UNIVERSIDAD MICHOACANA DE SAN NICOLÁS DE HIDALGO

División de Estudios de Posgrado

PIDCB

PROGRAMA INSTITUCIONAL DE DOCTORADO EN CIENCIAS BIOLÓGICAS

INSTITUTO DE INVESTIGACIONES QUÍMICO BIOLÓGICAS

OPCIÓN EN INVESTIGACIONES QUÍMICO BIOLÓGICAS

"Efecto de los fitosomas del extracto de hoja de *Callistemon citrinus* sobre el estrés oxidante en ratas alimentadas con una dieta hipercalórica"

TESIS

Que para obtener el grado académico de:

DOCTOR EN CIENCIAS BIOLÓGICAS

Que presenta:

M. C. LUIS GERARDO ORTEGA PÉREZ

Director: Dr. Daniel Godínez Hernández

Codirectora: Dra. Patricia Ríos Chávez

Comité Tutoral

Dr. Joel Edmundo López Meza. Facultad de Medicina Veterinaria y Zootecnia-UMSNH.

Dra. Martha Estrella García Pérez. IIQB-UMSNH.

Dr. Pedro López Sánchez. Escuela Superior de Medicina-IPN.

Morelia, Michoacán, México, noviembre de 2024

Instituto de Investigaciones Químico Biológicas

EL PRESENTE TRABAJO SE REALIZÓ EN EL LABORATORIO DE FITOBIOQUÍMICA (B4) DE LA FACULTAD DE BIOLOGÍA (U.M.S.N.H) BAJO LA DIRECCIÓN DE LA D.C. PATRICIA RÍOS CHÁVEZ Y EN EL LABORATORIO DE FARMACOLOGÍA DEL INSTITUTO DE INVESTIGACIONES QUÍMICO BIOLÓGICAS (U.M.S.N.H) BAJO LA DIRECCIÓN DEL DR. DANIEL GODÍNEZ HERNÁNDEZ.

AGRADECIMIENTOS

Quiero agradecer a mi alma mater, la Universidad Michoacana de San Nicolás de Hidalgo, la Facultad de Biología y el Instituto de Investigaciones Químico Biológicas por permitirme tener una sólida formación en un nivel profesional y ahora en esta ocasión, en mis estudios de posgrado en el Programa Institucional de Doctorado en Ciencias Biológicas.

Agradezco infinitamente a mis asesores de tesis, a la Dra. Patricia Ríos Chávez y el Dr. Daniel Godínez Hernández por ser unas excelentes guías en mi trayectoria profesional en la investigación científica, por su confianza brindada, gran paciencia y sus conocimientos invaluables para llevar a cabo esta fructífera investigación y lograr la gran calidad de mi tesis.

Sinceras gracias a: Dra. Martha Estrella García Pérez, Dr. Joel Edmundo López Meza y al Dr. Pedro López Sánchez, quienes me asesoraron y aportaron con sus valiosas observaciones, consejos y recomendaciones, de igual manera por su tiempo que dedicaron para revisar la presente tesis.

Agradezco al Consejo Nacional de Ciencia y Tecnología (CONACYT) por el otorgamiento de la beca con el número 880833 para la realización de mi Doctorado.

Por último y no menos importante, agradezco la amistad de mis compañeros y amigos de laboratorio.

A todos, mis más sinceros y humildes agradecimientos.

DEDICATORIA

A mi mamá,

Mi mayor inspiración, y la persona que me enseñó que no existen sueños imposibles. Porque siempre hiciste que el camino fuera más fácil de lo que realmente era. Por las palabras de aliento que atesoro en mi corazón, gracias por ser mi refugio al que siempre quiero volver.

Este logro es tan tuyo como mío, tu amor incondicional y tu confianza son los que me trajeron hasta aquí.

Con todo mi amor y gratitud eterna, hoy y siempre, esta tesis es para ti.

CONTENIDO

I. RESUMEN GENERAL

Antecedentes: *Callistemon citrinus* presenta varios efectos biológicos, entre ellos, propiedades antiinflamatorias, antiobesogénicas, antioxidantes, hepatoprotectoras y quimioprotectoras. Sus compuestos bioactivos incluyen terpenoides, ácidos fenólicos y flavonoides, los cuales poseen una baja biodisponibilidad y absorción oral. Los fitosomas son sistemas de administración de extractos vegetales que exhiben actividades biológicas y poseen una mayor biodisponibilidad, bioabsorción y menor toxicidad que los fármacos convencionales. La obesidad es un estado inflamatorio en el que se presenta estrés oxidante, lo que desencadena efectos graves en los órganos del cuerpo. El objetivo de este estudio fue desarrollar fitosomas de *C. citrinus* para mejorar la biodisponibilidad y absorción oral de sus compuestos, así como evaluar el impacto del extracto etanólico y de los fitosomas sobre el estrés oxidante y la inflamación en el hígado y corazón de ratas Wistar alimentadas con una dieta alta en grasas y fructosa.

Métodos: Los fitosomas fueron formulados utilizando fosfatidilcolina de soya y extracto de hojas de *C. citrinus*, mediante el método de ultrasonicación. Los fitosomas fueron evaluados mediante microscopía electrónica de barrido (SEM), eficiencia de encapsulación, solubilidad y determinación del tamaño de partícula. Asimismo, se midió la capacidad antioxidante y el contenido total de compuestos fenólicos, flavonoides y terpenoides. Se evaluó *in vivo* la actividad antiobesogénica y se analizaron las enzimas antioxidantes (SOD, CAT, GPx, GST, PON1), las enzimas proinflamatorias y los biomarcadores de estrés oxidante (MDA, HNE, AOPP, GSH).

Resultados: Los fitosomas cargados con el extracto de *C. citrinus* presentaron formas esféricas pequeñas. El tamaño promedio de las partículas fue de 129.98 ± 18.30 nm, la eficiencia de encapsulación fue del 80.49 ± 0.07%, y la solubilidad alcanzó el 90.00%. El estudio de estabilidad no mostró cambios significativos en el tamaño promedio de las partículas a 20°C. Los fitosomas de *C. citrinus* presentaron una alta capacidad antioxidante. Por primera vez, se reportó la presencia de ácido elágico en esta planta. En el estudio *in vivo*, los grupos de ratas fueron alimentados con una dieta hipercalórica durante 15 semanas, mientras que se administró Orlistat, extracto de *C. citrinus* y fitosomas en tres concentraciones diferentes (50, 100 y 200 mg/kg, respectivamente). Se

observó una marcada actividad antiobesogénica de los fitosomas de *C. citrinus*, con una reducción del 40% en el peso corporal, así como mejoras en los parámetros morfométricos y bioquímicos. Las actividades enzimáticas observadas fueron similares a las del grupo de control. Además, se observó que la concentración más baja de fitosomas tuvo un efecto comparable al de las otras concentraciones. El extracto de *C. citrinus* puede modular las actividades de las enzimas involucradas en los procesos de estrés oxidante e inflamación.

Conclusión: Los fitosomas de *C. citrinus* son un sistema eficaz para mejorar la biodisponibilidad de sus compuestos bioactivos, con un impacto positivo en la reducción del estrés oxidante y la inflamación asociados con la obesidad. Este enfoque novedoso de administración de extractos naturales representa una alternativa en el tratamiento de enfermedades relacionadas con la obesidad.

Palabras clave: *Callistemon citrinus*, fitosomas, estrés oxidante, inflamación, obesidad, biodisponibilidad.

II. ABSTRACT

Background: *Callistemon citrinus* exhibits various biological effects, including anti-inflammatory, anti-obesogenic, antioxidant, hepatoprotective, and chemoprotective properties. Its bioactive compounds, such as terpenoids, phenolic acids, and flavonoids, have low bioavailability and poor oral absorption. Phytosomes are delivery systems for plant extracts that demonstrate biological activities and provide enhanced bioavailability, improved bioabsorption, and reduced toxicity compared to conventional drugs. Obesity is an inflammatory state characterized by oxidative stress, leading to severe effects on body organs. The objective of this study was to develop *C. citrinus* phytosomes to improve the bioavailability and oral absorption of its compounds and evaluate the impact of the ethanolic extract and phytosomes on oxidative stress and inflammation in the liver and heart of Wistar rats fed a high-fat-fructose diet.

Methods: Phytosomes were formulated using soy phosphatidylcholine and *C. citrinus* leaf extract via the ultrasonic method. Phytosomes were evaluated through scanning electron microscopy (SEM), encapsulation efficiency, solubility, and particle size determination. Antioxidant capacity and the total content of phenolic compounds, flavonoids, and terpenoids were also measured. Anti-obesogenic activity was assessed *in vivo*, and antioxidant enzymes (SOD, CAT, GPx, GST, PON1), pro-inflammatory enzymes, and oxidative stress biomarkers (MDA, HNE, AOPP, GSH) were analyzed.

Results: *C. citrinus* phytosomes exhibited small spherical forms. The average particle size was 129.98 ± 18.30 nm, with an encapsulation efficiency of 80.49 ± 0.07% and solubility reaching 90.00%. Stability studies showed no significant changes in average particle size at 20°C. *C. citrinus* phytosomes demonstrated high antioxidant capacity. For the first time, the presence of ellagic acid in this plant was reported. In the *in vivo* study, rat groups were fed a hypercaloric diet for 15 weeks and treated with Orlistat, *C. citrinus* extract, and phytosomes at three different concentrations (50, 100, and 200 mg/kg, respectively). A marked anti-obesogenic activity of *C. citrinus* phytosomes was observed, with a 40% reduction in body weight and improvements in morphometric and biochemical parameters. Enzymatic activities were similar to those in the control group. Moreover, the lowest phytosome concentration showed an effect comparable to the higher

concentrations. The *C. citrinus* extract was found to modulate the activities of enzymes involved in oxidative stress and inflammatory processes.

Conclusion: *C. citrinus* phytosomes are an effective system for enhancing the bioavailability of its bioactive compounds, with a positive impact on reducing oxidative stress and inflammation associated with obesity. This innovative approach to natural extract delivery represents a potential alternative for treating obesity-related diseases.

Keywords: *Callistemon citrinus*, phytosomes, oxidative stress, inflammation, obesity, bioavailability.

III. INTRODUCCIÓN GENERAL

A nivel mundial, las tasas de obesidad están aumentando, lo que conduce a más de 2.8 millones de muertes anuales como resultado de tener sobrepeso u obesidad (WHO, 2021). Las proyecciones indican que una de cada cinco personas, lo que equivale a 1.5 mil millones de personas, se verá afectada por la obesidad para 2030 (World Obesity Atlas, 2023) y que este número se espera que aumente a 2 mil millones para el año 2035 (Belančić *et al*., 2023). El estilo de vida es importante en la prevención y tratamiento de la obesidad, la diabetes y las enfermedades relacionadas con el síndrome metabólico (SM). La obesidad se caracteriza por un índice de masa corporal (IMC) superior a 30 kg/m², mientras que un IMC entre 25 y 30 kg/m² se clasifica como sobrepeso (Sarma *et al*., 2021). Un estilo de vida sedentario y la malnutrición juegan un papel clave en el SM y las enfermedades relacionadas. El sobrepeso y la obesidad resultan de un desequilibrio crónico entre la ingesta de energía y el gasto energético. Una dieta rica en grasas y carbohidratos puede conducir a la obesidad y a la inflamación crónica a través del estrés oxidante y la supresión del sistema antioxidante (Bondia-Pons *et al*., 2012).

El estrés oxidante no solo es una característica común de la obesidad, las enfermedades cardiovasculares, neurológicas y autoinmunes, sino que también puede encontrarse en el envejecimiento (Simioni *et al*., 2018). Durante el estrés oxidante, hay un aumento en las especies reactivas de oxígeno (ROS) que son producidas por fuentes endógenas y exógenas (Martemucci *et al*., 2022).

El aumento de la inflamación, provocado por la alteración de las vías de señalización redox, la expresión génica de citocinas inflamatorias, quimiocinas y factores de crecimiento, puede llevar a la resistencia a la insulina, la diabetes y el daño cardiovascular (Reuter *et al*., 2010). Esto ocurre mediante mecanismos celulares y nucleares alterados, incluyendo la reparación deteriorada del daño al ADN y la regulación del ciclo celular (Gallagher *et al*., 2010).

Todos los radicales libres están involucrados en los procesos fisiopatológicos del cuerpo (Martemucci *et al*., 2022). Los antioxidantes efectivos pueden romper la reacción en cadena de los radicales libres, ya que contienen uno o más anillos aromáticos (a menudo fenólicos) con uno o más grupos -OH. Los ácidos fenólicos actúan como antioxidantes al atrapar radicales libres, mientras que los flavonoides pueden eliminar radicales libres y

quelar metales (Brewer, 2011). Los principales compuestos que se encuentran en las plantas y los alimentos son los polifenoles y los flavonoides. A pesar de que pueden encontrarse en altas concentraciones, también necesitan estar disponibles para su absorción gastrointestinal para tener efectos benéficos. Sin embargo, estos compuestos a menudo tienen baja bioaccesibilidad y biodisponibilidad, lo cual podría deberse a varios factores que afectan su absorción, estabilidad de los compuestos y el pH ácido del estómago y la microbiota (Rein *et al*., 2013; Rahman *et al*., 2020). El pH gastrointestinal tiene un papel importante en la absorción y biodisponibilidad de los fármacos orales. En estado de ayuno, el pH normal del estómago es aproximadamente 2.18 ± 0.18 (Alqahtani *et al*., 2021). Un cambio en el pH impacta en la disolución, solubilidad, liberación y estabilidad de los fármacos (Abuhelwa *et al*., 2017). Qin *et al*., (2022) encontraron que el contenido total de polifenoles y flavonoides de la infusión de té verde a pH 1.2 disminuyó a 65% y 60% respectivamente. Además, la actividad antioxidante también se redujo, lo que llevó a una baja bioaccesibilidad. Los polifenoles se transforman en fenoles oligoméricos por el pH ácido en el estómago. Los terpenos que contienen grupos polares a bajo pH gástrico permiten la bioaccesibilidad (Dajic Stevanovic *et al*., 2020).

Se ha informado que *Callistemon citrinus* (Myrtaceae) tiene muchos efectos biológicos, incluidos los antimicrobianos, antiinflamatorios, antioxidantes, hepatoprotectores y anticancerígenos (López-Mejía *et al*., 2019; Sowndhararajan *et al.,* 2021). Recientemente, Ortega-Pérez *et al*., (2022) informó que el extracto de hoja de *C. citrinus* tiene actividad anti-obesogénica y reduce el estrés oxidante observado en la obesidad.
Los fitosomas son un sistema de administración de fármacos o extractos de plantas preparados con fosfolípidos utilizando diferentes tipos de solventes (Sandhiya y Ubaidulla, 2020). En comparación con los fármacos o extractos de plantas, los fitosomas tienen un mejor perfil de estabilidad, evitan la destrucción de los fitoquímicos por las enzimas digestivas y la microbiota, aumentan la permeabilidad a través de las membranas, incrementan la biodisponibilidad y mejoran la eficiencia de los compuestos (Permana *et al*., 2020). Por consiguiente, el objetivo de este estudio fue evaluar el efecto de los fitosomas del extracto de hoja de *Callistemon citrinus* sobre el estrés oxidante en ratas alimentadas con una dieta hipercalórica.

3.1. Obesidad

La obesidad es un problema de salud global. En los estados insulares del Pacífico, el 50% de la población es obesa. En Estados Unidos, un tercio de los adultos son obesos (Chakhtoura *et al*., 2023). En 2030, más de mil millones de adultos y 50 millones de niños y adolescentes serán considerados obesos (Tham *et al*., 2023).

La obesidad se caracteriza como una enfermedad compleja marcada por una acumulación excesiva de grasa corporal que puede perjudicar la salud (WHO, 2024). Esta condición se asocia con un aumento de varios marcadores inflamatorios, lo que lleva a una inflamación crónica de bajo grado. Además, la obesidad puede aumentar el riesgo de desarrollar diabetes tipo 2 y enfermedades cardiovasculares; puede impactar negativamente la salud ósea y la reproducción y aumentar el riesgo de ciertos tipos de cáncer (Khanna *et al*., 2022)

Las causas de la obesidad son un conjunto de factores comunitarios e individuales. Aunque la mayoría de estos son modificables, controlar todos los factores contribuyentes es un verdadero desafío. Estos factores incluyen el entorno de la actividad física, el consumo de alimentos, la producción de alimentos, la psicología individual y la psicología social. Comprender estos factores de riesgo individualmente y apreciar su interconexión es clave para entender las causas de la obesidad y trabajar hacia una solución plausible para frenar esta pandemia mundial (Masood y Moorthy, 2023).

El tratamiento de la obesidad no se limita a modificaciones en el estilo de vida y dietas. Los fármacos más comunes utilizados para controlar la obesidad son orlistat (inhibidor de la lipasa pancreática), fentermina (amina simpaticomimética), liraglutida (agonista del receptor de péptido similar al glucagón 1) y naltrexona-bupropión (antagonista opioide e inhibidor de la recaptación de dopamina y noradrenalina). Sin embargo, todos ellos tienen efectos secundarios indeseados (Lee *et al*., 2023) que pueden reducirse utilizando productos naturales de plantas como una estrategia contra la obesidad (Raoof y Fawzy *et al*., 2022).

3.2. Estrés oxidante y obesidad

El estrés oxidante (EO) constituye un vínculo significativo entre la obesidad y sus complicaciones de salud asociadas. Dentro del entorno celular, las especies reactivas de

oxígeno (ROS) se producen como parte normal de los procesos celulares. Los niveles de ROS están finamente regulados por la actividad de enzimas antioxidantes, incluyendo la superóxido dismutasa (SOD), la glutatión peroxidasa (GPx) y la catalasa (CAT), manteniendo el equilibrio celular. Cualquier disrupción en este equilibrio, ya sea por una sobreproducción de ROS o defensas antioxidantes inadecuadas, resulta en estrés oxidante. Esta condición puede dañar las células y contribuir a una variedad de enfermedades. Además, este estado oxidativo puede actuar como un desencadenante primario para mecanismos adicionales que causan daño tisular, incluyendo inflamación, sobreproducción de matriz extracelular, activación del estrés del retículo endoplásmico y disrupción del flujo autofágico (Martínez-Martínez y Cachofeiro, 2022; Ragusa, 2023).

Las células cuentan con niveles basales de ROS necesarios para su funcionamiento normal. Sin embargo, la producción excesiva de ROS reduce la actividad del sistema de defensa enzimático antioxidante y también disminuye el contenido de glutatión reducido (GSH), afectando así el sistema de defensa antioxidante en general y volviéndolo incapaz de eliminar los radicales libres sobrantes. Estas ROS excesivas se producen en condiciones de hiperoxia e inflamación, y con un sistema de defensa antioxidante bajo o deteriorado, alterando finalmente la homeostasis de todo el sistema biológico (Stadtman y Levine, 2000). Uno de los biomarcadores para la evaluación del daño mediado por ROS es la peroxidación lipídica (LPO), que indica el grado de daño peroxidativo en los fosfolípidos de las membranas. La tasa de LPO aumenta rápidamente durante el estrés oxidativo debido al daño peroxidativo causado por los radicales libres en la membrana lipídica poliinsaturada. Esto a su vez da lugar a la formación de malondialdehído (MDA) y compuestos dienos conjugados; ambos son conocidos como compuestos citotóxicos y mutagénicos. Además, el EO también provoca cambios conformacionales en las proteínas, disminuyendo su eficiencia funcional y alterando sus actividades enzimáticas (Young y Woodside, 2001; Yasui *et al*., 2014).

El estrés oxidante activa diversas vías de señalización apoptótica debido al aumento en la producción de ROS o a la reducción en las actividades de las enzimas antioxidantes. El hígado es el objetivo principal del ataque de ROS debido a su participación directa en los procesos metabólicos y de filtración. En el hígado, las ROS inducen daños en las membranas de los hepatocitos y conducen a la degeneración, lo que a su vez provoca la

deposición de colágeno en los hepatocitos y, finalmente, causa fibrosis y cirrosis hepática. Además, la oxidación incompleta de las biomoléculas causa lipoapoptosis en las células hepáticas e induce reacciones inmunitarias en el hígado (Hajam *et al*., 2022). En el caso del corazón, la hipertrofia cardíaca (HC) es una respuesta adaptativa del corazón a la sobrecarga de presión. Es una característica patológica general en el curso natural de algunas enfermedades cardiovasculares importantes, como la hipertensión y el infarto de miocardio. La hipertrofia cardíaca está fuertemente asociada con un mayor riesgo de insuficiencia cardíaca y muerte súbita cardíaca. El OS se considera uno de los factores contribuyentes importantes en el desarrollo de la hipertrofia cardíaca (Higuchi *et al*., 2003; Satoh *et al*., 2006; Hajam *et al*., 2022).

3.3. Antioxidantes y obesidad

Los antioxidantes pueden inhibir, disminuir, retrasar o eliminar directamente los radicales libres y neutralizar los oxidantes. Actúan como agentes reductores y quelantes de metales, que convierten los hidroperóxidos en compuestos estables. La transferrina, la metalotioneína y la ceruloplasmina son proteínas específicas de unión a metales consideradas agentes antioxidantes, y sus mecanismos incluyen la unión a iones metálicos prooxidantes como el hierro y el cobre (Hussain y Kayani, 2020). La ingesta de antioxidantes puede contribuir a proteger contra el daño producido por las especies reactivas de oxígeno (Ali *et al*., 2020). La actividad antioxidante en las plantas se debe a los compuestos fenólicos, flavonoides y terpenos que se encuentran en ellas (Lee *et al*., 2017). Los productos químicos derivados de plantas pueden mejorar la condición del tejido adiposo en individuos obesos al mitigar el estrés oxidante intracelular (Pérez-Torres *et al*., 2021). Los avances recientes en la investigación alimentaria han aumentado significativamente el interés en la capacidad de los productos naturales para combatir la obesidad. Es ampliamente reconocido que una variedad de alimentos de origen vegetal es efectiva en la gestión del peso. Por lo tanto, una dieta equilibrada debe incorporar consistentemente una cantidad adecuada de frutas, verduras, especias y hierbas (Ofori *et al*., 2024). Se ha encontrado que son ricos en fitoquímicos, incluyendo terpenos y compuestos fenólicos, que poseen propiedades antioxidantes, antitumorales y antiinflamatorias demostradas. Como resultado, se proponen como opciones naturales

potenciales para el tratamiento terapéutico de diversas enfermedades crónicas donde los mecanismos de inflamación y estrés oxidante juegan un papel crucial (Singh *et al*., 2017; López-Mejía *et al*., 2019; Amerikanou y Papada, 2023).

3.4. *Callistemon citrinus* (Curtis) Skeels

El género *Callistemon* se encuentra entre los grupos prometedores de plantas medicinales reportadas por sus propiedades neuroprotectoras, quimiopreventivas, antioxidantes, antienvejecimiento, antimicrobianas y otras propiedades farmacéuticas críticas (Rathore y Rai, 2022). *Callistemon citrinus* (*C. citrinus*) (Curtis) Skeels, comúnmente conocido como "Crimson Bottlebrush" y nativo de Australia, se cultiva por su belleza ornamental. Además, es utilizado por curanderos tradicionales en la elaboración de formulaciones medicinales herbales (Singh *et al*., 2017; López-Mejía *et al*., 2019). *C. citrinus* ha sido identificado como una fuente potencial de fitoquímicos con varios efectos anti-obesogénicos, a través de mecanismos que incluyen la inhibición de la enzima α-glucosidasa (Fayemi *et al*., 2019), modulación del estrés oxidante (López-Mejía *et al*., 2021), actividad anti-lipasa, antioxidante (Ortega-Pérez *et al*., 2022) y propiedades antiinflamatorias (Piñón-Simental *et al*., 2024). *C. citrinus* tiene compuestos terpénicos como 1,8-cineol, limoneno y α-terpineol (Petronilho *et al*., 2013). Ayala-Ruiz *et al*., (2022) mostraron que el papel principal de estos terpenos es reducir el estrés oxidante generado por la obesidad en el modelo animal. Las hojas y tallos de *C. citrinus* presentaron compuestos fenólicos y flavonoides como eucaliptina, blumenol, ácido gálico y ácido protocatecuico (Khanh *et al*., 2016). A pesar de las grandes actividades biológicas de *Callistemon citrinus*, hay pocos estudios sobre la aplicación de nanopartículas con esta planta.

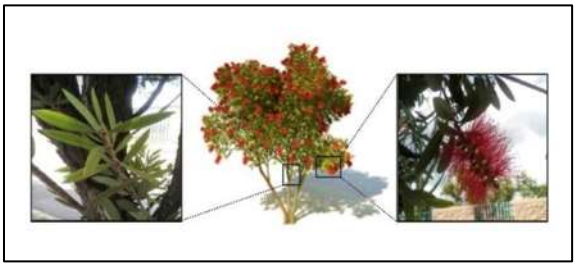

Figura 1. *Callistemon citrinus:* hojas lanceoladas (imagen izquierda) e inflorescencia (imagen derecha). Fuente: Elaboración propia.

3.5. Nanopartículas de *Callistemon citrinus*

La nanotecnología es un sistema de administración que puede clasificarse en dos grupos: inorgánicos como oro, plata y cobre y orgánicos como liposomas y nanopartículas poliméricas (Alharbi *et al*., 2021). Cuando se usa tecnología novedosa de administración de fármacos en lugar de la administración de fármacos tradicional, los efectos secundarios se reducen, mientras que la seguridad y la eficacia mejoran (Adiki *et al*., 2023).

La biosíntesis de nanopartículas de óxido de plata a partir del extracto acuoso de hoja de *Callistemon lanceolatus* (*C. citrinus*) demostró la capacidad antioxidante *in vitro* y la letalidad en camarones de salmuera (Ravichandran *et al*., 2016). Paosen *et al*., (2017) informaron sobre la síntesis de nanopartículas de plata con extractos de la familia Myrtaceae y la caracterización de su actividad antibacteriana. Las nanopartículas de plata obtenidas con extractos de hojas, flores y semillas de *C. citrinus* exhibieron actividad antiplasmodial y antibacteriana sin toxicidad (Rotimi *et al*., 2019a). Las nanopartículas de oro generadas con extractos de la semilla de *C. citrinus* tienen actividad antibacteriana, pero no actividad antitripanosómica, a diferencia del extracto obtenido de la misma semilla que exhibe ambas propiedades (Rotimi *et al*., 2019b). Las nanopartículas de ácido poli (láctico-co-glicólico) cargadas con fenólicos de *C. citrinus* mostraron actividad anticancerosa contra tres líneas celulares de cáncer de mama con un 69% de inhibición del crecimiento (Ahmed *et al*., 2019). Recientemente, se probó la actividad antibacteriana de nanopartículas de plata obtenidas con un extracto acuoso de hoja de C*. citrinus* (Gharibvand *et al*., 2022).

Los fitosomas se distinguen de la administración convencional de fármacos o extractos de plantas al proporcionar un perfil más estable, ofreciendo resistencia contra la degradación por enzimas digestivas y microbiota, mejorando la permeabilidad de la membrana, aumentando la biodisponibilidad y amplificando la efectividad de los fitoconstituyentes (figura 2) (Permana *et al*., 2020). Los compuestos bioactivos en los fitosomas ayudan a contrarrestar los factores de riesgo para los trastornos metabólicos. Durante las últimas dos décadas, los productos que utilizan compuestos bioactivos formulados en fitosomas han mostrado efectos terapéuticos mejorados *in vitro* e *in vivo*. Estos compuestos mejoran las enzimas antioxidantes, aumentan las respuestas

antiinflamatorias, reducen las citocinas proinflamatorias, inducen macrófagos M2, reducen los lípidos, aumentan la captación de glucosa, reducen las transaminasas hepáticas y regeneran el tejido hepático. Estas acciones conducen a una reducción de la grasa visceral, mejoran los parámetros hemodinámicos, la angiogénesis y la recuperación de los tejidos infartados (Toma *et al*., 2024).

Hasta la fecha, la literatura sobre la aplicación de formulaciones fitosomales en fitoquímicos antiobesogénicos sigue siendo limitada. Sin embargo, existe una necesidad urgente de desarrollar fármacos herbales anti-obesogénicos en formas fitosomales para mejorar la biodisponibilidad y minimizar los efectos adversos. Adoptar el enfoque fitosomal en la administración de fármacos podría superar las limitaciones enfrentadas por los mecanismos tradicionales de administración de fármacos (Adiki *et al*., 2023). El desarrollo de fitosomas de *C. citrinus* ha mostrado un potencial significativo en la gestión del peso en modelos de obesidad en roedores, mostrando una estrategia innovadora para utilizar *C. citrinus* en el tratamiento de la obesidad (Ortega-Pérez *et al*., 2023).

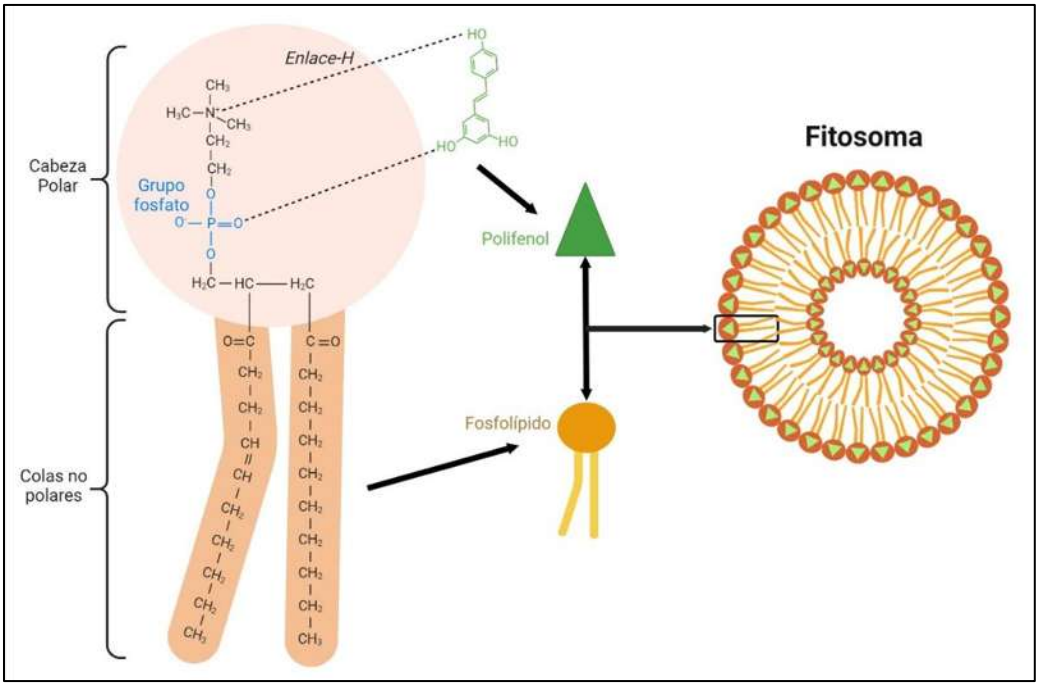

Figura 2. Estructura general de un fitosoma. Los fitosomas representan un sistema de innovación en la entrega de fitoquímicos (Barani *et al*., 2021). Imagen elaborada en Biorender.

IV. JUSTIFICACIÓN

La obesidad es un problema de salud global que afecta a millones de personas y es responsable de más de 2.8 millones de muertes anuales. Esta condición se asocia con diversas complicaciones de salud, incluyendo enfermedades cardiovasculares, neurológicas, autoinmunes y cáncer. El estrés oxidante y la inflamación crónica de bajo grado son factores clave en la patogénesis de la obesidad y sus complicaciones.

Callistemon citrinus es una planta que ha demostrado tener múltiples efectos biológicos benéficos, tales como propiedades antioxidantes, antiinflamatorias, hepatoprotectoras y anticancerosas. Estudios recientes han evidenciado su actividad anti-obesogénica y su capacidad para reducir el estrés oxidante e inflamación en la obesidad.

Los fitosomas son sistemas de administración de extractos de plantas preparados con fosfolípidos, que han mostrado mejorar la estabilidad, biodisponibilidad y efectividad de los compuestos bioactivos, ofreciendo una mejor alternativa para el tratamiento de la obesidad.

La presente investigación tiene como objetivo encapsular el extracto etanólico de hoja de *Callistemon citrinus* en fitosomas para mejorar su biodisponibilidad y absorción, y prevenir el aumento de peso en un modelo de obesidad inducida por dieta hipercalórica en ratas Wistar. Se busca determinar el efecto de los fitosomas sobre el estrés oxidante y los parámetros bioquímicos y morfométricos, proporcionando así una estrategia innovadora para el tratamiento de la obesidad utilizando productos naturales.

Esta investigación es relevante no solo por su potencial impacto en la salud pública, sino también porque contribuye al conocimiento científico sobre el uso de sistemas de administración avanzada de fármacos naturales para el manejo de enfermedades crónicas como la obesidad.

En este contexto, esta investigación aborda los siguientes aspectos:

Importancia Científica: Esta investigación contribuirá al conocimiento sobre el uso de fitosomas para mejorar la biodisponibilidad de compuestos bioactivos en el tratamiento de la obesidad.

Importancia Práctica: Podría ofrecer una nueva estrategia terapéutica basada en productos naturales, minimizando efectos secundarios de tratamientos farmacológicos tradicionales.

Impacto Social: Abordar la obesidad, una condición que afecta a millones de personas, mejorando la calidad de vida y reduciendo la carga en los sistemas de salud.

Necesidad de la Investigación: Existe una necesidad urgente de encontrar alternativas eficaces y seguras para el tratamiento de la obesidad, dadas las limitaciones de los tratamientos actuales y la creciente prevalencia de la enfermedad.

Finalmente, se conduce a la pregunta general de investigación, para lo cual se plantea lo siguiente: ¿Cuál es el efecto de los fitosomas del extracto de hoja de *Callistemon citrinus* en la modulación del estrés oxidante y la inflamación en un modelo de obesidad inducida por dieta hipercalórica en ratas Wistar?.

V. HIPÓTESIS

5.1. Hipótesis General

Los fitosomas del extracto etanólico de hoja de *Callistemon citrinus* atenúan el estrés oxidante y la inflamación, mejorando los parámetros bioquímicos y morfométricos en ratas alimentadas con una dieta hipercalórica.

5.1.1. Hipótesis Particulares

Capítulo 1: Los fitosomas preparados con el extracto etanólico de hoja de *Callistemon citrinus* presentan una mayor estabilidad, solubilidad y capacidad antioxidante en comparación con el extracto no encapsulado, mejorando su efectividad en estudios *in vitro* y biodisponibilidad *in vivo*.

Capítulo 2: La administración de fitosomas del extracto etanólico de hoja de *Callistemon citrinus* en ratas alimentadas con una dieta hipercalórica reduce significativamente los parámetros bioquímicos y morfométricos, así como el estrés oxidante y la inflamación.

VI. OBJETIVOS

6.1. Objetivo General

Determinar el efecto de los fitosomas del extracto etanólico de hoja de *Callistemon citrinus* sobre el estrés oxidante, inflamación, parámetros bioquímicos y morfométricos de ratas alimentadas con una dieta hipercalórica.

6.1.1. Objetivos Particulares

Capítulo 1.

- Determinar el perfil fitoquímico, la solubilidad y la capacidad antioxidante de los fitosomas de hojas de *Callistemon citrinus*, así como caracterizar los fitosomas mediante estudios microscópicos, de estabilidad de partículas, eficiencia de atrapamiento de vesicular y evaluar su efecto sobre los parámetros morfométricos y bioquímicos ratas alimentadas con una dieta hipercalórica.

Capítulo 2.

- Analizar el efecto de los fitosomas del extracto etanólico de hojas de *Callistemon citrinus* sobre los biomarcadores de estrés oxidante, enzimas antioxidantes y proinflamatorias en el hígado y corazón de ratas alimentadas con una dieta hipercalórica.

Cada uno de los objetivos específicos cuenta con la estructura de un artículo científico. Cabe mencionar que los objetivos planteados ya se encuentran publicados. Además, como parte de esta investigación, se está tramitando la patente de los fitosomas, la cual ha aprobado el examen de forma y se presenta en los anexos.

6.2. Estrategia Experimental

Para cumplir con los objetivos de esta investigación, el primer paso fue la obtención de las hojas de *Callistemon citrinus*. Luego, se procedió a la obtención del extracto etanólico, sometiendo las hojas a un proceso de extracción con etanol en una proporción de 1 g de tejido por 10 ml de etanol al 95%.

Para la preparación de los fitosomas, se utilizaron fosfolípidos de soya y el extracto etanólico para la formación de las vesículas. Posteriormente, se caracterizó la morfología de los fitosomas mediante microscopía electrónica de barrido (SEM). Adicionalmente, se llevó a cabo un estudio del tamaño de las partículas de los fitosomas y su estabilidad, evaluando la compactación de las partículas a lo largo del tiempo. Asimismo, se analizó la eficiencia de la encapsulación y la solubilidad de los fitosomas, y de manera *in vitro*, se midió su capacidad antioxidante mediante los métodos DPPH, ABTS y FRAP.

Se realizó un estudio fitoquímico para cuantificar fenoles, flavonoides y terpenos totales; e identificar terpenos y ácidos fenólicos utilizando GC/MS y HPLC, respectivamente. Posteriormente, se implementó un modelo *in vivo* de obesidad, donde se administraron los fitosomas durante 15 semanas a ratas Wistar de dos meses de edad, para evaluar sus efectos anti-obesogénicos. Para este fin, se contó con un grupo control y varios grupos experimentales. En este modelo *in vivo*, se llevaron a cabo mediciones de parámetros morfométricos y bioquímicos en los animales experimentales. Al término de la semana 15 del experimento, las ratas se sometieron a eutanasia. Finalmente, se realizó un análisis detallado de los órganos (hígado y corazón) *post mortem*, evaluando los marcadores de estrés oxidante y las determinaciones enzimáticas para completar la evaluación de los efectos de los fitosomas.

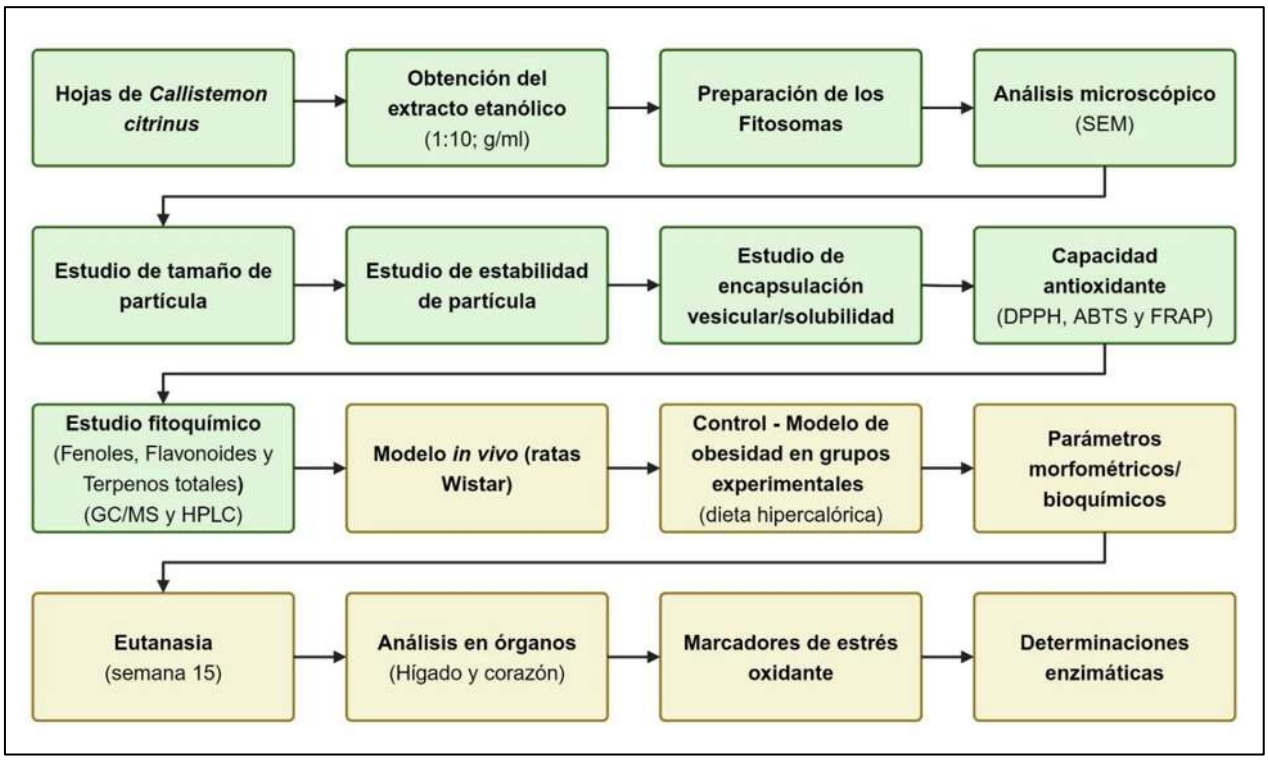

Figura 3. Estrategia experimental para evaluar el efecto de los fitosomas del extracto etanólico de la hoja de C*allistemon citrinus* en un modelo de obesidad inducida por dieta hipercalórica en ratas Wistar.

VII. RESULTADOS

Capítulo I: Development and Evaluation of Phytosomes Containing *Callistemon citrinus* Leaf Extract: A Preclinical Approach for the Treatment of Obesity in a Rodent Model.

Publicado en: *Pharmaceutics* **2023**, 15, 2178.
https://doi.org/10.3390/pharmaceutics15092178
https://www.mdpi.com/journal/pharmaceutics

pharmaceutics

MDPI

Article

Development and Evaluation of Phytosomes Containing *Callistemon citrinus* Leaf Extract: A Preclinical Approach for the Treatment of Obesity in a Rodent Model

Luis Gerardo Ortega-Pérez [1], Luis Alberto Ayala-Ruiz [1], Oliver Rafid Magaña-Rodríguez [1], Jonathan Saúl Piñón-Simental [1], Asdrubal Aguilera-Méndez [2], Daniel Godínez-Hernández [2] and Patricia Rios-Chavez [1,*]

[1] Facultad de Biología, Universidad Michoacana de San Nicolás de Hidalgo, Morelia 58000, Michoacán, Mexico; gerardo.ortega@umich.mx (L.G.O.-P.); 1232816g@umich.mx (L.A.A.-R.); oliver.rodriguez@umich.mx (O.R.M.-R.); 1434491c@umich.mx (J.S.P.-S.)

[2] Instituto de Investigaciones Químico Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Morelia 58000, Michoacán, Mexico; amendez@umich.mx (A.A.-M.); dgodinez@umich.mx (D.G.-H.)

* Correspondence: prchavez17@gmail.com

Abstract: *Callistemon citrinus* has several biological effects; it is anti-inflammatory, anti-obesogenic, antioxidant, hepatoprotection, and chemoprotective. Its bioactive compounds include terpenoids, phenolic acids, and flavonoids which have low oral bioavailability and absorption. This study aimed at developing phytosomes of *C. citrinus* to improve oral bioavailability and absorption. Phytosomes were formulated with soybean phosphatidylcholine and *C. citrinus* leaf extract using the thin layer sonication method. Phytosomes were evaluated by scanning electron microscopy (SEM), entrapment efficiency, solubility, and particle size determination. Antioxidant capacity and total phenolic, flavonoid, and terpenoid contents were also measured. The in vivo anti-obesogenic activity was evaluated. Phytosomes loaded with *C. citrinus* (P *C.c*) extract had small spherical shapes. The average particle size was 129.98 ± 18.30 nm, encapsulation efficiency 80.49 ± 0.07%, and solubility 90.00%; the stability study presented no significant changes in the average particle size at 20 °C. P *C.c* presented high antioxidant capacity. For the first time, ellagic acid is reported in this plant. The in vivo obesity study showed a strong anti-obesogenic activity of phytosomes with *C. citrinus* to reduce 40% body weight as well as morphometric and biochemical parameters.

Keywords: antioxidant capacity; bioavailability; anti-obesogenic; phosphatidylcholine

Citation: Ortega-Pérez, L.G.; Ayala-Ruiz, L.A.; Magaña-Rodríguez, O.R.; Piñón-Simental, J.S.; Aguilera-Méndez, A.; Godínez-Hernández, D.; Rios-Chavez, P. Development and Evaluation of Phytosomes Containing *Callistemon citrinus* Leaf Extract: A Preclinical Approach for the Treatment of Obesity in a Rodent Model. *Pharmaceutics* **2023**, *15*, 2178. https://doi.org/10.3390/pharmaceutics15092178

Academic Editor: Antonio Vassallo

Received: 4 July 2023
Revised: 18 August 2023
Accepted: 20 August 2023
Published: 22 August 2023

1. Introduction

Phytosomes are a delivery system of drug or plant extracts prepared with phospholipids using different types of solvents [1]. Compared with drug or plant extracts, phytosomes have a better stability profile, avoid destruction of the phytoconstituent by digestive enzymes and microbiota, increase permeability through membranes, increase bioavailability, and improve compound efficiency [2].

Oxidative stress is not only a common feature of obesity, cardiovascular, neurological, and autoimmune diseases but can also be found in aging [3]. During oxidative stress, there is an increase in reactive oxygen and nitrogen species (ROS/RNS) that are produced by endogenous and exogenous sources [4].

Antioxidants can inhibit, decrease, delay, or directly scavenge free radicals and neutralize oxidants. They act as reducing agents and metal chelators, which convert hydroperoxides into stable compounds. Transferrin, metallothionein, and ceruloplasmin are specific metal-binding proteins considered antioxidant agents and their mechanisms include binding pro-oxidant metal ions, such as iron and copper [5]. The intake of antioxidants may contribute to protecting against the damage produced by reactive oxygen species [6]. The

antioxidant activity in plants is due to the phenolic, flavonoid, and terpene compounds found in them [7].

The major compounds found in plants and food are polyphenols and flavonoids. Despite that they can be found in high concentrations, they also need to be available for absorption during gastrointestinal to have beneficial effects. However, these compounds often have low bioaccessibility and bioavailability, which could be due to a number of factors that affect their absorption, stability of the compounds, and the acidic pH of the stomach and microbiota [8,9]. Gastrointestinal pH has an important role in the absorption and bioavailability of oral drugs. In the fasting state, the normal stomach pH is approximately 2.18 ± 0.18 [10]. A change in pH has an impact on the dissolution, solubility release, and stability of drugs [11]. Quin et al. [12] found that the total polyphenol and flavonoid contents of green tea infusion, at pH 1.2, decreased to 65% and 60%, respectively. In addition, the antioxidant activity was reduced as well, leading to low bioaccessibility. Polyphenols are transformed into oligomeric phenols by acidic pH in the stomach. Terpenes containing polar groups at low gastric pH allow bioaccessibility [13]. In summary, pH changes have effects on bioaccessibility that have a strong connection with bioavailability.

Callistemon citrinus (Myrtaceae) has been reported to have many biological effects, including antimicrobial, anti-inflammatory, antioxidant, hepatoprotective, and anticarcinogenic [14,15]. Recently, Ortega-Pérez et al. [16] reported that *C. citrinus* leaf extract has anti-obesogenic activity and reduces the oxidative stress observed in obesity. *C. citrinus* has many terpene compounds such as 1-8-cineole, limonene, and α-terpineol [17]. Ayala-Ruiz et al. [18] showed that the main role of these terpenes is to reduce oxidative stress generated by obesity in the animal model. The leaves and stems of *C. citrinus* presented phenolic and flavonoid compounds, such as eucalyptine, blumenol, gallic acid, and protocatechuic acid [19].

Despite the great biological activities of *Callistemon citrinus*, there are few studies about the application of nanoparticles with this plant. The biosynthesis of silver oxide nanoparticles from the aqueous leaf extract of *Callistemon lanceolatus* (*C. citrinus*) proved the in vitro antioxidant capacity and brine shrimp lethality [20]. Paosen et al. [21] reported the synthesis of silver nanoparticles from the Myrtaceae family and the characterization of their antibacterial activity. Silver nanoparticles from leaves, flowers, and seeds of *C. citrinus* exhibited antiplasmodial and antibacterial activity without toxicity [22]. Gold nanoparticle from the seed of *C. citrinus* has antibacterial activity but no antitrypanosomal activity, unlike the extract obtained by the same seed which exhibits both properties [23]. Poly (lactic-co-glycolic acid) nanoparticles loaded with *C. citrinus* phenolics showed anticancer activity against three breast cancer cell lines with 69% growth inhibition [24]. Recently, the use of *C. citrinus* silver nanoparticles from leaf aqueous extract was tested for antibacterial activity [25]. Nanotechnology is a delivery system that can be classified into two groups: inorganic as gold, silver, and copper, and organic as liposomes and polymeric nanoparticles [26]. When novel drug delivery technology is used, instead of traditional drug delivery, side effects are reduced whereas safety and efficacy are improved [27].

Obesity is a global health problem. In the Pacific Island states, 50% of the population is obese. In the United States, one-third of adults are obese [28]. In 2030, more than one billion adults and 50 million children and adolescents will be considered obese [29]. The treatment of obesity is not limited to lifestyle modifications and diets. The most common drugs used to control obesity are orlistat (pancreatic lipase inhibitor), phentermine (sympathomimetic amine), liraglutide (glucagon-like peptide 1 receptor agonist), and naltrexone-bupropion (opioid antagonist and a dopamine and noradrenaline reuptake inhibitor). However, all of them have undesired side effects [30] that can be reduced using natural products from plants as a strategy against obesity [31].

This study aimed at encapsulating *Callistemon citrinus* leaf extract in a phosphatidylcholine complex to enhance its bioavailability and absorption and prevent weight gain. This paper demonstrates that the phytosomes of *Callistemon citrinus* extract had a small

size, high entrapment efficiency, and good solubility and stability. The anti-obesogenic activity was also evaluated using male Wistar rats fed with a hypercaloric diet.

2. Materials and Methods

2.1. Preparation of Callistemon citrinus Leaf Extract

Four-year-old leaves of *Callistemon citrinus* (Curtis) Skeels (Myrtaceae) plants were collected in the city of Morelia, Michoacán, Mexico. The plant voucher specimen EBUM23538 was identified by Professor Patricia Silva at the Biology School of Universidad Michoacana de San Nicolas de Hidalgo. The fresh leaves were macerated in a 1:10 ratio (*w*/*v* 96% ethanol) at room temperature for 5 days. Then, the extract was concentrated by a rotary evaporator at 45 °C. The yield was 20%. The extract of *Callistemon citrinus* was prepared according to the methodology reported by Lopez-Mejia et al. [32]. The authors concluded that the extract should be prepared with leaves of different four-year-old plants to ensure the highest concentration of its major compounds, as well as high antioxidant capacity.

2.2. Phytosome Preparation

To prepare phytosomal complex, the same concentration (200 mg/b.w.) of *Callistemon citrinus* and phospholipids were used. This dose has therapeutic efficacy against the inhibition of oxidative stress [15,32] and obesity amelioration [16,18].

Phytosomes were prepared using the assays reported by Baradan et al. [33] and Álvarez-Cortes [34], with slight modifications. The mixture contained 50 mL of hydration media (0.01 M phosphate buffer solution, 150 mM NaCl, pH 7.4), 1.25 g of *Callistemon citrinus* extract, 1.25 g of soybean phospholipids, and 0.72 g of Tween 80; 1% of ethyl acetate was added to improve solubility in the solution. The emulsion was formed using a VCX 500 ultrasonicator with an amplitude of 25% for 10 min at 10 °C. Phytosomes had a stoichiometric ratio of 1:1.

The phytosome complex was placed in an amber-colored glass bottle and stored at room temperature. Design Expert 11.0.5, an experimental design with response surface methodology of central composite design, was used to prepare phytosomes. Lecithin concentration (%*w*/*v*) and rotation speed (rpm) were selected as independent variables. Then, the effect of these variables on the vesicular size and entrapment efficiency of the phytosomes were assessed. All procedures were protected from light. Finally, to corroborate the preparation, the phytosomes were observed under optical microscopy.

2.2.1. Lyophilization and Scanning Electron Microscopy (SEM)

Phytosome samples were frozen at −80 °C overnight; afterward, lyophilized in a high vacuum of 34 Pa using a lyophilizer (Labconco Plus 12; Labconco, Kansas City, MO, USA) for 8 h with a condenser at −43 °C. Lyophilized phytosomes were stored in a sealed glass ampoule at 4 °C. One drop of lyophilized sample was placed on a brass electron microscope tube and coated with copper particles for sputtering. Representative images of the samples were taken and particle diameters were calculated using scanning electron microscopy (JEOL JSM-7600F SEM) with a voltage of 20.0 KV at a working distance of 15.1 mm. Details of the morphological structure of the phytosomes were observed at up to an amplitude of 10,000× and a working distance that allowed minute observations with increasing depth of focus.

2.2.2. Particle Size

Particle size was measured with a Nano Particle Analyzer SZ-100, based on the principle of dynamic light scattering; Ludox TM silica was used as reference material [35]. Ludox TM-50 was diluted to 10% using 0.01 M KCL. A total of 10 mL of KCl/LUDOX solution was filtered through a 2.5 µm filter. The samples were placed in a plastic cuvette and analyzed at a 90° scattering angle. All the batches were analyzed in a triplicate manner and mean and SD were calculated. Table 1 shows the measurement conditions to determine the particle size.

Table 1. Measurement conditions to determine the particle size.

Temperature	25 °C
Particle	LUDOX (1.45–0.000i)
Dispersion medium	Water
Cell	Plastic
Distribution type	Monodisperse narrow

2.2.3. Stability Study

The stability analysis was assessed by storing the phytosomes at 20 ± 2 °C and 4 ± 1 °C and the particle size was measured 1, 3, 5, and 10 days after storing. Later, it was measured after three and a half months.

2.2.4. Study of Vesicular Entrapment/Encapsulation and Solubility

The entrapment efficiency of *C. citrinus* phytosomes was measured using UV-visible spectrophotometer [36]. A total of 1 mL of dialyzed vesicular suspension was taken and diluted with 0.1 mL of Triton X-100. The solution was centrifuged at 1350× *g* for 5 min and the supernatant was diluted with ethanol. The amount of drug entrapped was analyzed spectrophotometrically at a maximum of 425 nm against ethanol containing Triton X-100 as blank. Equation (1) computes the efficiency of entrapment (EE); Tdrug is the total amount of drug; Edrug is the extract entrapment in the formulation (phytosome); and Udrug is the extract not entrapped in phytosomal formulation.

$$EE = \frac{Edrug}{Tdrug} \times 100\% = \frac{Edrug}{Edrug + Udrug} \times 100\% = \left(1 - \frac{Udrug}{Edrug\ +\ Udrug}\right) \times 100\% \quad (1)$$

Solubility analysis was calculated by dissolving 2 mg of each of the complexes formed (soybean phospholipid particles) and *C. citrinus* leaf extract in 5 mL of different solvents in small volumetric flasks. The solutions were stirred continuously for 1 h [37]. The experiments were performed in triplicate.

2.3. In Vitro Antioxidant Activity

2.3.1. DPPH Radical Assay

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay was performed as reported by Kamarac et al. [38]. The solution reaction contained 10 µL of the sample (*C. citrinus* leaf extract or phytosome at 200 mg), 90 µL of methanol, and 2 mL of methanolic solution of DPPH 0.1 mM, which were mixed and incubated in the dark for 60 min at room temperature; its absorbance was measured at 517 nm. Trolox (25–800 µM) was used as standard.

2.3.2. ABTS Radical Scavenging Assay

The 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay was performed as reported by Rufino et al. [39], with slight modifications. A total of 2.6 mM potassium persulfate solution was mixed in equimolar amounts with ABTS (ready to use, Sigma); then, the solution was stirred in the dark for 3 h at 27 °C. This working solution was diluted with ethanol to obtain an absorbance from 0.8–0.9 at 734 nm. For the tests, 1 µL of *C. citrinus* leaf extract (200 mg) and 1 µL of the phytosomes (200 mg) were used, and 49 µL of absolute ethanol and 950 µL of working solution were added. Subsequently, the absorbance at 734 nm was determined after 6 min of starting the reaction.

2.3.3. Ferric-Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was performed as reported by Thaipong et al. [40]. Working solution contained 10 mM 2,4,6-tri [2-pyridyl-s-triazine] (TPTZ) in 40 mM HCL, 20 mM ferric chloride ($FeCl_3$.6 H_2O), and 300 mM sodium acetate buffer (pH 3.6) in a 1:1:10 ratio. A total of 0.1 mL of sample was mixed with 1.5 mL working solution and allowed to stand at room temperature for 20 min in darkness. Then, the absorbance was measured at 593 nm. Results

were expressed as mean values ± one standard deviations. Trolox standards ranged from 25 to 800 µM.

2.3.4. Determination of Total Phenolic Content

The total phenolic content was determined using the reported by Pripdeevech et al. [41], with slight modifications; in brief, 0.2 mL of the sample and 1.0 mL of Folin–Ciocalteu reagent (1:9 *v*/*v*) were shaken vigorously for 5 min. Then, 1.0 mL of 7% Na_2CO_3 and 5.0 mL of distilled water were added. The reaction mixture was allowed to stand for 60 min at room temperature in darkness and its absorbance was measured at 765 nm. Gallic acid was used as standard (0.01–0.4 mM). Total phenolic content was expressed as mg gallic acid equivalent (mg GAE).

2.3.5. Total Flavonoid Content

The total flavonoid content was determined using the assay reported by Chang et al. [42]. In brief, 0.5 mL of the sample mixed with 1.5 mL of 95% methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water, stood for 30 min at room temperature in darkness, and the absorbance was measured at 415 nm. Water was used instead of aluminum chloride as blank. Rutin acid was used to calculate the standard curve (0.025–0.5 mg/mL).

2.3.6. Total Terpenoid Content

The total terpenoid content was determined using the methodology described by Chang and Lin [43]. A mixture containing 100 µL of sample (10 mg/mL), 150 µL of vanillin/glacial acetic acid (5% *w*/*v*), and 20 µL of sulfuric acid was incubated at 60 °C for 45 min. The mixture was left on ice for 7 min to stop reaction. Finally, 2.25 mL of glacial acetic acid was added and its absorbance was measured at 548 nm. A total of 1,8-Cineole at 1–6 mg/mL was used as standard.

2.4. GC-MS Determination

The samples were analyzed in an Agilent 7890A gas chromatography equipment (Agilent Technologies, Folsom, CA, USA) with an HP5MS30M column (5% phenyl polysilphenylene-siloxane, 30 × 0.25 × 0.25; Agilent Technologies, USA) coupled to an electronic impact ionization quadrupole mass analyzer mass spectrometer. Hewlett Packard 5975C (Hewlett Packard, Palo Alto, CA, USA, EEA). The initial temperature of the oven was 60 °C for 1 min and was increased to 280 °C at 8 °C/min. The injector temperature was 230 °C, the ionization source 230 °C, and the quadrupole temperature 150 °C. Helium was used as carrier gas at a constant flow of 1 mL/min. The mass spectrometer was operated in the EI mode at 70 eV using a range of *m*/*z* 50–500 and the voltage was −1737 V. Total ion chromatograms (TIC) were processed using the automated data processing Software MassHunter Workstation version B.06.00 (Agilent Technologies, Inc.). To identify the different compounds, the mass spectrum of each compound detected was compared to those in mass spectral databases (Wiley 275 and US National Institute of Science and Technology (NIST) V. 2.0. The quantities of compounds were calculated from a standard calibration curve using 1,8-cineole at range 1–0.2 mg/mL.

2.5. HPLC Determination

Phenolic acids were quantified by using a high-performance liquid chromatograph (HPLC, Agilent 1260 Infinity Series), equipped with a quaternary pump, auto sampler, column oven, diode array detector (DAD), and Express 90 analytical column. Å C18, 250 × 4.6, 5 µm. The column temperature was 40 °C with an injection volume of 5 µL, the flow was 0.7 mL/min. The mobile phases were A: methanol and B: 1% formic acid. The gradient elution was: 0–5 min: 2% A; 5–15 min: 2–15% A; 15–30 min: 15–25% A; 30–35 min: 25–35% A; 35–45 min: 35–55% A; 40–50 min: 55% A; 50–55 min: 55–2% A; 55–60 min: 2% A; Post time: 5 min. The DAD detector: 255, 270, 280, 310, 322, 355, 370 nm. Eleven

available HPLC grade phenolic markers were considered (gallic acid, 4-hydroxybenzoic acid, chlorogenic acid, caffeic acid, vinylic acid, syringic acid, p-coumaric acid, ferulic acid, synaptic acid, ellagic acid, t-cinnamic acid, quercetin, and rutin).

2.6. Anti-Obesity Evaluation of Phytosomes

2.6.1. In Vivo Study

Animals

Two-month-old male Wistar rats (180–200 g) were obtained from the laboratory animals of the Chemical-Biological Research Institute of UMSNH. All the animals were housed in plastic cages in the following conditions: 12 h light–dark cycle, relative humidity of 60–70%, and a temperature of (23–24 °C). They had ad libitum access to food and water. The animals were kept in the bioterium of the Chemical-Biological Research Institute of UMSNH. All protocols were approved and conducted in accordance with the guide for the care and use of laboratory animals by the Mexican Official Standard (NOM-062-ZOO-1999) and the Ethics Committee of the Universidad Michoacán de San Nicolás de Hidalgo.

2.6.2. Obesity Induction

A high-fat diet (HFD) containing 45.4% normal chow (Rodent diet brand Purina rat chow), 14.8% lard, 14.8% vegetable fat, and 25% fructose was daily prepared as reported in [16]. Fifty-four male Wistar rats were randomly divided into 9 (n = 6) groups to be fed. Group 1 (chow diet), Group 2 (chow diet plus vehicle), Group 3 (chow diet plus *C. citrinus* extract 200 mg/kg), Group 4 (HFD), Group 5 (HFD plus *C. citrinus* extract 200 mg/kg), Group 6 (HFD plus phytosomes loaded with *C. citrinus* (P *C.c*) 50 mg/kg), Group 7 (HFD plus P *C.c* 100 mg/kg), Group 8 (HFD plus P *C.c* 200 mg/kg) and Group 9 (HFD plus orlistat 5 mg/kg). Treatments were administered by oral gavage once daily at 9.00 a.m. in the home cage for 15 weeks. The animal's age at the end of the treatment was 23 weeks. All blood samples were collected after 12–13 h of fasting by cardiac puncture. After blood collection, the animals were anesthetized with pentobarbital sodium injection (150 mg/kg), and all tissues were taken, washed, and stored at −80 °C for subsequent analysis.

2.6.3. Measurement of Morphometric and Biochemical Parameters

Rats were weighed weekly. The percentage of weight gain, adiposity index, and Lee index were calculated as reported by Ortega-Pérez et al. [16]. Plasma glucose, triacylglycerol, and cholesterol were measured using enzymatic colorimetric kits SPINREACT® following the manufacturer's protocols.

2.7. Statistical Analysis

One-way ANOVA is a parametric method that can be used to determine if two or more groups of data are statistically different. Parametric tests make assumptions about the population distribution of the sample and in nonparametric tests the distribution of a population is unknown. This study selected a parametric test because it is more likely to detect significant differences with these methodologies than the use of nonparametric methods. The test results were expressed as mean ± standard error (SEM) or standard deviation (SD). Data were analyzed using GraphPad Prism (version 8.0) by one-way analysis of variance (ANOVA). To determine statistical differences (a, b, c) of nano-phytosomes, and morphometric and biochemical parameters between groups, Tukey's multiple comparison test was conducted. * $p \leq 0.05$ is a statistically significant result. Tukey's honestly significant difference (HSD) test, is a post hoc test used in ANOVA to compare all possible pairs of means. When conducting ANOVA and finding a significant difference among group means, a post hoc test like Tukey's is needed to determine whether the specific group means significantly differed from each other.

3. Results and Discussion

3.1. Morphology and Particle-Size Analysis

Phytosomes are a strategy used to improve the solubility and bioavailability of herbal extracts [44]. Particle size and phospholipid composition are important factors to obtain these parameters. This study used soybean phosphatidylcholine because this lipid is a main component of membranes and also provides choline, a substrate of choline acetyltransferase to produce the acetylcholine neurotransmitter. Xie et al. [45] reported that using soybean phosphatidylcholine to prepare curcumin-loaded phytosome presented small particle size, high-surface charge, stability, and drug-loading capacity. Figure 1 shows small spherical shapes of phytosomes loaded with *Callistemon citrinus* under an optical microscope at 40×.

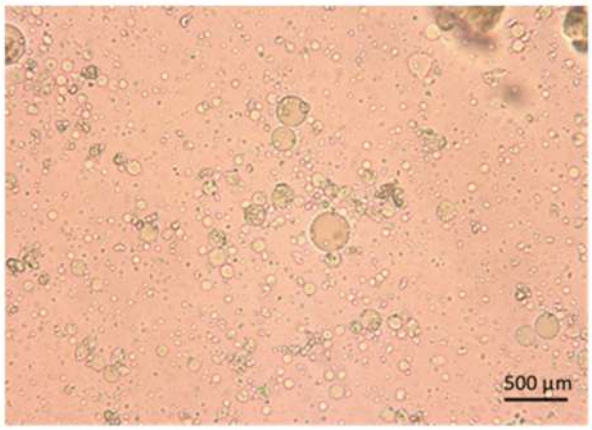

Figure 1. Optical microscope images at a 40× scale of *Callistemon citrinus* phytosomes.

Scanning electron microscope (SEM) was used to evaluate the size and surface morphology. Figure 2 shows the SEM image confirming that phytosomes have a highly spherical structure. The average particle size of the *Callistemon citrinus* phytosome was 129.98 nm ± 18.30 nm in the emulsion.

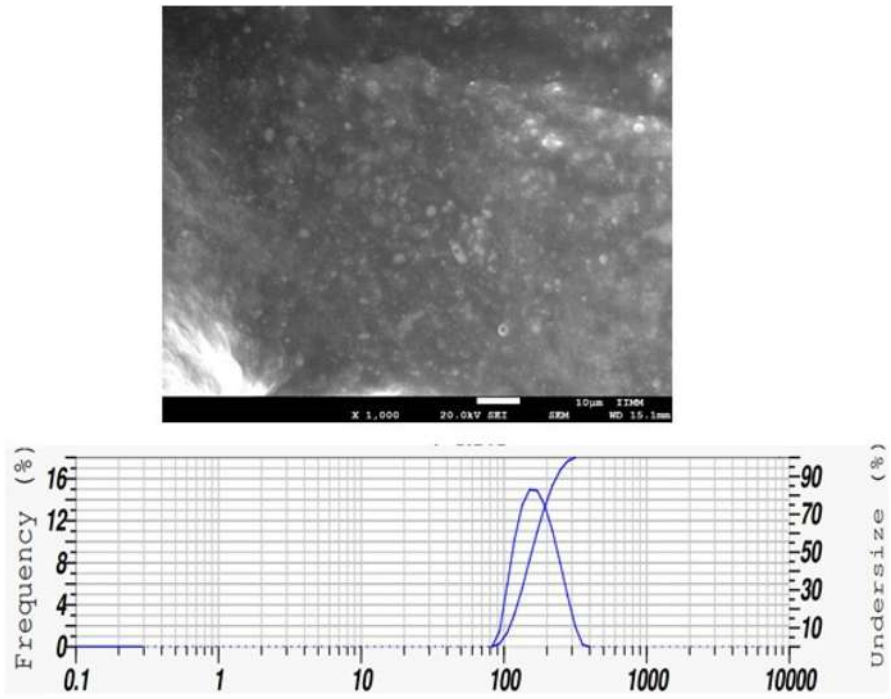

Figure 2. Scanning electron microscope images at scales of 1000×. Particle size was obtained through Nano Particle Analyzer SZ-100 of phytosomes loaded with *Callistemon citrinus* (200 mg/kg).

3.2. Study of Vesicular Entrapment/Encapsulation

The percentage drug entrapment was determined by extracting phytosomes with centrifugation and the supernatant was measured by UV-visible spectroscopy. Table 2 shows the drug entrapment. The results showed that the entrapment efficiency (EE) was about 80.49%. In this way, the encapsulation efficiency of phytosomes is represented by the concentration of unbound *C. citrinus* leaf extract (200 mg/kg); this indicates that the leaf extract and soybean phospholipids react to form the complex with a high degree of entrapment of the leaf extract.

Table 2. Entrapment efficiency of the *Callistemon citrinus* phytosomes.

Parameter	Abs
Tdrug	0.189 ± 0.01
Udrug	0.045 ± 0.07
EE	80.49 ± 0.07%

(Tdrug) is the total amount of drug, (EE) is the efficiency of entrapment, and (Udrug) is the extract not entrapped in phytosomal formulation. The data are expressed with the mean (n = 4) and standard deviation (±SD).

3.3. Study of Stability and Solubility

Table 3 shows the stability of the *C. citrinus* phytosome. During the storage period at 20 ± 2 °C, no significant changes in average particle size were observed for the phytosomes. However, low temperatures caused an increase in the particle size up to two folds. Our results indicate that a phytosome loaded with *C. citrinus* remained stable for three and a half months. This result is similar to a phytosome loaded with *Cuscuta reflexa* [46].

Table 3. Effect of the temperature on the stability of *Callistemon citrinus* phytosomes at 1, 3, 5, and 10 days and 3.5 months.

	Temperature	
Days	20 ± 2 °C	4 ± 1 °C
1	193.62 ± 27.33 ^{a}	285.07 ± 14.04 ^{ab}
3	218.06 ± 59.55 ^{a}	412.80 ± 248.22 ^{abc}
5	256.50 ± 29.00 ^{a}	454.23 ± 175.28 ^{abc}
10	279.64 ± 61.21 ^{a}	570.70 ± 132.73 ^{bc}
106	283.82 ± 51.87 ^{ab}	623.23 ± 142.18 ^{c}

Values are the particle size (nm) expressed as mean ± SD (ANOVA followed by Tukey, statistically different values (^{a}, ^{b}, ^{c}) between groups ($p \leq 0.05$, n = 6)).

The low lipid solubility of some compounds may be the reason for their weak absorption [47]. Thus, the solubility is an important parameter to study. Table 4 shows that *Callistemon citrinus* phytosomes were completely soluble in four solvents and partially soluble in one of them. Assuming 20% for the former and 10% for the latter, *Callistemon citrinus* phytosomes had a 90% of solubility. It follows that *C. citrinus* extract, without and with tween 80, shows 80% of solubility and finally soybean liposomes. The formation of phytosomes with plant extract is based on hydrogen-bonding interaction, which increases the bioavailability and stability of the compounds [48]. Consequently, phytosomes have better lipophilicity and hydrophilicity than bioactive compounds.

Table 4. Solubility profile of *Callistemon citrinus* leaf extract, *Callistemon citrinus* phytosomes, and soybean phospholipids.

Solvent	*C. citrinus* Extract (200 mg/kg)	*C. citrinus* Extract (200 mg/kg) + Tween 80	*C. citrinus* Phytosomes (200 mg/kg)	Soybean Liposomes + Tween 80	Soybean Liposomes-Tween 80
Distilled water	Partially	Partially	Soluble	Soluble	Micellar shape
Methanol	Soluble	Soluble	Partially	Unsolvable	Soluble
Dichloromethane	Soluble	Partially	Soluble	Soluble	Soluble
Chloroform	Soluble	Soluble	Soluble	Soluble	Partially
Hexane	Partially	Soluble	Soluble	Soluble	Soluble

5 mL of each solvent was added. The solutions were placed under continuous stirring for 1 h; $n = 4$.

3.4. In Vitro Antioxidant Activity of Callistemon citrinus Phytosomes

DPPH, ABTS, and FRAP are methodologies commonly used to evaluate the antioxidant capacity of plant extracts. DPPH and ABTS are based on the hydrogen or electron-donating capacity and FRAP on the capacity of reducing ferric to ferrous [49]. Ortega-Perez et al. [16] reported the strong antioxidant capacity and the total phenol, flavonoid, and terpene compounds of *Callistemon citrinus* leaf extract. Figure 3 shows that both *C. citrinus* extract and *C. citrinus* phytosomes exhibited significant inhibitory activity against the DPPH and ABTS radicals and a high ability to reduce ferric to ferrous. Many reports have demonstrated the correlation between total phenolic and flavonoid content and their antioxidant activities [50]. This study also found this correlation, suggesting that the compounds produced the antioxidant effect in *C. citrinus*, acting as hydrogen donators, singlet oxygen quenchers, and reducing agents [51].

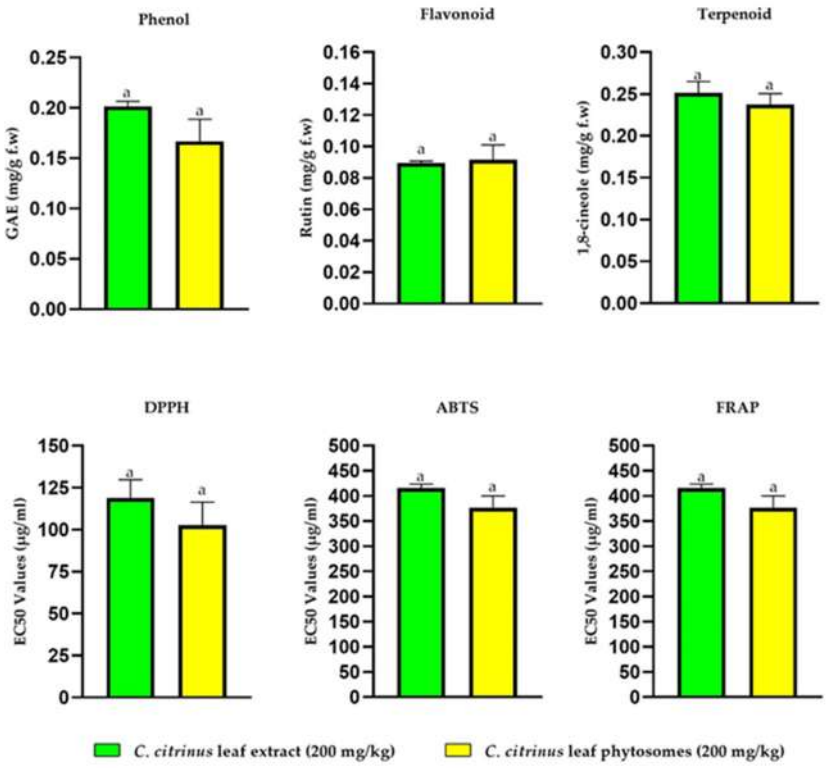

Figure 3. Determination of antioxidant capacity and total phenol, flavonoid, and terpenoid contents of *Callistemon citrinus* leaf extract and phytosomes. Data are expressed with the mean ± standard error (ANOVA followed by Tukey, $n = 6$). The same letter (a) meaning that there is no statistical differences.

Figure 3 shows that there are no significant differences between the bioactive compounds and the antioxidant capacity in the *Callistemon citrinus* extract and the phytosomes

of *C. citrinus*. This result shows that during the process of creating phytosomes, the bioactive compounds and antioxidant capacity were retained. However, the encapsulation did not significantly improve the activity. This result agrees with Saonere et al. [52], which found antioxidant capacity in a phytophospholipid complex of *Glycerrhiza glabra*.

Phytosomes containing extracts of mulberry and ginger used against the metabolic syndrome improved the antioxidant system and decreased inflammatory cytokines such as IL-6 and TNF-α [53]. The use of phytosome curcumin against paracetamol-induced liver toxicity in mice showed an increase in enzymatic antioxidant activities and the reduction in lipoperoxidation products [54]. Deleanu et al. [55] reported that phytosomes with the extract of ginger rhizomes and rosehips increase the bioavailability, antioxidant, and anti-inflammatory properties in LPS-induced systemic inflammation in mice. These results suggest that the use of phytosomes can improve enzymatic antioxidant properties and reduce inflammation during oxidative stress [33].

3.5. Gas Chromatography and Mass Spectrometry Analysis

Figure 4 shows the chromatogram of *C. citrinus* extract and *C. citrinus* phytosome analyzed by GC/MS to evaluate the terpenes profile. Terpenes were quantified according to GC/MS. Table 5 shows that 1,8-cineole and α-terpineol were the main compounds of the extract and phytosome. These two monoterpenes have been reported to have hepatoprotective, antiviral, antimicrobial, antioxidant, and anticarcinogenic effects [32,56], suggesting that *C. citrinus* may constitute an alternative pharmacological tool to treat oxidative stress in some diseases.

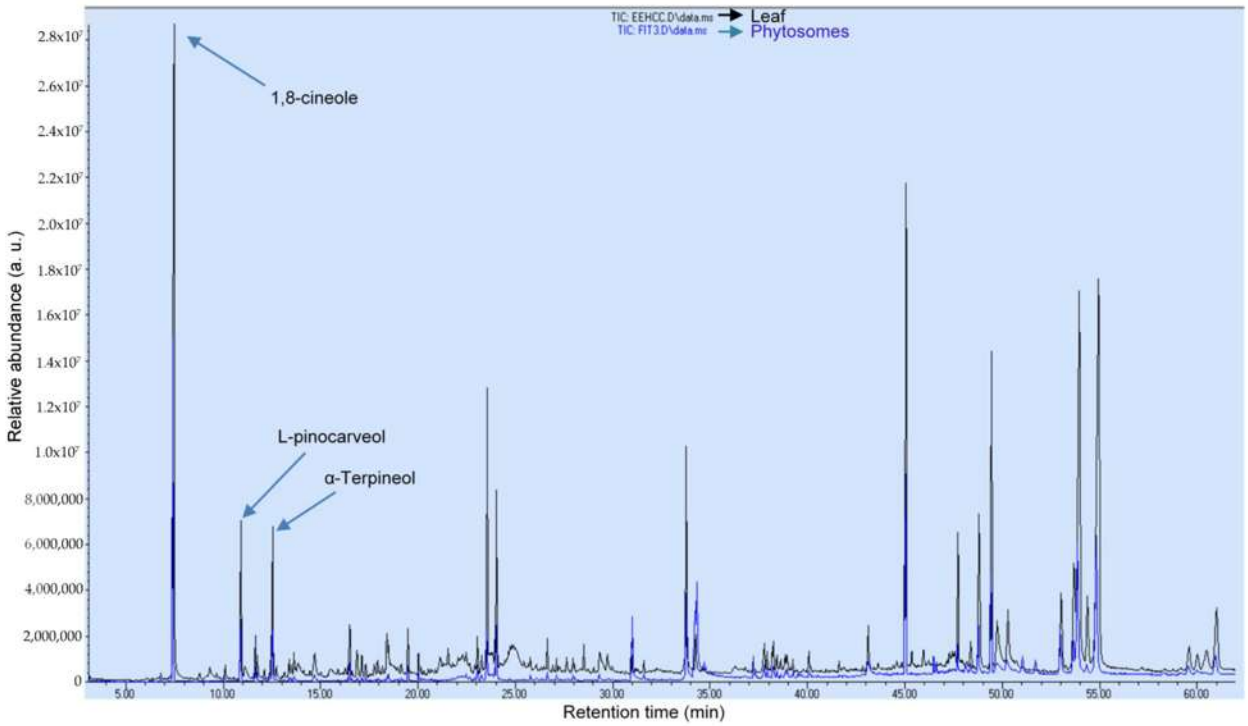

Figure 4. Comparison of terpenoid abundance (arbitrary units) in GC/MS total ion chromatogram of *Callistemon citrinus* leaf (black) and *C. citrinus* phytosomes (blue).

Table 5 shows the calculated retention indices and a comparison with retention indices found on the NIST home page (www.nistwebbook.com (accessed on 17 March 2023)). A total of 80% of the compounds were identified and only two of them could not be fully identified despite having a Match Factor above 800 (Good Match). According to the NIST library, Match Factor scores > 900 mean an Excellent Match, whereas Match Factors scores in the range of 800–900 are a Good Match. All identified compounds have Factor scores above 800.

Table 5. Terpene contents in *Callistemon citrinus* leaf extract and *C. citrinus* phytosomes (GC/MS).

RT	RI_{lit}	RI_{calc}	Ref. RI_{lit}	Match Factor	Prob (%)	Compounds	Extract	Phytosomes
7.47	1041	1059	Silva et al. [57]	972	93.8%	1,8-Cineole	0.613 ± 0.05	0.224 ± 0.04
10.91	1143	1131	Radulovic et al. [58]	950	67.2%	L-Pinocarveol	0.097 ± 0.007	0.030 ± 0.005
11.65	1140	1114	Muselli et al. [59]	883	68.6%	Pinocarvone	0.016 ± 0.003	nd
11.76	1170	1166	Al-Omar [60]	923	63.9%	Borneol	0.0081 ± 0.001	nd
12.54	1172	1143	Boti et al. [61]	952	74.5%	α-Terpineol	0.0894 ± 0.04	0.0233 ± 0.003
23.04	1567	1530	Babushok et al. [62]	929	55.5%	Globulol	0.011 ± 0.002	0.0012 ± 0.001
33.78	2099	2045	Babushok et al. [62]	894	81.2%	Phytol	0.1714 ± 0.03	0.0637 ± 0.01
45.04	2847	2914	Zhao et al. [63]	963	50.4%	Squalene	0.1041 ± 0.01	0.0044 ± 0.001
53.66	-	2886	-	886	59.9%	Unknown 1	0.0957 ± 0.02	0.0364 ± 0.006
54.94	-	2848	-	941	86.5%	Unknow 2	0.8505 ± 0.05	0.2187 ± 0.03

RT retention time (min). RI_{lit} retention index (iu) reported in the literature for 5% phenyl polysilphenylene-siloxane GC column. RI_{calc} retention index obtained through the modulated chromatogram. Ref. RI_{lit} retention index bibliography found in the literature for 5% phenyl polysilphenylene-siloxane GC column. Extract (mg/mL), phytosomes (mg/mL). nd = Not detected. Non-polar retention index (n-alkane scale). The values are the mean ± SD (n = 3). Fragment ions (m/z) of unknown 1: 218 (100), 203, 219, 69, 95, 426 [M+], 411.4. Unknown 2: 189 (100, 95,207,93,135, 426 [M+], 411.4.

3.6. High-Performance Liquid Chromatography Analysis

The phenolic and flavonoid compounds were identified according to their retention time in HPLC. Ellagic acid was found for the first time in *C. citrinus*. Figures 5 and 6 show that gallic acid, *p*-coumaric acid, and ellagic acid are the compounds identified in the *Callistemon citrinus* extract and phytosome. These phenolic acids have been reported to have anticancer, antiviral, antioxidant, and anti-inflammatory activities [64]. Table 6 shows that the concentration of gallic acid, p-coumaric acid, and ellagic acid was very similar in the *C. citrinus* extract and in the phytosome.

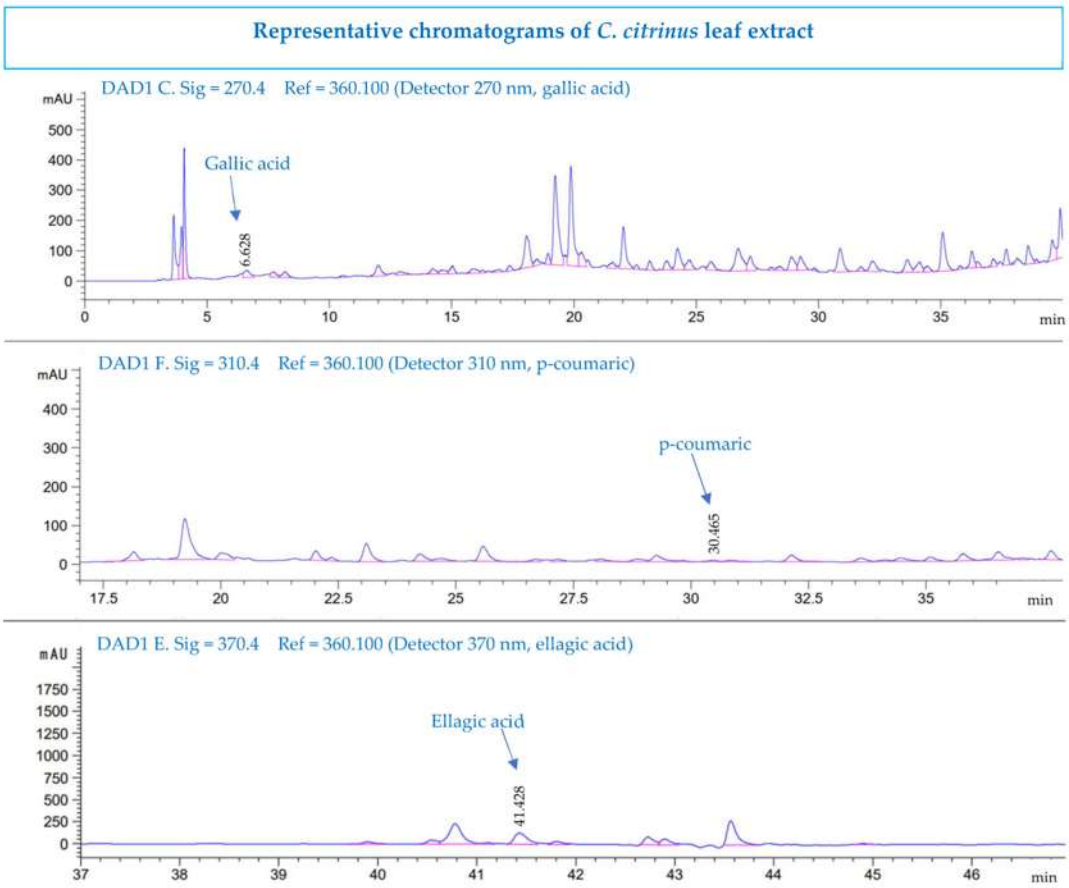

Figure 5. HPLC chromatograms of the *Callistemon citrinus* leaf extract.

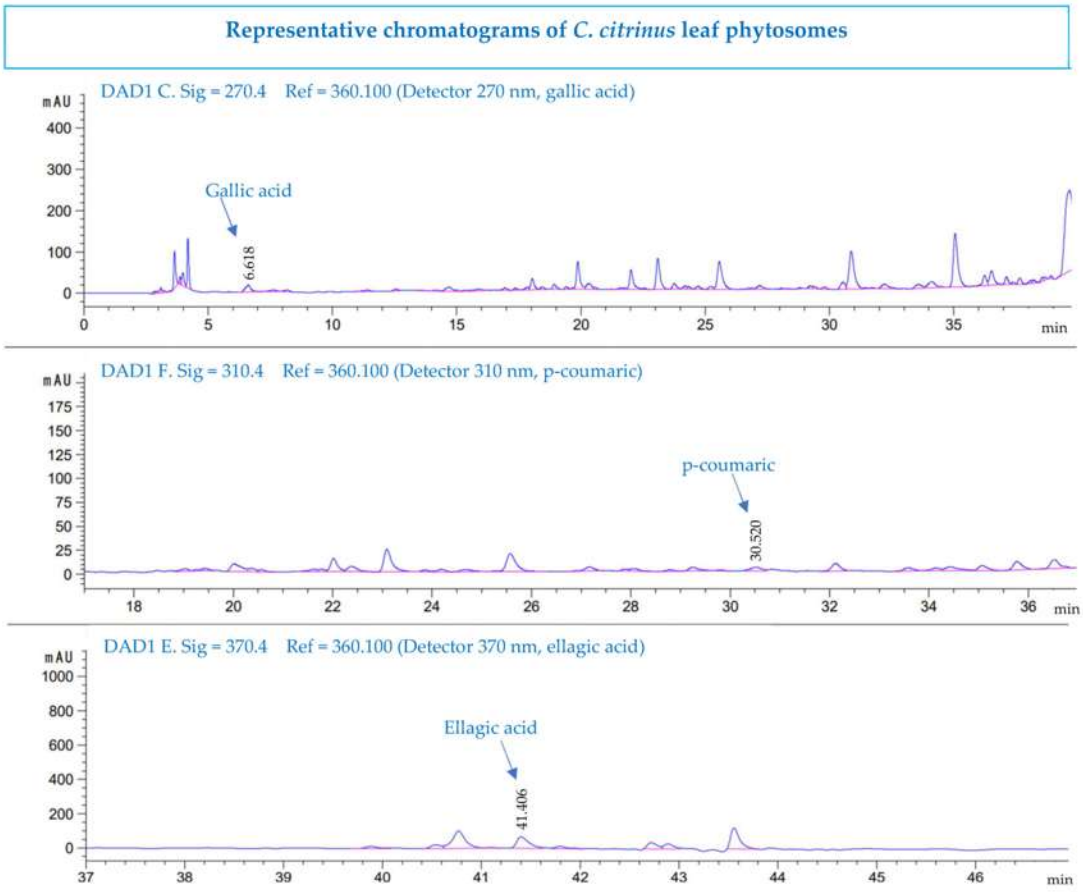

Figure 6. HPLC chromatograms of *Callistemon citrinus* leaf phytosome.

Table 6. HPLC analysis profile in *Callistemon citrinus* leaf extract and phytosomes.

Compounds	Extract (µg/mL)	Phytosomes (µg/mL)
gallic acid	6.94 ± 0.06	5.93 ± 0.0
4-hydroxybenzoic acid	nd	nd
chlorogenic acid	nd	nd
caffeic acid	nd	nd
Vanillic acid	nd	nd
Syringic acid	nd	nd
p-coumaric acid	0.47 ± 0.05	0.65 ± 0.07
ferulic acid	nd	nd
synaptic acid	nd	nd
ellagic acid	74.3 ± 1.3	67.3 ± 1.4
t-cinnamic acid	nd	nd
quercetin	nd	nd
rutin	nd	nd

nd = Not detected. Data expressed as mean ± SD (n = 3).

Until now, silver, gold, and poly (lactic-co-glycolic acid) nanoparticles loaded with *Callistemon citrinus* [22–24] have been reported. Nanoparticles have some characteristics that could affect their toxicity as nature, size, mobility, stability, surface aggregation, and storing time [65]. Metal oxide nanoparticles reduced the enzymatic activity of microorganisms [66].

However, the use of phytosomes as a delivery method of natural products has advantages. Phytosomes have an amphiphilic characteristic that allows the extract compounds to interact with the hydrophilic and hydrophobic parts, increasing the therapeutic effect.

3.7. Effect of Phytosomes on Morphometric and Biochemical Parameters

The rats fed with HFD showed increased body weight compared to the other groups. Conversely, the administration of phytosomes loaded with *C. citrinus* to animals fed with HFD showed significantly reduced body weight as compared to obese rats (Figure 7). These results agree with Ortega et al. [16]. A previous study showed that *C. citrinus* extract inhibited lipase activity in a dose-dependent manner [16]. Regarding the phytosomal dosage (50, 100, and 200 mg/kg) and *C. citrinus* extract (250 mg/kg), this study showed similar effects in all of them to reduce weight. This study suggests that phytosomes loaded with *C. citrinus* extract have stronger anti-obesogenic activity than *C. citrinus* extract itself; this result is probably due to the high bioavailability, which improves the solubility, allowing to reduce the dose. The Lee and adiposity indices in the HFD group were higher than those in other groups. Also, glucose and triacylglycerol levels increased in the HFD group, contrary to the rest of the groups (Table 7).

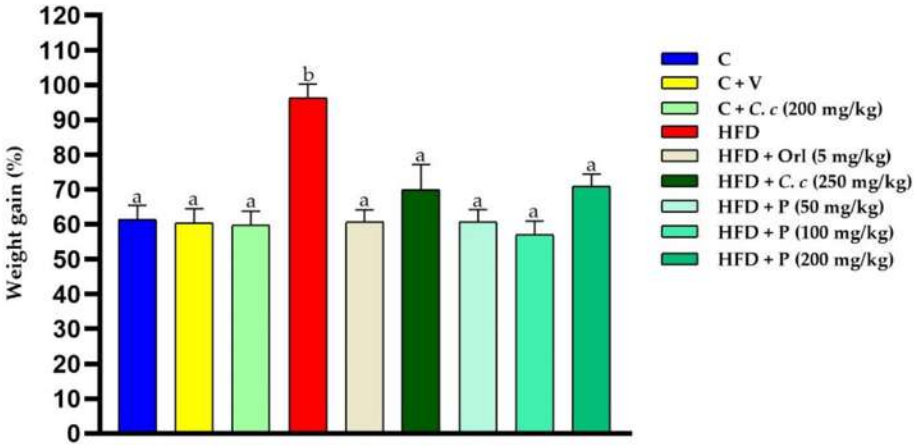

Figure 7. Final body weight percentage in control rats and the different experimental treatments during the 15 weeks. Values are presented as mean ± standard error (ANOVA followed by Tukey, $n = 6$). Statistically different values (a, b) between groups.

Table 7. Effect of *C. citrinus* on morphometric parameters, obesity markers, and biochemical determinations in rats.

Measurements	Control	Control + Vehicle	Control + *C. citrinus* Extract (200 mg/kg)	Hypercaloric-fat Diet (HFD)	HFD + Orlistat (5 mg/kg)	HFD + *C. citrinus* Extract (250 mg/kg)	HFD + Phytosomes (50 mg/kg)	HFD + Phytosomes (100 mg/kg)	HFD + Phytosomes (200 mg/kg)
Morphometric parameters									
Abdominal circumference (cm)	20.50 ± 0.45 ^{a}	20.50 ± 45 ^{a}	21.00 ± 0.45 ^{a}	25.50 ± 0.45 ^{b}	22.25 ± 0.45 ^{a}	20.33 ± 1.36 ^{a}	21.0 ± 0.52 ^{a}	21.20 ± 20 ^{a}	21.50 ± 0.45 ^{a}
Nose-to-anus length (cm)	25.25 ± 0.60 ^{a}	24.37 ± 0.60 ^{a}	24.60 ± 0.54 ^{a}	24.41 ± 0.91 ^{a}	23.66 ± 0.91 ^{a}	24.41 ± 0.91 ^{a}	23.80 ± 0.54 ^{a}	24.12 ± 0.60 ^{a}	23.50 ± 0.60 ^{a}
Nose-to-tail length (cm)	46.40 ± 0.42 ^{a}	46.40 ± 0.42 ^{a}	46.87 ± 0.47 ^{a}	45.66 ± 0.38 ^{a}	45.71 ± 0.47 ^{a}	44.66 ± 1.63 ^{a}	45.87 ± 0.47 ^{a}	45.75 ± 0.47 ^{a}	45.12 ± 0.47 ^{a}
Markers of obesity									
BMI (kg/m^2)	0.67 ± 0.03 ^{b}	0.72 ± 0.03 ^{ab}	0.70 ± 0.03 ^{ab}	0.88 ± 0.04 ^{a}	0.72 ± 0.04 ^{ab}	0.69 ± 0.09 ^{b}	0.66 ± 0.04 ^{b}	0.68 ± 0.04 ^{b}	0.76 ± 0.04 ^{ab}
Adiposity index	2.78 ± 0.55 ^{c}	2.77 ± 0.55 ^{c}	2.52 ± 0.62 ^{c}	9.43 ± 0.62 ^{a}	5.56 ± 0.71 ^{bc}	6.18 ± 0.39 ^{b}	5.98 ± 0.71 ^{b}	4.82 ± 0.71 ^{bc}	4.02 ± 0.62 ^{bc}
Lee index	0.30 ± 0.01	0.30 ± 0.02	0.30 ± 0.01	0.33 ± 0.01 ^{a}	0.30 ± 0.01	0.30 ± 0.01	0.29 ± 0.01	0.30 ± 0.01	0.31 ± 0.01

Table 7. *Cont.*

Measurements	Control	Control + Vehicle	Control + *C. citrinus* Extract (200 mg/kg)	Hypercaloric-fat Diet (HFD)	HFD + Orlistat (5 mg/kg)	HFD + *C. citrinus* Extract (250 mg/kg)	HFD + Phytosomes (50 mg/kg)	HFD + Phytosomes (100 mg/kg)	HFD + Phytosomes (200 mg/kg)
Biochemical parameters									
Triacylglycerol (mg/dL)	90.66 ± 11.64 ^{c}	103.66 ± 11.64 ^{c}	109.66 ± 11.64 ^{c}	202.66 ± 11.64 ^{a}	90.66 ± 11.64 ^{c}	136.33 ± 66.96 ^{b}	103.50 ± 11.64 ^{c}	105.33 ± 11.64 ^{c}	118.66 ± 11.64 ^{b}
Blood glucose (mg/dL)	93.99 ± 8.24 ^{a}	101.37 ± 8.24 ^{a}	95.59 ± 8.24 ^{a}	111.11 ± 8.24 ^{b}	100.24 ± 8.24 ^{a}	97.00 ± 4.24 ^{a}	104.18 ± 8.24 ^{a}	92.24 ± 8.24 ^{a}	96.65 ± 8.24 ^{a}
Total cholesterol (mg/dL)	161.33 ± 2.69 ^{a}	160.33 ± 2.69 ^{a}	162.00 ± 2.69 ^{a}	159.66 ± 2.69 ^{a}	156.66 ± 2.69 ^{a}	162.00 ± 2.69 ^{a}	155.66 ± 2.69 ^{a}	161.00 ± 2.69 ^{a}	159.30 ± 2.69 ^{a}

Values expressed as mean ± SEM (n = 6, ANOVA followed by Tukey test, statistically different values (^{a}, ^{b}, ^{c}) between groups; $p \leq 0.05$).

Callistemon citrinus phytosomal formulation improved oral bioavailability. Even the administration of low doses reduced the morphometrical and biochemical parameters in the treated animals.

4. Conclusions

The *Callistemon citrinus* phytosomal formulation improved oral bioavailability, retained the major compounds, and was stable for three and a half months when stored at 20 °C. Phytosomes of *C. citrinus*, even in low doses, reduced morphometrical and biochemical parameters in Wistar rats fed with a high-fat diet. The results also revealed that the supplementation of phytosomes of *Callistemon citrinus* reduced excessive weight in the animals.

Author Contributions: Conceptualization, formal analysis, investigation, and writing—original draft, L.G.O.-P.; performed experiments L.A.A.-R., O.R.M.-R. and J.S.P.-S.; collected and analyzed all the data, A.A.-M.; Funding acquisition and review, D.G.-H.; analyzed all the data, supervision, writing—review and editing, P.R.-C. All authors have read and agreed to the published version of the manuscript.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Institutional Review Board Statement: The animal study protocol was approval by the Institutional Bioethics and Biosecurity Committee in the Instituto de Investigaciones Químico Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, approval date: 1 September 2021; Protocol ID IIQB-CIBE-06-2021.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data availability under request.

Acknowledgments: The authors thank the Coordinación de la Investigación Científica de la Universidad Michoacana de San Nicolas de Hidalgo in Mexico for the partial support of the study. We also thank Eduardo Segundo-Jara and Mathieu Tétreault for the careful review of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Sandhiya, V.; Ubaidulla, U. A review on herbal drug loaded into pharmaceutical carrier techniques and its evaluation process. *Future J. Pharm. Sci.* **2020**, *6*, 51. [CrossRef]
2. Permana, A.D.; Utami, R.N.; Courtenay, A.J.; Manggau, M.A.; Donnelly, R.F.; Rahman, L. Phytosomal nanocarriers as platforms for improved delivery of natural antioxidant and photoprotective compounds in propolis: An approach for enhanced both dissolution behaviour in biorelevant media and skin retention profiles. *J. Photochem. Photobiol. B Biol.* **2020**, *205*, 111846. [CrossRef] [PubMed]
3. Simioni, C.; Zauli, G.; Martelli, A.M.; Vitale, M.; Sacchetti, G.; Gonelli, A.; Neri, L.M. Oxidative stress: Role of physical exercise and antioxidant neutraceutical in adulthood and aging. *Oncotarget* **2018**, *9*, 17181–17198. [CrossRef] [PubMed]

4. Martemucci, G.; Costagliola, C.; Mariano, M.; D'andrea, L.; Napolitano, P.; D'Alessandro, A.G. Free radical properties, source and targets, antioxidant consumption and health. *Oxygen* **2022**, *2*, 48–78. [CrossRef]
5. Warraich, U.M.; Hussain, F.; Kayani, H.U.R. Aging—Oxidative stress, antioxidant and computational modeling. *Heliyon* **2020**, *6*, e04107. [CrossRef]
6. Ali, S.S.; Ahsan, H.; Zia, M.K.; Siddiqui, T.; Khan, F.H. Understanding oxidant and antioxidants: Classical team with new players. *J. Food Biochem.* **2020**, *44*, e13145. [CrossRef]
7. Lee, M.Y.; Lin, W.C.; Yu, B.; Lee, T.T. Antioxidant capacity of phytochemicals and their potential effects on oxidative status in animals- A review. Asian-Australas. *J. Anim. Sci.* **2017**, *30*, 299–308.
8. Rein, M.J.; Renouf, M.; Cruz-Hernandez, C.; Actis-Goretta, L.; Thakkar, S.K.; Pinto, M.S. Bioavailability if bioactive food compounds: A challenging journey to bioefficacy. *Br. J. Clin. Pharmacol.* **2012**, *75*, 588–602. [CrossRef]
9. Rahman, H.S.; Othman, H.H.; Hammadi, N.I.; Yeap, S.K.; Amin, K.M.; Samad, N.A.; Alitheen, N.B. Novel drug delivery systems for loading of natural plant extracts and their biomedical applications. *Int. J. Nanomed.* **2020**, *15*, 2439–2483. [CrossRef]
10. Alqahtani, M.S.; Kazi, M.; Alsenaidy, M.A.; Ahmad, M.Z. Advances in oral drug delivery. *Front. Pharmacol.* **2021**, *12*, 618411. [CrossRef]
11. Abuhelwa, A.Y.; Williams, D.B.; Upton, R.N.; Foster, D.J.R. Food, gastrointestinal pH, and models of oral drug absorption. *Eur. J. Pharma. Biopharm.* **2017**, *112*, 234–248. [CrossRef] [PubMed]
12. Quin, W.; Ketnawa, S.; Ogawa, Y. Effect of digestive enzyme and pH on variation of bioavailability of green tea during simulated in vitro gastrointestinal digestion. *Food Sci. Hum. Wellness* **2022**, *11*, 669–675. [CrossRef]
13. Stevanovic, Z.D.; Sieniawska, E.; Glowniak, K.; Obradovoc, N.; Pajic-Lijakovic, I. Natural macromolecules as carriers for essential oils: From extraction to biomedical application. *Front. Bioeng. Biotechnol.* **2020**, *8*, 563. [CrossRef] [PubMed]
14. Sowndhararajan, K.; Deepa, P.; Kim, S. A review of the chemical composition and biological activities of *Callistemon lanceolatus* (Sm.) Sweet. *J. Appl. Pharm. Sci.* **2021**, *11*, 65–73. [CrossRef]
15. López-Mejía, A.; Ortega-Pérez, L.G.; Godínez-Hernández, D.; Nateras-Marin, B.; Meléndez-Herrera, E.; Rios-Chavez, P. Chemo-preventive effect of *Callistemon citrinus* (Curtis) Skeels against colon cancer induced by 1, 2-dimethylhydrazine in rats. *J. Cancer Res. Clin. Oncol.* **2019**, *145*, 1417–1426. [CrossRef]
16. Ortega-Pérez, L.G.; Piñón-Simental, J.S.; Magaña-Rodríguez, O.R.; López-Mejía, A.; Ayala-Ruiz, L.A.; García-Calderón, A.J.; Godínez-Hernández, D.; Rios-Chavez, P. Evaluation of the toxicology, anti-lipase, and antioxidant effects of *Callistemon citrinus* in rats fed with a high fat-fructose diet. *Pharm. Biol.* **2022**, *60*, 1384–1393. [CrossRef]
17. Petronilho, S.; Rocha, S.M.; Ramírez-Chávez, E.; Molina-Torres, J.; Rios-Chavez, P. Assessment of the terpenic profile of *Callistemon citrinus* (Curtis) Skeels from Mexico. *Ind. Crops Prod.* **2013**, *46*, 369–379. [CrossRef]
18. Ayala-Ruiz, L.A.; Ortega-Pérez, L.G.; Piñón-Simental, J.S.; Magaña-Rodríguez, O.R.; Meléndez-Herrera, E.; Rios-Chavez, P. Role of the major terpenes of *Callistemon citrinus* against the oxidative stress during a hypercaloric diet in rats. *Biomed. Pharmacother.* **2022**, *153*, 113505. [CrossRef]
19. Khanh, P.N.; Duc, H.V.; Huong, T.T.; Ha, V.T.; Van, D.T.; Son, N.T.; Kim, Y.H.; Viet, D.Q.; Cuong, N.M. Phenolic compounds from *Callistemon citrinus* leaves and stem. *J. Sci. Technol.* **2016**, *54*, 190–197. [CrossRef]
20. Ravichandran, S.; Paluri, V.; Kumar, G.; Loganathan, K.; Venkata, R.K. A novel approach for the biosynthesis of silver oxide nanoparticles using aqueous leaf extract of *Callistemon lanceolatus* (Myrtaceae) and their therapeutic potential. *J. Exp. Nanosci.* **2016**, *11*, 445–458. [CrossRef]
21. Paosen, S.; Saising, J.; Wira Septama, A.; Piyawan Voravuthikunchai, S. Green synthesis of silver nanoparticles using plants from Myrtaceae family and characterization of their antibacterial activity. *Mater. Lett.* **2017**, *209*, 201–209. [CrossRef]
22. Rotimi, L.; Ojemaye, M.O.; Okoh, O.O.; Okoh, A.I. Silver nanoparticles mediated by *Callistemon citrinus* extracts and their antimalaria, antitrypanosomal and antibacterial efficacy. *J. Mol. Liq.* **2019**, *273*, 615–625.
23. Rotimi, L.; Ojemaye, M.O.; Okoh, O.O.; Sadimennko, A.; Okoh, A.I. Synthesis, characterization, antimalarial, antitrypanocidal and antimicrobialproperties of gold nanoparticle. *Green Chem. Lett. Rev.* **2019**, *12*, 61–68. [CrossRef]
24. Ahmed, R.; Tariq, M.; Ahmad, I.S.; Fouly, H.; Abbas, F.I.; Hasan, A.; Kushad, M. Poly (lactic-co-glycolic acid) nanoparticles loaded with *Callistemon citrinus* phenolics exhibited anticancer properties against three breast cancer cell lines. *J. Food Qual.* **2019**, *2019*, 2638481. [CrossRef]
25. Gharibvand, Z.S.; Behbahani, B.A.; Noshad, M.; Jooyandeh, H. Green synthesis of silver nanoparticles using *Callistemon citrinus* leaf extract and evaluation of its antibacterial activity. *Iran. Food Sci. Technol. Res. J.* **2022**, *18*, 151–163.
26. Alharbi, W.S.; Almughem, F.A.; Almehmady, A.M.; Jarallah, S.S.; Alsharif, W.K.; Alzahrani, N.M.; Alshehri, A.A. Phytosomes as an emerging nanotechnology platform for the topical delivery of bioactive phytochemicals. *Pharmaceutics* **2021**, *13*, 1475. [CrossRef] [PubMed]
27. Adiki, S.K.; Sangeetha, S.; Kamireddy, S.; Katakam, P.; Obilineni, I. Phytosomes: A novel phytoconstituent delivery approach to improve the efficacy of obesity treatment. *Curr. Nutr. Food Sci.* **2023**, *19*, 229–237. [CrossRef]
28. Chakkhtoura, M.; Haber, R.; Ghezzawi, M.; Rhayem, C.; Tcheroyan, R.; Mantzoros, C.S. Pharmacotherapy of obesity: An update on the available medications and drugs under investigation. *Lancet* **2023**, *58*, 1–29. [CrossRef]
29. Tham, K.W.; Lim, A.Y.L.; Baur, L.A. The global agenda on obesity: What does this mean for Singapore. *Singapore Med. J.* **2023**, *64*, 182–187. [CrossRef]

30. Lee, P.C.; Lim, C.H.; Asokkumar, R.; Chua, M.W.J. Current treatment landscape for obesity in Singapore. *Singapore Med. J.* **2023**, *64*, 172–181. [CrossRef]
31. Gehan, F.; Raoof, A. Anti-obesity potential of natural products. *Egypt. J. Chem.* **2022**, *65*, 329–358.
32. López-Mejía, A.; Ortega-Pérez, L.G.; Magaña-Rodríguez, O.R.; Ayala-Ruiz, L.A.; Piñon-Simental, J.S.; Godínez-Hernández, D.; Rios-Chavez, P. Protective effect of *Callistemon citrinus* on oxidative stress in rats with 1,2-dimethylhydrazine-induced colon cancer. *Biomed. Pharmacother.* **2021**, *142*, 112070. [CrossRef] [PubMed]
33. Baradaran, S.; Hajizadeh Moghaddam, A.; Khanjani Jelodar, S.; Moradi-Kor, N. Protective effects of curcumin and its nano-phytosome on carrageenan-induced inflammation in mice model: Behavioral and biochemical responses. *J. Inflamm. Res.* **2020**, *13*, 45–51. [CrossRef]
34. Alvarez Cortes, O. Elaboración de una Biopelícula Comestible Incorporando Nanocápsulas de Aceite Esencial y Extractos Polifenólicos de *Aloysia citriodora* con Actividad Antiinflamatoria Evaluado en un Modelo Biológico. Master's Thesis, Facultad de Químico Farmacobiología, Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Mexico, 2020.
35. Horiba. Colloidal Silica as a Particle Size and Charge Reference Material. 2009. Available online: https://www.horiba.com/fileadmin/uploads/Scientific/Documents/PSA/TN158.pdf (accessed on 22 January 2023).
36. Jain, P.K.; Kharya, M.; Gajbhiye, A. Pharmacological evaluation of mangiferin herbosomes for antioxidant and hepatoprotection potential against ethanol induced hepatic damage. *Drug Dev. Ind. Pharm.* **2013**, *39*, 1840–1850. [CrossRef] [PubMed]
37. Jain, P.K.; Khurana, N.; Pounikar, Y.; Gajbhiye, A.; Kharya, M.D. Enhancement of absorption and hepatoprotective potential through soya-phosphatidylcholine-andrographolide vesicular system. *J. Liposome Res.* **2013**, *23*, 110–118. [CrossRef]
38. Karamac, M.; Kosiñska, A.; Pegg, R.B. Comparison of radical-scavenging activities for selected phenolic acids. *Pol. J. Food Nutr. Sci.* **2005**, *14*, 165–170.
39. Rufino, M.S.M.; Alves, R.E.; de Brito, E.S.; Perez-Jimenez, J.; Saura-Calixto, F.; Mancini-Filho, J. Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. *Food Chem.* **2010**, *121*, 996–1002. [CrossRef]
40. Thaipong, K.; Boonprakob, U.; Crosby, K.; Cisneros-Zevallos, L.; Byrne, D.H. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *J. Food Comp. Anal.* **2006**, *19*, 669–675. [CrossRef]
41. Pripdeevech, P.; Chumpolsri, W.; Suttiarporn, P.; Wongpornchai, S. The chemical composition and antioxidant activities of basil from Thailand using retention indices and comprehensive two-dimensional gas chromatography. *J. Serb. Chem. Soc.* **2010**, *75*, 1503–1513. [CrossRef]
42. Chang, C.C.; Yang, M.H.; Wen, H.M.; Chern, J.C. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Anal.* **2002**, *10*, 178–182.
43. Chang, C.L.; Lin, C.S. Phytochemical composition, antioxidant activity, and neuroprotective effect of *Terminalia chebula* Retzius extracts. *Evid.-Based Complement. Altern. Med.* **2012**, *2012*, 125247. [CrossRef] [PubMed]
44. Suryawanshi, J.A.S. Phytosome: An emerging trend in herbal drug treatment. *J. Genet. Genom.* **2011**, *3*, 109–114.
45. Xie, J.; Li, Y.; Song, L.; Pan, Z.; Ye, S.; Hou, Z. Design of a novel curcumin-soybean phosphatidylcholine complex-based targeted drug delivery system. *Drug Deliv.* **2017**, *24*, 707–719. [CrossRef] [PubMed]
46. Alshahrani, S.M. Optimization and characterization of *Cuscuta reflexa* extract loaded phytosomes by the box-behnken design to improve the oral bioavailability. *J. Oleo Sci.* **2022**, *71*, 671–683. [CrossRef] [PubMed]
47. Barani, M.; Sangiovanni, E.; Angarano, M.; Rajizadeh, M.A.; Mehrabani, M.; Piazza, S.; Gangadharappa, H.V.; Pardakhty, A.; Mehrbani, M.; Dell'Agli, M.; et al. Phytosomes as innovative delivery systems for phytochemicals: A comprehensive review of literate. *Int. J. Nanomed.* **2021**, *16*, 6983–7022. [CrossRef] [PubMed]
48. Surendra, T.; Patel, D.K.; Baro, L.; Nair, S.K. A review on phytosomes, their characterization, advancement & potential for transdermal application. *J. Drug Deliv. Ther.* **2013**, *3*, 147–152.
49. Boligon, A.A.; Machadi, M.M.; Athayde, M.L. Technical evaluation of antioxidant activity. *Med. Chem.* **2014**, *4*, 517–522. [CrossRef]
50. Khan, R.A.; Khan, M.R.; Sahreen, S.; Ahmed, M. Evaluation of phenolic contents and antioxidant activity of various solvent extracts of *Sonchus asper* (L.) Hill. *Chem. Cent. J.* **2012**, *6*, 12. [CrossRef]
51. Shan, B.; Cai, Y.Z.; Sun, M.; Corke, H. Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. *J. Agric. Food Chem.* **2005**, *53*, 7749–7759. [CrossRef]
52. Saonere, J.A.; Channawar, M.A.; Kochar, N.I.; Mohale, D.; Chandewar, A.V. Preparation and evaluation of phytophospholipid complex of phenolic fractions of *G. glabra* for antioxidant and antimicrobial activity. *Acta Sci. Pharm. Sci.* **2023**, *7*, 37–44.
53. Palachai, N.; Wattanathorn, J.; Muchimapura, S.; Thukham-Mee, W. Antimetabolic syndrome effect of phytosome containing the combined extracts of mulberry and ginger in an animal model of metabolic syndrome. *Oxidative Med. Cell. Longev.* **2019**, *2019*, 5972572. [CrossRef] [PubMed]
54. Tung, B.H.; Hai, N.T.; Son, P.K. Hepatoprotective effect of phytosome curcumin against paracetamol-induced liver toxicity in mice. *Braz. J. Pharm. Sci.* **2017**, *53*, e16136. [CrossRef]
55. Deleanu, M.; Toma, L.; Sanda, G.M.; Barbălată, T.; Niculescu, L.S.; Sima, A.V.; Deleanu, C.; Săcărescy, L.; Suciu, A.; Alexandru, G.; et al. Formulation of phytosomes with extracts of ginger rhizomes and rosehips with improved bioavailability, antioxidant and anti-inflammatory effects in vivo. *Pharmaceutics* **2023**, *15*, 1066. [CrossRef]
56. Wojtunik-Kulesza, K.A.; Kasprzak, K.; Oniszczuk, T.; Oniszczuk, A. Natural monoterpenes: Much more than only a scent. *Chem. Biodivers* **2019**, *16*, e1900434. [CrossRef]

57. Silva, J.C.; Barbosa, L.C.A.; Demuner, J.A.; Montanari, R.M.; Pinheiro, A.L.; Dias, I.; Andrade, N.J. Chemical composition and bacterial activities from the essential oils of Myrtaceae species planted in Brazil. *Química Nova* **2010**, *33*, 104–108. [CrossRef]
58. Radulovic, N.; Lazarevic, J.; Ristic, N.; Palic, R. Chemotaxonomic significance of the volatiles in the genus *Stachys* (Lamiaceae): Essential oil composition of four Balkan *Stachys* species. *Biochem. Syst. Ecol.* **2007**, *35*, 196–208. [CrossRef]
59. Muselli, A.; Rossi, P.-G.; Desjobert, J.-M.; Bernardini, A.-F.; Berti, L.; Costa, J. Chemical composition and antibacterial activity of *Otanthus maritimus* (L.) Hoffmanns. & Link essential oils from Corsica. *Flavour Fragr. J.* **2007**, *22*, 217–223.
60. Al-Omar, M.S. Phytochemical analysis, antioxidant, and anti-microbial activities of *Suaeda vermiculata* n-hexane extract in comparison to the plant's hydrodistilled volatile oil. *Pharmacogn. J.* **2021**, *13*, 853–859. [CrossRef]
61. Boti, J.B.; Koukoua, G.; N'Guessan, T.Y.; Casanova, J. Chemical variability of *Conyza sumatrensis* and *Microglossa pyrifolia* from Côte d'Ivoire. *Flavour Fragr. J.* **2007**, *22*, 27–31. [CrossRef]
62. Babushok, V.-I.; Linstrom, P.J.; Zenkevich, I.G. Retention indices for frequently reported compounds of plant essential oils. *J. Phys. Chem. Ref. Data* **2011**, *40*, 043101. [CrossRef]
63. Zhao, C.X.; Li, X.N.; Liang, Y.Z.; Fang, H.Z.; Huang, L.F.; Guo, F.Q. Comparative analysis of chemical components of essential oils from different samples of *Rhododendron* with the help of chemometrics methods. *Chemom. Intell. Lab. Syst.* **2006**, *82*, 218–222. [CrossRef]
64. Rahman, M.M.; Rahaman, M.S.; Islam, M.R.; Rahman, F.; Mithi, F.M.; Alqahtani, T.; Almikhlafi, M.A.; Alghamdi, S.Q.; Alruwaili, A.S.; Hossain, M.S.; et al. Role of phenolic compounds in human disease: Current knowledge and future prospects. *Molecules* **2022**, *27*, 233. [CrossRef] [PubMed]
65. Dikshit, P.K.; Kumar, J.; Das, A.K.; Sadhu, S.; Sharma, S.; Singh, S.; Gupta, P.K.; Kim, B.S. Green synthesis of metallic nanoparticles: Applications and limitations. *Catalysts* **2021**, *11*, 902. [CrossRef]
66. Das, S.R.; Kundu, C.N. Promising opportunities and potential risk of nanoparticle on the society. *IET Nanobiotechnol.* **2020**, *14*, 253–260. [CrossRef] [PubMed]

Capítulo II: Role of *Callistemon citrinus* Leaf Phytosomes Against Oxidative Stress and Inflammation in Rats Fed with a High-Fat-Fructose Diet.

Publicado en: *Antioxidants* **2024**, 13(10).
https://doi.org/10.3390/antiox13101263
https://www.mdpi.com/2076-3921/13/10/1263

antioxidants

MDPI

Article

Role of *Callistemon citrinus* Leaf Phytosomes Against Oxidative Stress and Inflammation in Rats Fed with a High-Fat-Fructose Diet

Luis Gerardo Ortega-Pérez [1,2], José Armando Hernández-Soto [1], Osvaldo Padilla-Avalos [1], Luis Alberto Ayala-Ruiz [1], Oliver Rafid Magaña-Rodríguez [1], Jonathan Saúl Piñón-Simental [1], Asdrúbal Aguilera-Méndez [2], Daniel Godínez-Hernández [2] and Patricia Rios-Chavez [1,*]

[1] Facultad de Biología, Universidad Michoacana de San Nicolás de Hidalgo (UMSNH), Morelia 58000, Michoacán, Mexico; gerardo.ortega@umich.mx (L.G.O.-P.); 1719780c@umich.mx (J.A.H.-S.); 1900289a@umich.mx (O.P.-A.); 1232816g@umich.mx (L.A.A.-R.); oliver.rodriguez@umich.mx (O.R.M.-R.); 1434491c@umich.mx (J.S.P.-S.)

[2] Instituto de Investigaciones Químico Biológicas, Universidad Michoacana de San Nicolás de Hidalgo (UMSNH), Morelia 58000, Michoacán, Mexico; amendez@umich.mx (A.A.-M.); daniel.godinez@umich.mx (D.G.-H.)

* Correspondence: prchavez17@gmail.com

Abstract: Phytosomes are used as vehicles that carry plant extracts. They exhibit biological activities and possess better bioavailability, bioabsorption, and lower toxicity than drugs. Obesity is an inflammatory state in which oxidative stress is present, which triggers severe effects on the body's organs. This study aimed to evaluate the impact of the extract and phytosomes of *Callistemon citrinus* on oxidative stress and inflammation in the liver and heart of Wistar rats fed with a high-fat-fructose diet. Phytosomes containing the extract of leaves of *C. citrinus* were prepared. The antioxidant, pro-inflammatory enzymes, and biomarkers of oxidative stress were evaluated. Among the groups, only the high-fat-fructose group presented an increase in the COX-2, 5-LOX, and MPO inflammatory enzymes, while the XO enzyme exhibited decreased activity. The groups were fed a hypercaloric diet for 15 weeks while orlistat, *C. citrinus* extract, and phytosomes were administered at three different concentrations, exhibiting enzyme activities similar to those of the control group. It was also observed that the lowest concentration of phytosomes had a comparable effect to the other concentrations. *Callistemon citrinus* extract can modulate the activities of enzymes involved in the inflammation process. Furthermore, small doses of phytosomes can serve as anti-inflammatory agents.

Keywords: *Callistemon citrinus*; anti-inflammatory; antioxidant

Citation: Ortega-Pérez, L.G.; Hernández-Soto, J.A.; Padilla-Avalos, O.; Ayala-Ruiz, L.A.; Magaña-Rodríguez, O.R.; Piñón-Simental, J.S.; Aguilera-Méndez, A.; Godínez-Hernández, D.; Rios-Chavez, P. Role of *Callistemon citrinus* Leaf Phytosomes Against Oxidative Stress and Inflammation in Rats Fed with a High-Fat-Fructose Diet. *Antioxidants* **2024**, *13*, 1263. https://doi.org/10.3390/antiox13101263

Academic Editors: Sanda Andrei, Mihai Cosmin Cenariu and Andrea Bunea

Received: 29 August 2024
Revised: 5 October 2024
Accepted: 16 October 2024
Published: 18 October 2024

1. Introduction

Obesity is a complex disease characterized by the excessive accumulation of body fat, which can be detrimental to health. It leads to an increase in inflammatory markers, causing chronic low-grade inflammation. Additionally, obesity increases the risk of developing type 2 diabetes and cardiovascular diseases, negatively affects bone health and reproduction, and raises the risk of certain cancers [1].

A high-fat-sugar diet can lead to obesity, activating innate immune cells and triggering chronic inflammation, especially in adipose tissue and the liver [2]. Obesity produces reactive oxygen species (ROS) [3]. The primary sources of ROS are the mitochondrial respiratory chain and nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase/NOX), which are byproducts of cellular metabolism. There is an enzymatic antioxidant defense system that includes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Additionally, non-enzymatic antioxidants like glutathione, thioredoxin, and vitamins C and E also protect cells from reactive oxygen species (ROS) by scavenging or neutralizing them. When there is an imbalance between ROS and the antioxidant system, oxidative stress is induced. It is implicated in many diseases, including

diabetes mellitus, atherosclerosis, and stroke. These oxidants attack lipids, proteins, sugar, and nucleic acid, producing molecules that can be used as oxidative biomarkers and lipid peroxidation products like malondialdehyde (MDA) and 4-hydroxynonenal (HNE) [4] and advanced oxidation protein products (AOPPs) [5].

Plant-based compounds may help reduce intracellular oxidative stress in obesity. Various plant-based foods are effective for weight management. Therefore, a balanced diet should consistently include adequate fruits, vegetables, spices, and herbs [6]. They contain various compounds, such as terpenes, phenolics, and flavonoids, which have antioxidant, antitumor, and anti-inflammatory properties. As a result, they can be used as a natural option for treating various chronic diseases in which inflammation and oxidative stress are important factors [7].

The *Callistemon* genus is a promising group of medicinal plants known for their neuroprotective, chemopreventive, antioxidative, anti-aging, antimicrobial, and other pharmaceutical properties [8,9]. *Callistemon citrinus* (Curtis) Skeels is cultivated for its ornamental beauty and is also utilized by traditional healers in crafting herbal medicinal formulations [10]. In a previous study by Petronilho et al. [11], the phytochemical characterization of *Callistemon citrinus* was reported. Over 77 terpene compounds were found in the ethanolic extract of *C. citrinus* leaves, and they were identified using two-dimensional gas chromatography with time-of-flight mass spectrometry detection (GC x GC-ToFMS). The main compounds identified were 1,8-cineole, limonene, and α-terpineol. Additionally, Ortega-Pérez et al. [12] reported the presence of gallic acid, p-coumaric acid, and ellagic acid using HPLC. Recent studies suggest that *C. citrinus* has a significant anti-obesogenic effect through mechanisms that include anti-lipase, antioxidant, and anti-inflammatory activities [9,12,13] and the modulation of oxidative stress [14].

Phytosomes are a delivery system for drugs or plant extracts formulated with phospholipids [15]. This method stands out from conventional drug or plant extract delivery methods by providing a more stable profile. It offers resistance against degradation by digestive enzymes and microbiota, enhances membrane permeability, increases bioavailability, and amplifies the effectiveness of the phytoconstituents [16]. There is limited research on using phytosomal formulations in anti-obesity drugs. Developing antioxidant and anti-inflammatory herbal drugs in phytosomal forms is essential to improving bioavailability and reducing adverse effects. Embracing the phytosomal approach in drug delivery could help overcome the limitations of traditional drug delivery methods [17]. The utilization of *Callistemon citrinus* phytosomes has shown promising potential in controlling weight in rodent models of obesity, presenting an innovative approach for utilizing *C. citrinus* in obesity treatment [12]. This research aims to assess the effectiveness of *C. citrinus* leaf phytosomes in regulating oxidative stress and inflammation in male Wistar rats fed a hypercaloric diet.

2. Materials and Methods

2.1. Chemicals

5,5-dithiobis-2-nitrobenzoic acid, blue nitrotetrazolium chloride, 1-chloro-2,4-dinitrobenzene, 4-nitrophenyl acetate,3,3′,5,5′-tetramethylbenzidine, N,N′,N″,N‴-tetramethyl-p-phenylenediamine and all other chemicals and reagents were of analytical grade and purchase from Sigma-Aldrich Company, Ciudad de México, México.

2.2. Plant Material and Preparation of Ethanolic Extract

To ensure the highest compound content and antioxidant effectiveness of the leaf extract [14], leaves of four-year-old *Callistemon citrinus* (Curtis) Skeels (Myrtaceae) were collected in Morelia, Michoacán, Mexico. The leaves were macerated in 96% ethanol (1:10 w/v) at room temperature for five days. Following maceration, the extract was concentrated using a rotary evaporator set at 45 °C. Standardization was made as specified by López-Mejía [14]. Our previous study [12] contains information about phytosome preparation.

2.3. Animals

Male Wistar rats, 2 months old and weighing 180–200 g, were obtained from the animal laboratory of Instituto de Investigaciones Químico Biológicas at UMSNH. The animals were kept in plastic enclosures in a controlled environment with a 12 h light-dark cycle, humidity levels maintained between 60 and 70%, and temperatures kept at 20–24 °C. Food and water were available at all times. These animals were maintained in the bioterium of the Instituto de Investigaciones Químico Biológicas at UMSNH. All experimental procedures were approved and adhered to the laboratory animal care guidelines of UMSNH Ethics Committee (approval date: 01/12/2023; protocol ID IIQB-CIBE-06-2023) and the established by the Official Mexican Norm (NOM-062-ZOO-1999) of Mexican secretary of Agriculture, livestock, rural development, fishing and food. Official Diary, Ciudad de Mexico, México, 2001.

2.4. Induction of Obesity by Feeding High-Fat-Fructose Diet

Obesity has been investigated in various animal models due to the consumption of high-calorie diets and the presence of oxidative stress [18]. A high-fat diet (HFD) comprised of 45.4% standard chow (Rodent diet brand: Purina® rat chow), 14.8% lard, 14.8% vegetable fat, and 25% fructose was prepared daily, as detailed in Ortega-Pérez et al. [12]. Fifty-four male Wistar rats were randomly divided into nine groups (n = 6). Group 1 received a chow diet; Group 2, a chow diet plus vehicle; Group 3, a chow diet plus *C. citrinus* extract (200 mg/kg); Group 4, HFD; Group 5, HFD plus orlistat (5 mg/kg); Group 6, HFD plus *C. citrinus* extract (200 mg/kg); Group 7, HFD plus phytosomes loaded with *C. citrinus* (P *C. c.*) (50 mg/kg); Group 8, HFD plus P *C. c.* (100 mg/kg) and Group 9, HFD plus P *C. c.* (200 mg/kg). Treatments were administered by oral gavage once daily at 9:00 a.m. for 15 weeks. Daily food and water intake measurements were taken, and the animals' weight was recorded every week to calculate the final body weight gain after the experimental model. The animals' age at the end of the treatment was 23 weeks. Blood samples were collected by cardiac puncture after 12–13 h of fasting. Then, the animals were anesthetized using an intraperitoneal injection of pentobarbital sodium at 150 mg/kg, and all tissues were harvested, washed, and stored at −80 °C for subsequent analysis.

2.5. Tissue Preparations

Liver and heart tissues weighing 0.25 g were homogenized in 1 mL of a 50 mM phosphate buffer at pH 7.4 with 0.1 M EDTA. Subsequently, they were centrifuged using a centrifuge 5424 R Eppendorf at 13,000 rpm for 20 min at 4 °C. The resulting supernatant was collected, frozen, and stored at −80 °C for further biochemical enzymatic and biomarker estimation. The protein concentration in all the homogenates was determined using Bradford's method, using a UV Vis Spectrophotometer genesis 50 [19].

2.6. Total Oxidative Status (TOS) Assay

The ferrous ion–o-dianisidine complex is oxidized to a ferric ion. At acidic pH levels, this procedure results in the formation of a colored complex with xylenol orange. Reagent 1 contained 150 µM of xylenol orange, 140 mM of NaCl, and 1.35 M of glycerol at pH 1.75. Reagent 2 contained 5 mM of ferrous ammonium sulfate and 10 mM of o-dianisidine dihydrochloride. Briefly, 450 µL of reagent 1, 70 µL of the sample, and 422 µL of reagent 2 were mixed. Absorbance readings were taken at two points: the first immediately before the combination of reagents 1 and 2 to serve as the sample blank, and the final reading when the reaction's progress flattens into a plateau, approximately 3–4 min post-mixing, indicating the end-point measurement. The assay is standardized using hydrogen peroxide, and results are presented as µmol H_2O_2 Equiv./L. [20].

2.7. Estimation of Malondialdehyde (MDA) and Hydroxyalkenals (HNE)

MDA levels were determined in a mixture consisting of 200 µL of homogenate (liver or heart), 5 µL of 5 mM butylated hydroxytoluene (BHT), 650 µL of 10 mM 1-methyl-2-

phenylindole, and 150 µL of 37% HCl. The reaction mixture was incubated at 45 °C for 60 min, then cooled on ice to stop the reaction and measured at 586 nm. The total HNE plus MDA assay was conducted similarly but with 37% methane sulfonic acid instead of hydrochloric acid, the blank without the sample. The levels of lipid peroxidation products were expressed in nmol MDA/g tissue and nmol HNE/g tissue [21].

2.8. Advanced Oxidation Protein Products Level (AOPP)

In brief, 50 µL of homogenate was mixed with 1 mL of 20 mM phosphate buffer at pH 7.4, followed by adding 50 µL of 1.16 M potassium iodide and 100 µL of acetic acid. After a 2 min wait, the absorbance was measured at 340 nm. The standard curve for chloramine-T ranged from 0 to 100 µmol/mL. The concentration of AOPP was reported in µmol/mL of chloramine-T equivalents [22].

2.9. Reduced Glutathione (GSH) Levels

The assay mixture comprised 62.5 µL of homogenate, 187.5 µL of 0.2 M phosphate buffer at pH 8.2, and 12.5 µL of 0.01 M 5,5-dithiobis-2-nitrobenzoic acid (DTNB). Following this, 987.5 µL of absolute methanol was added. The mixture was then placed in a laboratory mixer and agitated at 240 rpm for 15 min. After agitation, it was centrifuged at 1250 g for 15 min at room temperature. This process resulted in the development of a yellow color, and the absorbance was measured at 412 nm. The results are reported in units of mM GSH per gram of tissue [23].

2.10. SOD Activity

When exposed to light, superoxide dismutase (SOD) interacts with superoxide anions produced by riboflavin, thereby slowing down the formation of the formazan product. The assay mixture comprised 10 µL of homogenate (from the liver or heart), 641 µL of 0.067 M phosphate buffer at pH 7.0, 40 µL of 0.1 M EDTA, 20 µL of 1.5 mM blue nitrotetrazolium chloride (NTB), and 9 µL of 0.1 mM riboflavin. After gentle stirring, the mixture was illuminated from a distance of 15 cm using a 40-watt lamp for 15 min; the absorbance was measured at 560 nm. All components except the tissue sample were included in the blank, which was not illuminated. The specific activity of the enzyme was measured in U/mg protein. One unit of SOD is defined as the amount of enzyme required to achieve a 50% inhibition of the NBT reduction rate within one minute at room temperature [24].

2.11. CAT Activity

The catalase (CAT) activity was measured based on the rate at which hydrogen peroxide (H_2O_2) decomposed. The assay mixture consisted of 950 µL of 50 mM phosphate buffer at pH 7.0, 25 µL of homogenate (from liver and heart), and 25 µL of 30 mM H_2O_2 as the substrate. The change in absorbance at 240 nm was recorded every 30 s for three minutes. The enzyme-specific activity was expressed in µmol H_2O_2 consumed per minute per mg of protein, based on a molar extinction coefficient of 43.6 M^{-1} cm^{-1} [25].

2.12. GPx Activity

The working solution, totaling 975 µL, consisted of 0.5 mM NADPH, 100 mM reduced glutathione, 1 unit of the enzyme glutathione reductase, 50 mM phosphate buffer at pH 7.0, 30 mM cumene hydroperoxide, and 25 µL of homogenate from the liver and heart. Following stirring, the absorbance at 340 nm was measured every 30 s for five minutes. The activity of glutathione peroxidase (GPx) was defined as one unit per minute per mg of protein, based on the oxidation of one nmol of NADPH, using the molar extinction coefficient of 6.22 mM^{-1} cm^{-1} [26].

2.13. GST Activity

A 999 µL working solution was prepared by combining 980 µL of 0.1 M phosphate buffer at pH 6.5, 10 µL of 100 mM 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate, and

10 µL of 10 mM reduced glutathione. This solution was then incubated at 30 °C for 15 min. After incubation, 1 µL of homogenate from the liver and heart was added. The absorbance at 340 nm was measured every 30 s for five minutes. Glutathione-S-transferase (GST) activity was calculated as the amount of GSH-CDNB conjugate formed per minute per mg of protein (min/mg protein), using a molar extinction coefficient of 9.6 mM^{-1} cm^{-1} [14].

2.14. PON1 Activity

4-nitrophenyl acetate was employed to assess paraoxanase-1 (PON1) activity. A mixture comprising 5 µL of homogenate and 1 mL of working reagent, consisting of 25 mM Tris buffer at pH 8.0, 10 mM CaCl2, and 1 mM 4-nitrophenyl acetate, was prepared. The absorbance at 402 nm was monitored every 30 s for three minutes, with water serving as the blank to correct for non-enzymatic hydrolysis. PON1 activity was quantified as the production of 1 µM of phenol per minute per mg of protein, using the molar extinction coefficient of 14,000 M^{-1} cm^{-1} [27].

2.15. MPO Activity

Homogenates were prepared from 0.1 mg of liver or heart tissue in 50 mM phosphate buffer (pH 7.4) containing 0.5% hexadecyl-trimethyl ammonium bromide. After sonication for 15 s, the samples underwent three freeze–thaw cycles and were centrifuged at 17,000 rpm for 20 min at 4 °C. The supernatant obtained was used to measure myeloperoxidase (MPO) levels. The reaction mixture was composed of 425 µL of 200 mM phosphate buffer (pH 5.4), 10 µL of 15 mM H_2O_2, and 40 µL of 20 mM 3,3′,5,5′-tetramethylbenzidine (TMB). Subsequently, 25 µL of the supernatant was added. The mixture was then incubated at 37 °C for 3 min in darkness, followed by a 3 min incubation on ice. To halt the reaction, 1000 µL of 200 mM sodium acetate (pH 3) was added, and the absorbance was measured at 655 nm for 3 min [28].

2.16. COX-1 and COX-2 Activity

The peroxidase activity of cyclooxygenase (COX-1, COX-2) was assessed using the method outlined by Kumar et al. [29]. In brief, the reaction mixture comprised 712 µL of 100 mM Tris-HCl buffer (pH 8), 31 µL of 15 µM hematin, 31 µL of 3 µM EDTA, 100 µL of liver or heart homogenate, and 63 µL of 100 mM N,N′,N″,N‴-tetramethyl-p-phenylenediamine (TMPD). Subsequently, 63 µL of 133 µM arachidonic acid was introduced as a substrate, and the mixture was incubated for 20 min at 25 °C. The absorbance was then measured at 590 nm. Concurrently, to differentiate COX-1 activity, a tube was prepared for each sample containing an inhibitory substrate (etoricoxib, a selective COX-2 inhibitor). The extinction coefficient for TMPD was 0.00826 μM^{-1}. Enzyme activity was defined as the amount required to oxidize 1 nmol of TMPD per minute.

2.17. 5-LOX Activity

The 5-lipoxygenase (5-LOX) assay relies on the formation of the Fe^{3+}/xylenol orange salt complex. The reaction mixture comprises 490 µL of 50 mM Tris-HCl buffer at pH 7.4, 10 µL of sample homogenate, and 10 µL of 133 µM arachidonic acid. This mixture is then thoroughly mixed and left to incubate at room temperature in darkness for 10 min. Subsequently, 490 µL of FOX reagent is introduced. The FOX reagent is composed of 25 mM sulfuric acid, 100 µM xylenol orange, and 250 µM ferrous sulfate, diluted in water-methanol (1:9). Additionally, 100 µL of 4 mM butylhydroxytoluene is included as an antioxidant to prevent lipid oxidation. The solution is mixed and incubated as previously described. Finally, the absorbance at 590 nm is measured [29].

2.18. XO Activity

Blair's method [30] was used to assess xanthine oxidase (XO) activity. The assay mixture comprised 1000 µL of 33 mM phosphate buffer at pH 7.5, 500 µL of sample homogenate, and 100 µL of 0.17 mM xanthine. The reaction proceeded by incubating the

mixture at 37 °C for 1 h. Subsequently, 100 µL of 100% trichloroacetic acid was added, followed by centrifugation at 10,000× *g* for 15 min.

The uric acid was quantified in the transparent supernatants, and the absorbance was measured at 293 nm. Control blanks had the same composition as the reaction mixture without xanthine. Enzyme activity was determined by comparing the reaction rates with and without xanthine. Xanthine oxidase activity was measured in µmoles of uric acid generated per hour per gram of wet tissue weight. It was expressed as specific activity in µmoles per hour per milligram of protein.

2.19. Statistical Analysis

The test results were presented as the mean ± standard error (SEM) or standard deviation (SD). Data were analyzed using GraphPad Prism (version 8.0) with a one-way analysis of variance (ANOVA). Tukey's multiple comparison test was used to identify statistical differences (a, b, c) in biomarkers and enzymatic parameters between groups. A result of * $p \leq 0.05$ is considered statistically significant. Tukey's Honestly Significant Difference (HSD) test is a post hoc test used in ANOVA to compare all possible pairs of means. When a significant difference among group means is found through ANOVA, a post hoc test like Tukey's is essential to determine which group is significantly different.

3. Results

3.1. Effect of Phytosomes of C. citrinus on Morphometric Parameters and Serum Total Oxidative Status (TOS) of High-Fat-Fructose-Fed Rats

It was observed that rats fed a high-fat-fructose diet experienced weight gain, resulting in increased adipose tissue. In contrast, rats treated with orlistat showed almost 50% reduction in adipose tissue compared to the diet-only group. Treatment with *C. citrinus* extract and phytosomes significantly decreased adipose tissue, similar to the control group (Table 1).

Table 1. Effects of supplementation of *Callistemon citrinus* extract and phytosomes on body weight gain and tissues in rats fed a high-fat-fructose diet.

Group	Body Weight Gain (g)	Adipose tissue (g)	Liver (g)	Heart (g)	Kidney (g)	Stomach (g)
C	178.80 ± 27.28 ^{b}	12.01 ± 2.70 ^{c}	12.77 ± 0.61 ^{a}	1.07 ± 0.12 ^{a}	3.00 ± 0.10 ^{a}	1.85 ± 0.09 ^{a}
C + V	170.20 ± 13.19 ^{b}	12.12 ± 2.72 ^{c}	13.42 ± 0.79 ^{a}	1.37 ± 0.12 ^{a}	2.88 ± 0.10 ^{a}	1.85 ± 0.12 ^{a}
C + *C. c* (200 mg/kg)	179.40 ± 20.42 ^{b}	11.20 ± 2.70 ^{c}	13.77 ± 0.61 ^{a}	1.39 ± 0.14 ^{a}	3.03 ± 0.11 ^{a}	1.96 ± 0.10 ^{a}
HFD	220.50 ± 13.48 ^{a}	49.21 ± 3.02 ^{a}	13.30 ± 0.68 ^{a}	1.26 ± 0.12 ^{a}	2.84 ± 0.08 ^{a}	1.88 ± 0.09 ^{a}
HFD + Orl (5 mg/kg)	180.75 ± 16.54 ^{b}	27.12 ± 3.02 ^{b}	14.65 ± 0.68 ^{a}	1.27 ± 0.14 ^{a}	2.81 ± 0.10 ^{a}	1.86 ± 0.12 ^{a}
HFD + *C. c* (250 mg/kg)	168.83 ± 12.47 ^{b}	14.80 ± 3.49 ^{bc}	12.26 ± 0.79 ^{a}	1.30 ± 0.12 ^{a}	2.75 ± 0.10 ^{a}	2.01 ± 0.12 ^{a}
HFD + P (50 mg/kg)	165.40 ± 29.97 ^{b}	23.3 ± 3.49 ^{bc}	12.00 ± 0.79 ^{a}	1.30 ± 0.14 ^{a}	2.72 ± 0.10 ^{a}	1.90 ± 0.12 ^{a}
HFD + P (100 mg/kg)	169.60 ± 28.04 ^{b}	20.30 ± 3.49 ^{bc}	12.14 ± 0.68 ^{a}	1.25 ± 0.14 ^{a}	2.64 ± 0.11 ^{a}	1.81 ± 0.10 ^{a}
HFD + P (200 mg/kg)	188.80 ± 16.91 ^{b}	21.17 ± 3.49 ^{bc}	12.97 ± 0.61 ^{a}	1.26 ± 0.12 ^{a}	2.69 ± 0.10 ^{a}	1.99 ± 0.12 ^{a}

All values expressed as mean ± SEM ($n = 6$; values statistically different $^{(a, b, c)}$ among groups ($p \leq 0.05$) according to the Tukey test.

The rats fed a high-fat-fructose diet demonstrated a notably higher increase in TOS levels than the other groups. The control group exhibited the lowest TOS levels. Moreover, supplementation with *C. citrinus* at 200 mg/kg did not significantly affect TOS levels compared to the control group, indicating that *C. citrinus* does not induce oxidative stress independently. This result aligns with that of the vehicle group. The administration of *C. citrinus* extract and phytosomes resulted in significant decreases in TOS levels compared to the HFD group. Notably, the phytosomes at 50 mg/kg demonstrated an equivalent effect to those at 200 mg/kg and the *C. citrinus extract* (Figure 1).

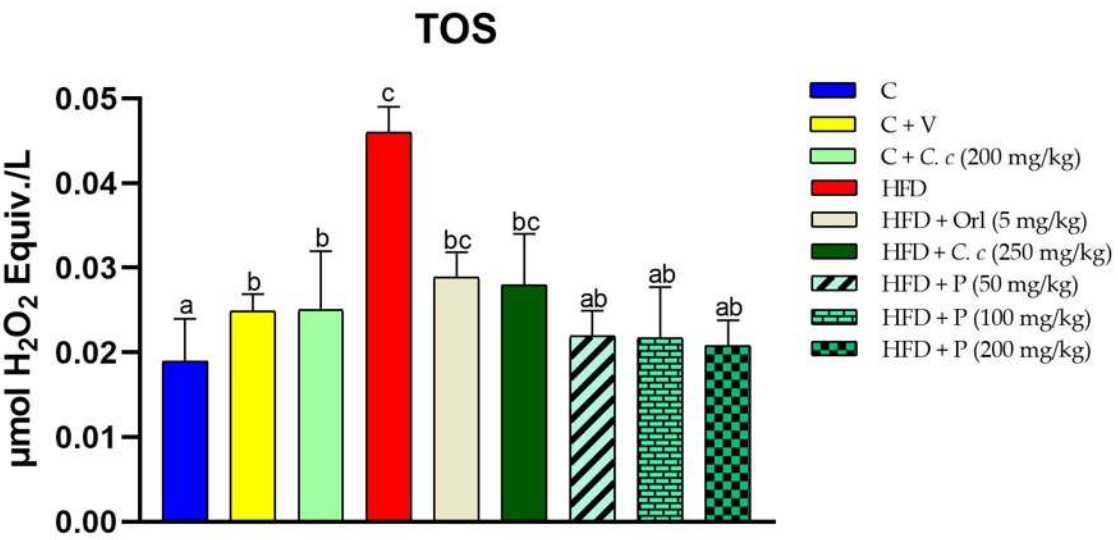

Figure 1. Total Oxidative Status (TOS) in various treatment groups. Values are expressed as mean ± standard error (ANOVA followed by Tukey's test, $n = 6$). Different letters (a, b, c) indicate statistically significant group differences.

3.2. Antioxidant Enzyme Activities

As illustrated in Figure 2, the SOD and CAT activities in the liver were lower in the HFD group compared to all other groups. However, when *C. citrinus* phytosomes were administered at three different doses, the SOD and CAT activities in the liver increased by 50% compared to the HFD group. Additionally, the administration of 200 mg/kg of *C. citrinus* phytosomes and Cc extract at 250 mg/kg resulted in CAT activity levels higher than those in the control group. This same pattern was observed in the SOD activity in the heart. On the other hand, CAT activity in the heart and GPx in the liver and heart showed an increase in the HFD group, unlike the other groups. Treatments with extracts and phytosomes exhibited protective potential, restoring or maintaining the activity of these enzymes at levels similar to the control. These findings are relevant for developing antioxidant-based therapeutic strategies to mitigate the effects of oxidative stress induced by a high-fat diet.

The rats fed a high-fat-fructose diet exhibited significantly higher GST activity in the liver and lower activity in the heart. Additionally, PON1 activity increased in both tissues in the HFD group. The supplementation of *C. citrinus* extract and phytosomes with the high-fat diet helped to maintain GST and PON activities in both tissues, similar to the control group (Figure 3).

3.3. Biomarkers of Oxidative Stress

Oxidative damage in the liver and heart was assessed using markers such as MDA, HNE, AOPP, and reduced glutathione (GSH). The results revealed a significant increase in MDA, HNE, and AOPP levels in the liver and heart of the high-fat diet (HFD) group, which showed a negative correlation with GSH levels in both tissues. However, the treatment with *C. citrinus* extract and three doses of phytosomes showed a protective effect by reducing MDA, HNE, and AOPP levels and increasing GSH levels in these tissues, similar to the control group (Table 2).

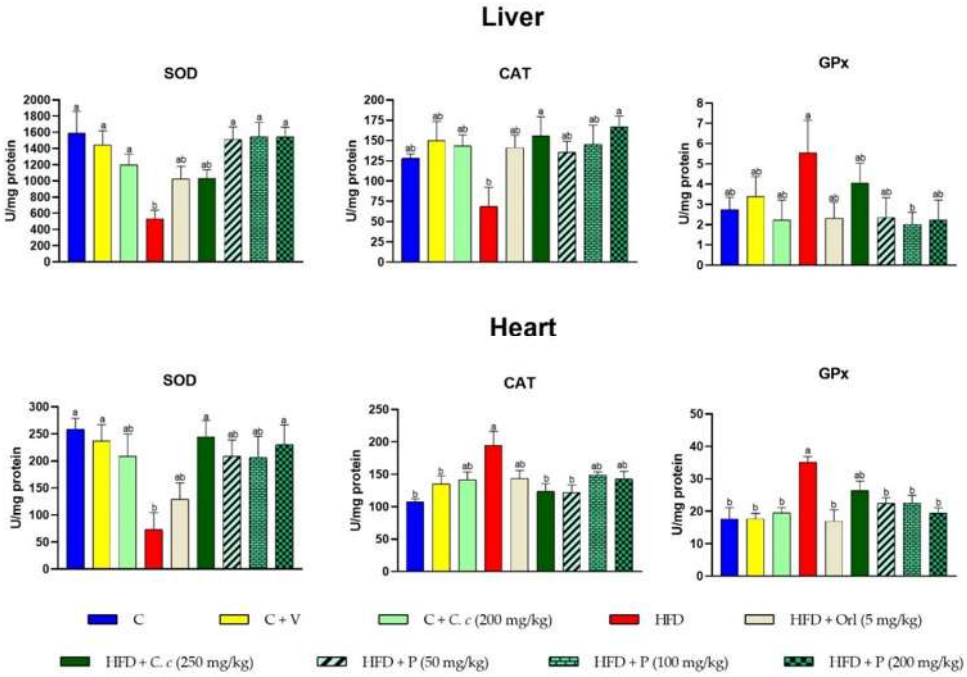

Figure 2. Effect of *C. citrinus* extract and phytosomes on the superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities in liver and heart tissues. Control (C), Control + Vehicle (C + V), Control + *C. citrinus* extract (C + *C. c*, 200 mg/kg), High-Fat Diet (HFD), HFD + Orlistat (HFD + Orl, 5 mg/kg), HFD + *C. citrinus* extract (HFD + *C. c.*, 250 mg/kg), HFD + Phytosome (HFD + P, 50 mg/kg, 100 mg/kg, and 200 mg/kg, respectively). Values are expressed as mean ± standard error (ANOVA followed by Tukey's test, $n = 6$). Different letters (a, b) indicate statistically significant differences between groups.

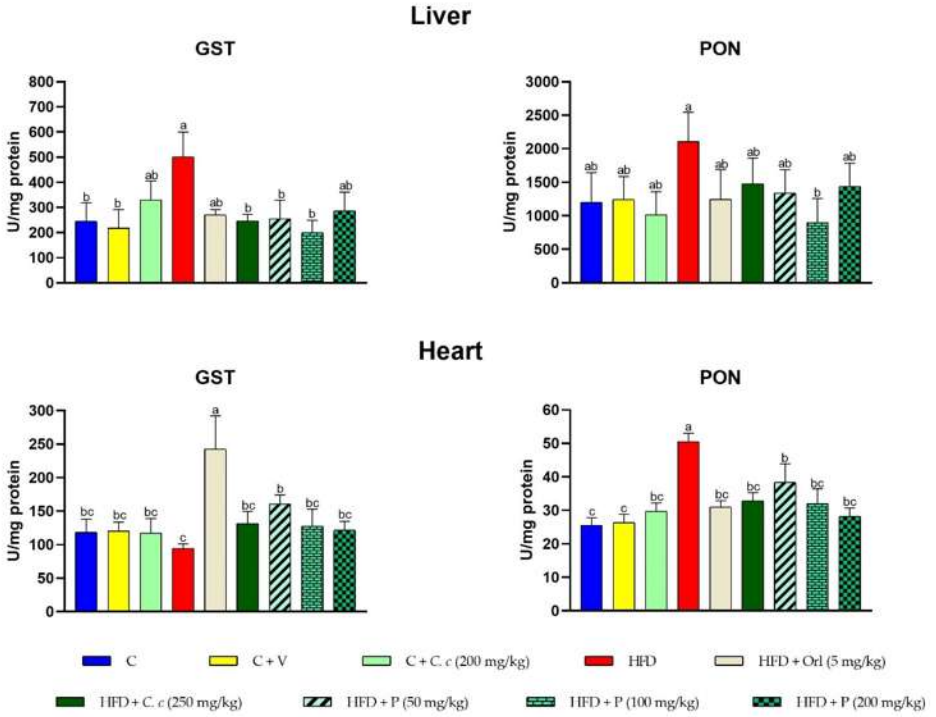

Figure 3. Effect of *C. citrinus* extract and phytosomes in glutathione S-transferase (GST) and paraoxonase-1 (PON1) in liver and heart tissues. Values are expressed as mean ± standard error (ANOVA followed by Tukey's test, $n = 6$). Different letters (a, b, c) indicate statistically significant group differences.

Table 2. Biomarkers of oxidative stress in the liver and heart in the control and experimental groups.

Biomarkers Oxidative Stress in Treatments					
Group	**Organ**	**GSH (mM GSH/g Tissue)**	**MDA (nmol/mg Protein)**	**HNE (nmol/mg Protein)**	**AOPP (nmol/mg Protein)**
C	Liver	8.72 ± 1.92 ^{a}	0.41 ± 0.09 ^{b}	0.35 ± 0.16 ^{b}	8.13 ± 0.95 ^{ab}
	Heart	17.34 ± 2.10 ^{a}	0.25 ± 0.025 ^{b}	0.18 ± 0.015 ^{b}	8.97 ± 2.09 ^{b}
C + V	Liver	6. 23 ± 1.63 ^{a}	0.55 ± 0.07 ^{b}	0.73 ± 0.31 ^{b}	9.91 ± 1.95 ^{ab}
	Heart	18.28 ± 0.44 ^{a}	0.25 ± 0.016 ^{b}	0.26 ± 0.053 ^{b}	8.55 ± 1.09 ^{b}
C + *C. c* (200 mg/kg)	Liver	6.55 ± 1.63 ^{a}	0.38 ± 0.07 ^{b}	0.57 ± 0.31 ^{b}	6.32 ± 2.95 ^{b}
	Heart	16.23 ± 0.88 ^{ab}	0.24 ± 0.012 ^{b}	0.22 ± 0.043 ^{b}	9.62 ± 0.85 ^{b}
HFD	Liver	2.08 ± 0.92 ^{b}	0.86 ± 0.09 ^{a}	2.55 ± 0.31 ^{a}	13.97 ± 2.95 ^{a}
	Heart	13.67 ± 0.88 ^{b}	0.41 ± 0.018 ^{a}	0.60 ± 0.018 ^{a}	17.18 ± 2.09 ^{a}
HFD + Orl (5 mg/kg)	Liver	6.17 ± 0.92 ^{a}	0.55 ± 0.07 ^{b}	1.17 ± 0.03 ^{ab}	10.70 ± 2.15 ^{ab}
	Heart	16.86 ± 0.57 ^{ab}	0.30 ± 0.016 ^{b}	0.13 ± 0.044 ^{b}	12.52 ± 0.95 ^{ab}
HFD + *C. c* (250 mg/kg)	Liver	6.85 ± 2.63 ^{a}	0.58 ± 0.09 ^{b}	0.87 ± 0.21 ^{ab}	7.55 ± 1.95 ^{b}
	Heart	15.14 ± 0.88 ^{ab}	0.23 ± 0.030 ^{b}	0.24 ± 0.020 ^{b}	10.10 ± 1.10 ^{b}
HFD + P (50 mg/kg)	Liver	6.36 ± 1.63 ^{a}	0.41 ± 0.08 ^{b}	1.20 ± 0.26 ^{ab}	8.89 ± 0.95 ^{ab}
	Heart	14.91 ± 0.46 ^{ab}	0.28 ± 0.016 ^{b}	0.26 ± 0.051 ^{b}	11.53 ± 1.09 ^{b}
HFD + P (100 mg/kg)	Liver	6.23 ± 0.92 ^{a}	0.43 ± 0.02 ^{b}	1.02 ± 0.31 ^{ab}	7.54 ± 1.95 ^{ab}
	Heart	15.92 ± 0.88 ^{ab}	0.27 ± 0.012 ^{b}	0.18 ± 0.043 ^{b}	9.63 ± 1.09 ^{b}
HFD + P (200 mg/kg)	Liver	6.09 ± 1.63 ^{a}	0.44 ± 0.07 ^{b}	0.33 ± 0.14 ^{b}	6.22 ± 0.47 ^{b}
	Heart	14.53 ± 1.88 ^{ab}	0.21 ± 0.016 ^{b}	0.18 ± 0.056 ^{b}	9.14 ± 0.95 ^{b}

All values expressed as mean ± SEM (n = 6; values statistically different $^{(a, b)}$ among groups ($p \leq 0.05$) according to Tukey's test.

3.4. Pro-Inflammatory Enzymes Activities

The results were further substantiated by examining the inflammatory activities, as shown in Figure 4. Myeloperoxidase (MPO), cyclooxygenase-2 (COX-2), and 5-lipoxygenase (5-LOX) activities were notably elevated in the livers of rats subjected to a high fat-fructose diet (HFD) compared to both the control group and the groups receiving *C. citrinus* extract and phytosomes supplementation. Conversely, xanthine oxidase (XO) activity was reduced in the HFD group (Figure 4). In heart tissue, COX-2, 5-LOX, and XO activities were higher in the HFD group, while MPO activity decreased compared to the control group (Figure 5).

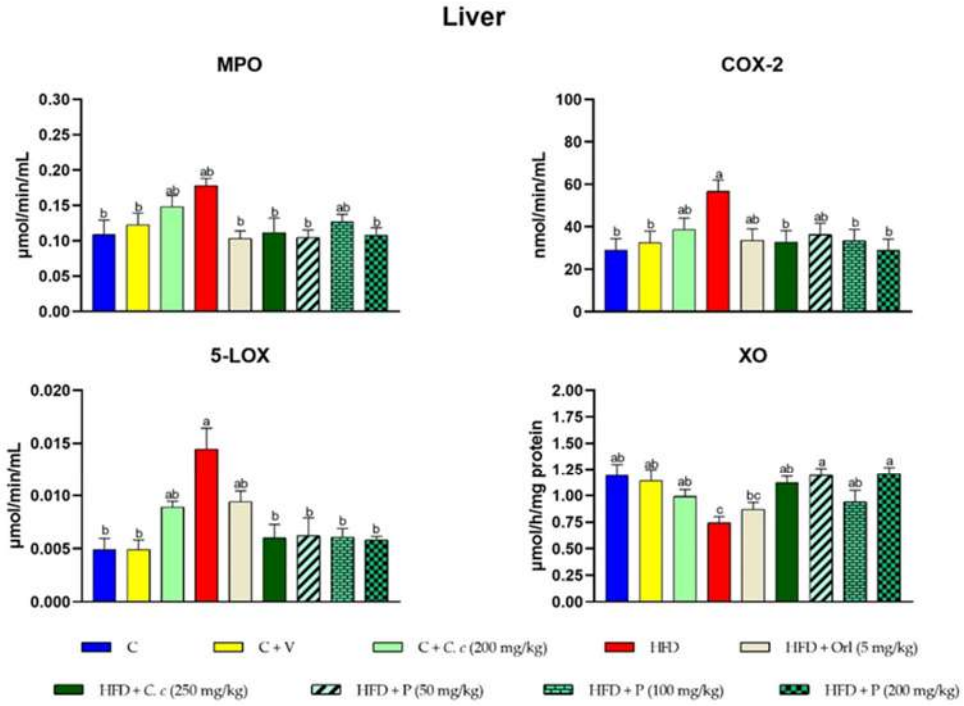

Figure 4. Effects of different treatments on the enzymatic activities of MPO, COX-2, 5-LOX, and XO in liver tissue. Values are expressed as mean ± standard error (ANOVA followed by Tukey's test, $n = 6$). Different letters (a, b, c) indicate statistically significant group differences.

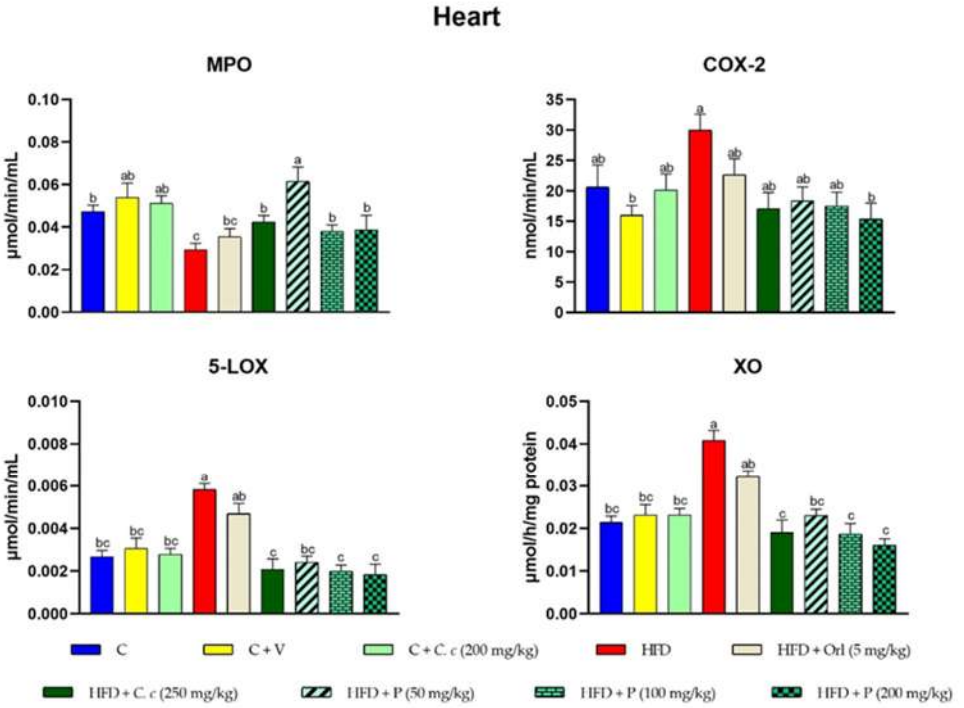

Figure 5. Effects of different treatments on the enzymatic activities of MPO, COX-2, 5-LOX, and XO in heart tissue. Values are expressed as mean ± standard error (ANOVA followed by Tukey's test, $n = 6$). Different letters (a, b, c) indicate statistically significant group differences.

4. Discussion

The study demonstrated that supplementing rats with *Callistemon citrinus* extract and phytosomes while feeding them a high-fat-fructose diet for 15 weeks reduced body weight gain and adipose tissue. Additionally, it improved antioxidant levels and decreased

markers of oxidative stress and inflammation in the liver and heart. Importantly, even a low concentration of phytosomes (50 mg/kg) was sufficient to produce beneficial effects. Ortega-Pérez et al. [12] previously reported that treatment with phytosomes significantly decreased plasma triacylglycerol levels in HFFD-fed rats, leading to improved lipid metabolism and reduced fat accumulation. This was evidenced by the Adiposity index, which was 9.4 ± 0.62 in the high-fat-fructose-diet group and 4.02 ± 0.62 in the group treated with phytosomes at 200 mg/kg.

The protective effect of *C. citrinus* extract and phytosomes is attributed to its main compounds, including 1,8-cineole, limonene, and α-terpineol, as reported in *C. citrinus* [11]. In addition, gallic acid, p-coumaric acid, and ellagic acid [12] possess scavenging free radical activities, as shown by López-Mejía et al. [14]. Additionally, Ayala-Ruiz et al. [31] have reported that these three terpenes boost the activities of antioxidant enzymes such as SOD, CAT, and GPx. Furthermore, they reduce the oxidative stress biomarkers' levels and lower the cytokines IL-6, TNF-α, and leptin concentrations in the livers of Wistar rats fed a high fat-sucrose diet for 15 weeks. Moreover, these terpenes demonstrate protective effects against the liver damage induced by a high-fat-sucrose diet. On the other hand, the *C. citrinus* extract and phytosomes exhibit gastroprotective properties by inhibiting anti-inflammatory enzymes MPO, COX-2, 5-LOX, and XO [13].

The *C. citrinus* extract and the phytosomes exhibit strong antioxidant activity against oxidative stress in rats fed with a high-fat-fructose diet. However, there are differences between them in terms of total oxidative status. The activities of SOD in the liver and GPx in the heart show that the three doses of phytosomes have better antioxidant capacity than the extract. Phytosomes (200 mg/kg) as the extract showed similar activities of SOD, GST, PON-1, MPO, XO, and 5-LOX in the heart, and CAT, GPx, PON-1, MPO, COX-2 in the liver. Concerning the levels of GSH and MDA in both tissues and the activity of 5-LOX in the liver, the three doses of phytosomes and the extract had the same values. An interesting finding was that the lowest levels of HNE and AOPP in both tissues were observed with the phytosomes at 200 mg/kg compared to the other two doses of phytosomes and the extract.

The effect of phytosomes at a dosage of 50 mg/kg was similar to those at dosages of 100 and 200 mg/kg, as well as that of the *C. citrinus* extract. This similarity can be explained by the fact that the compound or compounds responsible for producing the effect, which are contained in the extract, reach saturation at 50 mg/kg or even at lower concentrations. This suggests that the responsible compound or compounds may be highly potent, achieving the maximum effect even at the lowest concentration tested (50 mg/kg). However, further studies are needed to establish the therapeutic dosage range of phytosomes.

The *C. citrinus* phytosomes offer improved stability and solubility compared to *C. citrinus* extract due to their phosphatidylcholine formulation, which organizes the phytochemicals based on their functional groups. This arrangement increases the retention time of the phytosomes in the gastrointestinal tract, leading to improved bioavailability. Additionally, the phytosomes' structure allows for better absorption of polar compounds and provides a hydrophobic bilayer for non-polar compounds. This contrasts with the low bioavailability and absorption of phenolic and flavonoid compounds in *C. citrinus* extract. Also, the major compounds found in *C. citrinus* extract are 1,8 cineole, limonene, and α-terpineol, which have high volatility and low bioavailability when administered orally.

This study also revealed that the liver is the first organ to be damaged during a high-fat-fructose diet. Previous studies have reported that a fructose diet is more obesogenic than a sucrose diet [32]. Additionally, research on high-fat diets has shown that they are sufficient to induce an increase in reactive oxygen species (ROS) and inflammatory status [33] or to cause a decrease in the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) [34,35]. Other studies have reported that high-fat-sugar diets increase pro-inflammatory proteins more than high-fat or high-sugar diets [36].

Due to its heightened sweetening power, high fructose has been used instead of cane sugar in foods. However, this sugar is linked to significant health concerns, including the rise in obesity and its associated conditions. Fructolysis primarily occurs in the liver,

increasing the synthesis of fatty acids, triglycerides, uric acid, and oxidative stress [37]. Several studies have indicated that a high fructose diet can result in metabolic syndrome and hyperinsulinemia [38]. Comparing results across studies can be challenging due to variations in fat concentration and type, sugar form (liquid or solid), and administration duration. Studies [38,39] have shown that gut microbiota and the production of advanced glycation end products are influenced by whether fructose is consumed in solid or liquid form. Furthermore, Collotta et al. [40] demonstrated that administering fructose in liquid form led to elevated levels of pro-inflammatory cytokines (TNF-α and IL-6).

In a study by García-Beltrán et al. [41], it was observed that levels of catalase (CAT), superoxide dismutase (SOD), glutathione S-transferase (GST), glutathione peroxidase (GPx), and quinone reductase (QR) activities were similar to those of the control group after 13 weeks. However, after 21 weeks, all the enzyme activities decreased compared to the control group. In contrast, our study found decreased SOD and CAT levels and increased GPx and GST levels in the liver and heart of rats fed a high-fat diet (HFD). Additionally, CAT and GST activities were reduced in the hearts of rats fed the HFD for 15 weeks. While both diets contain high levels of fat and fructose, the differences in rodent types and fructose administration methods may explain the variation. Additionally, GPx is a primary defense antioxidant enzyme, while GST is a detoxification enzyme combating toxic compounds. Both enzymes play a crucial role in maintaining GSH homeostasis [42,43]. Our study found that the rise in GPx and GST activities directly correlated with the decreased GSH levels in the liver and heart. GSH, acting as a reducing agent, serves numerous functions, including being a substrate for GPx and GST enzymes and providing protection against ROS. Based on our research, a high-fat-fructose diet (HFD) may reduce GSH levels and raise MDA, HEN, and AOPP levels in tissues. This could be because of increased free radical generation, resulting in oxidative stress in the tissues.

Our results contrast those reported by Norman et al. [44], who observed reductions in GPx, GST, and PON1 activities in the livers and hearts of rats fed a diet containing 46% fat and 24% sucrose. In contrast, our study involved a diet comprising 30% fat and 25% fructose. Our study demonstrated a notable increase in PON1 activity in the livers and hearts of rats treated with HFD. PON1 is recognized as an antioxidant enzyme due to its protective effects in the serum, being bound to HDL and preventing the oxidation of LDL [45]. Malondialdehyde (MDA) and hydroxynonenal (HNE) are the primary products of lipid peroxidation, known for their reactivity towards proteins and DNA, causing damage to these biomolecules [46]. Additionally, these products can impact the recruitment of cytokines such as TNF-α, IL-1β, IL-6, and COX-2. Two types of COX enzymes have been identified: COX-1, a constitutive enzyme found in most tissues, and COX-2, an inducible enzyme expressed during inflammatory processes [47]. According to Silva Santi et al. [48], a high-carbohydrate diet is associated with higher levels of inflammatory markers than a high-fat diet. Combining a high-fat and high-sugar diet also leads to more significant oxidative stress than a high-fat or high-sugar diet alone [49]. Furthermore, Almasri et al. [50] found that a diet containing 20% fat and 25% fructose increased inflammatory markers in the liver and skeletal muscle of rats

In our study, we observed an inflammatory process occurring in the group that was fed a high-fat-fructose diet. This was accompanied by increased cyclooxygenase activity and high adipose tissue deposition in this group (Table 1). The adipose tissue produced prostaglandins, which are known to be involved in the inflammatory process [51]. In contrast, the other groups maintained a low COX-2 activity similar to the control group, indicating that the extracts and phytosomes of *C. citrinus* could potentially inhibit this enzyme and prevent the inflammatory process. Limonene, one of the main compounds in *C. citrinus*, has been reported to reduce adipose tissue [31]. Lipoxygenases (LOXs) are enzymes involved in producing leukotrienes (LTs). LTs are mediators that impact chemotaxis, vascular function, fluid balance, immunity, and pain responses and are also part of the body's inflammatory cascade [52]. The isoforms of LOX (5-LOX, 12-LOX, and 15-LOX) are present in the neuroinflammation process. The activity of LOX is regulated

during oxidative stress [53]. In a study by Rudrapal et al. [54], it was reported that certain Indian spices can inhibit COXs and platelet activities, indicating their potential as anti-inflammatory agents. Our study found that the extract and phytosomes of *C. citrinus* effectively reduced COX-2 and platelet activities. In a previous study, we observed that *C. citrinus* only inhibited COX-2 while activating COX-1 [13]. These findings suggest that *C. citrinus* could be utilized as an anti-inflammatory agent due to its dual action against COX-2 and platelet activities.

Myeloperoxidase (MPO) is primarily found in neutrophils and serves as a bactericidal agent by reacting with chlorine ions (Cl^-) and hydrogen peroxide (H_2O_2) to produce hypochlorous acid (HOCl). However, numerous reports suggest its involvement in various diseases due to its ability to produce reactive products such as chloramines, tyrosine radicals, and nitrogen dioxide. Additionally, it is considered a marker of inflammation [55]. Once again, the group that adhered to the high-fat-fructose diet exhibited an increase in MPO. Mazzoli et al. [56] observed that diets rich in fat and fructose are linked to enhanced oxidative stress in the liver, resulting in increased MPO activity. Furthermore, Lasker et al. [57] demonstrated that MPO activity rises in the livers of rats fed a high-fat diet for eight weeks. Van Leeuwen et al. [58] showed that MPO activity in the plasma initially increased in mice fed a high-fat diet but decreased after prolonged consumption. Our study revealed elevated MPO activity in the livers of rats fed a high-fat-fructose diet, while the same group exhibited low MPO activity in their hearts. These findings align with a report by Brennan et al. [59] indicating a protective role of MPO in atherosclerosis.

Xanthine oxidoreductase (XOR) plays a crucial role in purine catabolism, functioning as either a dehydrogenase (XDH) or an oxidase (XO). It is extensively present in the liver and intestines, generating the superoxide anion implicated in various inflammatory processes, including tissue damage and heart failure [60]. The liver exhibits the highest expression of XOR in its dehydrogenase activity. Under pathological conditions such as oxidative stress, the enzyme is an oxidant (XO) [61]. Our findings indicate a decrease in XO activity in the livers of rats fed a high-fat-fructose diet. The same group exhibited increased XO activity in the heart, possibly attributed to lard and vegetable shortening as fats, along with administering fructose in the food rather than in liquid form. Mastrocola et al. [39] reported varying results based on the method of fructose administration.

Orlistat is a medication used to treat obesity by inhibiting gastric and pancreatic lipase, preventing dietary triacylglycerol absorption. Recent studies have also demonstrated its antioxidant properties. Our study observed this antioxidant capacity in the liver and heart of rats fed a high-fat-fructose diet. This finding is consistent with the results presented by Hamza and Alsolami [62] and Othman et al. [63,64], who reported anti-atherogenic and antioxidant properties and regulated Nrf2 expression. Our study revealed a significant increase in glutathione-S-transferase activity in the hearts of the rats in the orlistat group compared to all other groups. Our study administered orlistat for 15 weeks, which is longer than most other studies. This could explain the result. This prolonged use of orlistat may lead to the generation of harmful compounds. The detoxification enzyme GST aids in transforming these compounds into GSH conjugates for elimination. Furthermore, overexpression of GST is detected in certain medical conditions [43].

The study revealed that orlistat positively impacts pro-inflammatory enzymes such as MPO, XO, COX-2, and LOX-5, as well as the levels of AOPP and HNE, which serve as biomarkers of oxidative stress. It has been reported that 1,8-cineole, the major compound in *C. citrinus*, increases the nuclear factor erythroid 2-related factor 2 (Nrf2) [65]. Additionally, 1,8-cineole, limonene, and α-terpineol reduce TNF-α, IL-6, and leptin levels [31].

The utilization of both the extract and phytosomes of *C. citrinus* in this study serves to validate its protective and anti-inflammatory properties, which can be attributed to the composition of its major phytoconstituents., 1,8-cineole, limonene, and α-terpineol [11,31]. The extract contains gallic acid, ellagic acid, p-coumaric acid, and phloroglucinol, which exhibit various biological activities such as antibacterial, antioxidant, anti-inflammatory, and antidiabetic properties [12]. These compounds work together to promote anti-inflammatory

activity in rats fed a high-fat-fructose diet. Based on the study, it was found that the extract and phytosomes of *C. citrinus* were effective in reducing the levels of MPO, 5-LOX, and COX-2. This indicates that *C. citrinus* may help decrease the production of chlorinated products and the infiltration of immune cells in the tissues. Additionally, it was observed that it also lowers the activity of COX-2, resulting in reduced levels of PGE2, which is attributed to the presence of limonene, 1.8-cineole, and α-terpineol [66]. Limonene diminishes inflammation by decreasing the activity of 5-LOX and lowering the levels of LTB4, thereby preventing the inflammatory process [67]. Additionally, both limonene and terpineol demonstrate anti-inflammatory effects by decreasing the levels of pro-inflammatory cytokines such as TNF, IL-6, leptin, and AOPP in a colitis induction model [68]. Limonene reduces the production of ROS and RNS by increasing the activity of the antioxidant defense system in cells of diabetic rats and an inflammatory state [67]. It also promotes a reduction in MDA [69].

In our study, we observed a significant increase in the levels of biomarkers of oxidative stress, such as MDA, HNE (indicating lipid peroxidation), and AOPP (indicating protein oxidation), in the livers and hearts of rats fed a high-fat diet. However, the administration of *C. citrinus* extract and phytosomes of *C. citrinus* led to a reduction in these levels compared to the control group, suggesting that *C. citrinus* may effectively scavenge free radicals. The results align with the functions of antioxidant enzymes. An imbalance in the antioxidant system and the generation of reactive oxygen species led to oxidative stress, which is associated with various disorders in obesity. Thus, treatment with compounds possessing higher antioxidant capacity may help mitigate the issues related to this condition (see Figure 6). It is essential to carefully consider the dosage and bioavailability of compounds intended for use as therapeutic agents. The present study observed that the lowest concentration of the *C. citrinus* phytosomes demonstrated a similar pattern to the higher concentration, thereby underscoring its significance. In the context of future studies, it is essential to consider using an additional analytical technique, such as Western blot or QPCR, to enhance further the understanding of the potential mechanism of action of phytosomes.

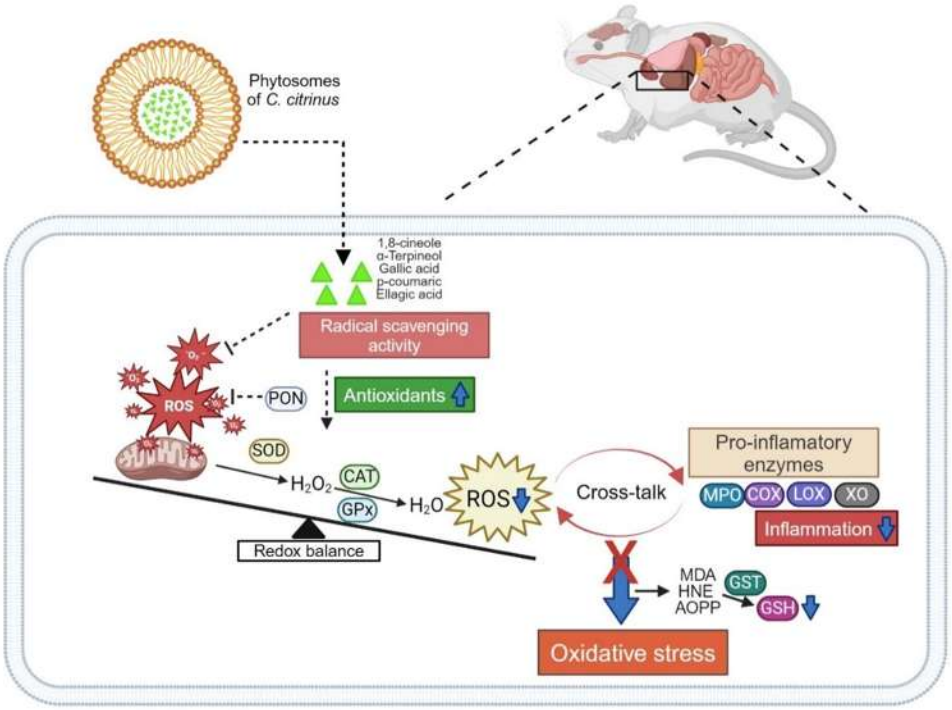

Figure 6. The proposed mechanism of action of *C. citrinus* phytosomes on oxidative stress and inflammation in rats induced by a high fat-fructose diet involves terpenes and phenolic acids. These components help reduce the increase in reactive oxygen species (ROS) by enhancing the activity of antioxidant and anti-inflammatory enzymes through enzyme induction and by lowering the biomarkers of oxidative stress. This information was created with BioRender.com.

5. Conclusions

Phytosomes extracted from *C. citrinus* demonstrate the capacity to mitigate risk factors associated with oxidative stress, diminish the inflammatory process, and enhance the activities of antioxidant enzymes. Notably, even at low doses, dietary supplementation with phytosomes effectively averted the harmful effects of high-fat-fructose consumption.

Author Contributions: Investigation, L.G.O.-P., J.A.H.-S. and O.P.-A.; visualization, L.G.O.-P. and P.R.-C.; formal analysis, L.G.O.-P., L.A.A.-R. and O.R.M.-R.; data curation, L.G.O.-P. and J.S.P.-S.; software, A.A.-M. and D.G.-H.; methodology, L.G.O.-P., J.A.H.-S. and O.P.-A.; writing—original draft, L.G.O.-P. and P.R.-C.; supervision, P.R.-C.; writing—review and editing, P.R.-C., D.G.-H. and A.A.-M. All authors have read and agreed to the published version of the manuscript.

Funding: This research did not receive a specific grant from public, commercial, or not-for-profit funding agencies.

Institutional Review Board Statement: All experimental procedures were approved and adhered to the laboratory animal care guidelines and use established by the Mexican Official Standard (NOM-062-ZOO-1999) and were approved by the Institutional Committee for the use of Animals of the UMSNH (approval date: 1 December 2023; protocol ID IIQB-CIBE-06-2023.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article further inquiries can be directed to the corresponding author.

Acknowledgments: The authors thank the Coordinación de la Investigación Científica de la Universidad Michoacana de San Nicolás de Hidalgo in México for the facilities provided for the study.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Khanna, D.; Khanna, S.; Khanna, P.; Kahar, P.; Patel, B.M. Obesity: A chronic low-grade inflammation and its markers. *Cureus* **2022**, *14*, e22711. [CrossRef] [PubMed]
2. Lee, Y.S.; Wollam, J.; Olefsky, J.M. An integrated view of immunometabolism. *Cell* **2017**, *172*, 1–19. [CrossRef] [PubMed]
3. Zhou, Y.; Li, H.; Xia, N. The interplay between adipose tissue and vasculature: Role of oxidative stress in obesity. *Front. Cardiovasc. Med.* **2021**, *8*, 650214. [CrossRef]
4. Nam, T.G. Lipid peroxidation and its toxicological implications. *Toxicol. Res.* **2011**, *27*, 1–6. [CrossRef]
5. Krzystek-Korpacka, M.; Patryn, E.; Boehm, D.; Berdowska, I.; Zielinski, B.; Noczynska, A. Advanced oxidation protein products (AOPPs) in juvenile overweight and obesity prior to and following weight reduction. *Clin. Biochem.* **2008**, *41*, 943–949. [CrossRef]
6. Nawaz, T.; Nawaz, S.; Khalid, W.; Mohamedahmed, K.A. Dietary phytochemicals beneficial to obesity and cancer management: A review. *Int. J. Food Prop.* **2013**, *26*, 2006–2024. [CrossRef]
7. Kim, E.; Jeon, S. The impact of phytochemicals in obesity-related metabolic diseases: Focus on ceramide metabolism. *Nutrients* **2023**, *15*, 703. [CrossRef]
8. López-Mejía, A.; Ortega-Pérez, L.G.; Godinez-Hernández, D.; Nateras-Marin, B.; Meléndez-Herrera, E.; Rios-Chavez, P. Chemopreventive effect of *Callistemon citrinus* (Curtis) Skeels against colon cancer induced by 1, 2-dimethylhydrazine in rats. *J. Cancer Res. Clin. Oncol.* **2019**, *145*, 1417–1426. [CrossRef]
9. Ortega-Pérez, L.G.; Piñón-Simental, J.S.; Magaña-Rodríguez, O.R.; Lopéz-Mejía, A.; Ayala-Ruiz, L.A.; García-Calderón, A.J.; Rios-Chavez, P. Evaluation of the toxicology, anti-lipase, and antioxidant effects of *Callistemon citrinus* in rats fed with a high fat-fructose diet. *Pharm. Biol.* **2022**, *60*, 1384–1393. [CrossRef]
10. Singh, R. A Review, Outlook, and Insight, of the Properties and Characteristics of *Callistemon citrinus*. *Bull. Pure Appl. Sci. Bot.* **2017**, *36*, 22–27. [CrossRef]
11. Petronilho, S.; Rocha, S.; Ramírez-Chávez, E.; Molina-Torres, J.; Rios-Chavez, P. Assessment of the terpenic profile of *Callistemon citrinus* (Curtis) Skeels from Mexico. *Ind. Crops Prod.* **2013**, *46*, 369–379. [CrossRef]
12. Ortega-Pérez, L.G.; Ayala-Ruiz, L.A.; Magaña-Rodríguez, O.R.; Piñón-Simental, J.S.; Aguilera-Méndez, A.; Godínez-Hernández, D.; Rios-Chavez, P. Development and Evaluation of Phytosomes Containing *Callistemon citrinus* Leaf Extract: A Preclinical Approach for the Treatment of Obesity in a Rodent Model. *Pharmaceutics* **2023**, *15*, 2178. [CrossRef] [PubMed]
13. Piñón-Simental, J.S.; Ayala-Ruiz, L.A.; Ortega-Pérez, L.G.; Magaña-Rodríguez, O.R.; Meléndez-Herrera, E.; Aguilera-Méndez, A.; Rios-Chavez, P. Use of *Callistemon citrinus* as a gastroprotective and anti-inflammatory agent on indomethacin-induced gastric ulcers in obese rats. *PeerJ* **2024**, *12*, e17062. [CrossRef] [PubMed]

14. López-Mejía, A.; Ortega-Pérez, L.G.; Magaña-Rodríguez, O.R.; Ayala-Ruiz, L.A.; Piñón-Simental, J.S.; Hernández, D.G.; Rios-Chavez, P. Protective effect of *Callistemon citrinus* on oxidative stress in rats with 1, 2-dimethylhydrazine-induced colon cancer. *Biomed. Pharmacother.* **2021**, *142*, 112070. [CrossRef] [PubMed]
15. Sandhiya, V.; Ubaidulla, U. A review on herbal drug loaded into pharmaceutical carrier techniques and its evaluation process. *Future J. Pharm. Sci.* **2020**, *6*, 51. [CrossRef]
16. Permana, A.D.; Utami, R.N.; Courtenay, A.J.; Manggau, M.A.; Donnelly, R.F.; Rahman, L. Phytosomal nanocarriers as platforms for improved delivery of natural antioxidant and photoprotective compounds in propolis: An approach for enhanced both dissolution behaviour in biorelevant media and skin retention profiles. *J. Photochem. Photobiol. B Biol.* **2020**, *205*, 111846. [CrossRef]
17. Adiki, S.K.; Sangeetha, S.; Kamireddy, S.; Katakam, P.; Obilineni, I. Phytosomes: A novel phytoconstituent delivery approach to improve the efficacy of obesity treatment. *Curr. Nutr. Food Sci.* **2023**, *19*, 229–237. [CrossRef]
18. Palma-Jacinto, J.A.; Santiago-Roque, I.; Arroyo-Helguera, O.E. Hypercaloric cafeteria diet-induces obesity, dyslipidemia, insulin resistance, inflammation and oxidative stress in Wistar rats. *J. Exp. Life Sci.* **2023**, *13*, 17–23.
19. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254. [CrossRef]
20. Erel, O. A new automated colorimetric method for measuring total oxidant status. *Clin. Biochem.* **2005**, *38*, 1103–1111. [CrossRef]
21. Johnston, J.W.; Horne, S.; Harding, K.; Benson, E.E. Evaluation of the 1-methyl-2-phenylindole colorimetric assay for aldehydic lipid peroxidation products in plants: Malodialdehyde and 4-hydroxynonenal. *Plant Physiol. Biochem.* **2007**, *45*, 108–112. [CrossRef] [PubMed]
22. Witko-Sarsat, V.; Friedlander, M.; Capeillère-Blandin, C.; Nguyen-Khoa, T.; Nguyen, A.T.; Zingraff, J.; Jungers, P.; Descamps-Latscha, B. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. *Kidney Int.* **1996**, *49*, 1304–1313. [CrossRef]
23. Sedlak, J.; Lindsay, R.H. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal. Biochem.* **1968**, *25*, 192–205. [CrossRef]
24. Bouhalit, S.; Kechrid, Z. Assessment of the potential role of l-methionine on nickel sulfate induced renal injury and oxidative stress in rat. *Asian J. Pharm. Clin. Res.* **2018**, *11*, 1–5. [CrossRef]
25. Aebi, H. Catalase in vitro. *Methods Enzymol.* **1984**, *105*, 121–126.
26. Prabhu, K.S.; Reddy, P.V.; Gumpricht, E.; Hildenbrandt, G.R.; Scholz, R.W.; Sordillo, L.M.; Reddy, C.C. Microsomal glutathione S-transferase A1-1 with glutathione peroxidase activity from sheep liver: Molecular cloning, expression and characterization. *Biochem. J.* **2001**, *360*, 345–354. [CrossRef] [PubMed]
27. Dantoine, T.F.; Debord, J.; Charmes, J.P.; Merle, L.; Marquet, P.; Lachatre, G.; Leroux-Robert, C. Decrease of serum paraoxonase activity in chronic renal failure. *J. Am. Soc. Nephrol.* **1998**, *9*, 2082–2088. [CrossRef]
28. Marquez, L.A.; Dunford, H.B. Mechanism of the oxidation of 3,3′,5,5′- tetramethylbenzidine by myeloperoxidase determined by transient- and steady-state kinetics. *Biochemistry* **1997**, *36*, 9349–9355. [CrossRef]
29. Kumar, K.A.; Reddy, T.C.; Reddy, G.V.; Reddy, D.B.; Mahipal, S.V.; Sinha, S.; Gaikwad, A.N.; Reddanna, P. High-throughput screening assays for cyclooxygenase-2 and 5-lipoxygenase, the targets for inflammatory disorders. *Indian J. Biochem. Biophys.* **2011**, *48*, 256–261.
30. Blair, D.G.; McLennan, B.D. Intensification of the xanthine oxidase activity of rat liver homogenates on storage. *Can. J. Biochem.* **1968**, *46*, 1047–1056. [CrossRef]
31. Ayala-Ruiz, L.A.; Ortega-Pérez, L.G.; Piñón-Simental, J.S.; Magaña-Rodríguez, O.R.; Meléndez-Herrera, E.; Rios-Chavez, P. Role of the major terpenes of *Callistemon citrinus* against the oxidative stress during a hypercaloric diet in rats. *Biomed. Pharmacother.* **2022**, *153*, 113505. [CrossRef] [PubMed]
32. DiNicolantonio, J.J.; Subramonian, A.M.; O´Keefe, J.H. Added fructose as a principal driver of non-alcoholic fatty liver disease: A public health crisis. *Open Heart* **2017**, *4*, e000631. [CrossRef] [PubMed]
33. Grujić-Milanovićet, J.D.; Miloradović, Z.Z.; Mihailović-Stanojević, N.D.; Banjac, V.V.; Vidosavljević, S.; Ivanov, M.S.; Karanović, D.J.; Vajić, U.J.V.; Jovović, D.M. Excessive consumption of unsaturated fatty acids leads to oxidative and inflammatory instability in Wistar rats. *Biomed. Pharmacother.* **2021**, *139*, 111691.
34. Das, S.; Choudhuri, D. Calcium supplementation shows a hepatoprotective effect against high-fat diet by regulating oxidative-induced inflammatory response and lipogenesis activity in male rats. *J. Tradit. Complement. Med.* **2019**, *10*, 511–519. [CrossRef] [PubMed]
35. Emami, S.R.; Jafari, M.; Haghshebas, R.; Ravasi, A. Impact of eight weeks endurance training on biochemical parameters and obesity-induced oxidative stress in high fat diet-fed rats. *JENB J. Exerc. Nutr. Biochem.* **2016**, *20*, 029–035. [CrossRef]
36. Masi, L.N.; Martins, A.R.; Crisma, A.R.; do Amaral, C.L.; Davanso, M.R.; Serdan, T.D.A.; de Sá, R.D.C.; Cruz, M.; Alonso-Vale, M.I.C.; Torres, R.P.; et al. Combination of a high-fat diet with sweetened condensed milk exacerbates inflammation and insulin resistance induced by each separately in mice. *Sci. Rep.* **2017**, *7*, 3937. [CrossRef]
37. Ackerman, Z.; Oron-Herman, M.; Grozovski, M.; Rosenthal, T.; Pappo, O.; Link, G.; Sela, B.A. Fructose-induced fatty liver disease: Hepatic effects of blood pressure and plasma triglyceride reduction. *Hypertension* **2005**, *45*, 1012–1018. [CrossRef]
38. Toop, C.R.; Gentili, S. Fructose beverage consumption induces a metabolic syndrome phenotype in the rat: A systematic review and meta-analysis. *Nutrients* **2016**, *8*, 577. [CrossRef]

39. Mastrocola, R.; Ferrocino, I.; Liberto, E.; Ciazza, F.; Cento, A.S.; Collotta, D.; Querio, G.; Nigro, D.; Bitonto, V.; Cutrin, J.C.; et al. Fructose liquid and solid formulations differently affect gut integrity, microbiota composition and related liver toxicity: A comparative in vivo study. *J. Nutr. Biochem.* **2018**, *55*, 185–199. [CrossRef]
40. Collotta, D.; Lucarini, L.; Chiazza, F.; Cento, A.S.; Durante, M.; Sgambellone, S.; Chini, J.; Baratta, F.; Aragno, M.; Mastrocola, R.; et al. Reduced susceptibility to sugar-induced metabolic derangements and impairments of myocardial redox signaling in mice chronically fed with D-tagatose when compared to fructose. *Oxid. Med. Cell Longev.* **2018**, *2018*, 5042428. [CrossRef]
41. García-Beltrán, A.; Martínez, R.; Porres, J.M.; Arrebola, F.; Ruiz Artero, I.; Galisteo, M.; Aranda, P.; Kapravelou, G.; López-Jurado, M. Novel insights and mechanisms of diet-induced obesity: Mid-term versus long-term effects on hepatic transcriptome and antioxidant capacity in Sprague-Dawley rats. *Life Sci.* **2023**, *324*, 121746. [CrossRef] [PubMed]
42. Pei, J.; Pan, X.; Wei, G.; Hua, Y. Research progress of glutathione peroxidase family (GPX) in redoxidation. *Front. Pharmacol.* **2023**, *14*, 1147414. [CrossRef] [PubMed]
43. Mazari, A.M.A.; Zhang, L.; Ye, Z.W.; Zhang, J.; Tew, K.D.; Townsend, D.M. The multifaceted role of glutathione S-transferase in health and disease. *Biomolecules* **2023**, *13*, 688. [CrossRef] [PubMed]
44. Noeman, S.A.; Hamooda, H.E.; Baalash, A.A. Biochemical study of oxidative stress markers in the liver, kidney, heart of high fat diet induced obesity in rats. *Diabetol. Metab. Syndr.* **2011**, *3*, 17. [CrossRef] [PubMed]
45. Marsillach, J.; Camps, J.; Ferré, N.; Beltran, R.; Rull, A.; Mackness, B.; Mackness, M.; Jove, J. Paraoxonase-1 is related to inflammation, fibrosis and PPAR delta in experimental liver disease. *BMC Gastroenterol.* **2008**, *9*, 3. [CrossRef]
46. Ayala, A.; Muñoz, M.F.; Argüelles, S. Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative Med. Cell. Longev.* **2014**, *2014*, 360438. [CrossRef]
47. Yu, J.; Ip, E.; de la Peña, A.; Hou, J.; Sesha, J.; Pera, N.; Farrell, G.C. COX-2 induction in mice with experimental nutritional steatohepatitis: Role as pro-inflammatory mediator. *Hepatology* **2006**, *43*, 826–836. [CrossRef]
48. Da Silva-Santi, L.G.; Antunes, M.M.; Caparroz-Assef, S.M.; Carbonera, F.; Masi, L.N.; Curi, R.; Visentainer, J.V.; Bazotte, R.B. Liver fatty acid composition and inflammation in mice fed with high-carbohydrate diet or high-fat diet. *Nutrients* **2016**, *8*, 682. [CrossRef]
49. Zaki, S.M.; Fattah, S.A.; Hassan, D.S. The differential effects of high-fat and high-fructose diets on the liver of male albino rat and the proposed underlying mechanisms. *Folia Morphol.* **2019**, *78*, 124–136. [CrossRef]
50. Almasri, F.; Collotta, D.; Aimaretti, E.; Sus, N.; Aragno, M.; Dal Bello, F.; Eva, C.; Mastrocola, R.; Landberg, R.; Frank, J.; et al. Dietary intake of fructooligosaccharides protects against metabolic derangements evoked by chronic expose to fructose or galactose in rats. *Mol. Nutr. Food Res.* **2024**, *68*, 2300476. [CrossRef]
51. Tsujimoto, S.; Kishina, M.; Koda, M.; Yamamoto, Y.; Tanaka, K.; Harada, Y.; Yoshida AHisatome, I. Nimesulide, a cyclooxygenase-2 selective inhibitor, suppresses obesity-related non-alcoholic fatty liver disease and hepatic insulin resistance through the regulation of peroxisome proliferator-activated receptor γ. *Int. J. Mol. Med.* **2016**, *38*, 721–728. [CrossRef] [PubMed]
52. Burnett, B.P.; Levy, R.M. 5-Lipoxygenase metabolic contributions to NSAID-induced organ toxicity. *Adv. Ther.* **2012**, *29*, 79–98. [CrossRef]
53. Czapski, G.A.; Czubowicz, K.; Strosznajder, J.B.; Strosznakder, R.P. The lipoxygenases: Their regulation and implication in Alzheimer´s disease. *Neurochem. Res.* **2016**, *41*, 243–257. [CrossRef]
54. Rudrapal, M.; Eltayeb, W.A.; Rakshit, G.; ElArabey, A.A.; Khan, J.; Aldosari, S.M.; Alshehri, B.; Abdalla, M. Dual synergistic inhibition of COX and LOX by potential chemicals from Indian daily spices investigated through detailed computational studies. *Sci. Rep.* **2023**, *13*, 8656. [CrossRef]
55. Lin, W.; Chen, H.; Chen, X.; Guo, C. The roles of neutrophil-derived myeloperoxidase (MPO) in diseases: The new progress. *Antioxidant* **2024**, *13*, 132. [CrossRef] [PubMed]
56. Mazzoli, A.; Crescenzo, R.; Cigliano, L.; Spagnuolo, M.S.; Cancelliere, R.; Gatto, C.; Iossa, S. Early hepatic oxidative stress and mitochondrial changes following western diet in middle aged rats. *Nutrients* **2019**, *11*, 2670. [CrossRef] [PubMed]
57. Lasker, S.; Rahman, M.M.; Parvez, F.; Zamila, M.; Miah, P.; Nahar, K.; Kabir, F.; Sharmin, S.B.; Subhan, N.; Ahsan, G.U.; et al. High-fat diet-induced metabolic syndrome and oxidative stress in obese rats are ameliorated by yogurt supplementation. *Sci. Rep.* **2019**, *9*, 20026. [CrossRef]
58. van Leeuwen, M.; Gijbels, M.J.; Duijvestijn, A.; Smook, M.; van de Gaar, M.J.; Heeringa, P.; de Winther, M.P.; Tervaert, J.W. Accumulation of myeloperoxidase-positive neutrophils in atherosclerotic lesions in LDLR$^{-/-}$ mice. *Arterioscler. Thromb. Vasc. Biol.* **2008**, *28*, 84–89. [CrossRef] [PubMed]
59. Brennan, M.L.; Anderson, M.M.; Shih, D.M.; Qu, X.D.; Wang, X.; Mehta, A.C.; Lim, L.L.; Shi, W.; Hazen, S.L.; Jacob, J.S.; et al. Increased atherosclerosis in myeloperoxidase-deficient mice. *J. Clin. Investig.* **2001**, *107*, 419–430. [CrossRef]
60. Kawachi, Y.; Fujishima, Y.; Nishizawa, H.; Nakamura, T.; Akari, S.; Murase, T.; Saito, T.; Miyazaki, Y.; Nagao, H.; Fukuda, S.; et al. Increased plasma XOR activity induced by NAFLD/NASH and its possible involvement in vascular neointimal proliferation. *JCL Insight* **2021**, *6*, e144762. [CrossRef]
61. Wang, B.; Yang, R.N.; Zhu, Y.R.; Xing, J.C.; Lou, X.W.; He, Y.J.; Ding, Q.L.; Zhang, M.Y.; Qiu, H. Involvement of xanthine oxidase and paraoxonase 1 in the process of oxidative stress in nonalcoholic fatty liver disease. *Mol. Med. Rep.* **2017**, *15*, 387–395. [CrossRef] [PubMed]

62. Hamza, R.Z.; Alsolami, K. Ameliorative effects of orlistat and metformin either alone or in combination on liver functions, structure, immunoreactivity and antioxidant enzymes in experimental induced obesity in male rats. *Heliyon* **2023**, *9*, e18724. [CrossRef] [PubMed]
63. Othman, Z.A.; Zakaria, Z.; Suleiman, J.B.; Ghazali, W.S.W.; Mohamed, M. Anti-atherogenic effects of orlistart on obesity-induced vascular oxidative stress rat model. *Antioxidant* **2021**, *10*, 251. [CrossRef]
64. Othman, Z.A.; Zakaria, Z.; Suleiman, J.B.; Mustaffa, K.M.F.; Jalil, N.A.C.; Wan Ghazali, W.S.; Zulkipli, N.N.; Mohamed, M. Orlistat mitigates oxidative stress-linked myocardial damage via NF-κβ and caspase-dependent activities in obese rats. *Int. J. Mol. Sci.* **2022**, *23*, 10266. [CrossRef] [PubMed]
65. Venkataraman, B.; Almarzooqi, S.; Raj, V.; Bhongade, B.A.; Patil, R.B.; Subramanian, V.S.; Attoub, S.; Rizvi, T.A.; Adrian, T.E.; Subramanya, S.B. Molecular docking identifies 1,8-cineole (eucalyptol) as a novel PPARγagonist that alleviates colon inflammation. *Int. J. Mol. Sci.* **2023**, *24*, 6160. [CrossRef]
66. Khaleel, C.; Tabanca, N.; Buchbauer, G. α-Terpineol, a natural monoterpene: A review of its biological properties. *Open Chem.* **2018**, *16*, 349–361. [CrossRef]
67. Vieira, A.J.; Beserra, F.P.; Souza, M.C.; Totti, B.M.; Rozza, A.L. Limonene: Aroma of innovation in health and disease. *Chem. Biol. Interact.* **2018**, *283*, 97–106. [CrossRef]
68. Alexandrino, T.D.; Moya, A.M.T.M.; de Medeiros, T.D.M.; Morari, J.; Velloso, L.A.; Leal, R.F.; Bicas, J.L. Anti-inflammatory effects of monoterpenoids in rats with TNBS- induced colitis. *PharmaNutrition* **2020**, *14*, 100240. [CrossRef]
69. Pop, R.M.; Sabin, O.; Suciu, Ș.; Vesa, S.C.; Socaci, S.A.; Chedea, V.S.; Bocsan, I.C.; Buzoianu, A.D. Nigella Sativa's Anti-Inflammatory and Antioxidative Effects in Experimental Inflammation. *Antioxidants* **2020**, *9*, 921. [CrossRef]

VIII. DISCUSIÓN GENERAL

Los fitosomas son una estrategia utilizada para mejorar la solubilidad y biodisponibilidad de los extractos herbales (Suryawanshi, 2011). El tamaño de las partículas y la composición de fosfolípidos son factores importantes para obtener estos parámetros. En este estudio se utilizó fosfatidilcolina de soya, ya que este lípido es un componente principal de las membranas y además aporta colina, un sustrato de la colina acetiltransferasa para producir el neurotransmisor acetilcolina. Xie *et al*., (2017) informaron que el uso de fosfatidilcolina de soya para preparar fitosomas cargados con curcumina mostró un tamaño de partícula reducido, una alta carga superficial, además de estabilidad y capacidad de carga del fármaco. De manera similar, los fitosomas generados en este trabajo tuvieron pequeñas formas esféricas, con un tamaño promedio de partícula de 129.8 nm ± 18.30 nm en la emulsión.

El porcentaje de atrapamiento de la sustancia activa se determinó extrayendo fitosomas mediante centrifugación y el sobrenadante se midió por espectroscopía UV-visible. Los resultados mostraron que la eficiencia de atrapamiento (EA) fue del 80.49%. De esta manera, la eficiencia de encapsulación de los fitosomas se representa mediante la concentración de extracto de hoja de *C. citrinus* no unido (200 mg/kg); esto indica que el extracto de hoja y los fosfolípidos de soya reaccionan para formar un complejo con un alto grado de atrapamiento del extracto de hoja.

La estabilidad del fitosoma de *C. citrinus* se mantuvo durante el período de almacenamiento a 20 ± 2°C, sin observarse cambios significativos en el tamaño promedio de las partículas. Sin embargo, las bajas temperaturas causaron un aumento en el tamaño de las partículas, hasta duplicarse. Estos resultados indican que los fitosomas cargados con *C. citrinus* a 20°C permanecieron estables en su tamaño durante tres meses y medio. Este resultado es similar al de un fitosoma cargado con *Cuscuta reflexa* (Alshahrani, 2022).

La limitada solubilidad lipídica de ciertos compuestos bioactivos puede ser una de las principales causas de su baja tasa de absorción (Barani *et al*., 2021). Por ello, el estudio de la solubilidad se configura como un parámetro crucial en el desarrollo de sistemas de liberación más eficientes. En el caso de los fitosomas de *C. citrinus*, estos demostraron una solubilidad completa en cuatro solventes y una solubilidad parcial en un quinto

solvente, alcanzando una solubilidad del 90%. En comparación, con el extracto de *C. citrinus*, tanto con y sin la adición de tween 80, presentó una solubilidad del 80%, mientras que los liposomas elaborados con fosfolípidos de soya lograron valores significativamente inferiores.

La formación de los fitosomas a partir de extractos vegetales se fundamenta en la interacción mediante enlaces de hidrógeno entre los fosfolípidos y los compuestos bioactivos, lo cual potencia la biodisponibilidad y la estabilidad estructural de estos últimos (Tripathy *et al*., 2013). En consecuencia, los fitosomas cargados con el extracto etanólico de *C. citrinus* presentan mejor lipofilicidad e hidrofobicidad que los compuestos bioactivos del extracto etanólico de *C. citrinus*.

En cuanto a los compuestos bioactivos totales (fenoles, flavonoides y terpenos) y la capacidad antioxidante en el extracto de *C. citrinus* y los fitosomas de *C. citrinus* no existen diferencias significativas. Este resultado indica que durante el proceso de creación de los fitosomas se conservaron tanto los compuestos bioactivos como la capacidad antioxidante. Este resultado coincide con los hallazgos de Saonere *et al*., (2023) quienes encontraron capacidad antioxidante en un complejo fitofosfolípido de *Glycyrrhiza glabra*.

Los principales compuestos del extracto y del fitosoma de *C. citrinus* fueron el 1,8-cineol y α-terpineol cuantificados mediante GC/MS. Se ha informado que estos dos monoterpenos poseen efectos hepatoprotectores, antivirales, antimicrobianos, antioxidantes y anticancerosos (Wojtunik-Kulesza *et al*., 2019; López-Mejía *et al*., 2021), lo que sugiere que *C. citrinus* podría constituir una herramienta farmacológica alternativa para tratar el estrés oxidante en algunas enfermedades. Los compuestos fenólicos y flavonoides fueron identificados según su tiempo de retención en HPLC. Se encontró ácido elágico en *C. citrinus* por primera vez. El ácido gálico, el ácido p-cumárico y el ácido elágico son los compuestos identificados en el extracto y el fitosoma de *C. citrinus*. Se ha informado que estos ácidos fenólicos poseen actividades anticancerosas, antivirales, antioxidantes y antiinflamatorias (Rahman *et al*., 2021). La concentración de ácido gálico, ácido p-cumárico y ácido elágico fue muy similar en el extracto y en el fitosoma de *C. citrinus*.

Las metodologías DPPH, ABTS y FRAP se utilizan comúnmente para evaluar la capacidad antioxidante de los extractos vegetales. DPPH y ABTS se basan en la capacidad de donación de hidrógenos o electrones, mientras que FRAP se basa en la capacidad de reducir hierro férrico a ferroso (Boligon *et al*., 2014). Ortega-Pérez *et al*., (2022) informó sobre la elevada capacidad antioxidante y el contenido total de compuestos fenólicos, flavonoides y terpenos en el extracto de hojas de *C. citrinus*. De igual manera, en este estudio tanto el extracto de *C. citrinus* como los fitosomas de *C. citrinus* exhibieron una actividad inhibitoria significativa contra los radicales DPPH y ABTS, así como una alta capacidad para reducir hierro férrico a ferroso. En un estudio realizado por Khan *et al*., (2012) ha demostrado la correlación entre el contenido total de fenoles, flavonoides y sus actividades antioxidantes. Nuestro estudio también encontró dicha correlación, sugiriendo que los compuestos en *C. citrinus* producen el efecto antioxidante al actuar como donadores de hidrógeno, atrapadores de oxígeno singlete y agentes reductores (Shan *et al*., 2005).

El uso de fitosomas de curcumina contra la toxicidad hepática inducida por paracetamol en ratones mostró un aumento en las actividades enzimáticas antioxidantes y una reducción en los productos de lipoperoxidación (Tung *et al*., 2017). Deleanu *et al*., (2023) informó que los fitosomas con extracto de rizomas de jengibre y escaramujos aumentan la biodisponibilidad, las propiedades antioxidantes y disminuyen la inflamación sistémica inducida por LPS en ratones. Estos resultados sugieren que el uso de fitosomas puede mejorar las propiedades enzimáticas antioxidantes y reducir la inflamación durante el estrés oxidante (Baradaran *et al*., 2020).

Las ratas alimentadas con una dieta alta en grasas (HFD) mostraron un aumento de peso corporal en comparación con los otros grupos. Por el contrario, la administración de fitosomas cargados con *C. citrinus* a los animales alimentados con HFD provocó una reducción significativa en el peso corporal. Estos resultados coinciden con los de Ortega-Pérez *et al*., (2022) quienes demostraron que el extracto de *C. citrinus* inhibe la actividad de la lipasa de manera dependiente de la dosis, lo cual puede explicar la reducción del peso. En cuanto a la dosificación de fitosomas (50, 100 y 200 mg/kg) y el extracto de *C. citrinus* (250 mg/kg), este estudio mostró efectos similares en todos ellos para reducir el

peso. Este estudio sugiere que los fitosomas cargados con el extracto de *C. citrinus* presentan una actividad antiobesogénica más fuerte que el extracto de *C. citrinus* por sí solo; este resultado es probablemente debido a la alta biodisponibilidad, la cual mejora la solubilidad, permitiendo reducir la dosis. La formulación fitosomal de *Callistemon citrinus* mejoró la biodisponibilidad oral. Incluso la administración de dosis bajas redujo los parámetros morfométricos y bioquímicos en los animales tratados.

Es importante destacar que incluso una baja concentración de fitosomas (50 mg/kg) fue suficiente para producir efectos benéficos. Ortega-Pérez *et al*., (2023) informó previamente que el tratamiento con fitosomas disminuyó significativamente los niveles de triacilglicéridos plasmáticos en ratas alimentadas con una dieta alta en grasas y fructosa, lo cual mejoró el metabolismo lipídico y redujo la acumulación de grasa. Esto se evidenció en el índice de adiposidad, que fue de 9.4 ± 0.62 en el grupo con dieta alta en grasas y fructosa y de 4.02 ± 0.62 en el grupo tratado con fitosomas a una dosis de 200 mg/kg.
El efecto de los fitosomas a una dosis de 50 mg/kg fue similar al de las dosis de 100 y 200 mg/kg, así como al del extracto de *C. citrinus*. Esta similitud puede explicarse por el hecho de que el compuesto o compuestos responsables de producir el efecto, contenidos en el extracto, alcanzan saturación a 50 mg/kg o incluso a concentraciones más bajas.
Sin embargo, el hecho de que el efecto persista incluso a esta dosis mínima indica la posible existencia de una ventana terapéutica por debajo de los 50 mg/kg, lo que abre un panorama para optimizar la dosis requerida y minimizar potenciales efectos adversos. Por lo que se necesitan estudios adicionales para establecer el rango de dosis terapéutico de los fitosomas.
Este estudio también reveló que el hígado es el primer órgano en dañarse durante una dieta alta en grasas y fructosa. Estudios previos han reportado que una dieta con fructosa es más obesogénica que una dieta con sacarosa (DiNicolantonio *et al*., 2017). Además, investigaciones sobre dietas altas en grasas han mostrado que estas son suficientes para inducir un aumento de las ROS y el estado inflamatorio (Grujić-Milanović *et al*., 2021), o para provocar una disminución en las actividades de la superóxido dismutasa (SOD), catalasa (CAT) y glutatión peroxidasa (GPx) (Emami *et al*., 2016; Das y Choudhuri, 2020). Otros estudios han reportado que las dietas altas en grasa y azúcar aumentan las

proteínas proinflamatorias más que las dietas altas en grasa o altas en azúcar (Masi *et al*., 2017). Debido a su elevado poder edulcorante, la fructosa ha sido utilizada en lugar de la azúcar de caña en los alimentos. Sin embargo, este azúcar está asociado a importantes problemas de salud, incluyendo el aumento de la obesidad y sus enfermedades relacionadas. La fructólisis ocurre principalmente en el hígado, lo que incrementa la síntesis de ácidos grasos, triglicéridos, ácido úrico y el estrés oxidante (Ackerman *et al*., 2005). Diversos estudios han indicado que una dieta alta en fructosa puede derivar en síndrome metabólico e hiperinsulinemia (Toop y Gentili, 2016).

Comparar los resultados entre estudios puede ser complicado debido a variaciones en la concentración y el tipo de grasa, la forma del azúcar (líquida o sólida) y la duración de la administración. Los estudios (Toop y Gentili, 2016; Mastrocola *et al*., 2018) han demostrado que la microbiota intestinal y la producción de productos finales de glicación avanzada están influenciados por la forma en la que se consume la fructosa, ya sea en forma sólida o líquida. Además, Collotta *et al*., (2018) demostró que la administración de fructosa en forma líquida conllevaba a niveles elevados de citocinas proinflamatorias (TNF-α e IL-6).

En un estudio realizado por García-Beltrán *et al*., 2023, se observó que los niveles de actividad de las enzimas catalasa (CAT), superóxido dismutasa (SOD), glutatión S-transferasa (GST), glutatión peroxidasa (GPx) y quinona reductasa (QR) eran similares a los del grupo de control tras 13 semanas. Sin embargo, después de 21 semanas, todas las actividades enzimáticas disminuyeron en comparación con el grupo de control. Por el contrario, nuestro estudio encontró niveles disminuidos de SOD y CAT y niveles aumentados de GPx y GST en el hígado y el corazón de las ratas alimentadas con una dieta alta en grasas (HFD). Además, las actividades de CAT y GST se redujeron en los corazones de las ratas alimentadas con HFD durante 15 semanas. Aunque ambas dietas contienen altos niveles de grasa y fructosa, las diferencias en los tipos de roedores y en los métodos de administración de fructosa pueden explicar la variación observada. Además, GPx es una enzima antioxidante de defensa primaria, mientras que GST es una enzima de detoxificación que combate compuestos tóxicos. Ambas enzimas desempeñan un papel crucial en el mantenimiento de la homeostasis de GSH (Mazari *et al*., 2023; Pei *et al*., 2023). Nuestro estudio encontró que el aumento en las actividades de GPx y GST

se correlaciona directamente con la disminución de los niveles de GSH en el hígado y el corazón. El GSH, actuando como un agente reductor, cumple numerosas funciones, incluyendo ser un sustrato para las enzimas GPx y GST, además de brindar protección contra las ROS. Basado en nuestra investigación, una dieta alta en grasas y fructosa (HFD) podría reducir los niveles de GSH y aumentar los niveles de MDA, HNE y AOPP en los tejidos. Esto podría deberse al aumento en la generación de radicales libres, resultando en estrés oxidativo en los tejidos.

Nuestros resultados contrastan con los reportados por Norman *et al*., (2011), quienes observaron reducciones en las actividades de GPx, GST y PON1 en los hígados y corazones de ratas alimentadas con una dieta que contenía 46% de grasa y 24% de sacarosa. En cambio, nuestro estudio involucró una dieta compuesta por 30% de grasa y 25% de fructosa. Nuestro estudio demostró un aumento notable en la actividad de PON1 en los hígados y corazones de ratas tratadas con HFD. PON1 es reconocida como una enzima antioxidante debido a sus efectos protectores en el suero, estando unida a HDL y previniendo la oxidación de LDL (Marsillach *et al*., 2009). El malondialdehído (MDA) y el hidroxinonenal (HNE) son los principales productos de la peroxidación lipídica, conocidos por su reactividad hacia proteínas y ADN, causando daño a estas biomoléculas (Ayala *et al*., 2014). Además, estos productos pueden influir en el reclutamiento de citocinas como TNF-α, IL-1β, IL-6 y COX-2. Se han identificado dos tipos de enzimas COX: COX-1, una enzima constitutiva presente en la mayoría de los tejidos, y COX-2, una enzima inducible expresada durante los procesos inflamatorios (Yu *et al*., 2006). Según Silva-Santi *et al*., (2016), una dieta alta en carbohidratos está asociada con niveles más altos de marcadores inflamatorios que una dieta alta en grasas. La combinación de una dieta alta en grasas y azúcares también conlleva un estrés oxidante mayor que una dieta alta en grasas o en azúcares por sí solas (Zaki *et al*., 2019). Además, Almasri *et al*., (2024) encontró que una dieta que contiene 20% de grasa y 25% de fructosa incrementaba los marcadores inflamatorios en el hígado y el músculo esquelético de las ratas.

El Orlistat es un medicamento utilizado para tratar la obesidad inhibiendo las lipasas gástrica y pancreática, previniendo la absorción de triacilglicéridos dietéticos. Estudios recientes también han demostrado sus propiedades antioxidantes. Nuestro estudio

observó esta capacidad antioxidante en el hígado y el corazón de las ratas alimentadas con una dieta alta en grasas y fructosa. Este hallazgo concuerda con los resultados presentados por Hamza y Alsolami (2023), Othman *et al*., (2021) y Othman *et al*., (2022), quienes informaron propiedades antiaterogénicas y antioxidantes y una regulación en la expresión de Nrf2. Nuestro estudio reveló un aumento significativo en la actividad de glutatión-S-transferasa en los corazones de las ratas del grupo de Orlistat en comparación con todos los demás grupos. Nuestro estudio administró Orlistat durante 15 semanas, lo cual es más prolongado que la mayoría de otros estudios. Esto podría explicar el resultado. Este uso prolongado de Orlistat puede conducir a la generación de compuestos nocivos. La enzima de detoxificación GST ayuda a transformar estos compuestos en conjugados de GSH para su eliminación. Además, se detecta sobreexpresión de GST en ciertas condiciones médicas (Mazari *et al*., 2023).

El estudio reveló que el Orlistat impacta positivamente en enzimas proinflamatorias como MPO, XO, COX-2 y LOX-5, así como en los niveles de AOPP y HNE, que sirven como biomarcadores de estrés oxidativo. Se ha informado que el 1,8-cineol, el compuesto principal en *C. citrinus*, incrementa el factor de transcripción relacionado con el factor 2 nuclear eritroide (Nrf2) (Venkataraman *et al*., 2023). Además, el 1,8-cineol, el limoneno y el α-terpineol reducen los niveles de TNF-α, IL-6 y leptina (Ayala-Ruiz *et al*., 2022).

El uso tanto del extracto como de los fitosomas de *C. citrinus* en este estudio sirve para validar sus propiedades protectoras y antiinflamatorias, que se pueden atribuir a la composición de sus principales fitoquímicos, 1,8-cineol, limoneno y α-terpineol (Petronilho *et al*., 2013; Ayala-Ruiz *et al.*, 2022). El extracto contiene ácido gálico, ácido elágico, ácido p-cumárico y floroglucinol, los cuales exhiben diversas actividades biológicas, como propiedades antibacterianas, antioxidantes, antiinflamatorias y antidiabéticas (Ortega-Pérez *et al*., 2023). Estos compuestos trabajan en conjunto para promover la actividad antiinflamatoria en ratas alimentadas con una dieta alta en grasas y fructosa. Con base en el estudio, se encontró que el extracto y los fitosomas de *C. citrinus* fueron efectivos para reducir los niveles de MPO, 5-LOX y COX-2. Esto indica que *C. citrinus* podría ayudar a disminuir la producción de productos clorados y la infiltración de células inmunes en los tejidos. Además, se observó que también disminuye la actividad de COX-2, lo que resulta en niveles reducidos de PGE2, atribuidos a la

presencia de limoneno, 1,8-cineol y α-terpineol (Khaleel *et al*., 2018). El limoneno reduce la inflamación al disminuir la actividad de 5-LOX y reducir los niveles de LTB4, previniendo así el proceso inflamatorio (Vieira *et al*., 2018). Además, tanto el limoneno como el terpineol demuestran efectos antiinflamatorios al disminuir los niveles de citocinas proinflamatorias como TNF, IL-6, leptina y AOPP en un modelo de inducción de colitis (Alexandrino *et al*., 2020). El limoneno reduce la producción de ROS y RNS al incrementar la actividad del sistema de defensa antioxidante en células de ratas diabéticas y en un estado inflamatorio (Vieira *et al*., 2018). También promueve una reducción de MDA (Pop *et al*., 2020).

En nuestro estudio, observamos un aumento significativo en los niveles de biomarcadores de estrés oxidativo, como MDA, HNE (indicativo de peroxidación lipídica) y AOPP (indicativo de oxidación de proteínas), en los hígados y corazones de ratas alimentadas con una dieta alta en grasas. Sin embargo, la administración de extracto de *C. citrinus* y fitosomas de *C. citrinus* condujo a una reducción en estos niveles en comparación con el grupo de control, lo que sugiere que *C. citrinus* puede eliminar eficazmente los radicales libres. Los resultados están en línea con las funciones de las enzimas antioxidantes. Un desequilibrio en el sistema antioxidante y la generación de especies reactivas de oxígeno provocaron estrés oxidativo, el cual se asocia con diversos trastornos en la obesidad. Por lo tanto, el tratamiento con compuestos que posean una alta capacidad antioxidante puede ayudar a mitigar los problemas relacionados con esta condición. Es esencial considerar cuidadosamente la dosis y la biodisponibilidad de los compuestos destinados a ser utilizados como agentes terapéuticos. El presente estudio observó que la concentración más baja de los fitosomas de *C. citrinus* mostró un patrón similar a la concentración más alta, lo que subraya su importancia.

IX. CONCLUSIÓN GENERAL

La formulación fitosomal de *Callistemon citrinus* mejoró la biodisponibilidad oral, conservó los compuestos principales y se mantuvo estable durante tres meses y medio cuando se almacenó a 20 °C. Los fitosomas de *C. citrinus*, incluso en dosis bajas, redujeron los parámetros morfométricos y bioquímicos en ratas Wistar alimentadas con una dieta alta en grasas. Los resultados también revelaron que la suplementación con fitosomas de *Callistemon citrinus* disminuyó el peso excesivo en los animales.

Los fitosomas extraídos de *C. citrinus* demuestran la capacidad de mitigar factores de riesgo asociados con el estrés oxidante, reducir el proceso inflamatorio y potenciar la actividad de las enzimas antioxidantes. Cabe destacar que, aun en dosis bajas, la suplementación con fitosomas previno eficazmente los efectos perjudiciales del consumo de una dieta alta en grasas y fructosa.

- Perspectivas y recomendaciones para futuros trabajos.

Para fortalecer la investigación futura sobre el *Callistemon citrinus* en la prevención y tratamiento de la obesidad, se sugieren las siguientes perspectivas y recomendaciones:

1. Optimización de la Formulación Fitosomal

La investigación adicional debería centrarse en mejorar la formulación de los fitosomas, evaluando la posibilidad de incorporar otros compuestos naturales sinérgicos, o centrándose en los ácidos fenólicos o terpenos mayoritarios, lo cual podría maximizar sus propiedades antioxidantes y antiinflamatorias. Esto incluiría el uso de tecnologías avanzadas de liberación para aumentar aún más la biodisponibilidad de los compuestos bioactivos.

2. Evaluación de Mecanismos Moleculares

Investigar los mecanismos moleculares precisos mediante los cuales los fitosomas de *C. citrinus* actúan sobre las vías inflamatorias y oxidativas proporcionará una comprensión más profunda de su efecto anti-obesogénico. Esto podría involucrar estudios a nivel de expresión génica y proteómica para identificar los mecanismos específicos en los tejidos afectados.

3. Estudio de Diferentes Modelos de Obesidad

A fin de comprender la aplicabilidad y eficacia de los fitosomas de *C. citrinus* en distintos tipos de obesidad, se recomienda extender los estudios a modelos animales variados, como aquellos con predisposición genética a la obesidad. Esto permitiría obtener una visión más amplia de sus efectos y optimizar su aplicación.

Estas recomendaciones contribuirán a que la investigación sobre *Callistemon citrinus* y sus formulaciones fitosomales se traduzca en estrategias terapéuticas más efectivas y seguras para el tratamiento de la obesidad y otras enfermedades crónicas relacionadas.

X. BIBLIOGRAFÍA COMPLEMENTARIA

Abuhelwa, A. Y., Williams, D. B., Upton, R. N. y Foster, D. J. 2017. Food, Gastrointestinal pH, and Models of Oral Drug Absorption. European Journal of Pharmaceutics and Biopharmaceutics, 112, 234-248.

Ackerman, Z., Oron-Herman, M., Grozovski, M., Rosenthal, T., Pappo, O., Link, G., y Sela, B. A. 2005. Fructose-Induced Fatty Liver Disease: Hepatic Effects of Blood Pressure and Plasma Triglyceride Reduction. Hypertension, 45(5), 1012-1018.

Adiki, S. K., Sangeetha, S., Kamireddy, S., Katakam, P. y Obilineni, I. 2023. Phytosomes: A Novel Phytoconstituent Delivery Approach to Improve the Efficacy of Obesity Treatment. Current Nutrition & Food Science, 19(3), 229-237.

Ahmed, R., Tariq, M., Ahmad, I. S., Fouly, H., Hasan, A., y Kushad, M. 2019. Poly (lactic-co-glycolic acid) Nanoparticles Loaded with *Callistemon citrinus* Phenolics Exhibited Anticancer Properties against Three Breast Cancer Cell Lines. Journal of Food Quality, 2019(1), 2638481.

Alexandrino, T. D., Moya, A. M. T. M., de Medeiros, T. D. M., Morari, J., Velloso, L. A., Leal, R. F., ... y Bicas, J. L. 2020. Anti-Inflammatory Effects of Monoterpenoids in Rats with TNBS-induced Colitis. PharmaNutrition, 14, 100240.

Alharbi, W. S., Almughem, F. A., Almehmady, A. M., Jarallah, S. J., Alsharif, W. K., Alzahrani, N. M. y Alshehri, A. A. 2021. Phytosomes as an Emerging Nanotechnology Platform for the Topical Delivery of Bioactive Phytochemicals. Pharmaceutics, 13(9), 1475.

Ali, S. S., Ahsan, H., Zia, M. K., Siddiqui, T. y Khan, F. H. 2020. Understanding Oxidants and Antioxidants: Classical Team with New Players. Journal of food Biochemistry, 44(3), e13145.

Almasri, F., Collotta, D., Aimaretti, E., Sus, N., Aragno, M., Dal Bello, F., ... y Collino, M. 2024. Dietary Intake of Fructooligosaccharides Protects against Metabolic Derangements Evoked by Chronic Exposure to Fructose or Galactose in Rats. Molecular Nutrition & Food Research, 68(4), 2300476.

Alqahtani, M. S., Kazi, M., Alsenaidy, M. A. y Ahmad, M. Z. 2021. Advances in Oral Drug Delivery. Frontiers in Pharmacology, 12, 618411.

Alshahrani, S. M. 2022. Optimization and Characterization of *Cuscuta reflexa* Extract Loaded Phytosomes by the Box-Behnken Design to Improve the Oral Bioavailability. Journal of Oleo Science, 71(5), 671-683.

Amerikanou, C. y Papada, E. 2023. Plant-Derived Natural Products and Their Biomedical Properties: Recent Advances and Future Directions. Life, 13(10), 2105.

Ayala, A., Muñoz, M. F., y Argüelles, S. 2014. Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-nonenal. Oxidative Medicine and Cellular Longevity, 2014(1), 360438.

Ayala-Ruiz, L. A., Ortega-Pérez, L. G., Piñón-Simental, J. S., Magaña-Rodríguez, O. R., Meléndez-Herrera, E. y Rios-Chavez, P. 2022. Role of the Major Terpenes of *Callistemon citrinus* against the Oxidative Stress During a Hypercaloric Diet in Rats. Biomedicine & Pharmacotherapy, 153, 113505.

Baradaran, S., Hajizadeh Moghaddam, A., Khanjani Jelodar, S., y Moradi-Kor, N. 2020. Protective Effects of Curcumin and its Nano-Phytosome on Carrageenan-Induced Inflammation in Mice Model: Behavioral and Biochemical Responses. Journal of Inflammation Research, 45-51.

Barani, M., Sangiovanni, E., Angarano, M., Rajizadeh, M. A., Mehrabani, M., Piazza, S., ... y Nematollahi, M. H. 2021. Phytosomes as Innovative Delivery Dystems for Phytochemicals: A Comprehensive Review of Literature. International Journal of Nanomedicine, 6983-7022.

Belančić, A., Klobučar, S. y Rahelić, D. 2023. Current Obstacles (With Solutions) in Type 2 Diabetes Management, Alongside Future Directions. Diabetology, 4(3), 376-378.

Boligon, A. A., Machado, M. M., y Athayde, M. L. 2014. Technical Evaluation of Antioxidant Activity. Med. Chem, 4(7), 517-522.

Bondia-Pons, I., Ryan, L. y Martinez, J. A. 2012. Oxidative Stress and Inflammation Interactions in Human Obesity. Journal of Physiology and Biochemistry, 68, 701-711.

Brewer, M. S. 2011. Natural Antioxidants: Sources, Compounds, Mechanisms of Action, and Potential Applications. Comprehensive Reviews in Food Science and Food Safety, 10(4), 221-247.

Chakhtoura, M., Haber, R., Ghezzawi, M., Rhayem, C., Tcheroyan, R. y Mantzoros, C. S. 2023. Pharmacotherapy of Obesity: An Update on the Available Medications and Drugs under Investigation. EClinicalMedicine, 58.

Collotta, D., Lucarini, L., Chiazza, F., Cento, A. S., Durante, M., Sgambellone, S., ... y Collino, M. 2018. Reduced Susceptibility to Sugar-Induced Metabolic Derangements and Impairments of Myocardial Redox Signaling in Mice Chronically Fed with D-Tagatose when Compared to Fructose. Oxidative Medicine and Cellular Longevity, 2018(1), 5042428.

Da Silva-Santi, L. G., Antunes, M. M., Caparroz-Assef, S. M., Carbonera, F., Masi, L. N., Curi, R., ... y Bazotte, R. B. 2016. Liver Fatty Acid Composition and Inflammation in Mice Fed With High-Carbohydrate Diet or High-Fat Diet. Nutrients, 8(11), 682.49

Dajic Stevanovic, Z., Sieniawska, E., Glowniak, K., Obradovic, N. y Pajic-Lijakovic, I. 2020. Natural Macromolecules as Carriers for Essential Oils: From Extraction to Biomedical Application. Frontiers in Bioengineering and Biotechnology, 8, 563.

Das, S., y Choudhuri, D. 2020. Calcium Supplementation Shows a Hepatoprotective Effect against High-Fat Diet by Regulating Oxidative-Induced Inflammatory Response and Lipogenesis Activity in Male Rats. Journal of Traditional and Complementary Medicine, 10(5), 511-519.

Deleanu, M., Toma, L., Sanda, G. M., Barbălată, T., Niculescu, L. Ş., Sima, A. V., ... y Stancu, C. S. 2023. Formulation of Phytosomes with Extracts of Ginger Rhizomes and Rosehips with Improved Bioavailability, Antioxidant and Anti-Inflammatory Effects *in vivo*. Pharmaceutics, 15(4), 1066.

Dikshit, P. K., Kumar, J., Das, A. K., Sadhu, S., Sharma, S., Singh, S., ... y Kim, B. S. 2021. Green Synthesis of Metallic Nanoparticles: Applications and Limitations. Catalysts, 11(8), 902.

DiNicolantonio, J. J., Subramonian, A. M., y O'Keefe, J. H. 2017. Added Fructose as a Principal Driver of Non-Alcoholic Fatty Liver Disease: A Public Health Crisis. Open Heart, 4(2), e000631.

Emami, S. R., Jafari, M., Haghshenas, R., y Ravasi, A. 2016. Impact of Eight Weeks Endurance Training on Biochemical Parameters and Obesity-Induced Oxidative Stress in High Fat Diet-Fed Rats. Journal of Exercise Nutrition & Biochemistry, 20(1), 29.

Fayemi, P. O., Ozturk, I., Kaan, D., Özcan, S., Yerer, M. B., Dokumaci, A. H., y Yetim, H. 2019. Bioactivities of Phytochemicals in *Callistemon citrinus* against Multi-Resistant Foodborne Pathogens, Alpha Glucosidase Inhibition and MCF-7 Cancer Cell Line. Biotechnology & Biotechnological Equipment, 33(1), 764-778.

Gallagher, E. J., Fierz, Y., Ferguson, R. D. y LeRoith, D. 2010. The Pathway from Diabetes and Obesity to Cancer, on the Route to Targeted Therapy. Endocrine Practice, 16(5), 864-873.

García-Beltrán, A., Martínez, R., Porres, J. M., Arrebola, F., Artero, I. R., Galisteo, M., ... y López-Jurado, M. 2023. Novel Insights and Mechanisms of Diet-Induced Obesity: Mid-Term versus Long-Term Effects on Hepatic Transcriptome and Antioxidant Capacity in Sprague-Dawley Rats. Life Sciences, 324, 121746.

Gharibvand, Z. S., Behbahani, B. A., Noshad, M. y Jooyandeh, H. 2022. Green Synthesis of Silver Nanoparticles using *Callistemon citrinus* Leaf Extract and Evaluation of its Antibacterial Activity.

Grujić-Milanović, J. D., Miloradović, Z. Z., Mihailović-Stanojević, N. D., Banjac, V. V., Vidosavljević, S., Ivanov, M. S., ... y Jovović, D. M. 2021. Excesive Consumption of Unsaturated Fatty Acids Leads to Oxidative and Inflammatory Instability in Wistar rats. Biomedicine & Pharmacotherapy, 139, 111691.

Hajam, Y. A., Rani, R., Ganie, S. Y., Sheikh, T. A., Javaid, D., Qadri, S. S. y Reshi, M. S. 2022. Oxidative Stress in Human Pathology and Aging: Molecular Mechanisms and Perspectives. Cells, 11(3), 552.

Hamza, R. Z., y Alsolami, K. 2023. Ameliorative Effects of Orlistat and Metformin Either Alone or in Combination on Liver Functions, Structure, Immunoreactivity and Antioxidant Enzymes in Experimentally Induced Obesity in Male Rats. Heliyon, 9(8).

Higuchi, Y., Otsu, K., Nishida, K., Hirotani, S., Nakayama, H., Yamaguchi, O. y Hori, M. 2003. The Small GTP-Binding Protein Rac1 Induces Cardiac Myocyte Hypertrophy Through the Activation of Apoptosis Signal-Regulating Kinase 1 and Nuclear Factor-κB. Journal of Biological Chemistry, 278(23), 20770-20777.

Hussain, F. y Kayani, H. U. R. 2020. Aging-Oxidative Stress, Antioxidants and Computational modeling. Heliyon, 6(5).

Khaleel, C., Tabanca, N., y Buchbauer, G. 2018. α-Terpineol, a Natural Monoterpene: A Review of Its Biological Properties. Open Chemistry, 16(1), 349-361.

Khan, R. A., Khan, M. R., Sahreen, S., y Ahmed, M. 2012. Evaluation of Phenolic Contents and Antioxidant Activity of Various Solvent Extracts of *Sonchus asper* (L.) Hill. Chemistry Central Journal, 6, 1-7.

Khanh, P. N., Đuc, H. V., Huong, T. T., Ha, V. T., Van, D. T., Kim, Y. H. y Cuong, N. M. 2016. Phenolic Compounds from *Callistemon citrinus* Leaves and Stems. Vietnam Journal of Science and Technology, 54(2), 190-197.

Khanna, D., Khanna, S., Khanna, P., Kahar, P. y Patel, B. M. 2022. Obesity: A Chronic Low-Grade Inflammation and Its Markers. Cureus, 14(2).

Lee, M. T., Lin, W. C., Yu, B. y Lee, T. T. 2017. Antioxidant Capacity of Phytochemicals and Their Potential Effects on Oxidative Status in Animals-A Review. Asian-Australasian Journal of Animal Sciences, 30(3), 299.

Lee, P. C., Lim, C. H., Asokkumar, R. y Chua, M. W. J. 2023. Current Treatment Landscape for Obesity in Singapore. Singapore Medical Journal, 64(3), 172-181.

López-Mejía, A., Ortega-Pérez, L. G., Godínez-Hernández, D., Nateras-Marín, B., Meléndez-Herrera, E. y Rios-Chavez, P. 2019. Chemopreventive Effect of *Callistemon citrinus* (Curtis) Skeels against Colon Cancer Induced by 1, 2-Dimethylhydrazine in Rats. Journal of Cancer Research and Clinical Oncology, 145, 1417-1426.

López-Mejía, A., Ortega-Pérez, L. G., Magaña-Rodríguez, O. R., Ayala-Ruiz, L. A., Piñón-Simental, J. S., Hernández, D. G., y Rios-Chavez, P. 2021. Protective Effect of *Callistemon citrinus* on Oxidative Stress in Rats with 1, 2-Dimethylhydrazine-Induced Colon Cancer. Biomedicine & Pharmacotherapy, 142, 112070.

Marsillach, J., Camps, J., Ferré, N., Beltran, R., Rull, A., Mackness, B., ... y Joven, J. 2009. Paraoxonase-1 is Related to Inflammation, Fibrosis and PPAR Delta in Experimental Liver Disease. BMC Gastroenterology, 9, 1-13.

Martemucci, G., Costagliola, C., Mariano, M., D'andrea, L., Napolitano, P. y D'Alessandro, A. G. 2022. Free Radical Properties, Source and Targets, Antioxidant Consumption and Health. Oxygen, 2(2), 48-78.

Martínez-Martínez, E. y Cachofeiro, V. 2022. Oxidative Stress in Obesity. Antioxidants, 11(4), 639.

Masi, L. N., Martins, A. R., Crisma, A. R., do Amaral, C. L., Davanso, M. R., Serdan, T. D. A., ... y Curi, R. 2017. Combination of a High-Fat Diet with Sweetened Condensed Milk Exacerbates Inflammation and Insulin Resistance Induced by Each Separately in Mice. Scientific Reports, 7(1), 3937.

Masood, B. y Moorthy, M. 2023. Causes of Obesity: A Review. Clinical Medicine, 23(4), 284-291.

Mastrocola, R., Ferrocino, I., Liberto, E., Chiazza, F., Cento, A. S., Collotta, D., ... y Collino, M. 2018. Fructose Liquid and Solid Formulations Differently Affect Gut Integrity, Microbiota Composition and Related Liver Toxicity: A Comparative *In Vivo* Study. The Journal of Nutritional Biochemistry, 55, 185-199.

Mazari, A. M., Zhang, L., Ye, Z. W., Zhang, J., Tew, K. D., y Townsend, D. M. 2023. The Multifaceted Role of Glutathione S-Transferases in Health and Disease. Biomolecules, 13(4), 688.

Noeman, S. A., Hamooda, H. E., y Baalash, A. A. 2011. Biochemical Study of Oxidative Stress Markers in the Liver, Kidney and Heart of High Fat Diet Induced Obesity in Rats. Diabetology & Metabolic Syndrome, 3, 1-8.

Ofori, E. K. 2024. Potentials of Food Phytochemicals in the Treatment and Management of Obesity. In Plant Food Phytochemicals and Bioactive Compounds in Nutrition and Health (pp. 331-363). CRC Press.

Ortega-Pérez, L. G., Ayala-Ruiz, L. A., Magaña-Rodríguez, O. R., Piñón-Simental, J. S., Aguilera-Méndez, A., Godínez-Hernández, D. y Rios-Chavez, P. 2023. Development and Evaluation of Phytosomes Containing *Callistemon citrinus* Leaf Extract: A Preclinical Approach for the Treatment of Obesity in a Rodent Model. Pharmaceutics, 15(9), 2178.

Ortega-Pérez, L. G., Piñón-Simental, J. S., Magaña-Rodríguez, O. R., Lopéz-Mejía, A., Ayala-Ruiz, L. A., García-Calderón, A. J., ... y Rios-Chavez, P. 2022. Evaluation of the Toxicology, Anti-Lipase, and Antioxidant Effects of *Callistemon citrinus* in Rats Fed with a High Fat-Fructose Diet. Pharmaceutical Biology, 60(1), 1384-1393.

Othman, Z. A., Zakaria, Z., Suleiman, J. B., Ghazali, W. S. W., y Mohamed, M. 2021. Anti-Atherogenic Effects of Orlistat on Obesity-Induced Vascular Oxidative Stress Rat Model. Antioxidants, 10(2), 251.

Othman, Z. A., Zakaria, Z., Suleiman, J. B., Mustaffa, K. M. F., Jalil, N. A. C., Wan Ghazali, W. S., ... y Mohamed, M. 2022. Orlistat Mitigates Oxidative Stress-Linked Myocardial Damage Via NF-κβ-and Caspase-Dependent Activities in Obese Rats. International Journal of Molecular Sciences, 23(18), 10266.

Paosen, S., Saising, J., Septama, A. W. y Voravuthikunchai, S. P. 2017. Green Synthesis of Silver Nanoparticles Using Plants from Myrtaceae family and Characterization of Their Antibacterial Activity. Materials Letters, 209, 201-206.

Pei, J., Pan, X., Wei, G., y Hua, Y. 2023. Research Progress of Glutathione Peroxidase Family (GPX) in Redoxidation. Frontiers in Pharmacology, 14, 1147414.

Pérez-Torres, I., Castrejón-Téllez, V., Soto, M. E., Rubio-Ruiz, M. E., Manzano-Pech, L. y Guarner-Lans, V. 2021. Oxidative Stress, Plant Natural Antioxidants, and Obesity. International Journal of Molecular Sciences, 22(4), 1786.

Permana, A. D., Utami, R. N., Courtenay, A. J., Manggau, M. A., Donnelly, R. F. y Rahman, L. 2020. Phytosomal Nanocarriers as Platforms for Improved Delivery of Natural Antioxidant and Photoprotective Compounds in Propolis: An Approach for Enhanced Both Dissolution Behaviour in Biorelevant Media and Skin Retention Profiles. Journal of Photochemistry and Photobiology B: Biology, 205, 111846.

Petronilho, S., Rocha, S. M., Ramírez-Chávez, E., Molina-Torres, J. y Rios-Chavez, P. 2013. Assessment of the Terpenic Profile of *Callistemon citrinus* (Curtis) Skeels from Mexico. Industrial Crops and Products, 46, 369-379.

Piñón-Simental, J. S., Ayala-Ruiz, L. A., Ortega-Pérez, L. G., Magaña-Rodríguez, O. R., Meléndez-Herrera, E., Aguilera-Méndez, A. y Rios-Chavez, P. 2024. Use of *Callistemon citrinus* as a Gastroprotective and Anti-Inflammatory Agent on Indomethacin-Induced Gastric Ulcers in Obese Rats. PeerJ, 12, e17062.

Pop, R. M., Sabin, O., Suciu, Ș., Vesa, S. C., Socaci, S. A., Chedea, V. S., ... y Buzoianu, A. D. 2020. Nigella Sativa's Anti-Inflammatory and Antioxidative Effects in Experimental Inflammation. Antioxidants, 9(10), 921.

Qin, W., Ketnawa, S. y Ogawa, Y. 2022. Effect of Digestive Enzymes and pH on Variation of Bioavailability of Green Tea During Simulated *In Vitro* Gastrointestinal Digestion. Food Science and Human Wellness, 11(3), 669-675.

Ragusa, A. 2023. Secondary Metabolites for the Reduction of Oxidative Stress. Molecules, 28(22), 7555.

Rahman, H. S., Othman, H. H., Hammadi, N. I., Yeap, S. K., Amin, K. M., Abdul Samad, N. y Alitheen, N. B. 2020. Novel Drug Delivery Systems for Loading of Natural Plant Extracts and Their Biomedical Applications. International Journal of Nanomedicine, 2439-2483.

Rahman, M. M., Rahaman, M. S., Islam, M. R., Rahman, F., Mithi, F. M., Alqahtani, T., ... y Uddin, M. S. 2021. Role of Phenolic Compounds in Human Disease: Current Knowledge and Future Prospects. Molecules, 27(1), 233.

Raoof, A. y Fawzy, G. 2022. Anti-Obesity Potential of Natural Products. Egyptian Journal of Chemistry, 65(10), 329-358.

Rathore, R. y Rai, N. 2022. Pharmacological Action and Underlying Molecular Mechanism of *Callistemon*: A Genus of Promising Medicinal Herbs. Phytomedicine, 99, 154013.

Ravichandran, S., Paluri, V., Kumar, G., Loganathan, K. y Kokati Venkata, B. R. 2016. A Novel Approach for the Biosynthesis of Silver Oxide Nanoparticles Using Aqueous Leaf Extract of *Callistemon lanceolatus* (Myrtaceae) and Their Therapeutic Potential. Journal of Experimental Nanoscience, 11(6), 445-458.

Rein, M. J., Renouf, M., Cruz-Hernandez, C., Actis-Goretta, L., Thakkar, S. K. y da Silva Pinto, M. 2013. Bioavailability of Bioactive Food Compounds: A Challenging Journey to Bioefficacy. British Journal of Clinical Pharmacology, 75(3), 588-602.

Reuter, S., Gupta, S. C., Chaturvedi, M. M. y Aggarwal, B. B. 2010. Oxidative Stress, Inflammation, and Cancer: How are They Linked?. Free Radical Biology and Medicine, 49(11), 1603-1616.

Rotimi, L., Ojemaye, M. O., Okoh, O. O. y Okoh, A. I. 2019a. Silver Nanoparticles Mediated by *Callistemon citrinus* Extracts and Their Antimalaria, Antitrypanosomal and Antibacterial Efficacy. J. Mol. Liq, 273, 615-625.

Rotimi, L., Ojemaye, M. O., Okoh, O. O., Sadimenko, A., y Okoh, A. I. 2019b. Synthesis, Characterization, Antimalarial, Antitrypanocidal and Antimicrobial Properties of Gold Nanoparticle. Green Chemistry Letters and Reviews, 12(1), 61-68.

Sandhiya, V. y Ubaidulla, U. 2020. A Review on Herbal Drug Loaded into Pharmaceutical Carrier Techniques and Its Evaluation Process. Future Journal of Pharmaceutical Sciences, 6, 1-16.

Saonere, J.A.; Channawar, M.A.; Kochar, N.I.; Mohale, D.; Chandewar. 2023. A.V. Preparation and Evaluation of Phytophospholipid Complex of Phenolic Fractions of *G. glabra* for Antioxidant and Antimicrobial Activity. Acta Scientific Pharmaceutical Sciences, 7, 37-44.

Sarma, S., Sockalingam, S. y Dash, S. 2021. Obesity as a Multisystem Disease: Trends in Obesity Rates and Obesity-Related Complications. Diabetes, Obesity and Metabolism, 23, 3-16.

Satoh, M., Ogita, H., Takeshita, K., Mukai, Y., Kwiatkowski, D. J. y Liao, J. K. 2006. Requirement of Rac1 in the Development of Cardiac Hypertrophy. Proceedings of the National Academy of Sciences, 103(19), 7432-7437.

Shan, B., Cai, Y. Z., Sun, M., y Corke, H. 2005. Antioxidant Capacity of 26 Spice Extracts and Characterization of Their Phenolic Constituents. Journal of Agricultural and Food Chemistry, 53(20), 7749-7759.

Simioni, C., Zauli, G., Martelli, A. M., Vitale, M., Sacchetti, G., Gonelli, A. y Neri, L. M. 2018. Oxidative Stress: Role of Physical Exercise and Antioxidant Nutraceuticals in Adulthood and Aging. Oncotarget, 9(24), 17181.

Singh, R. 2017. A Review, Outlook, and Insight, of the Properties and Characteristics of *Callistemon Citrinus*. Bulletin of Pure & Applied Sciences-Botany, 36(1), 22-27.

Sowndhararajan, K., Deepa, P., y Kim, S. 2021. A Review of the Chemical Composition and Biological Activities of *Callistemon lanceolatus* (Sm.) Sweet. Journal of Applied Pharmaceutical Science, 11(12), 065-073.

Stadtman, E. R. y Levine, R. L. 2000. Protein Oxidation. Annals of the New York Academy of Sciences, 899(1), 191-208.

Suryawanshi, J. S. 2011. Phytosome: An Emerging Trend in Herbal Drug Treatment. J Med Genet Genomics, 3(6), 109-114.

Tham, K. W., Lim, A. Y. L. y Baur, L. A. 2023. The Global Agenda on Obesity: What Does this Mean for Singapore?. Singapore Medical Journal, 64(3), 182-187.

Toma, L., Deleanu, M., Sanda, G. M., Barbălată, T., Niculescu, L. Ş., Sima, A. V. y Stancu, C. S. 2024. Bioactive Compounds Formulated in Phytosomes Administered as Complementary Therapy for Metabolic Disorders. International Journal of Molecular Sciences, 25(8), 4162.

Toop, C. R., y Gentili, S. 2016. Fructose Beverage Consumption Induces a Metabolic Syndrome Phenotype in the Rat: A Systematic Review and Meta-Analysis. Nutrients, 8(9), 577.

Tripathy, S., Patel, D. K., Barob, L., y Naira, S. K. 2013. A Review on Phytosomes, Their Characterization, Advancement & Potential for Transdermal Application. Journal of Drug Delivery and Therapeutics, 3(3), 147-152.

Tung, B. T., Hai, N. T., y Son, P. K. 2017. Hepatoprotective Effect of Phytosome Curcumin against Paracetamol-Induced Liver Toxicity in Mice. Brazilian Journal of Pharmaceutical Sciences, 53(1), e16136.

Venkataraman, B., Almarzooqi, S., Raj, V., Bhongade, B. A., Patil, R. B., Subramanian, V. S., ... y Subramanya, S. B. 2023. Molecular Docking Identifies 1,8-Cineole (Eucalyptol) as a Novel PPARγ Agonist that Alleviates Colon Inflammation. International Journal of Molecular Sciences, 24(7), 6160.

Vieira, A. J., Beserra, F. P., Souza, M. C., Totti, B. M., y Rozza, A. L. 2018. Limonene: Aroma of Innovation in Health and Disease. Chemico-Biological Interactions, 283, 97-106.

Wojtunik-Kulesza, K. A., Kasprzak, K., Oniszczuk, T., y Oniszczuk, A. 2019. Natural Monoterpenes: Much More than Only a Scent. Chemistry & Biodiversity, 16(12), e1900434.

World Health Organization (WHO). 2021. Obesity. Disponible: https://www.who.int/news-room/facts-in-pictures/detail/6-facts-on-obesity [citado: 28 de marzo de 2024].

World Health Organization (WHO). 2024. Obesity and Overweight. Disponible: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight [citado: 28 de marzo de 2024].

World Obesity Atlas. 2023. World Obesity Federation. Disponible: https://data.worldobesity.org/publications/?cat=19 [citado: 28 de marzo de 2024].

Xie, J., Li, Y., Song, L., Pan, Z., Ye, S., y Hou, Z. 2017. Design of a Novel Curcumin-Soybean Phosphatidylcholine Complex-Based Targeted Drug Delivery Systems. Drug Delivery, 24(1), 707-719.

Yasui, M., Kanemaru, Y., Kamoshita, N., Suzuki, T., Arakawa, T. y Honma, M. 2014. Tracing the Fates of Site-Specifically Introduced DNA Adducts in the Human Genome. DNA Repair, 15, 11-20.

Young, I. S. y Woodside, J. V. 2001. Antioxidants in Health and Disease. Journal of Clinical Pathology, 54(3), 176-186.

Yu, J., Ip, E., dela Peña, A., Hou, J. Y., Sesha, J., Pera, N., ... y Farrell, G. C. 2006. COX-2 Induction in Mice with Experimental Nutritional Steatohepatitis: Role as Pro-Inflammatory Mediator. Hepatology, 43(4), 826-836.

Zaki, S. M., Fattah, S. A., y Hassan, D. S. 2019. The Differential Effects of High-Fat and High-Fructose Diets on the Liver of Male Albino Rat and the Proposed Underlying Mechanisms. Folia Morphologica, 78(1), 124-136.

XI. ANEXOS

Anexo 1. Documento de patente en trámite de los fitosomas derivados del extracto etanólico de *Callistemon citrinus*.

No. de expediente	Título de Patente	Titulares	Inventores	Fecha de ingreso	Estatus	Vigencia
MX/a/2024/002793	Fitosomas derivados del extracto etanólico de Callistemon citrinus	Universidad Michoacana de San Nicolás de Hidalgo	Dr. Patricia Ríos Chávez, Dr. Luis Gerardo Ortega Pérez, Dr. Daniel Godínez Hernández	4/03/2024	Aprobado el examen de forma	

Anexo 2. Certificado de análisis con Software Turnitin

Luis Gerardo Ortega Pérez

Efecto de los fitosomas del extracto de hoja de Callistemon citrinus sobre el estrés oxidante en rat

Universidad Michoacana de San Nicolás de Hidalgo

Detalles del documento

Identificador de la entrega
trn:oid:::3117:404716751

Fecha de entrega
12 nov 2024, 12:40 p.m. GMT-6

Fecha de descarga
12 nov 2024, 12:59 p.m. GMT-6

Nombre de archivo
Efecto de los fitosomas del extracto de hoja de Callistemon citrinus sobre el estrés oxidante en r....pdf

Tamaño de archivo
7.5 MB

87 Páginas

33,985 Palabras

189,323 Caracteres

74% Similitud general

El total combinado de todas las coincidencias, incluidas las fuentes superpuestas, para ca...

Grupos de coincidencias

473 Sin cita o referencia 69%
Coincidencias sin una citación ni comillas en el texto

14 Faltan citas 5%
Coincidencias que siguen siendo muy similar al material fuente

2 Falta referencia 0%
Las coincidencias tienen comillas, pero no una citación correcta en el texto

0 Con comillas y referencia 0%
Coincidencias de citación en el texto, pero sin comillas

Fuentes principales

69% Fuentes de Internet

68% Publicaciones

0% Trabajos entregados (trabajos del estudiante)

Marcas de integridad

N.º de alerta de integridad para revisión

Texto oculto
68 caracteres sospechosos en N.º de página
El texto es alterado para mezclarse con el fondo blanco del documento.

Los algoritmos de nuestro sistema analizan un documento en profundidad para buscar inconsistencias que permitirían distinguirlo de una entrega normal. Si advertimos algo extraño, lo marcamos como una alerta para que pueda revisarlo.

Una marca de alerta no es necesariamente un indicador de problemas. Sin embargo, recomendamos que preste atención y la revise.

Anexo 3. Formato de declaración de originalidad y uso de Inteligencia Artificial

Formato de Declaración de Originalidad y Uso de Inteligencia Artificial

Coordinación General de Estudios de Posgrado
Universidad Michoacana de San Nicolás de Hidalgo

A quien corresponda,

Por este medio, quien abajo firma, bajo protesta de decir verdad, declara lo siguiente:

- Que presenta para revisión de originalidad el manuscrito cuyos detalles se especifican abajo.
- Que todas las fuentes consultadas para la elaboración del manuscrito están debidamente identificadas dentro del cuerpo del texto, e incluidas en la lista de referencias.
- Que, en caso de haber usado un sistema de inteligencia artificial, en cualquier etapa del desarrollo de su trabajo, lo ha especificado en la tabla que se encuentra en este documento.
- Que conoce la normativa de la Universidad Michoacana de San Nicolás de Hidalgo, en particular los Incisos IX y XII del artículo 85, y los artículos 88 y 101 del Estatuto Universitario de la UMSNH, además del transitorio tercero del Reglamento General para los Estudios de Posgrado de la UMSNH.

Datos del manuscrito que se presenta a revisión		
Programa educativo	Programa Institucional de Doctorado en Ciencias Biológicas	
Título del trabajo	Efecto de los fitosomas del extracto de hoja de *Callistemon citrinus* sobre el estrés oxidante en ratas alimentadas con una dieta hipercalórica	
	Nombre	**Correo electrónico**
Autor/es	M. C. Luis Gerardo Ortega Pérez	gerardo.ortega@umich.mx
Director	Dr. Daniel Godínez Hernández	daniel.godinez@umich.mx
Codirector	Dra. Patricia Ríos Chávez	patricia.rios@umich.mx
Coordinador del programa	Dr. Omar Chassin Noria	doc.ciencias.biologicas@umich.mx

Uso de Inteligencia Artificial		
Rubro	**Uso (sí/no)**	**Descripción**
Asistencia en la redacción	No	

Formato de Declaración de Originalidad y Uso de Inteligencia Artificial

Coordinación General de Estudios de Posgrado
Universidad Michoacana de San Nicolás de Hidalgo

Uso de Inteligencia Artificial		
Rubro	**Uso (sí/no)**	**Descripción**
Traducción al español	NO	
Traducción a otra lengua	NO	
Revisión y corrección de estilo	NO	
Análisis de datos	NO	
Búsqueda y organización de información	NO	
Formateo de las referencias bibliográficas	NO	
Generación de contenido multimedia	NO	
Otro	NO	

Datos del solicitante	
Nombre y firma	M. C. Luis Gerardo Ortega Pérez
Lugar y fecha	Morelia, Mich., a 08 de noviembre de 2024.