Flores et al., 2018).

From identification and quantification of phenolic acids and flavonoids, the water 717 plus diode array detector, with column Symmetry C18 (4.6*250 mm*5mm) was used. The mobile phase was acetonitrile (A) and water acid/acetic acid pH 2.8-3.0, to a Flow 1 mL/min with injection of 20 µL (Diaz-Batalla, et al., 2018).

Vitamin C

For extraction of vitamin C, 250 mg sample was weighed and homogenized in metaphosphoric acid at 4.5 % (25 mL), shaked for 15 min in the dark. Then centrifuge at 10,000 rpm for 10 min and was filtered (0.22 µm). From identification and quantification of vitamin C, the water 717 plus diode array detector, with column Symmetry C18 (4.6*250 mm*5 mm) was used. The mobile phase was acid water/ sulphuric acid (A) pH 2.8-3.0, to a Flow 0.9 mL/min with injection of 20 µL (Sánchez-Mata.,2000).

Results and discussion

Total phenolic compounds and antioxidant activity

The table 3 shows the content of total polyphenol compounds and antioxidant activity in Mallow leafs and garambullo flowers before and after thermal treatment. The content of total phenolic compounds from raw mallow was 464.32 mg GAE/ 100 g db, while almost twice as much content was presented by garambullo flowers (755.37 mg GAE/100 g db). A high amount of TPC in flowers in comparison with leaves could be that the flowers contain a diversity of phytochemicals which give attractive visual aspects through its characteristic colors given by the content of carotenoids, betalains, xanthophylls, etc. (Pires, E. 2021; Abd El-Salam and Morsy, 2019; Petkova et al., 2019; Solís-Ramírez and García-Vieyra, 2017). Therefore, the content of polyphenols had a relation with the antioxidant, in the flowers the high antioxidant capacity measured as ABTS, DPPH, FRAP methods) had a relation with the high content of polyphenols. The antioxidant capacity in flowers was higher on DPPH (13661.7 mg TE/100 g db) and ABTS 2344.66 TE/100 g db raw. In FRAP, the value was 95.72 mmol Fe(II).

The antioxidant activity by DPPH and ABTS on Mallow leaves had values of 1268.69 mg TE/100 g db and 331.01 mg TE/100 g db respectively, while the capacity measured by FRAP was very low (5.55 mmol Fe(II)).

On the other hand the thermal treatment provoking an effect in food vegetables, a high retention percentage of total polyphenol compounds of 78-89 % and therefore the antioxidant activity was shown between 70 -77 % from DPPH, ABTS and FRAP in plants both, with exception in garambullo flowers where the antioxidant activity by DPPH had only 28 % of retention. The low antioxidant activity of garambullo flower by DPPH method after of the exposure to the thermal treatment, could be due to slow reaction of DPPH with low lipophilic antioxidant species extracted according with different authors (Teow et al., 2007 and Iqbal et al., 2012; Prevc et al., 2013).

On the other hand, Arias-Rico et al (2020) and Nicoli (1999) indicate that after a thermal treatment there is a disruption of cellular structures of the plant. Therefore, there is the output of soluble compounds of these structures. These compounds can be captured by the matrix

and form complexes or they could be released on water boiling and be exposed to reactions of degradation. Sandoval-Gallegos et al., (2021) mentions that after boiling originated the decrease of soluble compounds, carbohydrates, total phenolic compounds, antioxidant capacity, physicochemical properties and changes in the morphology. All these changes could be attributed to modifications in plant structures. Therefore, short thermal treatments are recommended to minimize changes of these plants or inclusive intake raw from these.

Identification and quantification of phenolic acid, flavonoids and vitamin C

Regarding the content of phenolic compounds determined by HPLC, compounds such as coumarin and ferulic acid were found in both raw studied samples. While only caffeic acid was present in the sample of mallow sample and quercetin in garambullo flowers (table 4)

Table 3. Antioxidant activity during thermic treatment 100 g /db

		TPC (mg GAE)	DPPH (mg TE)	ABTS (mg TE)	FRAP [µmol Fe (II)]
Mallow	Raw	464.32 ± 1.14*	1268.69 ± 67.7*	331.01 ± 4.45*	5.55 ± 0.17*
	Boiled	364.32 ± 1.97	980.45 ± 11.49	243.37 ± 3.37	3.99 ± 0.07
	Retention %	78	77	74	72
Garambullo flower	Raw	755.37 ± 0.74*	13661.7±370.08*	2344.66 ± 9.81*	95.72 ± 0.94*
	Boiled	672.53 ± 0.74	3808.8±169.81	1639 ± 79.73	68.73 ± 0. 72
	Retention %	89	28	70	72

Values represent mean ± standard deviation (n=3) *The asterisk indicates a significant difference between raw and boiled of each plant.

Table 4. Identification and quantification phenolic acids, flavonoids and vitamin C by HPLC (mg/100g)

	Mallow			Flor Garambullo		
	Raw	Boiled	Retention %	Raw	Boiled	Retention %
Caffeic acid	2.04 ± 0.02	-	0	-	-	
Coumaric acid	7.90 ± 0.05*	5.36 ± 0.14	67.84	1.24 ± 0.00*	14.96 ± 0.68	+100
Ferulic acid	1.19 ± 0.02*	0.56 ± 0.00	47.05	0.15 ± 0.00*	0.60 ± 0.00	+100
Gallic acid	-	-		-	-	
Quercetin	-	-		4.03 ± 0.44	0	
Apigenin	-	0.87 ± 0.87	-	-	-	-
Vitamin C	101.13 ± 2.83*	252.44 ± 1.17	-	325.35 ± 2.72*	143.69 ± 1.30	-

Values represent mean ± standard deviation (n=3) *The asterisk indicates a significant difference between raw and boiled of each plant.

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The compounds identified and quantified on mallow in our study were ferulic acid (1.19 mg/100g) and p-coumaric acid (7.90 mg/100g) in raw samples. These compounds found were also identified in the study carried out by Abd El-Salam et al., (2019) (ferulic 0.399 mg/100g and p-coumaric acid 37.186 mg/100g) and other compounds such as p-coumaric, cinnamic acids, apigenin-7-glycoside, luteolin, naringenin, apigenin, kaempferol.

Garambullo flowers identified and quantified compounds as coumaric (1.24 mg/100g) and ferulic acids (0.60 mg/100g). This result was similar to the report by Pinedo-Espinoza (2021) on garambullo flowers (*p*-coumaric 27 mg/100 g and ferulic acid 9.6 mg/100g) and concentration phenolic acids such as gallic, chlorogenic, sirinic, vanyl, caffeic, hydroxybenzoic, and flavonoids as rutin, florizidine, floretina, myricetin (Table 2).

After thermal treatment mallow leaves had a diminution of compounds (ferulic and coumaric acid), with a retention of these compounds of 47 and 68 % in mallow respectively. In addition, after treatment was identified and quantified apigenin (0.87mg /100g).

In garambullo flowers after thermal treatment had an increase in the compounds of ferulic and coumaric acid with an amount of 0.60 mg/100g and 14.96 m/100g with retention percentage of more than 100 %.

Our belief that structures in both plants had release of compounds in some cases were matrix captured and therefore present a good scavenging and on the other hand others were degraded provoking the loss of total compound phenolic.

The content of bioactive compounds is variable between study plants due to several factors as the botanical characteristics, antioxidants composition, time and use of different solvents for extraction (Abd El-Salam and Morsy, 2019; Farhan et al., 2012; Messaoudi et al., 2015; Mohammed et al., 2014; Petkova et al., 2019; Solís-Ramírez and García-Vieyra, 2017, Sandoval-Gallegos, 2021).

On the other hand, the content of vitamin C in the raw mallow product was 101.13 mg/100 g, while that raw garambullo flower had values of 325.35 mg/100 g.

After thermal treatment an increase in vitamin C concentration on mallow was observed (2,5 fold), in comparison with garambullo flowers that had a loss of 44 %. Among characteristics vitamina C, it is known to be water-soluble and temperature-sensitive. In this study thermal treatment was of 1 min. to 1.5 min, therefore the thermal treatment shorter time resulted with higher content vitamin C (Lee S, 2017). In addition, vitamin C is stored in all cell compartments, cell wall and extracellular space, as in chloroplasts (Paciolla C, 2019). Therefore, after thermal treatment applied to the mallow caused disruption to the cellular compounds plant and increased vitamin content in the soluble fraction. Garambullo flowers lose vitamin C content after treatment, which could be due to the less rigid structure that flowers have and therefore released easily of the compartment cells and lose vitamin C in the coccion liquid.

The leaves of Mallow had a concentration of 252.44 ± 1.17 mg/100 g db and in the garambullo flower 143.69 ± 1.30 mg/100 g db, after thermal treatment as the vegetables are eaten. The content of vitamin C on a wet basis was 7.56 mg/100g on mallow and in garambullo flower 2.88 mg /100g wb. These values are lower in comparison to other vegetables as chilis (43 and 247 mg/100 g wb) (Olatunji, et al., 2018), guavas (62.3 to 168.9

mg wb) (Ordoñez-Santos et al., 2010), pepper, green peas, spinach, pumpkin and carrot (25.72 - 61.56 mg/100 mg wb) (Igwemmar, et al.,2013). However, in places where there is no availability of fruits and vegetables rich in vitamin C, these plants may be an important contribution of vitamin C.

According to NOM-051-SCFI/SSA1-2010 el IDR of vitamin C 60 mg/day to adults. Therefore, these plants maintained an important supply of vitamin C on boiled plants (252.44 mg/100g in mallow and 143.64 mg/100 g in garambullo flowers), contributing at least 2.38 fold of IDR on boiled samples of vitamin C.

Some studies had mentioned that the boiled water used in the preparations of foods, is important due the diffusion of water of low molecular compounds such as phenolic compounds or vitamins, affecting the nutritional composition of the foods. Therefore, it is important to use the water of vegetables in other food preparations to take advantage of the released soluble compounds (Arias- Rico 2021).

Conclusion

The results herein reported on the effect of cooking on mallow and garambullo flower following are found an important percentage of retention of compounds phenolic maintaining a good scavenging capacity, which could be caused by compounds such as coumarin, ferulic acid, quercetin and apigenin in the mallow as in the garambullo flower. In addition, an important content of vitamin C was shown after the thermal treatment on mallow, although in the garambullo flower it had a diminution, it continues providing an important concentration of vitamin C. On the other hand, it is very important to mention that water boils could have soluble compounds, and therefore it is suggested to consume the liquid in the same ways.

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Conflict of interests

The authors have declared no conflicts of interest for this article.

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Capítulo IV

Efecto del consumo del liofilizado y extracto de *Malva parviflora* en un modelo de obesidad inducido en ratas Wistar.

Efecto del consumo de un liofilizado y extracto de *Malva parviflora* en un modelo de obesidad inducido en ratas Wistar.

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Resumen

La malva es una planta herbácea que crece de manera espontánea durante todo el año, de acuerdo con algunos estudios la malva por su composición encontrada se le ha atribuido efectos antinflamatorio y antiobesidad (estado inflamatorio crónico de bajo grado) debido a su composición encontrada (polifenoles, minerales, fibra, etc), los cuales coadyuvan al mejoramiento de este padecimiento. Por tal motivo el objetivo del presente trabajo radica en evaluar en un modelo obesogénico en ratas Wistar, el efecto de la administración del liofilizado y extracto hidroalcohólico de *Malva parviflora* sobre parámetros antropométricos (peso, longitud, IMC e índice de Lee), tolerancia a la glucosa, estrés oxidativo, tránsito intestinal e histopatología de hígado y tejido adiposo. Los resultados demostraron que la administración de malva liofilizada y extracto hidroalcohólico de malva presentaron disminución en el peso al final del estudio. En cuanto al índice de masa corporal e índice de Lee, se observaron valores similares al encontrado en el grupo control (0.67 ± 0.48 g/cm^2 y 310.27 ± 8.95 respectivamente) mientras que el grupo de inducción siguió presentado un incremento de tejido adiposo catalogado como obesidad. Para el análisis de tolerancia a la glucosa al final del estudio se observaron concentraciones entre 128-134 mg/dL (120 min) de los grupos grupo de inducción, grupo inducción tratamiento con liofilizado de malva y grupo inducción tratamiento con acarbosa. En el estrés oxidativo se presentaron disminuciones de las enzimas oxidantes (GPx, CAT y SOD) en los grupos tratados con malva liofilizada y extracto de malva. De acuerdo con los análisis realizados del tránsito intestinal

se observó un mayor incrementó en la peristalsis en las ratas del grupo de inducción tratadas con malva liofilizada (89 %) con respecto al grupo de inducción.

Los resultados obtenidos de los estudios histopatológicos hacen remarcar que en tejido hepático no se desarrolló daño, mientras que el tejido adiposo del grupo de inducción presentó alteraciones morfológicas (hipertrofia) indicando el comienzo de daño en el adipocito cerca de las 21 semanas en el grupo con inducción a la obesidad. Mientras que en los demás grupos con los diferentes tratamientos no se observó hipertrofia o alguna otra alteración aunada al tejido adiposo indicando un mejoramiento con el tratamiento con malva liofilizada y el extracto de malva.

Palabras clave: Obesidad, peso, estrés oxidativo, antioxidantes, tránsito intestinal, hígado, tejido adiposo

Introducción

La alimentación de la población mexicana se centraba en la preparación de alimentos con granos, leguminosas y verduras de los cuales destacaban alimentos como las tortillas, frijoles, calabaza, jitomate, chile y cebolla. Sin embargo, tras la “transición alimentaria” la alimentación entre la población mexicana se modifica fuertemente ocasionando un alto consumo de alimentos altamente calóricos y pobres en nutrientes, provocando así el desplazamiento de algunos alimentos del patrón tradicional (Valerio-Pera, S., 2019).

La malva es una planta herbácea del género malva, de la familia de las Malváceas, las cuales forman parte de la alimentación debido a que posee buenas características sensoriales y que comúnmente pueden ser consumidas cocidas o en ensaladas. Por otra parte, en la medicina tradicional la malva suele utilizarse para contrarrestar síntomas del resfriado como fiebre y tos, cataplasma en heridas, hinchazones, dolor abdominal, gastritis por su efecto gastroprotector, diarrea, antihelmíntico, estreñimiento, úlcera de vejiga, antiinflamatorio, entre otros (Akbar S.,2014, Dugani A. 2016, Munir et al., 2021, Altyar, A. E., et al., 2021). Debido a los múltiples efectos que se les adjudican a las hojas de malva entre los que presenta el efecto antiinflamatorio pudiera ser utilizada para contrarrestar los efectos desarrollados en la obesidad y ser utilizada como una alternativa terapéutica de este padecimiento. Por tal motivo el objetivo radica en evaluar en un modelo obesogénico en ratas

Wistar, el efecto de la administración del liofilizado y extracto hidroalcohólico de *Malva parviflora* sobre parámetros antropométricos (peso, longitud, IMC e índice de Lee), tolerancia a la glucosa, estrés oxidativo, tránsito intestinal, así como sus características histopatológicas en tejido hepático y adiposo.

Material y métodos

Muestras

Las muestras de hojas frescas de malva fueron obtenidas en un mercado local localizado en Pachuca de Soto Hidalgo, México durante el mes de mayo del 2019. En la tabla 1 se presenta una fotografía de la planta, su denominación y las partes comestibles.

Tabla 1: Plantas de estudio

Nombre científico	Nombres comunes	Partes comestibles	Usos
Malva parviflora 	Malva, malva de quesitos, malva de campo, malva de castilla, malva alta, malva rosa, malva real, y en inglés mallow	Hojas y flores	Comestible

Preparación de liofilizado y extracto

Para el análisis de la muestra se llevó a cabo un liofilizado y la extracción hidroalcohólica del liofilizado de malva. A continuación, se describen las condiciones de cómo se llevaron a cabo

- Liofilizado: Las muestras crudas de las plantas de estudio se liofilizaron, y homogeneizaron en un mortero a un tamaño de poro de 500 mm. Se almacenaron en bolsas herméticas resguardadas de la luz y a una temperatura de -30°C, hasta su uso.
- Extracto: la muestra liofilizada se pesó (10 g) y se macero con una solución hidroalcohólica (30/70 v/v), durante 3 días a temperatura ambiente, posteriormente fue rotoevaporado a 40 °C y almacenado a -30°C.

Animales

Se utilizaron ratas Wistar macho de 8 semanas de edad (n=30) con peso de 180-220 g de peso al inicio del estudio. Todos los animales fueron proporcionados por el Bioterio de la Universidad Autónoma del Estado de Hidalgo (Pachuca, México). Los animales se mantuvieron en cajas de plástico en un lugar ventilado a temperatura de 23-25 °C, con una humedad de 45 ± 5 % y un ciclo de 12 horas de luz/12 de oscuridad. Los animales tenían acceso a alimento (formula diet 5008 labdiet) y agua *ad libitum*.

El protocolo experimental fue aprobado por el Comité Institucional de Ética para el Cuidado de Animales (CIECUAL) de la Universidad Autónoma del Estado de Hidalgo. Además, todos los experimentos se realizaron en cumplimiento de las normas oficiales mexicanas (NOM062-ZOO-1999) sobre especificaciones técnicas para la producción, cuidado y uso de animales de laboratorio. Los animales fueron anestesiados y con Tiletamina-Zolazepam 0.1 ml/80g de peso corporal (Anexo CIECUAL)

Dosis letal media

Para la determinación DL_{50}, se realizó de acuerdo a la guía de OECD (2001). En esta prueba se emplearon animales adultos jóvenes sanos de cepas Wistar de laboratorio de uso común de entre 8 y 12 semanas de edad y un peso entre 200 – 220 g, se agruparon en 3 lotes (n=3). Los primeros 2 lotes fueron administrados con dosis de 2000 mg/kg de liofilizado de malva y extracto hidroalcohólico de malva respectivamente, vía intragástrica y al grupo control se le administro un vehículo (agua), se mantuvieron en observación durante 14 días. Posteriormente al término de estos 14 días, la administración de la siguiente dosis inferior (300 mg/Kg) o mayor (5000 mg/Kg), se determinó de acuerdo con las observaciones y sobrevivencia de los animales durante la primera administración que se realizó. Al final los animales fueron sacrificados (cámara de CO_2), para la obtención de órganos con la finalidad de realizar el análisis macroscópico.

Dieta de Inducción de Obesidad

La inducción de obesidad se realizó con la administración de sacarosa al 30 % en agua durante 17 semanas y el tratamiento consistió en la administración de un liofilizado de malva, un extracto hidroalcohólico de malva y un fármaco de referencia (acarbosa), Jaramillo-Morales, 2016.

Grupos. Se formaron de manera aleatoria 5 grupos experimentales (n=6) (figura 1)

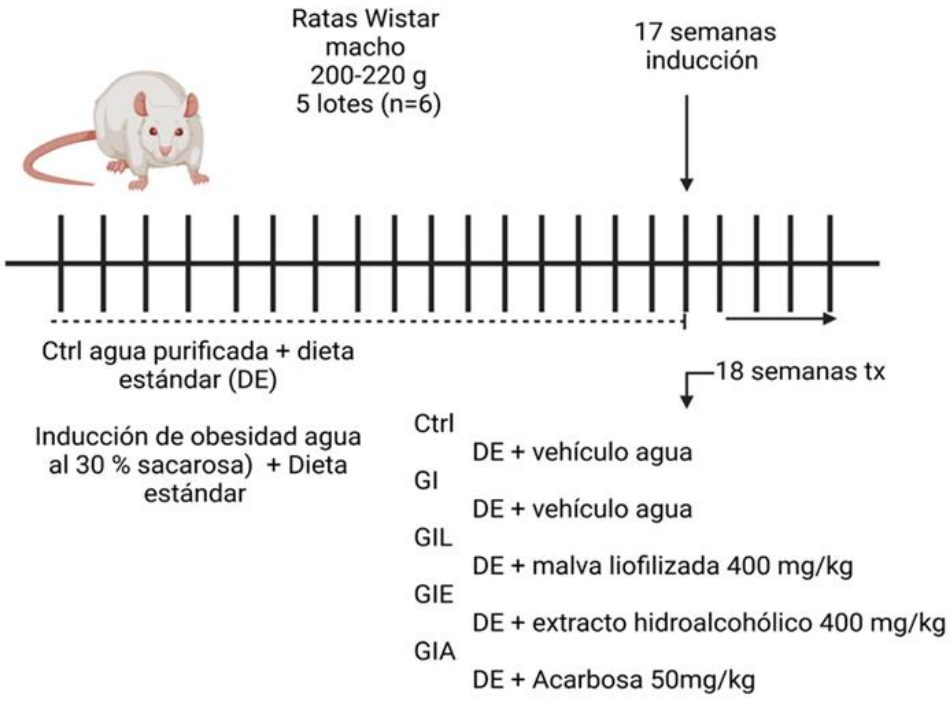


Figura 1. Grupos experimentales de inducción y tratamiento de la obesidad (grupo control: Ctrl, grupo de inducción: GI, grupo de inducción tratada con liofilizado de malva: GIL, grupo de inducción tratada con extracto hidroalcohólico de malva (GIE), grupo de inducción tratada con fármaco acarbosa: GIA)

.

Análisis de efecto bio-clínico de Obesidad

Parámetros antropométricos

- Peso corporal: Se tomó el peso cada 15 días a las ratas
- Longitud: se tomó la longitud naso-anal

- Nivel de adiposidad por el índice de Lee: se expresó como la raíz cúbica de peso corporal en gramos dividida por la longitud naso-anal en milímetros multiplicada por 10^4 (Sruthi Kaveripakam, Sreedevi, & Mathi, 2017)
- IMC. Para la determinación de IMC se tomó el peso y la longitud del cuerpo y se expresó como el peso en g entre la longitud al cuadrado naso-anal $(cm)^2$

Determinación de tolerancia a la glucosa

La determinación de la tolerancia a la glucosa se midió después de las 16 semanas del período de inducción a la obesidad mediante la administración de glucosa al 50 % (2 g de glucosa/kg peso corporal) a través de sonda gastrointestinal. Se tomaron muestras de sangre de la cola a los 0, 30, 60, 90 y 120 min y la glucosa en sangre se analizó con un glucómetro (marca One-Touch).

Marcadores del estrés oxidativo en eritrocitos

Recolección de muestras y análisis bioquímico se obtuvo sangre total a partir de punción cardiaca al momento del sacrificio, la cual se centrifugó a 1500 × g durante 10 min a 4 °C. del residuo obtenido se lavaron 250 µL de eritrocitos con 250 µL de solución fisiológica y se diluyeron a una proporción 1:1. Posteriormente se midieron los siguientes parámetros.

Como marcadores del estado redox se evaluó la capacidad de absorción de radicales de oxígeno (ORAC), así como el contenido de malondialdehido (MDA) como marcador de daño oxidante lipídico. El ensayo ORAC fluorométrico se realizó de acuerdo con Huang et al. (2002) utilizando una curva estándar de 1,1,3,3-tetrametoxipropano y los datos se expresaron como micromol equivalente de Trolox (uM TE). Finalmente, el ensayo espectrofotométrico de MDA se realizó según el procedimiento descrito por Gérard-Monnier et al. (1998) y las mediciones se expresaron como nanomol de malondialdehido por mL (nmol MDA/mL). Todas las determinaciones se obtuvieron utilizando el lector de microplacas multimodo Synergy HT (BioTek Instruments Inc., Winooski, VT, EE. UU.).

La actividad de glutatión peroxidasa (GPx) se midió usando H_2O_2 como sustrato y la actividad de GR se determinó usando glutatión oxidado (GSSG) como sustrato y ambos midiendo la desaparición de NADPH a 340 nm cada minuto durante 3 min. Una unidad de GPx o GR se define como la cantidad de enzima que oxida 1 µmol de NADPH/min. Las actividades de ambas enzimas se expresaron como U/ul de plasma.

La actividad de Superóxido dismutasa 1 (SOD-1) se midió usando xantina-xantina oxidasa para la generación de anión superóxido y nitroazul-tetrazolio (NBT) como reactivo indicador que se reduce a formazán detectable a 586 nm. Una unidad de SOD-1 se define como la cantidad de proteína que inhibe la reducción de NBT en un 50%.

Se determinó la actividad de catalasa (CAT) midiendo el consumo de peróxido de hidrógeno en el eritrocito a 240 nm (Aebi, H., 1984.), la actividad enzimática se expresó como κ/mg de proteína. El contenido total de proteínas fue determinado utilizando el método colorimétrico de Lowry et al. (1951).

Determinación del tránsito gastrointestinal

El movimiento en el tracto intestinal se determinó por el método de carbón activado con algunas modificaciones. La solución de carbono se preparó disolviendo 5 g de carbón vegetal en polvo y 5 g de goma arábiga. La solución de carbono preparada se administró por vía oral a las ratas 30 min antes de ser sacrificadas. Posteriormente al sacrificio se recolectó el intestino (delgado y colon) de cada rata para evaluar la longitud del colon y la distancia recorrida por el bolo alimenticio coloreado por el carbón activado. Lo cual nos indicará la motilidad intestinal calculado por el porcentaje del tránsito intestinal, es decir la distancia recorrida por el carbón activado dividida entre la longitud total del intestino delgado de la rata evaluada, multiplicado por 100 (Han et al., 2017)

$$T\ (\%) = B / A \times 100$$

Dónde:

T = relación de tránsito del tracto intestinal

A = longitud total del tracto intestinal

B = La distancia de movimiento de la parte más distal.

porción final del carbón

Análisis histopatológico

Las muestras de hígado y de tejido adiposo de cada grupo fueron fijadas por inmersión en formaldehído al 3.8%, en solución acuosa, amortiguada con fosfatos, al menos por 24 horas. Posteriormente fueron procesadas por el método de inclusión en parafina (Prophet, 1995) mediante un procesador automatizado de tejidos marca Microm, modelo TP1020, cortadas a 6 µm en un micrótomo marca Leica modelo 2125RT a 4 µm de grosor y coloreadas con la técnica de Hematoxilina-Eosina (H-E) (Prophet, 1995). Las preparaciones histológicas resultantes fueron observadas y analizadas con un microscopio compuesto de campo claro marca Olympus, modelo BX41. Las imágenes seleccionadas fueron capturadas con una cámara digital MediaCybernetics, Modelo Evolution VF, mediante el software Image-Pro Express 6.0 (MediaCybernetics), instalado en una computadora marca Vaio con procesador Pentium 4 y 1 Gb de RAM.

Análisis estadístico

Los resultados se presentaron con la media por desviación estándar de cada medición. Los datos fueron analizados mediante el Análisis de Varianza [ANOVA] y una prueba post test de Tukey, con un nivel de significancia de $p > 0.05$ como suficiente para establecer las diferencias entre los diferentes grupos de estudio. Para este análisis se utilizó el programa GraphPad Prisma versión 5.03 y SPPS statistics 19.

Resultados

Estudios *in vivo*

Peso

En la figura 2 se graficó el seguimiento de ganancia de peso en gramos de los animales en estudio durante 21 semanas con inducción con sacarosa al 30 %. Todos los grupos de estudio incrementaron a partir de la semana 3 en comparación al grupo control. Estos incrementos fueron del 24 % para el grupo GI, y de forma similar para los grupos GIL, GIE, GIA (19, 14 y 10 % respectivamente). Al final del estudio los animales en estudio con el tratamiento GI tuvieron un incremento de 19 % (con un peso de 485 g) con respecto al CTRL (con un peso de 406 g), mientras que los grupos tratados (GIL, GIE, y GIA) mostraron una disminución entre 9-14 % con respecto al GI.

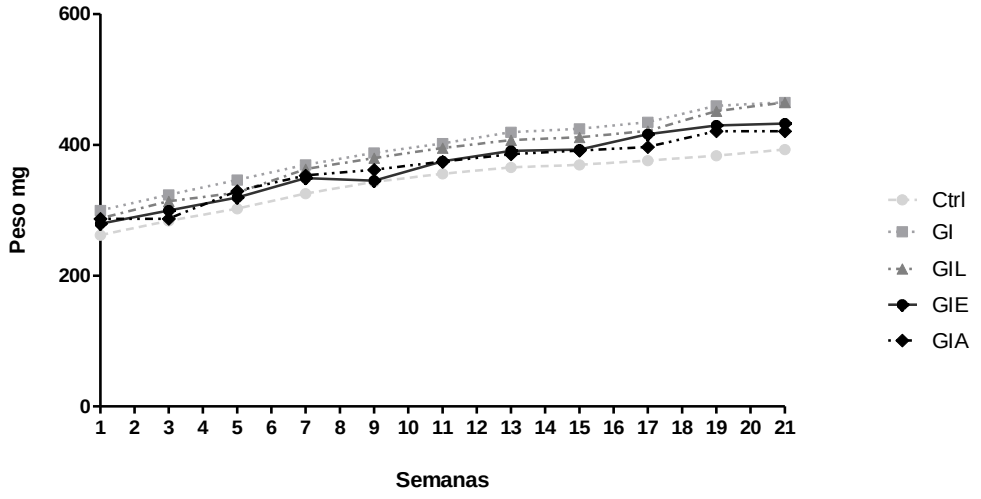


Figura 2. Ganancia de peso (g) de CTRL, GI, GIL, GIE y GIA por 21 semanas inducidas por una dieta alta en sacarosa.
Control (Ctrl),
Grupo inducción (GI)
Grupo tratado con malva liofilizada (GIL)
Grupo tratado con extracto hidroalcohólico de malva (GIE)
Grupo tratado con Acarbosa (GIA)

Tabla 2. Parámetros antropométricos (IMC e Índice de Lee) en ratas Wistar

Grupo	IMC (g/cm^2)	IL (g/cm)
Ctrl	0.67 ± 0.48^a	310.27 ± 8.95^a
GI	0.84 ± 0.68^b	340.94 ± 9.03^b
GIL	0.68 ± 0.01^a	312.27 ± 8.99^a
GIE	0.69 ± 0.09^a	312.85 ± 13.86^a
GIA	0.68 ± 0.07^a	313.51 ± 12.71^a

[a,b,c] las letras muestran diferencias significativas entre los grupos:
Control (Ctrl),
Grupo inducción (GI)
Grupo tratado con malva liofilizada (GIL)
Grupo tratado con extracto hidroalcohólico de malva (GIE)
Grupo tratado con Acarbosa (GIA)

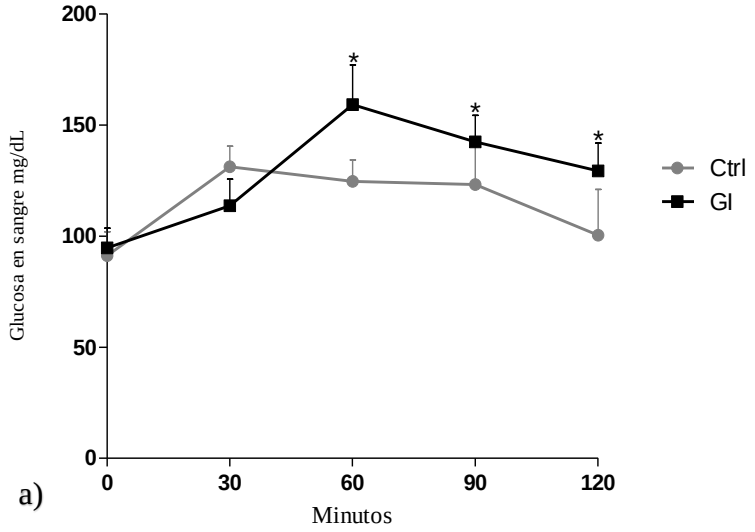
Parámetros IMC e índice de Lee

En la tabla 2 se muestran los parámetros IMC e índice de Lee. El grupo Control presentó un IMC 0.67 g/cm^2 y un IL 310.27 g/cm. Estos valores son considerados normales de acuerdo con Novelli et al., (2007) y Chikopela et al., (2021), Bastías-Pérez et al., (2020), valores superiores son considerados como sobrepeso u obesidad. Los valores de los tratamientos en donde se utilizó la hoja de malva en liofilizado y extracto se mantuvieron sin diferencias significativas, al igual que el fármaco de referencia la acarbosa. Esto indica que la malva si tiene la función de antiobesidad a diferencia del grupo que tuvo una inducción con glucosa y que sí presenta diferencias estadísticas significativas. Nuestros resultados son similares a los reportados por Novelli et al., (2007) donde mostró que el grupo tratado con sacarosa más la ingesta de alimentos (control chow) durante 30 días mantuvieron un aporte mayor de energía transformado lo en un incremento de IMC de 0.82 g/cm^2 al final de estudio.

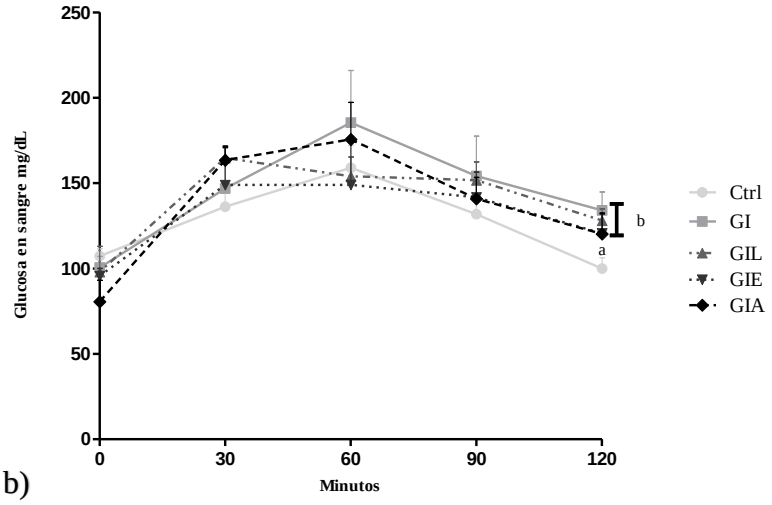
Tolerancia a la glucosa

La determinación de tolerancia a la glucosa se determinó en los grupos Ctrl y GI antes de iniciar tratamiento para establecer cómo el organismo regula las cargas de un estado de homeostasis como es el control y en el grupo que se le administró sacarosa y que se desarrolló obesidad. El control tuvo un comportamiento esperado con un incrementó de glucosa a los 30 min baja a valores normales (< 110 mg/dL). El grupo de inducción mostró un incremento de glucosa a los 30 min, con un mayor incremento a los 60 min con valores superiores a los 150 mg/dL sin lograr bajar a valores basales (figura 3).

Posteriormente, al final del estudio se determinó la tolerancia de glucosa a todos los grupos. Todos los grupos iniciaron con niveles basales normales al inicio. No se observó diferencias significativas debido a que todos los grupos mantuvieron niveles de glucosa basales normales, sin embargo, a los 60 min se presentó un incremento en el nivel de glucosa en todos los grupos, a los 120 min los valores basales solo se presentaron al grupo Ctrl. Los demás grupos sometidos con los diferentes tratamientos presentaron concentraciones de glucosa entre 120-134 mg/dL (120 min). Sin embargo, no se mostraron diferencias estadísticas entre los grupos. Por otro lado, los valores de referencia apuntan a que los grupos GI, GIL, GIE y GIA podrían presentar alteración en el metabolismo de la glucosa, que podría deberse a la disminución en la secreción de la insulina o a la alteración funcional de la insulina por el exceso en el consumo de sacarosa administrada en los tratamientos (figura 3b) (Kaveripakam, S. S., et al., 2017).

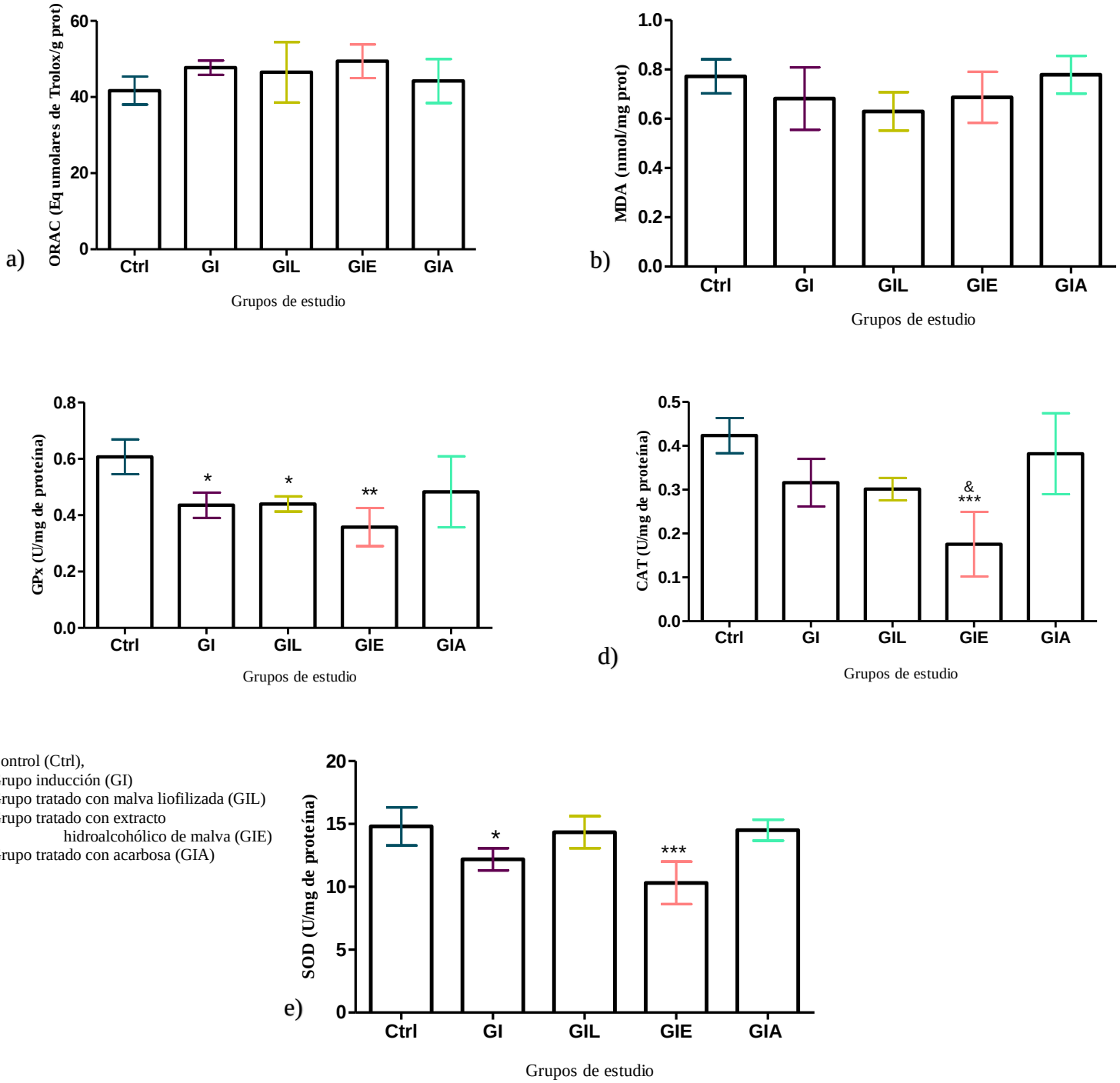


* indican diferencias significativas entre los grupos.
Control (Ctrl)
Grupo inducción (GI)



a,b,c indican diferencias significativas entre los grupos.
Control (Ctrl)
Grupo inducción (GI)
Grupo tratado con malva liofilizada (GIL)
Grupo tratado con extracto hidroalcohólico de malva (GIE)
Grupo tratado con Acarbosa (GIA

Figura 3. Curva tolerancia a la glucosa



Control (Ctrl),
Grupo inducción (GI)
Grupo tratado con malva liofilizada (GIL)
Grupo tratado con extracto hidroalcohólico de malva (GIE)
Grupo tratado con acarbosa (GIA)

.

Indica Diferencia Estadísticamente Significativa vs grupo control (*) y de inducción (&). ANOVA y t-Tukey con un * o & *p<0.05, ** p<0.01 y ***p<0.001

Figura 4. Efecto de la malva sobre los marcadores del estrés oxidativo en un modelo de obesidad inducido con sacarosa al 30 %

Marcadores del estrés oxidativo en eritrocitos

El estrés oxidativo en la obesidad origina una variedad de alteraciones con las que se va deteriorando la calidad de vida de la persona con esta patología, entre estas afectaciones son: alteración de factores reguladores de la actividad mitocondrial, modificación de la concentración de mediadores de la inflamación asociados al número y tamaño de adipocitos, promoción de la lipogénesis, estimulando la diferenciación de preadipocitos a adipocitos maduros y regulación del efecto energético en neuronas hipotalámicas en el control del apetito (Pérez-Torres et al., 2021). Dicho estrés oxidativo puede ser evaluado en el laboratorio por la determinación de marcadores de la peroxidación

En la figura 4 se muestran los resultados del estrés oxidativo determinado por ORAC (Figura 4A), MDA (Figura 4B), GPx (Figura 4C), CAT (Figura 4D) y SOD (Figura 4E).

De acuerdo a los resultados para ORAC no se observaron diferencias significativas entre las muestras de estudio durante 4 semanas de tratamiento. En un estudio por Hogan *et al.*, (2010) estableció que la suplementación del extracto de orujo de uva durante 12 semanas en ratones alimentados con dieta alta en grasa no mostró cambios significativos en los valores de ORAC.

La concentración de MDA no presentó diferencias significativas en el grupo control entre las muestras. Por otra parte, el MDA, el cual es un producto final secundario de la peroxidación lipídica se relaciona con la alteración oxidativa, su incremento indica la presencia de daños a nivel tisular, causado por el estrés oxidativo. Aunado a esto, el estrés oxidativo genera radicales libres, los cuales pueden estar involucrados con componentes celulares cruciales como los lípidos de membrana, ADN y proteínas que están implicados en el cambio de la función normal de estos componentes y por lo tanto provocan daño a nivel celular, por lo que MDA es considerado como un factor asociado a la obesidad (Adnan, M. T. et al., 2018).

Por otra parte, el organismo cuenta con un sistema protector antioxidante integrado por las enzimas superóxido dismutasa (SOD), catalasa (CAT), y el glutatión peroxidasa (GPX), las cuales funcionan mediante la desintoxicación de hidroperóxidos H_2O_2 se realiza por la glutatión peroxidasa y catalasa, mientras que los radicales libres de superóxido O^{-2} (mediante SOD) son considerados como marcadores antioxidantes en la célula. Las bajas

concentraciones de las enzimas antioxidantes se traducen en la pérdida de lípidos y proteínas, daño y disfunción celular. En el presente estudio el GPx en eritrocitos en la muestra control presentó valores de 0.6 U/mg de proteína. Con excepción del tratamiento con acarbosa, los tratamientos de inducción (GI), liofilizado (GIL) y el extracto (GIE) presentaron concentraciones más bajas de GPx en comparación con el control (figura 4c). De acuerdo a lo encontrado por Valdecantos, *et al*., (2009) en personas obesas determinaron las concentraciones de GPx, encontrandouna disminución en la actividad como en su expresión presente cuando está establecida la obesidad, de manera similar a lo que se encontró en este estudio.

En el análisis realizado de CAT el grupo control presentó concentración de 0.04 U/mg de proteína, presentando solo diferencias significativas en el grupo de GIE, donde se observó una disminución de este parámetro en eritrocito. Por otro lado, en SOD el grupo control presentó concentraciones de 15 U/mg de proteína, observándose diferencias significativas con GI y GIE, debido a los detrimentos presentados de esta enzima (figura 4d y 4e). En el estudio ya referido (Valdecantos *et al*., 2009) se encontró que en la obesidad aunado a una patología se encontró una disminución de ambas enzimas (CAT y SOD).

En todos los parámetros podemos observar un detrimento en las concentraciones de las enzimas antioxidantes, podríamos creer que estos valores que se presentan bajos pudieran deberse a la función que realizan las enzimas antioxidantes ante el efecto del estrés oxidativo que se presenta en la obesidad, además se cree que se requerirá de más tiempo de administración de malva para tener un mayor efecto sobre el incremento de las enzimas.

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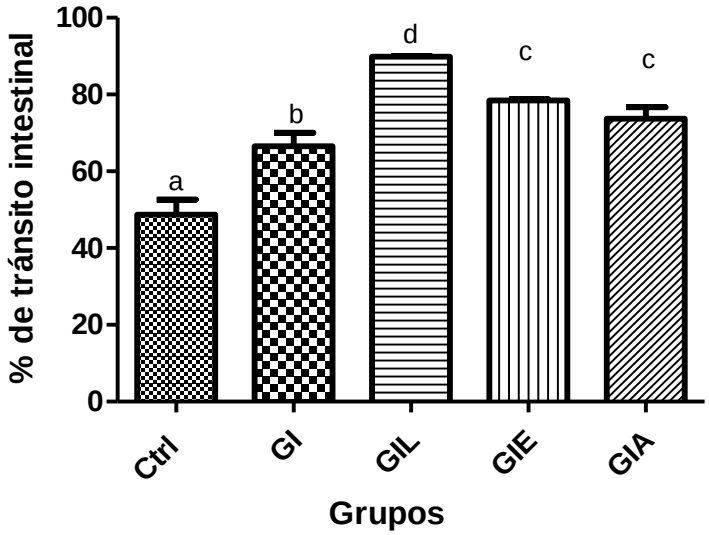
Tránsito intestinal

En la figura 5, se presentan resultados de la medición de los centímetros recorridos del bolo alimenticio en el intestino de los animales de experimentación. Lo anterior es expresado como el porcentaje de tránsito gastrointestinal en relación con la totalidad del intestino delgado. El grupo control presentó un porcentaje de tránsito intestinal de 49 %, mientras que el grupo GI mostró un mayor porcentaje (67 %) de tránsito intestinal recorrido por el bolo alimenticio. Por otra parte, se observó un mayor porcentaje de tránsito intestinal en el grupo que llevó por tratamiento malva liofilizada (GIL) con un 89%. En un estudio previo realizado por el grupo de investigación se determinó que el contenido de fibra dietética total es alto (36.47 g/100 g bs) con un importante aporte de fibra insoluble (31.32 g/100 g bs) en comparación con la soluble (5 g/100 g bs). (Sandoval-Gallegos., *et al*.., 2021). El alto contenido de fibra dietética insoluble de la malva en GIL, provocó que el tránsito intestinal del bolo alimenticio se acelerara. Esta fibra insoluble es capaz de retener agua en su matriz estructural formando mezclas de baja viscosidad, por lo que contribuye a la formación de heces abundantes y de más rápido tránsito intestinal (Sánchez *et al*., 2015).

Tanto la fibra dietética insoluble como la presencia de mucílagos presentes (fibra soluble) en las hojas de malva, son las responsables de las propiedades emolientes y reguladoras intestinales (Calderón *et al*., 2011). El mucílago funciona como coloide hidrófilo, que origina un gel no digerible, espeso y viscoso al contacto con el agua incrementando los movimientos peristálticos, actuando como un laxante suave (López, L. M. T., 2000), característico de la fibra dietética (Taghipoor *et al.,* 2014, Mudgil, 2007). Los otros grupos GIE y GIA no presentaron fibra en su composición, sin embargo, tuvieron un mayor incremento en el tránsito intestinal que el control, posiblemente por la cantidad de fibra soluble en GIE y en la acarbosa a los efectos secundarios que pudiera causar. La acarbosa es un pseudotetrasacárido de origen microbiano. Inhibe alfa-glucosidasas intestinales, retrasa de modo dosis dependiente la digestión de disacáridos, oligosacáridos y polisacáridos. Por lo que debido a esta inhibición estos carbohidratos son fermentados y en ocasiones causan alteraciones gastrointestinales tales como flatulencias, distensión abdominal o diarrea esta última provocando un incremento en el tránsito intestinal (Balfour, J.A. and McTavvish, D., 1993).



[a,b,c] las letras muestran diferencias significativas entre los grupos:
Control (CTRL),
Grupo inducción (GI)
Grupo tratado con malva liofilizada (GIL)
Grupo tratado con extracto hidroalcohólico de malva (GIE)
Grupo tratado con Acarbosa (GIA)

Figura 5. Tránsito gastrointestinal (%) del grupo control, inducción y los grupos experimentales administrados

Análisis histopatológico

Hígado

En la figura 6 se muestra una micrografía de un corte del hígado del grupo control, en el cual se observa una arquitectura normal, con un citoplasma granuloso, producto de la degeneración hidrópica, posiblemente como resultado del manejo de la muestra. De forma similar fué observado en los cortes de tejidos de los demás grupos de estudio, sin daños en el tejido. Es importante resaltar que, a pesar de la administración de sacarosa en los grupos de estudio durante 21 semanas, estas células hepáticas pudieron establecer una regeneración del tejido por diferentes mecanismos. Un estudio realizado por Lima ML *et al*., (2015) mencionan que una dieta con una alta cantidad de carbohidratos simples (33% de comida estándar para ratas compactada en polvo Nuvilab-CR1 Nuvital-Colombo, Brasil, 33% de leche condensada (Moça, Nestlé, Brasil), 7% de sacarosa (refinada azúcar, União, Brasil), y 27% de agua), similar al modelo dietético que ha adoptado el humano, causó daño hepático (hígado graso no alcohólico) en ratas wistar, el cual se relaciona con la obesidad inducida (a

partir de la semana 10). Histopatológicamente el hígado que analizaron presentó esteatosis hepática y hepatocitos balonizados, provocado por el incremento de circunferencia toráxica e IMC relacionado con hiperleptinemia, hiperglucemia, hipertrigliceridemia, aumento de VLDL, agotamiento de enzimas hepáticas antioxidantes e incremento de los niveles de MDA.

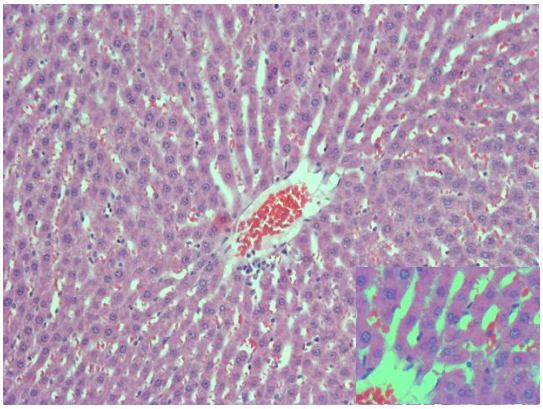


Figura 6. **Efecto del extracto de malva sobre la actividad lipogénica. Tinción: Hematoxilina-Eosina. 100X. A. Hígado.** Sin cambios patológicos aparentes en todos los grupos estudiados. Aquí se muestra el hígado del grupo inducido sin tratamiento. Inserto 400X.

Tejido adiposo

En la figura 7, se observan fotografías de los adipocitos analizados de los animales de experimentación. En la figura 7a se observa el grupo Ctrl donde el tejido adiposo presenta un estado normal, con adipocitos bien definidos, de tamaño uniforme y con una ligera hiperemia pasiva asociada a los vasos sanguíneos del tejido.

El tejido adiposo del grupo GI se muestra en la figura 7b, donde se observa una ligera hipertrofia zonal de los adipocitos con una ligera hiperemia pasiva asociada a los vasos sanguíneos del tejido, con un infiltrado leucocitario donde se vislumbra el inicio de un proceso inflamatorio.

Las fotografías de las figuras 7c y 7d corresponden al tejido de los animales sacrificados al final del tratamiento del grupo inducido con administración de liofilizado y extracto de malva (GIL, GIE), en donde hay una involución del tejido graso hipertrofiado por la inducción a un tejido normal muy similar al grupo ctrl. La Figura 7e es una fotografía del tratamiento con acarbosa,en donde la involución de la hipertrofia adipocitaria no es tan completa como con

los tratamientos en donde se administra el extracto y el liofilizado de Malva. Por lo tanto, podríamos concluir que la utilización del extracto de malva tiene un efecto positivo en tejido hipertrofiado por la obesidad inducida en los animales de experimentación.

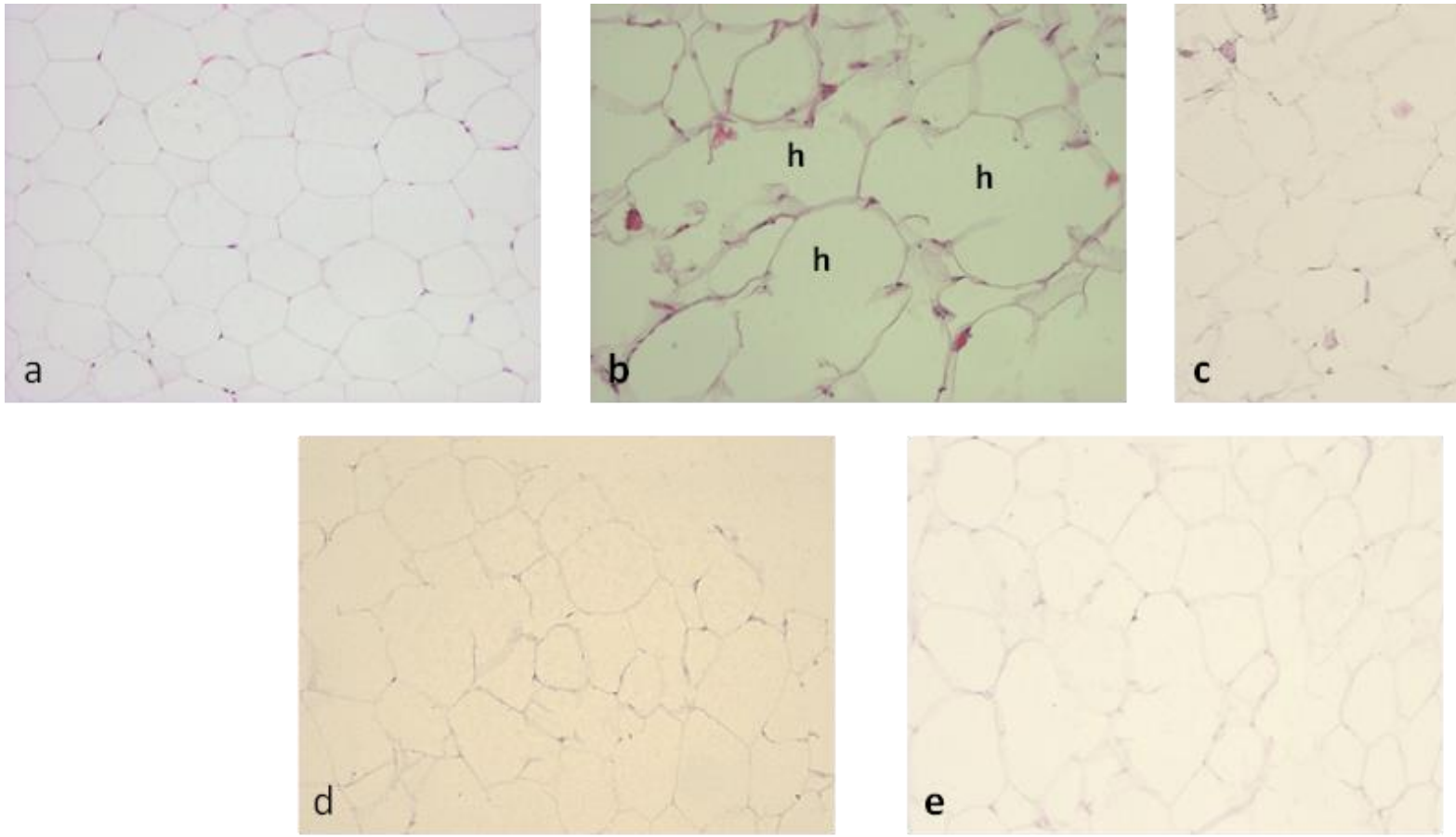


Figura 7. **Efecto del extracto de malva sobre la actividad lipogénica. Tinción: Hematoxilina-Eosina. 100X.** Inserto 400X. a**. Tejido adiposo.** Grupo control sin inducción. b**. Tejido adiposo.** Grupo inducido. c**. Tejido adiposo.** Grupo inducido con extracto de malva. d**. Tejido adiposo.** Grupo inducido y tratado con malva liofilizada. e**. Tejido adiposo.** Grupo inducido y tratado con fármaco acarbosa. Nótese el tamaño uniforme de todas las poblaciones de adipocitos, con relación al grupo control sin inducción **(a)**, excepto en el grupo inducido sin tratamiento **(b)**, que presenta adipocitos hipertrofiados **(h)**

Conclusión

El tratamiento con malva mostró detrimento en peso, mientras que el Índice de masa corporal e Índice de Lee, se observaron valores similares al encontrado en el grupo control (0.67 ± 0.48 g/cm^2 y 310.27 ± 8.95 respectivamente) valores normales, mientras que el grupo de inducción prosiguió con un incremento de tejido adiposo catalogando como obesidad, para la tolerancia a la glucosa al final del estudio se observaron concentraciones entre 128-134 mg/dL (120 min) de los grupos GI, GIL y GIA. En el estrés oxidativo se presentaron disminuciones de las enzimas oxidantes. En el tránsito intestinal se observó incrementó mayor en la peristalsis de las ratas (89 %) con respecto al grupo GI. Por otra parte, los estudios histopatológicos hacen remarcar que en tejido hepático no desencadenó daño, mientras que el tejido adiposo del grupo de indicción presentó alteraciones morfológicas (hipertrofia) indicando el comienzo de daño en el adipocito. Es importante mencionar que se deben de realizar más estudios preclínicos con los que se puedan corroborar los efectos benéficos de la malva.

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VIII. Conclusiones generales

I. Las plantas de la malva y la flor de garambullo son productos vegetales que se encuentran de manera silvestre en el territorio nacional en México, y se caracterizan por la presencia de antioxidantes, minerales, vitaminas, además de una importante proporción de fibra, por lo cual son considerados importantes en la alimentación y como plantas medicinales, por lo cual es importante continuar con estudios, de tal manera que puedan ser apreciadas y revalorizadas.

II. Diversos compuestos bioactivos presentes en los productos de origen vegetal, tales como flavonoides, ácido fenólicos o fibra, pueden contribuir a reducir la incidencia de la obesidad. Por lo tanto, es importante continuar con trabajos de investigación que permitan conocer la eficacia y la seguridad de dichos componentes.

III. En el estudio de los alimentos debe ser considerado el procesamiento como el tratamiento térmico. El tratamiento térmico aplicado a la Malva y la Flor de garambullo ocasionó un menor contenido de compuestos solubles como minerales, compuestos fenólicos y por lo tanto una disminución de la actividad antioxidante. Lo anterior debido a que el tratamiento térmico ocasiona modificaciones en la fibra dietética (componente estructural de la pared celular) y por lo tanto reacciones de degradación, liberación o formación de complejos. Por tal motivo es recomendable utilizar tratamientos térmicos cortos que minimicen las alteraciones o el consumo de vegetales crudos.

IV. Una cantidad importante de compuestos fenólicos (cumarina, ácido ferúlico, quercetina y apigenina) y de vitamina C fueron retenidos en la matriz del alimento después de la cocción en la flor de malva y garambullo, contribuyendo con una buena capacidad antioxidante.

V. La administración de la malva presentó efectos benéficos sobre la obesidad inducida en ratas Wistar, disminución de peso, decremento del daño en tejido adiposo hipertrofiado, además de un mejor tránsito intestinal, con una gran diferencia por la administración de la muestra liofilizada. Sin embargo, deben de realizarse más estudios preclínicos con los que se puedan corroborar los efectos positivos del consumo de la malva.

Este trabajo contribuye a demostrar que los vegetales de cultivo silvestre como la hoja de Malva y la flor de garambullo en México pueden ser considerados importantes fuentes de nutrientes, bien en sus formas habituales de consumo (en fresco o cocinado), o como ingredientes en otros alimentos (como en el caso del enriquecimiento de alimentos con muestra liofilizada). Esto unido a su alta disponibilidad, hacen de estas plantas silvestres, recursos alimenticios de gran interés para la población mexicana, por lo que su consumo como alimento tradicional debe ser conservado y revalorizado, contribuyendo así a mejorar el estado de salud de la población mexicana.

IX. Anexos

UNIVERSIDAD AUTÓNOMA DEL ESTADO DE HIDALGO
COORDINACIÓN ACADÉMICA
DIRECCIÓN DE SERVICIOS ACADÉMICOS
DIRECCIÓN DEL BIOTERIO
COMITÉ INTERNO PARA EL USO Y CUIDADO DE ANIMLAES DE LABORAORIO

DICTAMEN DE EVALUACIÓN

11 de septiembre de 2020

Estimada Dra. Esther Ramírez Moreno (investigador principal).

Investigadores participantes: Dr. José Arias Rico y Dr. Osmar Jaramillo Morales, Alumnas participantes: Mtra. Eli Mireya Sandoval Gallegos, Pasante en nutrición Iris Cristal Hernández Ortega y Pasante en nutrición Jessica Lizbeth Ramírez Téllez.

Después de haber realizado la evaluación del protocolo intitulado **"Evaluación de las características nutrimentales, antioxidantes y su efecto anti-obesidad *in vivo* de *Malva parviflora* L."**, el Comité Interno para el Uso y Cuidado de Animales de Laboratorio (CIECUAL), ha resuelto **aceptar** el protocolo.

Sin embargo, para completar el trámite deberá realizar las siguientes modificaciones al protocolo y enviar la versión final, así como la carta compromiso.

1) El nombre científico de los seres vivos deben escribirse en itálicas. Corrija en el título y en el texto.
2) La primera vez que escriba el nombre científico de la planta, deberá apaercer completo (*Malva parviflora* L.), posteriormente escriba el solo género abreviado (*M. parviflora* L.)
3) Revise la redacción. En los antecedentes de estudio, corrija el siguiente párrafo, "Además de presentar efectos antiinflamatorios, antioxidantes, antiulcerogénicosa, hepatoprotector, neuroprotector y anti y antidiabético."
4) La experiencia en el manejo de animales, por parte de los participantes se debe evidenciar presentando una copia de la caratula del documento correspondiente en el anexo, el cual deberá colocar después de las referencias.
5) La palabra *in vivo* se escribe en cursivas, revise el apartado de "Antecedentes experimentales *in vivo*" y corrija.
6) En el objetivo, cambie "Evaluar el efecto del consumo de *Malva parviflora*…", por "Evaluar el efecto del consumo de **un liofilizado de** *Malva parviflora* **L.**,…"
7) Revise la redacción del tercer objetivo específico. La redacción de la frase "…en 2 ocasiones: (al inicio y al final del estudio) y al final se realizara el efecto del tránsito gastrointestinal…" no es clara.
8) En el apartado de "Modelo de toxicidad (DL50)", especifique:
   a) como obtendrá la dosis de 2000 mg/Kg.
   b) si la muestra liofilizada será tamizada, de ser el caso cual será el tamaño de partícula a utilizar. Además, si al ser diluida en agua será filtrada o no.

c) Si el control de peso se realizará periódicamente.

9) En el apartado de "Modelo de inducción la obesidad", al mencionar el número de animales y formación de los diferentes grupos, mencionan que formarán dos grupos con una *n* de 9 animales (total de 18 animales) cada uno, posteriormente mencionan 5 grupos con una *n* de 6 animales (total de 30 animales), esto crea confusión porque al principio del párrafo mencionan que utilizarán 36 animales. Tampoco se entiende cómo calculará la dosis del liofilizado que administrará a los animales. Revise y explique de una forma clara.

10) Corrija "cánula esofágica de acero inoxidable calibre 18"3" por "cánula esofágica de acero inoxidable calibre 18x3" especificando las unidades y anote las la dosis que probará.

11) En el apartado de "Evaluación de deposiciones de las ratas", por favor especifique si la prueba se hará a todos los animales o solo a un lote en específico.

12)Especifique como administrará el carbón activado y la concentración que utilizará.

13)Explique la técnica ya que no se entiende como determinará el % de tránsito intestinal del carbón activado.

14) El párrafo "Se administrarán las ratas con el vehículo y la solución de malva, posteriormente se administrará el carbón," es confuso. Revise la redacción.

15) Especifique el número total de animales en este ensayo.

16)Se menciona que se prevén un 15 % de perdida de animales, explique cómo realizó este cálculo, si es con base a bibliografía, cite las referencias.

17)En Los datos de los animales solicitados, escriba el número total de animales, sin sepáralos por grupos. Revise que el número sea correcto.

Esperamos el documento con los cambios y adecuaciones solicitadas, así como la carta compromiso para entregarle la carta oficial de aceptación.

ATENTAMENTE

"AMOR, ORDEN y PROGRESO"

PRESIDENTE DEL CIECUAL

Dr. Luis Delgado Olivares