Becerril Campos Adriana Araceli

Efecto del consumo de hoja de frijol (*Phaseolus vulgaris L.*) en la lipotoxicidad de ratas Wistar con alteraciones metabólicas e hígado graso no alcohólico: vía Nrf2/ PPARα/ NFKB

2023

Universidad Autónoma de Querétaro

Facultad de Ciencias Naturales

Efecto del consumo de hoja de frijol (*Phaseolus vulgaris L.*) en la lipotoxicidad de ratas Wistar con alteraciones metabólicas e hígado graso no alcohólico: vía Nrf2/ PPARα/ NFKB

Que como parte de los requisitos para
obtener el Grado de

Doctora en Ciencias Biológicas

Presenta

Adriana Araceli Becerril Campos

Dirigido por:

Dra. Santiaga Marisela Ahumada Solórzano

Querétaro, Qro. a Septiembre de 2023

REPOSITORIO INSTITUCIONAL DGBSDI-UAQ

Dirección General de Bibliotecas y Servicios Digitales de Información

Efecto del consumo de hoja de frijol (Phaseolus vulgaris L.) en la lipotoxicidad de ratas Wistar con alteraciones metabólicas e hígado graso no alcohólico: vía Nrf2/ PPARα/ NFκB

por

Adriana Araceli Becerril Campos

se distribuye bajo una Licencia Creative Commons Atribución-NoComercial-SinDerivadas 4.0 Internacional.

Clave RI: CNDCC-263954

Universidad Autónoma de Querétaro
Facultad de Ciencias Naturales
Doctorado en Ciencias Biológicas

Efecto del consumo de hoja de frijol (*Phaseolus vulgaris L.*) en la lipotoxicidad de ratas Wistar con alteraciones metabólicas e hígado graso no alcohólico: vía Nrf2/ PPARα/ NFKB

Opción de titulación: Artículos de investigación

Que como parte de los requisitos para obtener el Grado de
Doctora en Ciencias Biológicas

Presenta:
Adriana Araceli Becerril Campos

Dirigido por:
Dra. Santiaga Marisela Ahumada Solórzano

Dra. Santiaga Marisela Ahumada Solórzano
Presidente
Dra. Margarita Teresa de Jesús García Gasca
Secretario
Dra. Minerva Ramos Gómez
Vocal
Dra. Yazmín Macotela Guzmán
Suplente 1
Dr. Jesús Eduardo Castro Ruiz
Suplente 2

Centro Universitario, Querétaro, Qro.
Septiembre 2023
México

Dedicatoria

Por elegir seguir mis sueños y no rendirme: a mí.

Por ser mi inspiración y mi brújula: a mi abuela.

.

Por motivarme y recordarme mis fortalezas, por sacarme de mi zona de confort e impulsarme a crecer: a Iván.

Por hacerme sentir que puedo conseguir todo lo que me proponga con trabajo y esfuerzo; por creer en mí y ser mi círculo de apoyo siempre: a mi hermana, mi mamá y mi papá.

Por su amor y alegría incondicional, que me han ayudado a navegar durante la frustración y el miedo: a Kiwi y Sumi.

Por su contención y herramientas: a mi dermatóloga y a mi psicóloga.

A todos los corazones que señalaron mi camino y me han traído a este momento, a este lugar.

"Ni la vida, ni la justicia social, ni los logros personales nacen del individualismo… son resultado de lo colectivo"

-Anónimo-

"Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less"

-Marie Skłodowski-Curie-

Agradecimientos

Todo el trabajo fue realizado gracias al apoyo de mi directora de tesis, Dra. Santiaga Marisela Ahumada Solórzano; el conocimiento y guía de mi comité de tesis, Dra. Margarita Teresa de Jesús García Gasca, Dra. Minerva Ramos Gómez, Dra. Yazmín Macotela Guzmán y Dr. Jesús Eduardo Castro Ruiz.

Por la experiencia científica, conocimiento técnico y apoyo para la obtención de la hoja de frijol y realización de experimentos; agradezco a Viridiana Ocampo, Bridget Vigoroux, Dra. Aracely Anaya, Dra. Mariela Camacho, Laura Muñoz, MNH. Josué López, Dr. Konisgmar Escobar, Dra. Xóchitl Zambrano, Dra. Adriana González, Dra. Ericka de los Ríos, Ing. Nydia Hernández Ríos, Oswaldo Salinas, Aurora Jáuregui, Leticia Castillo, MVZ. Verónica Andrade, Zurisadai Soto, Montse Rodríguez, Karen Arguijo, Andrea Castro, Evelyn Maya, Dr. Candelario Mondragón, Dr. Luis Concha y Dr. Juan Ortiz

También agradezco a los laboratorios y unidades de investigación por permitirme usar su infraestructura; Laboratorio Nacional de Imagenología por Resonancia Magnética (LANIREM, INB, UNAM), Unidad de Proteogenómica (INB, UNAM), Laboratorio de Biología Celular y Molecular (FCN, UAQ), Unidad de Microscopia (INB, UNAM), Bioterio (FCN, UAQ), Laboratorio de Estrés oxidativo y Salud (FQ, UAQ).

Y por el financiamiento al proyecto, agradezco al Fondo de Proyectos Especiales de Rectoría-UAQ (FOPER: 2019–00798; 2019–00594; 2020-FCN01886; 2021-FCN02473); Programa para el Desarrollo del Profesorado-UAQ (PRODEP-UAQ-2018–2020); Fondo para el Desarrollo del Conocimiento-UAQ (FONDEC–UAQ 2021). Al Consejo Nacional de Ciencia y Tecnología por la beca (CONACYT: 734854).

Índice

Contenido

Índice de figuras y tablas

Resumen

El consumo elevado de grasa saturada e hidratos de carbono simples favorece el desarrollo de enfermedades metabólicas. Por otro lado, los compuestos bioactivos presentes en los alimentos de origen vegetal han mostrado efectos protectores contra las alteraciones metabólicas, incluida la enfermedad del hígado graso no alcohólico (NAFLD). Las hojas de frijol cultivadas ampliamente en el mundo son una fuente de fibra dietética y polifenoles. El objetivo del presente estudio fue evaluar el efecto protector de las hojas de frijol sobre las alteraciones metabólicas y la lipotoxicidad hepática presentes en la NAFLD, así como el papel de la vía Nrf2/ PPARα/ NFκB como posible mecanismo de acción. Para ello se utilizó un modelo *in vivo* de ratas Wistar macho con 8 grupos experimentales: 1-2) S= Dieta estándar, 3-4) SBL= S+ 10% hoja de frijol, 5-6) H= dieta alta en grasa (20% de manteca) y alta en fructosa (20%), 7-8) HBL= H+ 10% hoja de frijol. El tratamiento de los grupos 1, 3, 5 y 7 tuvo una duración de 6 semanas (Capítulo 1), para el resto el tratamiento fue de 13 semanas (Capítulo 2). Después de 6 semanas de tratamiento, la hoja de frijol previno la ganancia de peso, la acumulación de grasa visceral (circunferencia abdominal) y mejoró la respuesta postprandial a la glucosa, probablemente mediada por un incremento en la producción de ácidos grasos de cadena corta (AGCC). Mientras que, la suplementación de hoja de frijol mejoró la sensibilidad a la insulina (HOMA-IR), redujo la acumulación de grasa corporal e incrementó el glutatión reducido en suero después de 13 semanas. A nivel hepático, la suplementación con hoja de frijol previno la acumulación de triglicéridos (esteatosis) y disminuyó la lipoperoxidación. Por lo tanto, el consumo de las hojas de frijol es una alternativa de bajo costo con efecto anti-lipotoxicidad en la prevención de NAFLD y alteraciones metabólicas asociadas a obesidad.

Palabras clave: hígado graso, lipotoxicidad, quelites, MAFLD, AGCC

Abstract

High saturated fat and high simple carbohydrate diets lead to metabolic diseases. Meanwhile, bioactive compounds of plant-based food exert protective effects against metabolic alterations, included NAFLD (nonalcoholic fatty liver disease). Bean leaves are a source of dietary fiber and polyphenols and are widely cultivated around the world. The aim of this work was to evaluate the effect of bean leaves consumption against metabolic alterations and hepatic lipotoxicity present in NAFLD; as well as the role of Nrf2/ PPARα/ NFκB pathway as a possible mechanism of action of bean leaves in the liver. In this regard, an i*n vivo* model of male Wistar rats with 8 experimental groups was used: 1-2) S= Standard diet, 3-4) SBL= S+ 10% of bean leaves, 5-6) H= high fat (lard 20%) and high fructose (20%) diet, 7-8) HBL= H+ 10% of bean leaves. Groups 1, 3, 5 and 7 received a 6-weeks treatment (Chapter 1) and the other groups (2, 4, 6 and 8) were treated for 13 weeks (Chapter 2). After 6 weeks of treatment, bean leaves supplementation prevented body weight gain, visceral fat accumulation (abdominal circumference) and enhanced postprandial glucose response. These findings are probably related to the increase in SCFA (short chain fatty acids) production. After 13 experimental weeks, the supplementation bean leaves (HBL) improved insulin sensibility (HOMA-IR), ameliorated body fat accumulation (MRI) and increased GSH (reduced glutathione) in serum. In liver, bean leaves supplementation prevented steatosis development (decrease on hepatic triglycerides) and lipid peroxidation. Therefore, bean leaves intake is a low-cost alternative that showed antilipotoxicity effect leading to the prevention of NAFLD and metabolic alterations.

Key words: fatty liver, lipotoxicity, quelites, MAFLD, SCFA.

I. Introducción

La globalización ha causado cambios en los patrones alimentarios, dejando atrás el consumo de productos locales y propiciando el desarrollo de alteraciones metabólicas (Mateos-Maces et al., 2020). Dentro de las alteraciones metabólicas más complejas y con una prevalencia global de 25% se encuentra la enfermedad del hígado graso no alcohólico (EHGNA o del inglés NAFLD) (Younossi et al., 2016). En México se estima que la prevalencia de NAFLD podría sobrepasar el 50% debido al elevado número de casos de obesidad, síndrome metabólico, enfermedades cardiovasculares y diabetes mellitus tipo 2, factores estrechamente relacionados con su aparición y evolución (Bernal-Reyes et al., 2019).

A nivel hepático, las dietas altas en grasa saturada e hidratos de carbono promueven la síntesis de triglicéridos y ácidos grasos de novo y la proporción de ácidos grasos saturados en las VLDL, lo que favorece la acumulación de lípidos en hígado (Ferramosca y Zara, 2014; Letexier et al., 2003; Perez-Martinez et al., 2010). Además, las dietas altas en hidratos de carbono y grasa saturada han mostrado incrementar la producción de EROs, nitrógeno o azufre, y generar daños al ADN. La acumulación de lípidos en tejidos ectópicos (lipotoxicidad), como el hígado, en conjunto con la inactividad física, favorece la obesidad y los daños metabólicos asociados (Yu et al., 2016).

La NAFLD[1] es una alteración metabólica hepática caracterizada por la acumulación de grasa macrovesicular (≥5% hepatocitos) en ausencia de consumo excesivo de alcohol, medicamentos hepatotóxicos u otras enfermedades hepáticas que pudieran favorecer la acumulación hepática de grasa (Bernal-Reyes et al., 2019; Younossi et al., 2016). Cuando la acumulación de grasa es ≥5% sin evidencia de daño hepatocelular se denomina esteatosis (primera etapa de NAFLD). La esteatosis es una etapa reversible con riesgo muy bajo de desarrollar complicaciones como cirrosis o falla hepática, pero puede progresar rápidamente a la segunda etapa de NAFLD, esteatohepatitis (Chalasani et al., 2018; Eslam et al., 2020). El paso de esteatosis a esteatohepatitis no tiene una causa única, por ello algunos autores

[1] En 2020, con el fin de resaltar la importancia de alteraciones metabólicas como la diabetes, la obesidad, la hipertensión arterial y la dislipidemia en el desarrollo de hígado graso y su progresión a cirrosis se propuso un nuevo concepto enfermedad del hígado graso asociada a disfunción metabólica (EHMet o del inglés MAFLD) (Eslam et al. 2020). A diferencia de la NAFLD, la MAFLD incluye como factores etiológicos hepatitis B o C, el consumo crónico de alcohol o alguna sustancia hepatotóxica. Dado que es un concepto nuevo, aún no hay información suficiente, lo que genera falta de consenso entre la comunidad científica (Fouad et al. 2021).

plantean a NAFLD como la manifestación hepática del síndrome metabólico, donde la resistencia a la insulina, la dislipidemia y la obesidad juegan un papel clave (Brooks et al., 2017). Mientras que, la inflamación y el estrés oxidativo son efectos negativos ocasionados por la acumulación ectópica (hepática) de lípidos que caracteriza a la lipotoxicidad (Symons y Dale Abel, 2013).

La esteatosis es curable, por lo que es importante desarrollar tratamientos para retardar su progresión. En las últimas décadas, la alimentación basada en plantas se ha estudiado por promover una alimentación sostenible y proveer de un variado contenido de compuestos bioactivos, los cuales han mostrado tener un papel protector contra el estrés oxidativo, la resistencia a la insulina, la inflamación y la acumulación de lípidos y, por tanto, prevenir enfermedades metabólicas como NAFLD.

II. Antecedentes

2.1 Hoja de frijol (Phaseolus vulgaris L.)

La planta de frijol es ampliamente cultivada en el mundo, no sólo por sus semillas, también por sus hojas y vainas verdes (Jones y Mejia, 1999). La semilla seca del frijol común (*Phaseolus vulgaris L.*) es la leguminosa de mayor consumo en el mundo, tiene un alto contenido de compuestos bioactivos como proteínas, fibra, hierro y fitoquímicos (Martínez-Zavala et al., 2016). Mientras que, la hoja de frijol (HF) es consumida como quelite en comunidades rurales en México (Linares et al., 2017) y ha mostrado ser fuente de compuestos bioactivos como polifenoles, proteína, fibra y hierro (Figura 1). Debido al creciente interés por revalorizar el consumo de alimentos locales (Slow Food, 2023) e incentivar su inclusión en la dieta, este proyecto tiene como finalidad impulsar el consumo de la hoja de frijol al ampliar el conocimiento sobre sus posibles efectos en la prevención de alteraciones metabólicas tempranas y esteatosis hepática.

La HF variedad Flor de Mayo Eugenia fue diseñada en 2003 por el INIFAP (Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias) con el fin de mejorar el rendimiento y la resistencia a plagas de cultivo (Acosta Gallegos et al., 2010). Previamente, en un modelo in vivo con protocolo de restricción alimentaria (RA) de 7 h expuesto a una dieta alta en grasa y alta en fructosa, se evaluó el efecto de la inclusión de 10% de HF de la variedad Flor de Mayo Eugenia durante 8 semanas, encontrando que la HF mejora la

sensibilidad a la insulina (HOMA-IR), la glucemia y la trigliceridemia. Además, el consumo de la HF disminuyó la expresión hepática de la enzima Scd1 (estearoil-CoA desaturasa 1) causando 38% menor acumulación de grasa hepática (Becerril Campos et al., 2018; Ramírez-Venegas et al., 2021).

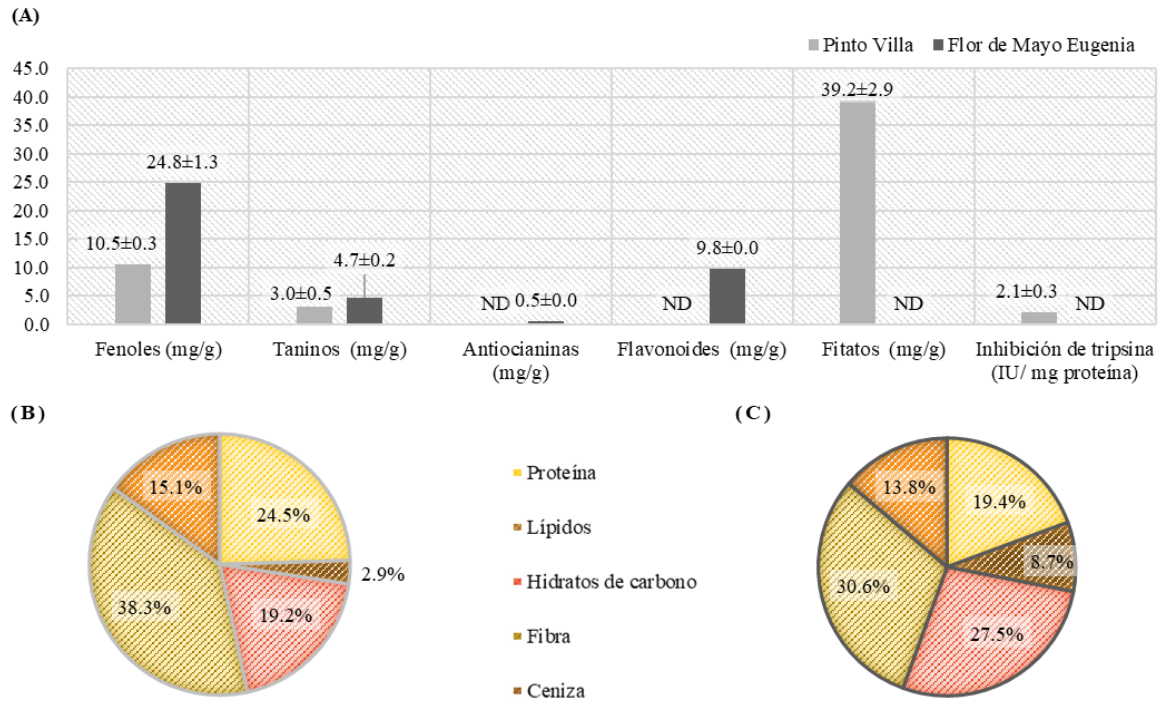

Figura 1 Análisis de compuestos antinutricios (A) y químico proximal de la hoja de frijol *(Phaseolus vulgaris L.)* variedades (B) Pinto Villa y (C) Flor de Mayo Eugenia.

Los analisis fueron realizados en base seca (Guzman Maldonado 2017; Martínez-Zavala 2012).

Los mecanismos de acción de los compuestos bioactivos descritos en la prevención de enfermedades metabólicas son diversos, incluso tienen diferentes efectos cuando se administran de manera aislada o como parte de un alimento (efecto sinérgico). Este trabajo se enfocó en determinar el efecto de los compuestos bioactivos provenientes de la inclusión 10% de HF en una dieta alta en grasa y alta en fructosa, en alteraciones metabólicas y lipotoxicidad hepática, así como en explorar la vía Nrf2/ PPARα/ NFκB como posible mecanismo de acción de la HF (Figura 2).

2.2 Mecanismos moleculares de la regulación de la respuesta inflamatoria y del estrés oxidativo

El factor de transcripción Nrf2 (factor 2 relacionado con el factor nuclear eritroide 2) regula cerca de 250 genes que buscan mantener la homeostasis celular en condiciones

fisiológicas y patológicas. Nrf2 regula genes del metabolismo de glucosa, lípidos y xenobióticos, y la producción de ácido sulfhídrico, la generación de NADPH, la síntesis de GSH (glutatión reducido), la síntesis de SOD (superóxido dismutasa), CAT (catalasa) y GPx (glutatión peroxidasa), entre otros genes (Li et al. 2019; Tebay et al. 2015).

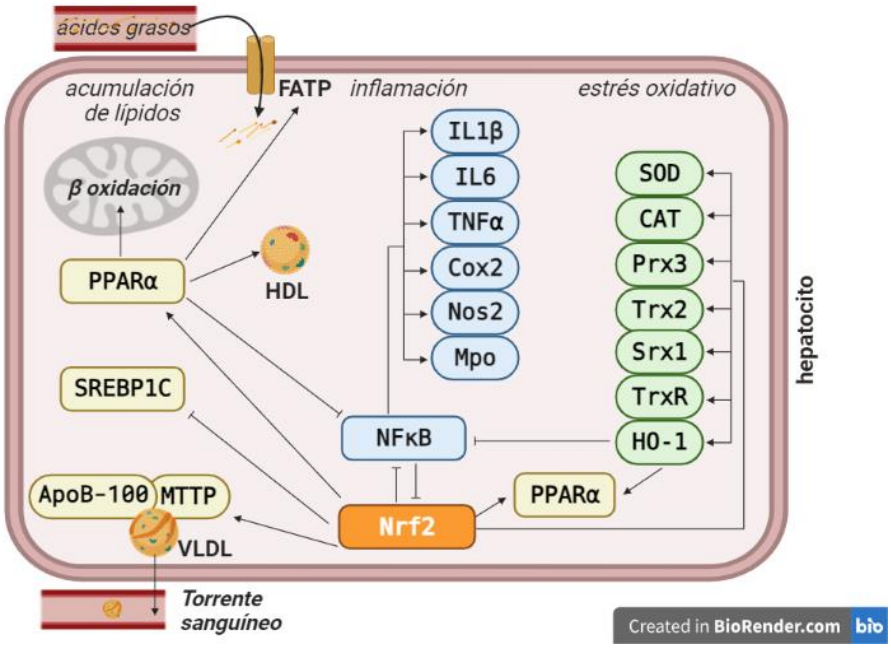

Figura 2 Vía Nrf2/ PPARα/ NFκB en la modulación de la acumulación de lípidos, estrés oxidativo e inflamación.

Nrf2 participa en la regulación de tres procesos claves en el desarrollo de NAFLD, siendo por tanto un intersante blanco terapeútico. Mecanismos: 1) Disminuye la acumulación de lípidos: incrementa la exportación de triglicéridos activando a MTTP, inhibe la lipogénesis a través de SREBP1C y activar a PPARα promoviendo la β oxidación, el transporte reverso de colesterol y regula la captura de ácidos grasos. 2) Disminuye la inflamación: inhibe a NFκB, importante regulador de procesos inflamatorios mediados por citocinas (IL1β, IL6, TNFα), enzimas productoras de especies reactivas (Nos 2, Mpo) y la síntesis de prostaglandinas (Cox 2). 3) Disminuye el estrés oxidativo: regula la expresión de enzimas antioxidantes con el fin de reducir las especies reactivas.y participa en la reducción del peroxinitrito (Prx3), de proteínas y ácidos nucleícos (Trx2). Cataliza el el superóxido en H2O2 (SOD) y a su vez el peróxido de hidrogeno en agua y oxígeno(CAT) . Además de la activación de enzimas como peroxirredoxinas (Srx1), tiorredoxinas (TrxR) y la degradación del grupo hemo (HO-1). Nrf2= nuclear factor erythroid-derived 2-like 2, ApoB-100= Apolipoproteína B-100, VLDL= lipoproteína de muy baja densidad, SREBP1C= proteína de unión al elemento regulador de esterol 1c, PPARα= receptor del activador del proliferador de peroxisoma alfa, HDL= lipoproteína de baja densidad, FATP= proteína transportadora de ácidos grasos, NFκB= factor nuclear kappa B, Mpo= mieloperoxidasa, Nos 2= sintasa de oxido nitrico 2, Cox 2= ciclooxigenasa 2, IL1β= interleucina 1 beta, IL6= interleucina 6, TNFα= factor de necrosis tumoral alfa, HO-1= hemo oxigenasa 1, TrxR/TR= tioredoxina reductasa, Srx1= sulfirredoxina 1, , Trx2= tiorredoxina 2, Prx3= peroxirredoxina 3, CAT= catalasa, SOD, superóxido dismutas (Li et al., 2019; Ma, 2013; Tebay et al., 2015; Wardyn et al., 2015).

Nrf2 ha mostrado tener un rol importante en la NAFLD (Galicia-Moreno et al., 2020; Liu et al., 2021). En condiciones de estrés, Nrf2 puede ser activado por la vía canónica o la vía no canónica (Figura 3) (Liu et al., 2021). En la vía canónica, la actividad de Nrf2 es regulada por la presencia de Keap 1 (Kelch-like ECH-associated protein 1) favoreciendo la poli ubiquitinación de Nrf2 (Tebay et al., 2015; Vysakh et al., 2016). A través de esta vía, Keap1 actúa como un sensor de electrófilos, por lo que, en condiciones con altas concentraciones de electrófilos (especies reactivas o antioxidantes provenientes de la dieta), libera a Nrf2 para que se traslade al núcleo (Tebay et al., 2015). Mientras que, por la vía no canónica, los PPIDs (disruptores de la interacción proteína-proteína) como diversos péptidos, moléculas pequeñas y p21 provocan una acumulación de p62 (Lian et al., 2023; Silva-Islas y Maldonado, 2018; Zhao et al., 2018). La acumulación de p62 está relacionada con la activación crónica de Nrf2 y el desarrollo de enfermedades crónicas como diabetes mellitus tipo II, NAFLD y cáncer (Dodson y Zhang, 2017; Liu et al., 2021).

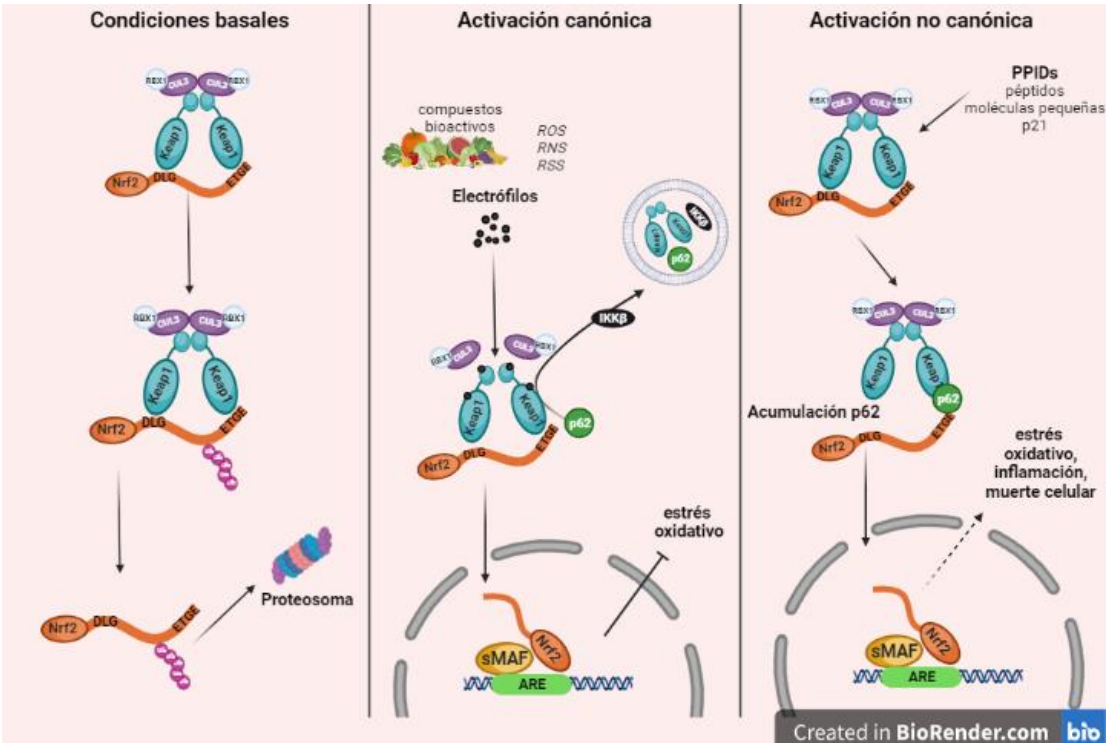

Figura 3 Mecanismos de activación de Nrf2

Nrf2 tiene diversos mecanismos de activación. En condiciones basales, Nrf2 es degradado por el proteosoma. La activación canónica es dependitente de Keap1 y su capacidad de sensar electrófilos, provenientes de la dieta o especies reactivas. La activación no canónica surge de una acumulación de p62 asociada a PPIDs. Nrf2= nuclear factor erythroid-derived 2-like 2, Keap 1= Kelch-like ECH-associated protein 1, PPIDs=disruptores de la interacción proteína-proteína. (Lian et al., 2023; Silva-Islas y Maldonado, 2018; Zhao et al., 2018).

Cuando Nrf2 se traslada al núcleo regula positivamente la oxidación de ácidos grasos mediada por la activación de PPARα (receptor del activador del proliferador de peroxisoma alfa) (Figura 4) (Li et al., 2019; Valenzuela et al., 2017). El factor de transcripción PPARα regula la expresión de acil-CoA oxidasa, tiolasa, esterol 12-alfa hidroxilasa (CYP8B1), proteína transportadora de ácidos grasos (FATP), traslocasa de ácidos grasos (FAT/CD36) y las apolipoproteínas A-I y A-II (Cave et al., 2016). Por tanto, PPARα regula el transporte reverso de colesterol (HDL), la entrada y captura de ácidos grasos libres, la síntesis de sales biliares y la obtención de energía a partir de ácidos grasos (β oxidación). Además, PPARα ha mostrado regular la respuesta inflamatoria mediada por NFκB; por un lado, induce la expresión de IκB y, por el otro, inhibe la expresión de la subunidad p50- NFκB (Zambon et al., 2006).

En cuanto a la regulación de NFκB (factor nuclear kappa B) mediada por Keap-1/Nrf2, una vez que Nrf2 se traslada al núcleo, Keap1 queda libre para inhibir a IKKβ (IκB cinasa beta). La fosforilación de IKKα (IκB cinasa alfa) mediada por IKKβ es necesaria para que NFκB se transloque al núcleo y se active una respuesta inflamatoria (Stefanson y Bakovic, 2014; Wardyn et al., 2015).

En el capítulo 1 de este trabajo describimos el efecto de la suplementación de 10% de hoja de frijol en las alteraciones metabólicas tempranas y en el incremento en la producción de ácidos grasos de cadena corta después de 6 semanas de tratamiento (Becerril-Campos et al., 2022). Mientras que, en el capítulo 2 nos enfocamos en el efecto de la suplementación de 10% de hoja de frijol en la lipotoxicidad en la enfermedad por hígado graso no alcohólico, además de explorar el papel de la vía Nrf2/ PPARα/ NFκB como posible mecanismo de acción (Becerril-Campos et al., 2023).

La suplementación con 10% de hojas de frijol en una dieta alta en grasas/alta en fructosa mostró efectos preventivos contra obesidad, alteraciones en el metabolismo de glucosa y modulación de la exportación de triglicéridos. Estos efectos podrían estar relacionados con un incremento en la producción de AGCC, los cuales han mostrado favorecen una modulación en la ingesta energética, disminuyendo la acumulación de lípidos y favoreciendo el control glucémico. Adicionalmente, a nivel hepático evitó el desarrollo de esteatosis y peroxidación lipídica, activando el sistema de defensa antioxidante y mostrando un efecto antiinflamatorio, reduciendo la lipotoxicidad hepática. La realización de más

estudios enfocados en evaluar el efecto de la hoja de frijol en alteraciones metabólicas contribuirá en elucidar los mecanismos de acción involucrados en la prevención de alteraciones metabólicas, producción de ácidos grasos de cadena corta y disminución de la acumulación de grasa hepática, así como definir si la inclusión de la hoja de frijol en la alimentación solo tiene la capacidad de prevenir enfermedades o podría usarse también como parte del tratamiento de éstas.

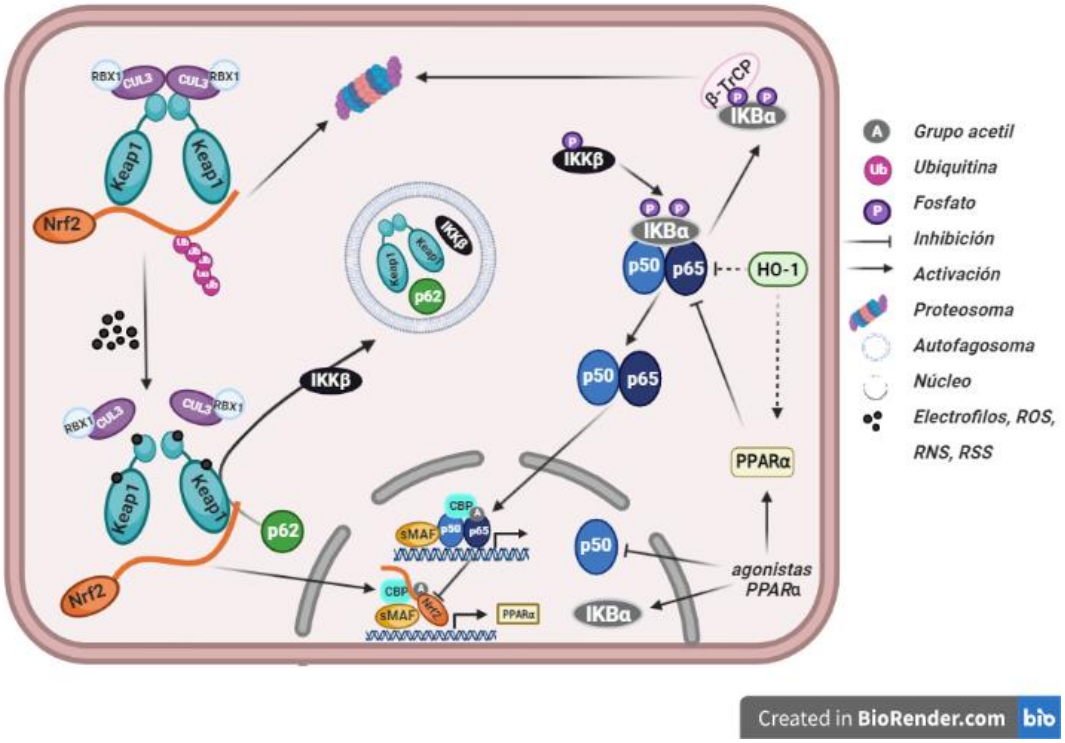

Figura 4 Regulación molecular de la vía Nrf2/ PPARα/ NFκB

La actividad de Nrf2 es regulada por Keap1. En ausencia de estímulos, Nrf2 es poliubiquitinado por el complejo Keap1-CUL3-RBX1 y degradado en el proteosoma. Keap1 es sensible a electrófilos y especies reactivas de oxígeno, nitrógeno y azufre, permitiendo la translocación al núcleo de Nrf2. En este punto. p62, Keap1 e IKKβ son degradados en el autofagosoma. IKKβ es clave en la movilización de NFκB al núcleo. Nrf2 puede inhibir a NFκB mediante Keap 1, NFκB también puede inhibir a Nrf2. La subunidad p65 de NFκB tiene mayor afinidad por CBP que Nrf2, inhibiendo la actividad de Nrf2 por competencia. Los agonistas de PPARα pueden inhibir la expresión de la subunidad p50 y promover la expresión de IKBα. Además, PPARα puede unirse a p65 inhibiendo su movilización al núcleo. El papel de HO-1 aún no está tan claro, pero se sabe que su regulación positiva incrementa la actividad de PPARα.RBX1= RING-box 1, CUL3= culina 3, Keap 1= Kelch-like ECH-associated protein 1, Nrf2= nuclear factor erythroid-derived 2-like 2, p62/SQSTM1= prteína reguladora inducible por estrés, sMAF= proteína pequeña Maf (reguladora génica, región tipo zipper de leucina), CBP= proteínas de unión a CREB, PPARα= receptor del activador del proliferador de peroxisoma alfa, IKBα= IKB cinasa alfa, p50= subunidad de NFκB, p65/RelA= subunidad de NFκB, HO-1= hemo oxigenasa 1, IKKβ= cinasas de células B beta (Alcaraz et al., 2004; Ganesh Yerra et al., 2013; Hinds et al., 2014; Keleku-Lukwete et al., 2018; Stefanson y Bakovic, 2014; Wardyn et al., 2015; Yamamoto et al., 2018; Zambon et al., 2006).

III. Capítulo 1. Efecto de la hoja de frijol en las alteraciones metabólicas tempranas y en el incremento en la producción de ácidos grasos de cadena corta

Copyright © 2022, Licencia 5547730923168 (Becerril-Campos et al. 2022)

Plant Foods for Human Nutrition
https://doi.org/10.1007/s11130-022-00992-1

ORIGINAL PAPER

Phaseolus vulgaris L. Leaves Increase Short-Chain Fatty Acid (SCFA) Production, Ameliorating Early Metabolic Alterations

Adriana Araceli Becerril-Campos[1] · Perla Viridiana Ocampo-Anguiano[1] · Candelario Mondragón-Jacobo[2] · Konisgmar Escobar-García[3] · Mariela Camacho-Barrón[4] · Miriam Aracely Anaya-Loyola[4] · Ana Angélica Feregrino-Perez[5] · Teresa García-Gasca[1] · Santiaga Marisela Ahumada-Solórzano[1]

Accepted: 2 July 2022
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2022

Abstract
High-fat/high-fructose diets promote early metabolic disorders in weight and lipid and glucose metabolism. Bioactive compounds such as polyphenols and fiber present in plant-based food prevent the development of metabolic disorders. The objective of the present study was to evaluate the effect of *Phaseolus vulgaris* L. Flor de Mayo Eugenia (FME) bean leaves on early metabolic alterations in male Wistar rats fed a high-fat/high-fructose diet. After proximate and chemical analysis of FME bean leaves, thirty-six male Wistar rats (ethical approval 06FCN2019 and 77FCN2019) were randomly assigned to one of four groups: 1) standard diet (S) fed with Rodent Laboratory Chow 5001®; 2) standard diet + 10% dry FME bean leaves (SBL); 3) high-fat (lard) and high-fructose diet (H); and 4) high-fat/high-fructose diet + 10% dry FME bean leaves (HBL). The study was carried out for six weeks. Group H exhibited early metabolic alterations compared to Group S: final weight gain (↑15%), abdominal fat accumulation (waist circumference, ↑11%), triglycerides (↑30%), glucose (↑16%), insulin resistance (HOMA-IR, ↑32%), and fecal triglycerides (↑284%) and decreased total short-chain fatty acids (SCFAs, ↓17%). FME bean leave supplementation (HBL) prevented body weight gain (↓12%), abdominal fat accumulation (waist circumference, ↓10%), and early insulin resistance (glucose area under the curve, ↓6%) compared to Group H. The supplementary bean leave diet increased SCFA production (↑54%), most likely mediated by the fiber and polyphenols present in the leaves. Therefore, bean leaves are a low-cost alternative for human nutritional care and prevention of early metabolic alterations.

Keywords Insulin resistance · Polyphenols · Fiber · SCFA · Visceral fat · Plant-based foods

Introduction

High consumption of saturated fat and fructose promotes early metabolic disorders, such as alterations in lipid and glucose metabolism, weight gain, abdominal fat accumulation and intestinal dysbiosis, in humans and in animal models [1]. Diet is one of the most important environmental factors that could be modified to prevent metabolic disorders. Fiber and polyphenols in plant-based food have been shown to have an important role against metabolic alterations [2]. When fiber and polyphenols reach the colon, they are fermented by

Adriana Araceli Becerril-Campos and Perla Viridiana Ocampo-Anguiano contributed equally to this work.

✉ Santiaga Marisela Ahumada-Solórzano
santiaga.marisela.ahumada@uaq.mx

[1] Laboratorio de Biología Celular y Molecular, Facultad de Ciencias Naturales (FCN), Universidad Autónoma de Querétaro (UAQ), Campus Juriquilla, Av. de las Ciencias S/N., Juriquilla, Querétaro C.P. 76230, México

[2] Horticultura y Producción Agrícola, FCN, UAQ, Campus Amazcala. Carretera Chichimequillas. S/N km 1, El Marqués, Querétaro C.P. 76265, México

[3] Laboratorio de Nutrición Animal, FCN, UAQ. Campus Juriquilla, Av. de las Ciencias S/N, Juriquilla, Querétaro C.P. 76230, México

[4] Laboratorio de Nutrición Humana, FCN, UAQ. Campus Juriquilla, Av. de las Ciencias S/N, Juriquilla, Querétaro C.P. 76230, México

[5] Laboratorio Metabolitos Secundarios y Nutraceúticos, Facultad de Ingeniería, UAQ, Campus Aeropuerto. Carretera a Chichimequillas S/N, Ejido Bolaños, Querétaro C.P. 76140, México

Published online: 12 July 2022

the microbiota, producing short-chain fatty acids (SCFAs), mainly acetate, propionate and butyrate [2]. SCFA activation regulates food intake, activates intestinal gluconeogenesis and increases the expression of anorectic hormones in the ileum and colon [3].

Phaseolus vulgaris L. bean leaves are a source of bioactive compounds, including protein, fiber, iron and polyphenols [4, 5], and their tender leaves, called quelites, are consumed in rural communities due to their accessibility and cooking versatility [6]. Previously, rats were supplemented with FME bean leaves for eight weeks with a 7-h daytime-restricted-feeding protocol (RFP) and showed improvements in insulin sensitivity, hepatic fat accumulation and lipid profiles compared to those of rats fed a high-fat/high-fructose diet (HFFD) with RFP [5]. Thus, the aim of this study was to evaluate the effect of bioactive compounds (fibers and polyphenols) from FME bean leaves on early metabolic alterations in male Wistar rats fed a HFFD *ad libitum*. This will provide an affordable option for health care, early metabolic disorder prevention and greater use of the whole crop.

Materials and Methods

This section is detailed in the Supplementary Material (Table S1).

Results and Discussion

Chemical Composition of *Phaseolus vulgaris* L. Bean Leaves This subsection is described in the Supplementary Material (Table S2).

Effect of *Phaseolus vulgaris* L. Bean Leaves on Anthropometric Parameters Increases in body weight gain and the abdominal and thoracic circumferences are low-cost measurements associated with early metabolic alteration development [7]. After week twos (Fig. 1), rats in Group H showed 8% higher weight with a 15% increase in weight gain, 11% in abdominal circumference (AC) and 7% in the abdominal/thoracic circumference index (AC/TC) compared with those of rats on the other diets (Table 1). As reported, the increases in the AC and AC/TC index in the high-fat or high-fructose model are related to higher visceral fat accumulation, the development of obesity (weight gain > 10%) and an increase in the risk of metabolic diseases [7, 8]. Moreover, fiber and polyphenol intake are associated with low weight gain and abdominal fat deposition [1], which could explain the lower weight (Fig. 1) and AC in the HBL group (Table 1), preventing obesity development.

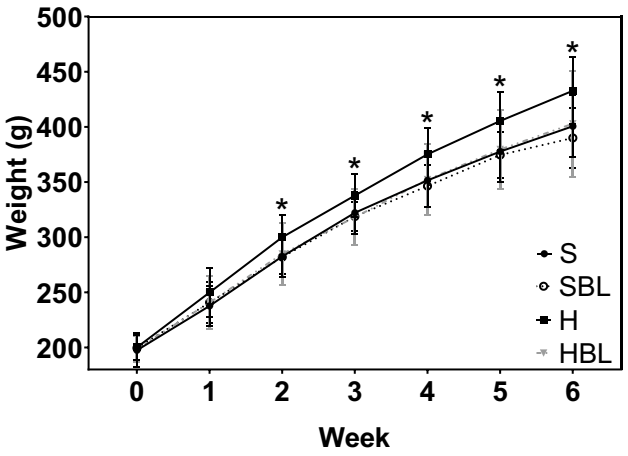

Fig. 1 Effect of *Phaseolus vulgaris* L. bean leaves on weight *per* week. Values are the mean ± SD ($n = 9$). ANOVA and the Duncan *post hoc* analysis were performed, and significant differences of $^*p \leq 0.05$ for H *versus* S, and H *versus* HBL were identified. S = standard diet, SBL = S + 10% FME bean leaves, H = high-fat/high-fructose diet, HBL = H + 10% FME bean leaves

Food, Water and Energy Intake In accordance with a previous report, [5], there was no significant difference in daily food and energy intake between the groups (Table 2). However, the water intake in the S group was higher than those of the H and HBL groups, without differences in urine excretion (Table 2). As was observed in other studies, a HFFD reduces water intake, perhaps due to the effect of fat on satiety or the absence of a sweet taste in the water [1]. It should be noted that the groups treated with bean leaves (SBL and HBL) showed a lower energy density intake compared to that of the control diet (Table 2). Fat has a high energy density, low satiety capacity, and great flavor, thus favoring the intake of greater amounts of energy [9].

Effect of *Phaseolus vulgaris* L. Bean Leaves on Glucose and Lipid Parameters Rats fed a HFFD (H and HBL) exhibited increases in total cholesterol and triglycerides (Table 3) compared to levels in the SBL and S groups. The SBL group had decreased triglyceride levels and hepatic accumulation (Table 3). This effect on serum and hepatic triglycerides was shown earlier by supplementation with 10% FME bean leaves in a HFFD with a 7-h RFP [10]. The quantity of hepatic triglycerides and MTP (microsomal transfer protein) activity regulate very-low-density lipoprotein cholesterol (VLDL-C) maturation. MTP has multiple regulators, and insulin and SREBP-1c (sterol regulatory element binding protein-1c) downregulate MTP, diminishing triglyceride exportation [11]. Increases in fasting insulin in the SBL group could explain the higher VLDL-C serum levels compared to those of the S group (Table 3). The HFFD upregulates SREBP-1c and SCD1 (stearyl-coenzyme A desaturase

Table 1 Effect of *Phaseolus vulgaris* L. bean leaves on anthropometric parameters

	S	SBL	H	HBL
Weight gain (g)	203.2 ± 25.8	193.0 ± 34.6	232.7 ± 30.3*	204.0 ± 41.8
Body length (cm)	24.4 ± 2.7	24.1 ± 1.6	24.0 ± 1.9	24.0 ± 2.0
Abdominal circumference (cm)	20.8 ± 2.1	20.3 ± 1.9	23.0 ± 2.0*	20.9 ± 2.2
Thoracic circumference (cm)	17.7 ± 1.6	17.3 ± 1.4	18.3 ± 1.5	17.6 ± 1.7
AC/TC index	1.18 ± 0.1	1.18 ± 0.1	1.26 ± 0.1*	1.19 ± 0.1
BMI (g/cm^2)	0.70 ± 0.2	0.68 ± 0.1	0.75 ± 0.1	0.71 ± 0.1

Values represent the mean ± SD ($n = 9$). ANOVA with the *post hoc* Duncan's test was performed, significant difference $^{*}p \leq 0.05$ H *versus* S, SBL and HBL was determined. S = standard diet, SBL = S + 10% FME bean leaves, H = high-fat/high-fructose diet, HBL = H + 10% FME bean leaves, AC = abdominal circumference, TC = thoracic circumference, BMI = body mass index, weight gain = final weight–initial weight

Table 2 Food, energy and water intake and their effect on urine excretion

	S	SBL	H	HBL
Energy density (kcal*g/ml)	2.29 ± 0.04	2.27 ± 0.03	2.76 ± 0.04 *	2.67 ± 0.05 *$^{\#}$
Food intake (g/day)	26.6 ± 4.7	25.7 ± 5.6	24.1 ± 8.4	20.7 ± 8.3
Energy intake (kcal/day)	87.4 ± 15.6	87.3 ± 19.2	106.2 ± 37.1	91.2 ± 36.6
Feed efficiency (%)	16.3 ± 5.9	16.8 ± 7.6	23.9 ± 15.6	22.1 ± 10.8
Water intake (ml/day)	49.0 ± 7.5	50.3 ± 5.9	39.8 ± 6.9*	42.1 ± 6.9*
Urine excretion (ml/day)	17.7 ± 10.4	17.9 ± 3.2	16.5 ± 6.3	18.1 ± 7.0

Values represent the mean ± SD ($n = 9$). ANOVA and Duncan's post hoc test were performed, and significant differences $^{*}p \leq 0.05$ H versus S and SBL, $^{\#}p \leq 0.05$ H versus HBL were determined. S = standard diet, SBL = S + 10% FME bean leaves, H = high-fat/high-fructose diet, HBL = H + 10% FME bean leaves, feed efficiency (%) = weight gain/food intake × 100. Urine excretion ml/day ($n = 6$)

Table 3 Effect of *Phaseolus vulgaris* L. bean leaves on biochemical parameters

	S	SBL	H	HBL
Total cholesterol (mg/dl)	62.2 ± 7.3	59.8 ± 7.9	76.1 ± 9.6$^{\#}$	74.4 ± 7.2$^{\#}$
Triglycerides (mg/dl)	102.3 ± 16.2	76.2 ± 12.9*	132.8 ± 41.8$^{\$}$	139.9 ± 28.4$^{\$}$
VLDL-C (mg/dl)	21.9 ± 2.6	14.4 ± 6.5$^{\$}$	17.8 ± 8.5	26.5 ± 5.7*
LDL-C (mg/dl)	7.3 ± 1.3	7.5 ± 1.3	7.2 ± 5.9	7.9 ± 2.6
HDL-C (mg/dl)	44.5 ± 1.9	41.2 ± 4.7	47.1 ± 5.5$^{\$}$	47.4 ± 2.9$^{\$}$
Glucose (mg/dl)	92.8 ± 20.1	88.2 ± 15.6	107.4 ± 44.0	104.7 ± 33.3
Insulin (mUI/ml)	11.5 ± 4.6	25.8 ± 4.9*	11.1 ± 6.9	12.0 ± 8.8
HOMA-IR	2.5 ± 0.9	4.9 ± 1.2*	3.3 ± 2.7	2.8 ± 2.0
Hepatic triglycerides	139.8 ± 71.8	81.1 ± 56.0*	155.0 ± 44.1	189.1 ± 71.7

Values represent the mean ± SD ($n = 6$). ANOVA and Duncan's analysis were performed, significant difference $^{*}p \leq 0.05$ *versus* S, H and HBL, $^{\#}p \leq 0.05$ *versus* S and SBL, $^{\$}p \leq 0.05$ *versus* S. S = standard diet, SBL = S + 10% FME bean leaves, H = high-fat/high-fructose diet, HBL = H + 10% FME bean leaves, VLDL-C = very-low-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, HDL-C = high-density lipoprotein cholesterol, HOMA-IR = homeostatic model assessment. Hepatic triglycerides mg/100 mg of tissue ($n = 4$)

1) by the AMPK/SREBP1c/SCD1 pathway [11], but bean leave supplementation with RFP downregulates SCD1, leading to VLDL-C exportation [11, 12]. This, together with higher hepatic triglycerides, could increase VLDL-C in HBL rats compared to H rats.

The HFFD promotes insulin resistance development [1]; in the early stages, hyperinsulinemia could be a mechanism to compensate for normal glycemia until lipotoxicity damages pancreatic β cells, decreasing insulin secretion [13]. In short-term treatments (< 6 weeks), polyphenols enhanced insulin secretion and glycemia, but long-term HFFD damage lessened some of the protective effect [14], which could explain the lack of a difference in fasting insulin in the HBL *versus* the H group. On the other hand, the SBL group had increased fasting insulin levels compared to those of the S

group, which could be related to an increase in SCFA production by bean leave supplementation (Fig. 3C-F), which may stimulate insulin secretion by FFA2/3 (free fatty acid receptors) [15].

The glycemic response after intraperitoneal administration of glucose (2 g/kg of weight) is shown in Fig. 2. At 30, 60, 90, and 120 min, the H and HBL groups presented increased glucose levels compared with the S group (Fig. 2A). It is known that alterations in the area under the curve (AUC) are an abnormal response to postprandial glycemia, which is an early stage of insulin resistance [16]. With respect to the global response, that is, the glucose AUC (Fig. 2B), Group H exhibited higher AUC values than those of Groups S and HBL. The data suggested that even when the HFFD could attenuate alterations in lipid and glucose metabolism, the supplementation of FME bean leaves improved the postprandial glycemia response [17]. The observations could be related to the fiber (Table S2) and polyphenol contents in the diets supplemented with bean leaves due to the increase in the production of SCFA and its reported effects, such as the regulation of food intake, decrease in adiposity and increase in insulin secretion [3].

Effect of *Phaseolus vulgaris* L. Bean Leaves on Fecal Excretion, Fat and Triglycerides The animals fed with SBL excreted 29% more feces than the levels of Group S, without differences between Groups H and HBL (Fig. 3A). The increase in fecal excretion could be related to a higher fiber content (Table S1) and greater water intake (Table 2). Moreover, fecal triglycerides (Fig. 3B) in total fecal fat showed no difference between Groups S and SBL, which was related to the high content of fat in the diet. Groups H and HBL presented higher levels of fecal triglycerides than those of Group S. Insoluble fiber intake promotes fecal excretion by mechanical stimulation and lower absorption of nutrients, including glucose and fat [18]. Because FME bean leaves have a high quantity of insoluble fiber (Table S2), the HBL and SBL treatments may not have an effect on fat absorption, in addition to the similar amounts of crude fiber in the H and HBL diets (Table S1).

Effect of *Phaseolus vulgaris* L. Bean Leaves on Short-chain Fatty Acid (SCFA) Analysis in the Colon Luminal Contents Finally, the production of SCFAs (acetate, propionate, isobutyrate, butyrate, isovalerate, valeric and isocaproic) was determined in the colon luminal contents (Fig. 3). In general, rats in Group S presented higher levels of total SCFAs than those in Group H. Furthermore, the FME bean leave supplementary diet boosted the concentration of SCFAs (Fig. 3C) in the SBL group to levels that were 42% higher than those in the S group, while the HBL group had levels that were 54% higher than those of the H group. Additionally, acetate, propionate and butyrate concentrations (Fig. 3D-F) increased with FME bean leave supplementation (SBL and HBL). It is well known that a HFFD decreases SCFA production [19], while fiber and polyphenols from plant-based food increase their concentration [2] by increasing the bacterial quantity and variety [20]. The protective effect of FME bean leave supplementation on weight gain, postprandial glycemia response, and visceral adiposity could be related to increases in SCFAs (Fig. 3C). The beneficial effects of increasing the SCFA concentration are attributed to their role in pancreatic β cells as agonists of FFA2 and FFA3, thus promoting insulin synthesis [15]. This could explain the serum insulin increases in SBL (Table 3). Additionally, FFA2 and FFA3 intestinal activation is associated with increases in PYY (peptide YY) and GLP-1 (glucagon-like peptide 1) synthesis, which promotes satiety [2, 21].

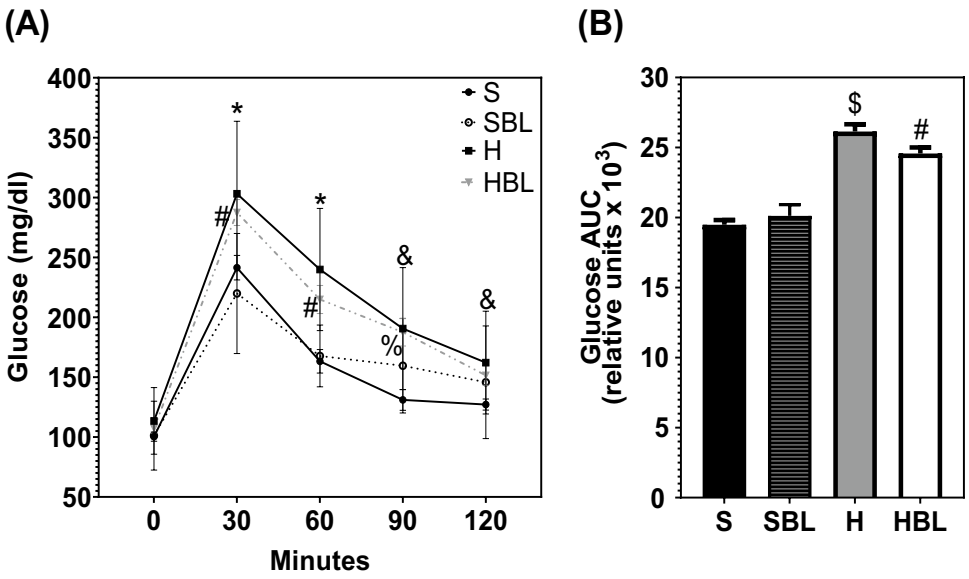

Fig. 2 Effect of *Phaseolus vulgaris* L. bean leaves on (**A**) the postprandial glycemia response and (**B**) the area under the curve in the intraperitoneal glycemic tolerance test. Values are the mean ± SD ($n = 9$). ANOVA and Duncan *post hoc* analysis were performed, significant difference $^{*}p \leq 0.05$ H vs. S and SBL, $^{\&}p \leq 0.05$ H vs. S, $^{\$}p \leq 0.05$ H vs. S, SBL and HBL; $^{\#}p \leq 0.05$ HBL vs. S and SBL $^{\%}p \leq 0.05$ HBL vs. S. S = standard diet, SBL = S + 10% FME bean leaves, H = high fat-high/high-fructose diet, HBL = H + 10% FME bean leaves

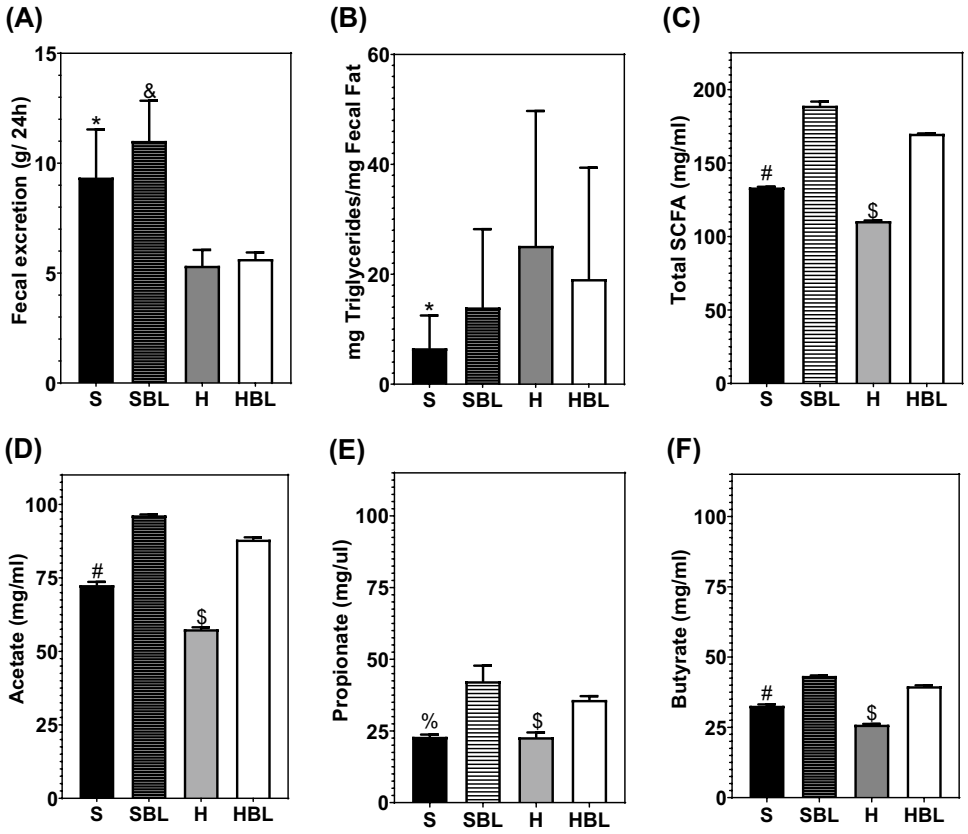

Fig. 3 Effect of *Phaseolus vulgaris* L. bean leaves on (**A**) fecal excretion *per* day, (**B**) triglycerides in fecal fat ($n=6$), and (**C-F**) the production of short-chain fatty acids in the colon luminal contents ($n=3$). Values correspond to the mean ± SD. ANOVA and Duncan *post hoc* analysis were performed, significant difference $^{*}p \leq 0.05$ S *vs*. H and HBL, $^{\&}p \leq 0.05$ SBL *vs*. H and HBL, $^{\#}p \leq 0.0001$ S *vs*. SBL, H and HBL, $^{\$}p \leq 0.0001$ H *vs*. SBL and HBL, $^{\%}p \leq 0.0001$ S *vs*. SBL and HBL. S = standard diet, SBL = S + 10% FME bean leaves, H = high-fat/high-fructose diet, HBL = H + 10% FME bean leaves, total SCFA: acetate, propionate, isobutyrate, butyrate, isovalerate, valeric and isocaproic

Conclusion

Globalization has affected food choices and their production, promoted a homogenized diet and reduced local food consumption [22]. Documented information about the nutritional, nutraceutical and functional potential of quelites could encourage their intake and increase the value of the crop. In terms of bioactive compounds, bean leaves are sources of iron [4], protein, insoluble fiber and polyphenols. This study is the first report that provides evidence of the protective effect of bean leave supplementation in the prevention of early metabolic alterations with *ad libitum* HFFD feeding. Fiber and polyphenols, as bioactive compounds present in FME bean leaves, prevent obesity and impairments in glucose metabolism and modulate hepatic triglyceride exportation in a HFFD model. Interestingly, these effects could be related to the fermentation of the indigestible fraction of bean leaves and an increase in SCFA production, which are involved in the modulation of food intake, adipose tissue accumulation, and lipidic and glycemic control. Bean leaves are a low-cost alternative with nutraceutical and functional potential in human health. Further studies of the protective effects of the bioactive compounds in bean leaves in *ad libitum* diets should be conducted. In addition, future research will allow us to understand the mechanism through which bean leaves affect metabolic alterations through liver function, gut health and modulation of energetic metabolism.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11130-022-00992-1.

Acknowledgements We are grateful to Verónica Andrade, Josué López, Aurora Jáuregui and Brigitte Vigouroux for technical support.

Author Contribution AABC, PVOA and SMAS conceived and designed the analysis, collected the data, performed the analysis and wrote the paper. CMJ cultivated and harvested bean leaves. KEG performed the proximal analyses of bean leaves and the short-chain fatty acid quantification in luminal contents. MCB and MAAL performed chemical parameter analysis in blood. AAFP contributed to the phytochemical quantification of bean leaves. TGG contributed analysis tools, equipment and infrastructure. All authors reviewed the manuscript.

Funding This work was supported by the Fondo de Proyectos Especiales de Rectoría-UAQ [FOPER: 2019–00798; 2019–00594; 2020-FCN01886; and 2021-FCN02473]; Programa para el Desarrollo del Profesorado-UAQ [PRODEP-UAQ-2018–2020]; and Fondo para el Desarrollo del Conocimiento-UAQ [FONDEC–UAQ 2021]. AABC and PVOA were fellows of Consejo Nacional de Ciencia y Tecnología [CONACYT: 734854 and 711939].

Data Availability All relevant data are included in the article. The datasets are available from the corresponding author on reasonable request.

Declarations

Ethics Approval All performed procedures were previously approved by the institutional bioethics committee (06FCN2019 and 77FCN2019). Additionally, bioethical standards were applied: NOM-062-Z00-1999 and ARRIVE guidelines.

Consent to Participate Not applicable.

Consent for Publication All authors consent to the publication of this article.

Conflicts of Interest The authors declare no conflicts of interest.

References

1. Pahua-Ramos ME, Garduño-Siciliano L, Dorantes-Alvarez L, Chamorro-Cevallos G, Herrera-Martínez J, Osorio-Esquivel O, Ortiz-Moreno A (2014) Reduced-calorie avocado paste attenuates metabolic factors associated with a hypercholesterolemic-high fructose diet in rats. Plant Foods Hum Nutr 69(1):18–24. https://doi.org/10.1007/s11130-013-0395-4
2. Hernández MAG, Canfora EE, Jocken JWE, Blaak EE (2019) The short-chain fatty acid acetate in body weight control and insulin sensitivity. Nutrients 11(8):1943. https://doi.org/10.3390/NU11081943
3. De Vadder F, Kovatcheva-Datchary P, Goncalves D, Goncalves D, Vinera J, Zitoun C, Duchampt A, Bäckhed F, Mithieux G (2014) Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. Cell 156(1–2):84–96. https://doi.org/10.1016/j.cell.2013.12.016
4. Martínez-Zavala M, Mora-Avilés MA, Anaya-Loyola MA, Guzmán-Maldonado H, Aguilera-Barreyro A, Blanco-Labra A, García-Gasca T (2016) Common bean leaves as a source of dietary iron: Functional test in an iron-deficient rat model. Plant Foods Hum Nutr 71(3):259–264. https://doi.org/10.1007/s11130-016-0554-5
5. Ramírez-Venegas G, De Ita-Pérez DL, Díaz-Muñoz M, Méndez I, García-Gasca T, Ahumada-Solórzano M, Zambrano-Estrada X, Vázquez-Martínez O, Guzmán-Maldonado H, Luna-Moreno D (2021) Supplementation with Phaseolus vulgaris leaves improves metabolic alterations induced by high-fat/fructose diet in rats under time-restricted feeding. Plant Foods Hum Nutr 76(3):297–303. https://doi.org/10.1007/S11130-021-00904-9
6. Linares E, Bye R, Ortega N, Arce AE (2017) Quelites: sabores y saberes, del sureste del Estado de México, Primera. Universidad Nacional Autónoma de Mexico, Ciudad de México. https://doi.org/10.22201/ib.9786073016667e.2019
7. Gerbaix M, Metz L, Ringot E, Courteix D (2010) Visceral fat mass determination in rodent: Validation of dual-energy x-ray absorptiometry and anthropometric techniques in fat and lean rats. Lipids Health Dis 9:140. https://doi.org/10.1186/1476-511X-9-140
8. Buettner R, Schölmerich J, Bollheimer LC (2007) High-fat diets: Modeling the metabolic disorders of human obesity in rodents. Obesity 15(4):798–808. https://doi.org/10.1038/OBY.2007.608
9. Ello-Martin JA, Ledikwe JH, Rolls BJ (2005) The influence of food portion size and energy density on energy intake: implications for weight management. The Am J Clin Nutr 82(1 Suppl):236S–41S. https://doi.org/10.1093/AJCN/82.1.236S
10. Becerril Campos AA, Luna Moreno AD, GarcíaGasca MTdeJ, Ahumada Solórzano SM (2018) Prevención del riesgo metabólico en un modelo *in vivo* con el consumo de quínoa roja (*Chenopodium Berlandieri* Spp. Nuttalliae) y hoja de frijol (*Phaseolus vulgaris*). Revista de la Conferencia Científica Anual sobre Síndrome Metabólico 4:13
11 Adeli K, Xiao C, Higgins V et al (2021) Diabetic dyslipidaemia. In: Ridgway ND, McLeod RS (eds) Biochemistry of lipids, lipoproteins and membranes, 7th edn. Elsevier, pp 667–6935
12. Zhu X, Bian H, Wang L, Sun X, Xu X, Yan H, Xia M, Chang X, Lu Y, Li Y, Xia P, Li X, Gao X (2019) Berberine attenuates nonalcoholic hepatic steatosis through the AMPK-SREBP-1c-SCD1 pathway. Free Radic Biol Med 141:192–204. https://doi.org/10.1016/J.FREERADBIOMED.2019.06.019
13. Engin AB (2017) What is lipotoxicity? In: Advances in experimental medicine and biology. pp 197–220
14. Ghorbani A, Rashidi R, Shafiee-Nick R (2019) Flavonoids for preserving pancreatic beta cell survival and function: A mechanistic review. Biomed Pharmacother 111:947–957. https://doi.org/10.1016/J.BIOPHA.2018.12.127
15. Liu JL, Segovia I, Yuan XL, Gao ZH (2020) Controversial roles of gut microbiota-derived short-chain fatty acids (SCFAs) on pancreatic β-cell growth and insulin secretion. Int J Mol Sci 21(3):910. https://doi.org/10.3390/IJMS21030910
16. Sakaguchi K, Takeda K, Maeda M, Ogawa W, Sato T, Okada O, Ohnishi Y, Nakajima H, Kashiwagi A (2016) Glucose area under the curve during oral glucose tolerance test as an index of glucose intolerance. Diabetology Int 7(1):53–58. https://doi.org/10.1007/s13340-015-0212-4
17. Hosseinpour-Niazi S, Mirmiran P, Sohrab G, Hosseini-Esfahani F, Azizi F (2011) Inverse association between fruit, legume, and cereal fiber and the risk of metabolic syndrome: Tehran Lipid and Glucose Study. Diabetes Res Clin Pract 94(2):276–283. https://doi.org/10.1016/j.diabres.2011.07.020
18. McRorie JW, McKeown NM (2017) Understanding the physics of functional fibers in the gastrointestinal tract: An evidence-based approach to resolving enduring misconceptions about insoluble and soluble fiber. J Acad Nutr Diet 117(2):251–264. https://doi.org/10.1016/j.jand.2016.09.021
19. Sulistyowati E, Handayani D, Soeharto S, Rudijanto A (2021) A high fat-fructose diet lowers the cecal digesta weight and short chain fatty acid level of Sprawe Dawley rat model. Turk J Med Sci 52(1). https://doi.org/10.3906/sag-1911-14
20. Parkar SG, Trower TM, Stevenson DE (2013) Fecal microbial metabolism of polyphenols and its effects on human gut microbiota. Anaerobe 23:12–9. https://doi.org/10.1016/J.ANAEROBE.2013.07.009
21. Edwards CA, Havlik J, Cong W, Mullen W, Preston T, Morrison DJ, Combet E (2017) Polyphenols and health: Interactions between fibre, plant polyphenols and the gut microbiota. Nutr Bull 42(4):356–360. https://doi.org/10.1111/nbu.12296
22. Mateos-Maces L, Chávez-Servia JL, Vera-Guzmán AM, Aquino-Bolaños EN, Alba-Jiménez JE, Villagómez-González BB (2020) Edible leafy plants from Mexico as sources of antioxidant compounds, and their nutritional, nutraceutical and antimicrobial potential: A review. Antioxidants 9(6):541. https://doi.org/10.3390/antiox9060541

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Supplementary material.

Abbreviations

AC	abdominal circumference
AC/TC	abdominal/thoracic circumferences index
ACD	acid detergent fiber
AUC	area under the curve
BMI	body mass index
FME	Flor de Mayo Eugenia
GLP-1	glucagon-like peptide 1
H	high-fat/high-fructose diet group
HBL	high-fat/high-fructose diet +10% of dry bean leaves group
HDL-C	high-density lipoprotein cholesterol
HFFD	high-fat/high-fructose diet
HOMA-IR	homeostatic model assessment
LDL-C	low-density lipoprotein cholesterol
NDF	neutral detergent fiber
Pklr	pyruvate kinase gene
PV	Pinto Villa
PYY	peptide YY
RFP	daytime restricted feeding protocol
S	standard diet group
SBL	standard diet+10% of dry bean leaves group
Scd1	stearoyl-CoA desaturase-1 gene
SCFA	short-chain fatty acids

TC	thoracic circumference
VLDL-C	very-low-density lipoprotein cholesterol

Materials and Methods

Materials and Methods

Plant material. The improved variety of bean seeds, Phaseolus vulgaris L. Flor Mayo Eugenia (FME) [25], was grown in Universidad Autónoma de Querétaro, campus Amazcala. The improvements in the FME variety are disease resistance to common illnesses, larger-sized seeds with a short cooking time and greater contents of iron and zinc [25]. The plants were grown under irrigation conditions without adding fertilizer, animal manure, herbicides or pesticides. FME bean leaves were harvested until the plant reached the maximum canopy cover (60-70 days), coincident with flowering initiation. They were rinsed in distilled water, dried to constant weight (40 °C), ground (2 mm), packed in sealed bags, and stored in dark containers at room temperature until processed.

Chemical analysis of *Phaseolus vulgaris L.* bean leaves. The chemical composition of dried FME bean leaves was analyzed according to the AOAC official methods: moisture (AOAC 930.15), ash (AOAC 942.05), protein by the Kjeldahl method (AOAC 2001.11), lipids by ether extract (AOAC 920.39), crude fiber (AOAC 962.09), and neutral and acid detergent fiber [26]. Carbohydrates were calculated by the difference. Dietary fiber analysis was performed by enzymatic assay (Total Dietary Fiber Assay Kit, Sigma–Aldrich) [27]. Total phenols were determined in the dried sample by the Folin–Ciocalteu method with modifications. Gallic acid was used as a standard for the calibration curve. The sample and standard solutions were read

at 750 nm in a UV–Vis spectrophotometer (Genesys 10S UV–Vis, Thermo Fischer Scientific, USA) [28].

Animal model and experimental design. For the animal model, 36 male Wistar rats were randomly divided into one of four experimental groups: 1) standard diet (S) fed with Rodent Laboratory Chow 5001®; 2) S+10% dry FME bean leaves (SBL); 3) high-fat (lard) and high-fructose diet (H); and 4) high-fat/high-fructose diet with 10% dry FME bean leaves (HBL). The control group contained 3.4 kcal/g, and the high-fat/high-fructose diets had 4.4-4.6 kcal/g [14], as detailed in Table S1.

Animals were housed in individual cages under controlled temperature and moisture conditions, with a 12-h light/dark cycle and water and food provided *ad libitum* [29]. Weight gain and water and food consumption were registered weekly during the 6 experimental weeks. At the last week, under fasting conditions (6-8 h), intraperitoneal glucose tolerance was evaluated with an Accu Check Active® glucometer [30]. After 6 weeks, blood samples were collected and maintained at -80 °C. Anthropometric measures were taken, including body length, abdominal circumference (AC), thoracic circumference (TC), AC/TC index, and body mass index (BMI) [11].

Biochemical markers in serum and liver tissue. Glucose, total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) levels were evaluated through enzymatic-colorimetric assays in the clinical analyzer Spin 120® with Spinreact® reagents. Insulin was determined by immunoassay (ELISA Rat insulin kit Alpco®, RRID: AB_2820242) on a Molecular Devices Spectramax 250 Microplate Reader. Very-low-density lipoprotein (VLDL-C) and the homeostasis model assessment of insulin

resistance (HOMA-IR) were calculated [31]. For hepatic triglyceride quantification, 300 mg of liver tissue was mixed with 2:1 (W/V) Folch's reagent (chloroform: methanol 2:1, V/V). Four milliliters of saline solution (0.9% NaCl) were added and mixed. The mixture was centrifuged at 2000 × g for 10 min. After 24 h (25 °C) of evaporation, the remaining pellet was reconstituted with saline solution and determined by enzymatic-colorimetric assay on a 96-well plate at 37 °C with Spinreact® reagent on a Molecular Devices Spectramax 250 Microplate Reader at 505 nm [32].

Analysis of excreted total fecal fat and triglycerides. Fresh feces were collected weekly for 24 h and weighed. The analysis of fecal fat was performed on 400 mg of fresh feces by the Folch method. Once the moisture from the sample was evaporated, the remaining pellet was weighed [33]. For triglyceride quantification, an enzymatic-colorimetric assay was performed on a Molecular Devices Spectramax 250 Microplate Reader at 505 nm. Hepatic triglyceride quantification is described elsewhere [32].

Short-chain fatty acid analysis in the colon luminal contents. Colon luminal samples stored at -80 °C were thawed, and approximately 2 g was weighed into centrifuge tubes. HPLC water was added according to the amount of sample in the tube (2 g of sample, 3 mL of water). Each tube was vortexed for 30 s. The tubes were placed in the centrifuge (Beckman Coulter) for 30 min at 4000 rpm and 4 °C. The previously filtered contents were poured into a small amber tube. Up to 1 µL of supernatant was injected into the gas chromatograph coupled to a flame ionization detector (Agilent, 6850 series GC system) under the following conditions: helium (40 mL/s) was used as the carrier gas, and the oven started at a temperature of 100 °C

for 5 min and increased by 10 °C until reaching 250 °C. To determine the individual SCFAs (acetate, propionate, isobutyrate, butyrate, isovalerate, valeric and isocaproic) of the sample, the retention times were determined using standard solutions [34].

Statistical analysis. All experimental results are expressed as the mean ± standard deviation (SD). Differences between the groups were analyzed by one-way ANOVA and Duncan's post hoc test. This is a more powerful (in the statistical sense) alternative to almost all other post hoc methods [19]. $p \leq 0.05$ was considered to be statistically significant. Data were analyzed using IBM SPSS Statistics v.25 (RRID:SCR_019096) and GraphPad Prism 8 (RRID:SCR_002798) for the graphics.

Results and Discussion

Chemical composition of *Phaseolus vulgaris L*. bean leaves. According to other authors, *quelites* such as bean leaves contribute significantly to the nutrient supply, which is comparable to the commonly consumed vegetable leaf supply [7]. All the analyses were calculated using the dry matter basis (Table S2) of FME bean leaves containing 25.7% protein, 25.9% dietary fiber, 29.33% neutral detergent fiber (NDF) and 16.7% acid detergent fiber (ADF). The amount of protein was similar to that observed in *Phaseolus vulgaris L.* Pinto Villa (PV) bean leaves [4] and higher than that in different varieties of bean seeds [8]. Dietary fiber is composed of soluble and insoluble fiber. FME bean leaves had higher amounts of insoluble fiber (NDF and ADF) than soluble fiber (Table S2), in accordance with previous reports.[9]. Regarding the total phenolic content, FME bean leaves had 2.9 mg gallic acid equivalent (Table S2). PV bean leaves contain twice the amount of total phenolic compounds [4], while black and speckled bean seed reports showed similar amounts

of total phenolic compounds [7, 8]. In a previous study, the total phenolic content and antioxidant capacity increased 53% and 31%, respectively, when 10% FME bean leaves were added to a HFFD diet [5]. A low-sugar diet with a high content of protein, dietary fiber and polyphenols has been related to a lower risk of developing noncommunicable chronic diseases, such as dyslipidemia, diabetes, and obesity [10].

References

23. Acosta Gallegos JA, Jiménez Hernández Y, Sánchez García BM, et al (2010) Flor de Mayo Eugenia, new bean cultivar for irrigated and rainfall conditions in central Mexico. Revista Mexicana de Ciencias Agrícolas 5:
24. Horwitz W (2000) Official methods of analysis of AOAC International, 17th ed. The Association of Official Analytical Chemists, Gaithersburg, MD, USA
25. Heredia Ortega J, Aguilera Barreyo A, Reis de Souza T, et al (2014) Fibra dietética total: métodos de determinación en pastas de oleaginosas. In: Cantó Alarcón G, Elton Puente JE (eds) Desarrollo de la investigación en la Facultad de Ciencias Naturales, Primer Edi. D.R. Facultad de Ciencias Naturales, Querétaro, México, pp 90–102
26. Dewanto V, Xianzhong W, Adom KK, Liu RH (2002) Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J Agric Food Chem. https://doi.org/10.1021/jf0115589
27. Percie du Sert N, Ahluwalia A, Alam S, et al (2020) Reporting animal research: Explanation and elaboration for the arrive guidelines 2.0. PLoS Biol. https://doi.org/10.1371/journal.pbio.3000411

143 28. Ghezzi AC, Cambri LT, Botezelli JD, et al (2012) Metabolic syndrome markers in
144 wistar rats of different ages. Diabetol Metab Syndr. https://doi.org/10.1186/1758-
145 5996-4-16
146 29. Antunes LC, Elkfury JL, Jornada MN, et al (2016) Validation of HOMA-IR in a
147 model of insulin-resistance induced by a high-fat diet in Wistar rats. Arch
148 Endocrinol Metab. https://doi.org/10.1590/2359-3997000000169
149 30. Eggers LF, Schwudke D (2016) Liquid extraction: Folch. In: Encyclopedia of
150 Lipidomics. Springer Netherlands, pp 1–6
151 31. Shang W, Si X, Zhou Z, et al (2017) Characterization of fecal fat composition and
152 gut derived fecal microbiota in high-fat diet fed rats following intervention with
153 chito-oligosaccharide and resistant starch complexes. Food Funct.
154 https://doi.org/10.1039/C7FO01244F
155 32. García KE, de Souza TCR, Landín GM, et al (2014) Microbial fermentation
156 patterns, diarrhea incidence, and performance in weaned piglets fed a low protein
157 diet supplemented with probiotics. Food and Nutrition Sciences 05:1776–1786.
158 https://doi.org/10.4236/FNS.2014.518191
159 33. Hernández-Guerrero CJ, Villa-Ruano N, Zepeda-Vallejo LG, et al (2021) Bean
160 cultivars (Phaseolus vulgaris L.) under the spotlight of NMR metabolomics. Food
161 Res Int. https://doi.org/10.1016/J.FOODRES.2021.110805
162 34. Moghaddam S, Brick M, Echeverria D, et al (2018) Genetic architecture of dietary
163 fiber and oligosaccharide content in a middle american panel of edible dry bean.
164 Plant Genome.https://doi.org/10.3835/PLANTGENOME2017.08.0074
165

Tables

Table S1 Composition of the experimental diets consumed for 6 weeks.

	Energy (kcal/g)	Protein (%)	Fat (%)	Carbohydrate (%)	Fiber (%)
Standard diet	3.4	29.8	13.4	56.7	5.3
Standard diet + 10% FME bean leaves	3.6	29.3	13.2	57.5	5.1
High-fat/high-fructose	4.4	20.3	39.3	40.4	3.6
High-fat/high-fructose + 10% FME bean leaves	4.6	19.2	37.7	43.1	3.7

Table S2 Nutritional composition of dry *Phaseolus vulgaris L.* bean leaves

Determination	
Moisture (%)	6.88 ± 0.02
Ash (%)	13.62 ± 0.22
Protein (%)	25.69 ± 0.18
Carbohydrates (%)	49.33 ± 0.67
Crude fiber (%)	9.94 ± 0.21
Dietary fiber (%)	25.85 ± 1.37
Neutral detergent fiber (%)	29.33 ± 0.71
Acid detergent fiber (%)	16.72 ± 0.59
Lipids (%)	1.42 ± 0.05
Total phenols (mg GAE/g)	2.924 ± 0.004

Values represent the mean ± SD of triplicate analyses. GAE= gallic acid equivalent

IV. Capítulo 2. Efecto de la hoja de frijol en la lipotoxicidad en la enfermedad por hígado graso no alcohólico.

(Becerril-Campos et al. 2023)

nutrients

IMPACT FACTOR 5.9

Indexed in: PubMed

CITESCORE 9.0

Article

Bean Leaves Ameliorate Lipotoxicity in Fatty Liver Disease

Adriana Araceli Becerril-Campos, Minerva Ramos-Gómez, Ericka Alejandra De Los Ríos-Arellano , Perla Viridiana Ocampo-Anguiano, Adriana González-Gallardo, Yazmín Macotela, Teresa García-Gasca and Santiaga Marisela Ahumada-Solórzano

Special Issue

The Benefits of Natural Products for Disease Treatments

Edited by

Prof. Dr. Sang J. Chung and Prof. Dr. Ki Hyun Kim

MDPI

https://doi.org/10.3390/nu15132928

nutrients

MDPI

Article

Bean Leaves Ameliorate Lipotoxicity in Fatty Liver Disease

Adriana Araceli Becerril-Campos [1], Minerva Ramos-Gómez [2], Ericka Alejandra De Los Ríos-Arellano [3], Perla Viridiana Ocampo-Anguiano [1,2], Adriana González-Gallardo [4], Yazmín Macotela [5], Teresa García-Gasca [1] and Santiaga Marisela Ahumada-Solórzano [6,*]

1 Laboratory of Cellular and Molecular Biology, Faculty of Natural Sciences, Autonomous University of Queretaro, Campus Juriquilla, Av. De las Ciencias S/N, Queretaro 76230, Mexico; adi.becerril@gmail.com (A.A.B.-C.); tggasca@uaq.edu.mx (T.G.-G.)
2 Food Research and Graduate Department, School of Chemistry, Autonomous University of Queretaro, Centro Universitario, Cerro de las Campanas S/N, Queretaro 76010, Mexico
3 Microscopy Unit, Institute of Neurobiology (INB), National Autonomous University of Mexico (UNAM), Campus UNAM-Juriquilla, Queretaro 76230, Mexico; erios.histologia.inb@gmail.com
4 Proteogenomic Unit, Neurobiology Institute, National Autonomous University of Mexico, Campus UNAM-Juriquilla, Queretaro 76230, Mexico; gallardog@unam.mx
5 Instituto de Neurobiología, Universidad Nacional Autónoma de México (UNAM), Campus UNAM-Juriquilla, Queretaro 76237, Mexico; macotelag@unam.mx
6 Interdisciplinary Research in Biomedicine, Faculty of Natural Sciences, Autonomous University of Queretaro, Campus Juriquilla, Av. De las Ciencias S/N, Queretaro 76230, Mexico
* Correspondence: santiaga.marisela.ahumada@uaq.mx; Tel.: +52-1-442-562-3388

Abstract: Bioactive compounds in plant-based food have protective effects against metabolic alterations, including non-alcoholic fatty liver disease (NAFLD). Bean leaves are widely cultivated in the world and are a source of dietary fiber and polyphenols. High fat/high fructose diet animal models promote deleterious effects in adipose and non-adipose tissues (lipotoxicity), leading to obesity and its comorbidities. Short-term supplementation of bean leaves exhibited anti-diabetic, anti-hyperlipidemic, and anti-obesity effects in high-fat/high-fructose diet animal models. This study aimed to evaluate the effect of bean leaves supplementation in the prevention of lipotoxicity in NAFLD and contribute to elucidating the possible mechanism involved for a longer period of time. During thirteen weeks, male Wistar rats (n = 9/group) were fed with: (1) S: Rodent Laboratory Chow 5001® (RLC); (2) SBL: 90% RLC+ 10% dry bean leaves; (3) H: high-fat/high-fructose diet; (4) HBL: H+ 10% of dry bean leaves. Overall, a HBL diet enhanced impaired glucose tolerance and ameliorated obesity, risk factors in NAFLD development. Additionally, bean leaves exerted antioxidant (↑serum GSH) and anti-inflammatory (↓mRNA TNFα in the liver) effects, prevented hepatic fat accumulation by enhanced ↑mRNA PPARα (β oxidation), and enhanced lipid peroxidation (↓liver MDA). These findings suggest that bean leaves ameliorated hepatic lipotoxicity derived from the consumption of a deleterious diet.

Keywords: fatty liver; bean leaves; MAFLD; lipotoxicity; bioactive compounds; dietary fiber; polyphenols; Nrf2; PPARs; NFκB

check for updates

Citation: Becerril-Campos, A.A.; Ramos-Gómez, M.; De Los Ríos-Arellano, E.A.; Ocampo-Anguiano, P.V.; González-Gallardo, A.; Macotela, Y.; García-Gasca, T.; Ahumada-Solórzano, S.M. Bean Leaves Ameliorate Lipotoxicity in Fatty Liver Disease. *Nutrients* **2023**, *15*, 2928. https://doi.org/10.3390/nu15132928

Academic Editors: Ki Hyun Kim and Sang J. Chung

Received: 2 June 2023
Revised: 26 June 2023
Accepted: 27 June 2023
Published: 28 June 2023

Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is a metabolic hepatic disease. The disease spectrum begins in steatosis, is characterized by macrovesicular lipid accumulation (≥5% hepatocytes), and over time it progresses to steatohepatitis (NASH) with hepatocellular ballooning, oxidative stress, inflammation, and fibrosis, leading to advanced cirrhosis and hepatocarcinoma [1,2]. About 25% of the worldwide population is affected by NAFLD and its onset involves genetic factors, insulin resistance, obesity, lipotoxicity, and gut dysbiosis [2]. Lipotoxicity drives the onset and progression of NAFLD, and is the result of excessive accumulation of lipids in the liver (steatosis) and other peripheral tissues affecting

their storage and oxidative capabilities. Even though steatosis is considered an early stage, it is usually accompanied by other comorbidities that can stimulate the rapid progression of the disease [3]. Steatosis is a reversible stage where the control of the comorbidities can favor its reversal and prevent the progression of the disease.

Epidemiological and experimental studies demonstrated that plant-based foods, such as common beans, (fruits, legumes, vegetables, spices, coffee, and tea) and their bioactive compounds have protective effects against steatosis, oxidative stress, inflammation, and gut dysbiosis [4]. *Phaseolus vulgaris* L. is highly worldwide consumed and cultivated because of its seeds, pods, and leaves [5]. The main nutritional compounds in bean leaves are protein (24.5–25.7%) and dietary fiber (25.9%, most of which was insoluble). Regarding polyphenols and micronutrients, bean leaves reported iron (275 mg/kg), total phenolic compounds (2.14–5.79 mg/g), and tannins (3 mg/g). Like many pulses, bean leaves had antinutritional compounds such as phytates (39.3 mg/g) and protease inhibitors (2.1 IU/mg of protein) [6,7]. Bean leaves are a low-cost alternative with nutraceutical and functional potential in human health due to their content of bioactive compounds [7].

In this regard, the effect of bean leaves supplementation in the high-fat/high-fructose feeding model was evaluated. After 8 weeks, under a 7-h daytime-restricted-feeding protocol (RFP), 10% of bean leaves supplementation improved insulin sensitivity, and diminished hepatic fat accumulation and hyperlipidemia [8]. Additionally, ad libitum bean leaves supplementation (10%) for 6 weeks without RPF prevented obesity and impairments in glucose metabolism, possibly related to an increase of 54% in SCFA (short-chain fatty acids) production [7]. However, the effect of bean leaves on oxidative stress and inflammation related to lipotoxicity in fatty liver disease has not been studied.

Nrf2 (nuclear factor erythroid-derived 2-like 2) had aroused interest as a therapeutic target in the treatment of metabolic diseases because of its ability to regulate about 250 genes involved in the adaptive response to antioxidants and xenobiotics, under physiological and pathological conditions [9,10]. Due to Keap 1 (Kelch-like ECH-associated protein 1) sensitivity to electrophiles, mainly regulators of Nrf2, including exogenous antioxidants from plant-based food, interest has grown in understanding the role of Nrf2 as a therapeutic target for NAFLD [10]. The crosstalk between Nrf2 and PPARα (peroxisome proliferator-activated receptor alpha) promotes the oxidation of fatty acids (β oxidation) to avoid hepatic lipid accumulation [10,11]. Additionally, Nrf2 prevents the progression to steatohepatitis by modulating oxidative stress, increasing the expression of antioxidant enzymes, and by delaying the inflammatory response mediated by NFκB (nuclear factor kappa B) [10,12].

Based on the reported beneficial effects of bioactive compounds and the interest in improving people's health, through the revaluing and encouraging the inclusion of vegetables in the diet such as highly cultivated bean leaves, this study aims to evaluate the effect of 10% bean leaves dietary supplementation to prevent lipotoxicity in fatty liver disease and related comorbidities as obesity, insulin resistance, and hyperlipemia.

2. Materials and Methods

2.1. Diet Design

Bean leaves (*Phaseolus vulgaris* L.) from the Flor Mayo Eugenia variety were cropped after 60–70 cultivating days at the experimental campus of the Autonomous University of Queretaro, Amazcala, Mexico. Bean leaves were dried at 40 °C (to constant weight), ground, and stored in darkness at room temperature (RT), to further analyze their chemical composition. Based on the proximal analysis outcome, four diets were designed (Table 1):

(1) S: Rodent Laboratory Chow 5001® (RLC), 3.4 kcal/g;
(2) SBL: mixture of 90% RLC+ 10% dry bean leaves, 3.6 kcal/g;
(3) H: high fat (lard) and high fructose diet, 4.4 kcal/g;
(4) HBL: H was supplemented with 10% of dry bean leaves, 4.6 kcal/g [7].

Table 1. Composition of experimental diets.

Diet	Energy (kcal/g)	Protein (g/100 g)	Fat (g/100 g)	Carbohydrate (g/100 g)	Fiber (g/100 g)	Ingredients
S	3.4	25.3	11.4	48.2	5.3	RLC 5001®
SBL	3.6	26.4	11.9	51.8	5.1	RLC 5001®, bean leaves, calcium caseinate, soy oil
H	4.6	22.3	43.2	44.4	3.6	RLC 5001®, lard, fructose, calcium caseinate, wheat bran
HBL	4.8	22.1	43.4	49.6	3.7	RLC 5001®, lard, fructose, calcium caseinate, bean leaves

RLC = Rodent Laboratory Chow, S = standard diet, SBL = S + 10% bean leaves, H = high-fat/high-fructose diet, HBL = H + 10% bean leaves.

2.2. Experimental Design

The experimental design and procedures were previously approved by the Ethics Committee of the Faculty of Natural Sciences, Autonomous University of Queretaro (77FCN2019); and the number of rats was minimized following the 3Rs principles [13].

Thirty-six male Wistar rats (198.4 ± 1.6 g) were aleatory randomized after one week of acclimatization in four experimental groups: (1) S, (2) SBL, (3) H, and (4) HBL. The rats were housed in individual plastic cages for thirteen weeks, kept under fully controlled conditions (temperature and moisture), 12/12 h dark/light cycle, with water and food ad libitum. After 13 weeks, blood and liver samples were collected, processed, and maintained at −80 °C until their analysis.

2.3. Body Measurements and Body Composition

Body weight, food intake, and water intake were registered weekly. At the thirteenth week, body length, abdominal (AC) and thoracic circumferences (TC), AC/TC ratio, and body mass index (BMI) [14] were measured in awake, unanesthetized rats. Total body weight gain was calculated as the difference between the final and the initial body weight. The energy and food intake per day are the averages of the week's registration, and the total energy and total food intake are cumulative sums of the whole period.

In the last experimental week, five animals from each group were selected to perform magnetic resonance imaging (MRI). The rats were anesthetized with 1.5–2% isoflurane in combination with medical air. MRI analyses were performed using Teslas Bruker Pharmascan 70/16US MR scanner (MA, USA) in the National Laboratory for magnetic resonance imaging (LANIREM-INB-UNAM). The image was acquired by water-suppressed Turbo Rapid Imaging with Refocused Echos in two dimensions (RARE, Rapid Imaging with Refocused Echoe factor = 8). The scanning time was 1:40 min with motion compensation by respiratory-triggered acquisition and considering the following parameters: matrix size 209 × 191, 54 slices with 3 mm thickness and slice gap 2 mm, repetition time 4359 ms, echo time 25 ms, and 5 averages. The field of view was 65 × 47 mm, with a resolution of 311 × 247 μm and a flip angle of 90°. Coronal and transversal images were used for fat quantification [3]. Fat quantification was performed by segmentation using the 3D Slicer® program [15,16].

2.4. Analysis of Biochemical Parameters

Postprandial glucose tolerance was evaluated during the thirteenth week by an intraperitoneal glucose tolerance test (IpGTT) on 4–6 h fasting rats. Baseline glycemia (0 min) was measured in the caudal vein with the Accu-Check Active® glucometer (Chennai, India). Intraperitoneal injection of 20% glucose solution (2 g/kg) was administrated and glycemia was measured at 30, 60, 90, and 120 min [17].

Enzymatic-colorimetric assays were used to determine circulating glucose, total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-c), and low-density lipoprotein cholesterol (LDL-c) parameters. In order to evaluate liver function, the levels of

aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin (A), and globulin (G) were determined. All the analyses were carried out in serum samples by using Spinreact® reagents in the clinical analyzer Spin 120® [7].

Insulin (ELISA Rat insulin kit Alpco® 2820242), oxidized LDL (Rat Ox-LDL ELISA kit MYBioSurce MBS2501477), and C-reactive protein (Rat CRP SimpleStep ELISA Kit Abcam, ab256398) were analyzed by immunoassay on a microplate reader (Molecular Devices Spectramax 250). AST/ALT ratio, A/G ratio (albumin/globulin), very-low-density lipoprotein (VLDL-c), and the triglycerides/ HDL-c ratio were calculated. Homeostasis model assessment of insulin resistance (HOMA-IR) and homeostasis model assessment of pancreatic β cell function (HOMA-β) were estimated considering fasting levels, with the following equations [18,19]:

$$\text{HOMA-IR} = [\text{insulin } (\mu\text{IU/mL}) \times \text{glucose (mmol/L)}]/22.5$$

$$\text{HOMA-}\beta = [20 \times \text{insulin } (\mu\text{IU/mL})/\text{glucose (mmol/L)} - 3.5]$$

2.5. Macroscopic and Microscopic Liver Examination

After the animals were euthanized, each liver was removed, weighed, and examined. Left lateral lobe samples were taken for further analysis. For histological analysis samples were fixed in phosphate-buffered 10% formalin. Paraffin-embedded sections were sliced (5 µm) and stained with hematoxylin and eosin (H&E). Histological evaluation was performed following Brunt's scoring system, under 400× magnification at Velab (VE-BC3PLUS) microscope [20,21].

2.6. Liver Triglyceride and Antioxidant Enzyme Determination

Liver samples (0.3 g) were pulverized using liquid nitrogen. For liver triglyceride extraction Folch reagent (2 chloroform: 1 methanol) was added to the pulverized tissue (20:1 v/w). Samples were vortexed for 3 min at RT and sonicated for 20 min at 4 °C. To induce phases separation, NaCl 0.9% (1:5 v/v) was added to each sample and samples were centrifuged (1000× g × 10 min at 4 °C). The remaining chloroform/methanol/water phase was evaporated from the lower phase to get a lipid pellet. Once the pellet was reconstituted (NaCl 0.9%), triglycerides were measured by enzymatic-colorimetric assay (Spinreact® reagent, Catalonia, Spain) [7,22,23].

For lipid peroxidation, 25 mg of liver were homogenized with 250 µL of RIPA buffer and centrifuged 1600× g × 10 min at 4 °C. Malondialdehyde (MDA 36357, Merck, Darmstadt, Germany) was used as standard (0, 0.625, 1.25, 5, 25, 75 µM). Thiobarbituric acid (TBA 10%), sodium hydroxide (3.5 M), and trichloroacetic acid (10%) were added to the samples and standards. After boiling (90–100 °C) for 1 h, MDA-TBA adducts were formed and measured at 540 nm on a microplate reader (Molecular Devices Spectramax 250, Marshall Scientific, NH, USA) [24].

For glutathione (GSH), 500 mg were homogenized with 3 mL of Tris-sucrose buffer (pH 6.5) and centrifuged (8000× g × 20 min at 4 °C). For the preparation of the cytosolic supernatant serum and liver samples were ultracentrifuged (100,000× g × 1 h at 4 °C) and precipitated with TCA (20%) to get concentrated cytosols. GSH determination was performed by Ellman's method and GSH reagent (Sigma Aldrich PHR1359, Darmstadt, Germany) was used as standard. After 5 min of incubation at RT, the 96-well plate was read on a Varioskan LUX Multimode Microplate Reader (Thermo Fisher Scientific, MA, USA) at λ 412 nm [25].

Liver samples (500 mg) were homogenized with PBS (50 mM, pH 7) and centrifuged (8000× g × 20 min at 4 °C) to obtain the cytosolic supernatants for antioxidant enzymes measurements. Catalase (CAT) activity was determined by Aebi´s method, using 30 mM of H_2O_2. Optical density absorbance measurements were recorded for 30 s (6 × 5 s) at 240 nm [26]. Glutathione peroxidase (GPx) activity was analyzed with Glutathione Peroxidase Assay Kit (Merck 353919, Darmstadt, Germany). The decreasing rate in the absorbance (340 nm) is directly proportional to the oxidation of NADPH to NADP+ [27].

Protein concentrations in liver homogenates were quantified by the bicinchoninic acid (BCA) assay (Thermo Fisher Scientific 23227, MA, USA), using BSA (bovine serum album) as a standard.

2.7. Expression Analysis

Total RNA from liver samples was isolated employing the TRIzol reagent (Invitrogen 15596026) [28]. RNA integrity was evaluated by electrophoresis and its concentration was determined by spectrophotometric analysis (NanoDrop 1000, Wilmington, DE, USA). To be able to amplify and quantify the RNA expression, cDNA was synthesized by reverse transcription reaction. To unwind RNA, 1 µg of total RNA was heated at 70 °C for 5 min. First, 1 µL of each of the following reagents were mixed and preincubated for 2 min at 42 °C, heat-denatured RNA, antisense oligonucleotides (15 bases synthesized by IDT), dNTP (Thermo Fisher Scientific R0181, MA, USA R0181), random primers (Promega C1181). Then, reverse transcriptase (Promega M1701) was added and the mixture was heated at 70 °C for 15 min. cDNA was kept at −20 °C and used to quantify gene expression of *Tnfa*, *Nfe2l2*, *Ppara,* and *Hmox1* (Table 2) by quantitative PCR (qPCR) analyses in the LightCycler® 2.0 instrument with the LightCycler FastStart DNA Master Sybr Green I kit (Roche Applied Science, Mannheim, Germany). *Sod2* and *Ywhaz* were used as housekeeping genes after their validation by NormFinder [29]. Gene expressions were analyzed by the $2^{-\Delta\Delta CT}$ method [30]. Amplicon identity was corroborated by sequences and BLAST (NIH) analysis.

Table 2. List of primers for real-time PCR analysis.

Gene **(Bank Number)**	**Primer Sequence 5′ to 3′**
Sod2 (NM_017051.2)	Fwd: TGGACAAACCTGAGGCCTAA Rev: GACCCAAAGTCACGCTTGATA
Ywhaz (NM_013011.4)	Fwd: TTGAGCAGAAGACGGAAGGT Rev: GAAGCATTGGGGATCAAGAA
Tnfa (AY427675.1)	Fwd: TGGGCTGTACCTTATCTACTCC Rev: GGCTGACTTTCTCCTGGTATG
Nfe2l2 (BC061724.1)	Fwd: CAGAAGGAACAGGAGAAGGC Rev: TCAACGTGGCTGGGAATATC
Ppara (NM_013196.2)	Fwd: GGGTCATACTCGCAGGAAAG Rev: ACCTGGTCATTCAAGTCCAAG
Hmox1 (NM_012580.2)	Fwd: ACAGAGGAACACAAAGACCAG Rev: GAGAAGGCTACATGAGACAGAG

Sod2 = Superoxide dismutase 2, *Ywhaz* = Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta, *Tnfa* = Tumor necrosis factor-alpha, *Nfe2l2* = Nuclear factor erythroid 2-related factor 2, *Ppara* = Peroxisome proliferator-activated receptor alpha, *Hmox1* = Heme oxygenase 1.

Liver sections, previously paraffin-embedded and sliced (5 µm), were dewaxed and rehydrated. After antigen retrial with HCl 2 M for 30 min and permeabilization, the samples were incubated for 2 h with normal goat serum (1:20) for blocking [31]. Slices were incubated for 24 h with primary Nrf2-antibody (Abcam 89443, Cambridge, UK) and PPARα (Abcam215270, Cambridge, UK). To avoid non-specific fluorescence, a 15 min incubation with Sudan Black B (0.1%) was performed [32]. After 12 h of secondary antibody incubation (Abcam150113 and 150077, Cambridge, UK), slices were stained with DAPI (Sigma-AldrichSLCB0123, Darmstadt, Germany). Nine images per slice were captured on an Apotome Zeiss microscope. Pearson's correlation coefficient (PCC) and Mander's correlation coefficient (MCC) were performed to evaluate colocalization [33] with the image analyzer Fiji [34].

2.8. Statistical Analysis

The data are presented as mean± standard error of the mean (SEM). The Shapiro–Wilk test was executed in the continuous variables to assess their normal distribution. In order to know the similarity of SBL and HBL to the S group and describe the NAFLD model, statistical analysis was performed by one-way ANOVA, and the differences against the

control group (S) were analyzed by Dunnet's post hoc test, * $p \leq 0.05$. Further, to determine the preventive effect of bean leaves supplementation in a high-fat and high-fructose model, the statistical differences between H and HBL groups were analyzed by Student's *t*-test, # $p \leq 0.05$. Graphics were carried out with GraphPad Prism 8 (Dotmatics, San Diego, CA, USA), and SPSS Statistics v.25 (IBM, NY, USA) was used for the statistical analysis.

3. Results

3.1. Effect of Bean Leaves on Body Fat Accumulation

The rats were fed with a standard diet (S) and simultaneously supplemented with 10% of bean leaves (SBL). Meanwhile, experimental rats were fed with a high-fat/high-fructose diet (H) to induce obesity and hepatic lipotoxicity; and in order to prevent these metabolic alterations another group was supplemented with 10% of bean leaves (HBL).

Body weight was measured weekly (Figure 1). The main statistical differences in weekly body weight were between weeks 4 to 6 (Figure 1), H gained higher weight (↑4–6%), compared to that of the S group. After 13 weeks, total body weight ↑8% (Table 3) and body fat accumulation by MRI ↑160% (Figure 2) in the H group were higher than those of the S group; due to ↑30% total energy intake and 32% in daily energy intake (Table 4). Water intake total food intake (Table 4) and body measurements as length, AC, TC, AC/TC ratio, or BMI (Table 3) in the H group do not have differences compared to the S group. H rats developed obesity, by the increase in energy intake, body weight gain, and body fat accumulation.

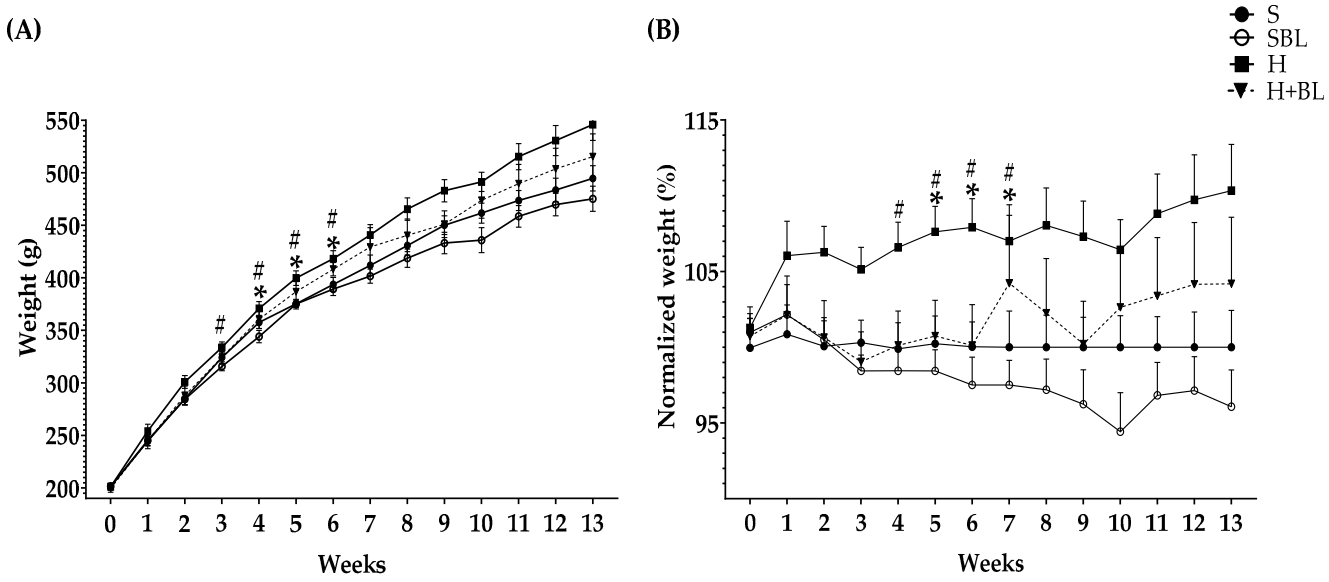

Figure 1. Effect of common bean leaves in (**A**) weight per week and (**B**) normalized weight % against the correspondent weight of standard diet group or S-diet group. Values represent the mean ± SEM (n = 9). ANOVA post hoc Dunnet's test was performed to compare groups versus S * $p \leq 0.05$. Student's *t*-test was performed to compare H versus HBL # $p \leq 0.05$. S = standard diet, SBL = S + 10% bean leaves, H = high-fat/high-fructose diet, HBL = H + 10% bean leaves.

Interestingly, the supplementation with 10% of bean leaves (HBL) lead to ↓2–4% less body weight between weeks 3 and 7 and ↓48% decreased body fat accumulation by MRI (Figure 2) at the end of the 13 weeks, ↓7% reduced thoracic circumference and ↓5% shorter length (Table 3), compared to H. Food, water, or energy intake, and AC, AC/TC ratio, or BMI (Table 4) in HBL rats did not show differences to those in H group. Even though the HBL group showed less water intake (total and daily) compared to the S group, the water intake of all groups was in the recommended range (8–12 mL of water/100 g body weight) [35,36].

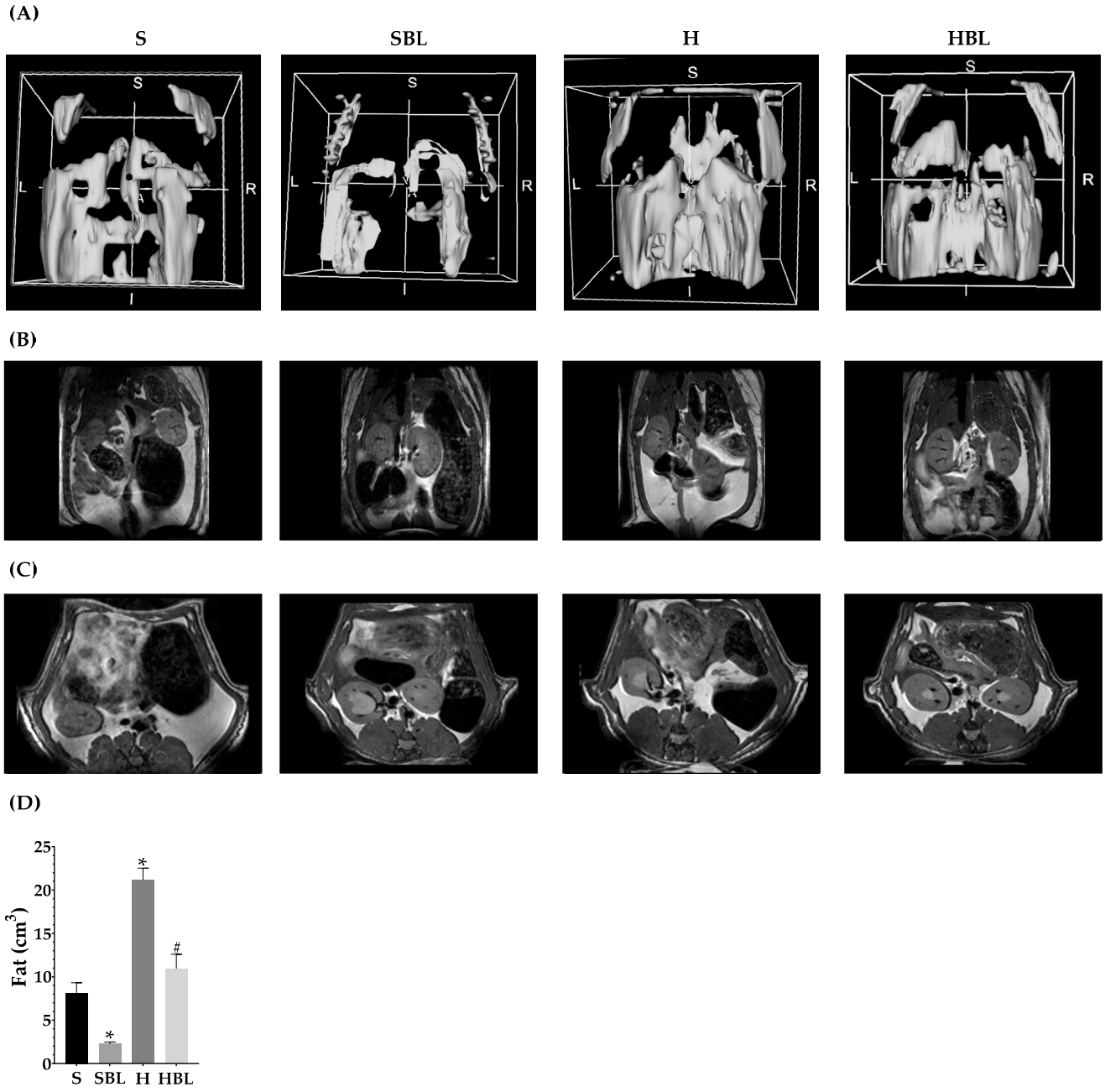

Figure 2. Effect of common bean leaves in body fat accumulation. Body fat accumulation was evaluated by magnetic resonance (**B**) in coronal and (**C**) in transversal plane. Analysis was performed on 3D Slicer®, (**A**) shows a 3D reconstruction, and (**D**) fat quantification. Values represent the mean ± SEM (n = 5). ANOVA post hoc Dunnet's test was performed to compare groups versus S * $p \leq 0.05$. Student's t-test was performed to compare H versus HBL # $p \leq 0.05$. S = standard diet, SBL = S + 10% bean leaves, H = high-fat/high-fructose diet, HBL = H + 10% bean leaves.

Additionally, HBL showed a protective role against obesity development (↓body fat accumulation by MRI) in spite of total energy intake being similar to H and ↑38% more than S ($p \leq 0.05$). Additionally, the supplementation with 10% of bean leaves in the standard diet (SBL) reduced ↓72% of body fat accumulation by MRI (Figure 2), without evidence of a possible negative effect of bean leaves intake for 13 weeks.

Table 3. Effect of bean leaves in body measurements.

	S	SBL	H	HBL
Total body weight gain (g)	293.31 ± 10.67	275.39 ± 12.7	344.61 ± 15.3 *	315.83 ± 19.11
Body length (cm)	24.74 ± 0.85	23.68 ± 0.61	24.6 ± 0.27	23.38 ± 0.44 #
Abdominal circumference (cm)	21.67 ± 0.47	20.93 ± 0.57	22.98 ± 0.54	21.36 ± 0.65
Thoracic circumference (cm)	19.39 ± 0.26	18.59 ± 0.46	20.00 ± 0.48	18.61 ± 0.39 #
AC/TC ratio	1.12 ± 0.03	1.13 ± 0.05	1.16 ± 0.04	1.15 ± 0.01
BMI (g/cm^2)	0.80 ± 0.04	0.86 ± 0.04	0.90 ± 0.02	0.89 ± 0.04

Values represent the mean ± SEM (n = 9). ANOVA post hoc Dunnet's test was performed to compare groups versus S, * $p \leq 0.05$. Student's t-test was performed to compare H versus HBL, # $p \leq 0.05$. S = standard diet, SBL = S + 10% bean leaves, H = high-fat/high-fructose diet, HBL = H + 10% bean leaves, AC = abdominal circumference, TC = thoracic circumference, BMI = body mass index.

Table 4. Food, energy and water intake.

	S	SBL	H	HBL
Food intake (g/day)	38.0 ± 2.0	37.9 ± 1.7	38.8 ± 1.4	38.7 ± 2.2
Total food intake (g)	494.7 ± 26.1	492.3 ± 22.1	503.9 ± 18.7	503.0 ± 28.0
Energy intake (kcal/day)	129.4 ± 6.8	136.3 ± 6.1	170.6 ± 6.3 *	178.0 ± 9.9 *
Total energy intake (kcal)	1681.9 ± 88.9	1772.2 ± 79.4	2191.6 ± 77.1 *	2313.8 ± 129.0 *
Water intake (mL/day)	55.7 ± 2.8	55.2 ± 3.2	44.8 ± 4.2	41.0 ± 2.6 *
Total water intake (mL)	723.7 ± 36.7	717.8 ± 41.5	583.5 ± 55.3	533.4 ± 34.8 *

Values represent the mean ± SEM (n = 9). ANOVA post hoc Dunnet's test was performed to compare groups versus S, * $p \leq 0.05$. Student's t-test was performed to compare H versus HBL, ($p \leq 0.05$), not statistically different. S = standard diet, SBL = S + 10% bean leaves, H = high-fat/high-fructose diet, HBL = H + 10% bean leaves.

3.2. Effect of Bean Leaves on Insulin Resistance, Impaired Glucose Tolerance, and Dyslipidemia

Alteration in the oxidative and storage capacity of lipids in peripheral tissues (lipotoxicity) due to excessive fat accumulation [3] has an important role in insulin resistance and an increase in circulating lipids [37]. Therefore, we analyzed fasting levels of lipids, glucose, and glucose tolerance in the rats after thirteen weeks. Fasted glucose levels (Table 5) showed by all groups were normal [38]. Moreover, fasted glucose level (Table 5) in the H group was similar to that in the S group, but fasted insulin was higher (↑142%) in H rats. These results suggested compensatory hyperinsulinemia in H animals, related to the increase in the pancreatic β-cell function (HOMA-β ↑259%) due to a diminished capacity of the tissues to utilize insulin (HOMA-IR ↑116%) compared to S.

Table 5. Effect of bean leaves in glucose metabolism.

	S	SBL	H	HBL
Glucose (mg/dL)	130.67 ± 6.69	126.38 ± 5.60	129.91 ± 9.17	125.02 ± 9.10
Insulin (pM)	93.97 ± 9.62	112.51 ± 10.98	227.19 ± 38.03 *	177.47 ± 20.74 *
HOMA-IR	4.29 ± 0.47	4.81 ± 0.40	9.29 ± 1.63 *	7.71 ± 1.07
HOMA-β	32.1 ± 4.0	47.0 ± 6.9	115.1 ± 26.4 *	81.7 ± 13.4

Values represent the mean ± SEM (n = 9). ANOVA post hoc Dunnet's test was performed to compare groups versus S, * $p \leq 0.05$. Student's t-test was performed to compare H versus HBL ($p \leq 0.05$), not statistically different. S = standard diet, SBL = S + 10% bean leaves, H = high-fat/high-fructose diet, HBL = H + 10% bean leaves, HOMA-IR = homeostatic model assessment for insulin resistance, HOMA-β = homeostatic model assessment for pancreatic β cell function.

Besides during IpGTT, postprandial glycemia (Figure 3A) in H rats rose ↑27% at 30 min and ↑65% at 60, 90, and 120 min, and glycemic global response (AUC, Figure 3B) increased ↑48%, compared to those of S rats. This impaired glucose tolerance and compensatory hyperinsulinemia in the H animals was accompanied by hyperlipidemia (Table 6). Circulating lipids increased in the H group, ↑26% in total cholesterol, ↑58% in triglycerides, ↑58% in VLDL-c, and ↑17% HDL-c, compared to those of the S group.

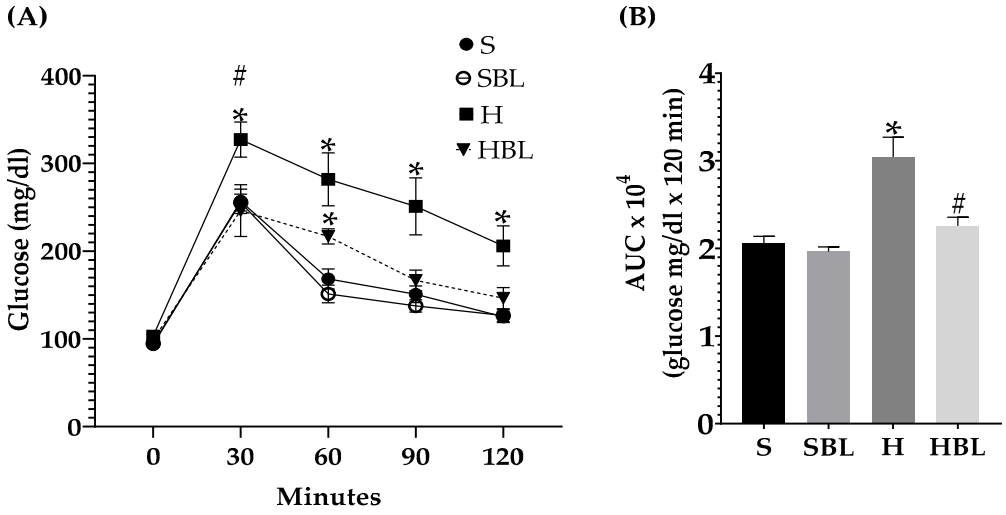

Figure 3. Effect of common bean leaves in (**A**) postprandial glycemic response and (**B**) area under the curve in intraperitoneal glycemic tolerance test. Values represent the mean ± SEM (n = 9). ANOVA post hoc Dunnet's test was performed to compare groups versus S * $p \leq 0.05$. Student's t-test was performed to compare H versus HBL # $p \leq 0.05$. S = standard diet, SBL = S + 10% bean leaves, H = high-fat/high-fructose diet, HBL = H + 10% bean leaves, AUC = area under the curve.

Table 6. Effect of bean leaves in lipid profile.

	S	SBL	H	HBL
Total cholesterol (mg/dL)	69.95 ± 2.93	64.87 ± 2.31	87.88 ± 2.82 *	78.64 ± 2.99 #
Triglycerides (mg/dL)	104.61 ± 6.55	99.97 ± 9.17	165.00 ± 12.03 *	147.99 ± 9.59 *
VLDL-c (mg/dL)	20.92 ± 1.31	19.99 ± 1.83	33.00 ± 2.41 *	29.60 ± 1.92 *
LDL-c (mg/dL)	13.33 ± 2.05	14.25 ± 1.72	12.80 ± 1.18	9.90 ± 1.00
OxLDL (ng/mL)	28.48 ± 1.71	30.43 ± 1.42	32.03 ± 1.74	32.83 ± 1.40
HDL-c (mg/dl)	46.31 ± 1.93	43.82 ± 1.00	54.43 ± 1.94 *	49.46 ± 2.03
Triglycerides/ HDL-c ratio	4.17 ± 0.70	3.24 ± 0.57	5.26 ± 1.06	5.04 ± 0.92

Values are mean ± SEM (n = 9), comparison against S was analyzed by ANOVA post hoc Dunnet * $p \leq 0.05$, and differences between H and HBL was analyzed using Student's t-test (# $p \leq 0.05$), not statistically different. S = standard diet, SBL = S + 10% bean leaves, H = high-fat/high-fructose diet, HBL = H + 10% bean leaves, VLDL-C = very low-density lipoprotein, LDL-C = low-density lipoprotein, OxLDL = oxidized low-density lipoprotein, HDL-C = high-density lipoprotein.

Fasted glucose of HBL rats was similar to that of the H group, but insulin was lower ↓22% (without statistical differences). However fasted insulin of HBL increased ↑88%, but glucose, HOMA-IR, and HOMA-β (Table 5) did not show statistical differences against those of the S group, leaving HBL in a middle point between H and S, without effects on fasting glucose.

Meanwhile, in postprandial glucose metabolism (Figure 3), during IpGTT, glucose tolerance improved (↓26%AUC) in HBL rats compared to H rats. Moreover, the similarity of the shape of the curve, resulting from IpGTT, between the HBL curve to S and SBL curves could suggest an improved glucose metabolism [39].

Regarding circulating lipids, total cholesterol (↓11%) was lower in the HBL group compared to the H group (Table 6), these could be associated with less accumulation of body fat. Bean leaves supplementation (HBL group) did not show an effect on hypertriglyceridemia (↑41%) and increased VLDL-c (↑41%) compared to S levels. The data suggested that bean leaves supplementation in a high-fat/high-fructose diet (HBL) improved insulin resistance without increasing β pancreatic cells function, and enhanced impaired glucose tolerance.

3.3. Effect of Bean Leaves on the Silent Stage of NAFLD, Steatosis

Besides the effect of bean leaves preventing obesity and insulin resistance, metabolic alterations that play a key role in NAFLD development, we further explore the effect of bean

leaves in steatosis, the earliest stage of NAFLD. As expected, the H diet promoted fatty liver accumulation and lipoperoxidation, as evidenced by the increase in hepatic triglycerides accumulation ↑303%, liver weight ↑16%, and MDA ↑75% (Figure 4), compared to those of the S diet. The increase in hepatic triglycerides matches with the macroscopic appearance of the liver, pale red color with yellowish spots (Figure 5), and the histopathological analysis, macrovesicular steatosis (<33%) in the centrilobular zone (Table 7), without loss of hepatic zonation (Figure 5). H rats developed steatosis grade I (Table 7, Figure 5) without changes in liver function serum parameters and neither in protein C reactive (Table 8).

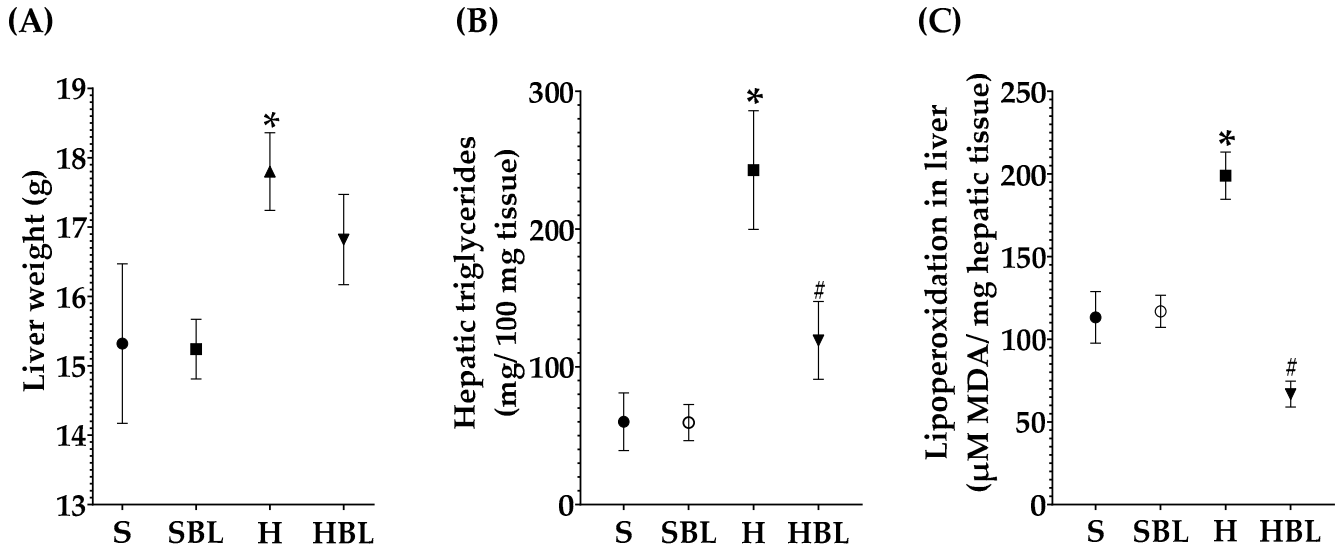

Figure 4. Effect of common bean leaves in (**A**) hepatic weight, (**B**) triglycerides quantification, and (**C**) lipid peroxidation. Values are mean ± SEM (n = 9), comparison against S was analyzed by ANOVA post hoc Dunnet * $p \leq 0.05$, comparison between H and. HBL was analyzed with Student's t-test # $p \leq 0.05$. S = standard diet, SBL = S + 10% bean leaves, H = high-fat/high-fructose diet, HBL = H + 10% bean leaves, MDA = malondialdehyde.

Even though there were no statistical differences in liver antioxidant enzymes activity between the H and S rats (Figure 6), there are some changes that could have biological importance; as a defense mechanism in the H group GSH increased ↑83% in the liver and ↑38% in serum (Figure 6A,B) compared to those of the S group, trying to keep the redox homeostasis. Meanwhile, *Hmox1* mRNA expression increased ↑147% (Figure 7B) and *Tnfa* mRNA ↑20% (Figure 7D) in the H rats, compared to those of the S rats. The findings suggested that the livers of our high-fat/high-fructose animals (H group) had lipid peroxidation and inflammation.

Moreover, the H diet increased *Nfe2l2* mRNA expression ↑44% (Figure 7A) and increased the signal intensity of Nrf2 nuclear localization ↑48% (Figure 8), these could be due to the development of steatosis grade I, insulin resistance, and obesity in H group.Moreover, *Ppara* mRNA expression increased ↑80% (Figure 7C) and PPARα nuclear translocation ↑112% (Figure 9) were higher in H rats compared to those of the S group, as expected in murine steatosis models, where *Ppara* overexpression is a defense mechanism of the liver to delay NAFLD progression to NASH [40].

Therefore, the data showed that our high fat/ high fructose animals (H group) had obesity, insulin resistance, impaired glucose tolerance, and hyperlipidemia, and as a consequence of those, the livers of H animals developed steatosis I, lipid peroxidation, and inflammation. Because steatosis is an early stage of NAFLD, the livers of H rats kept defense mechanisms against fat accumulation, inflammation, and oxidative stress.

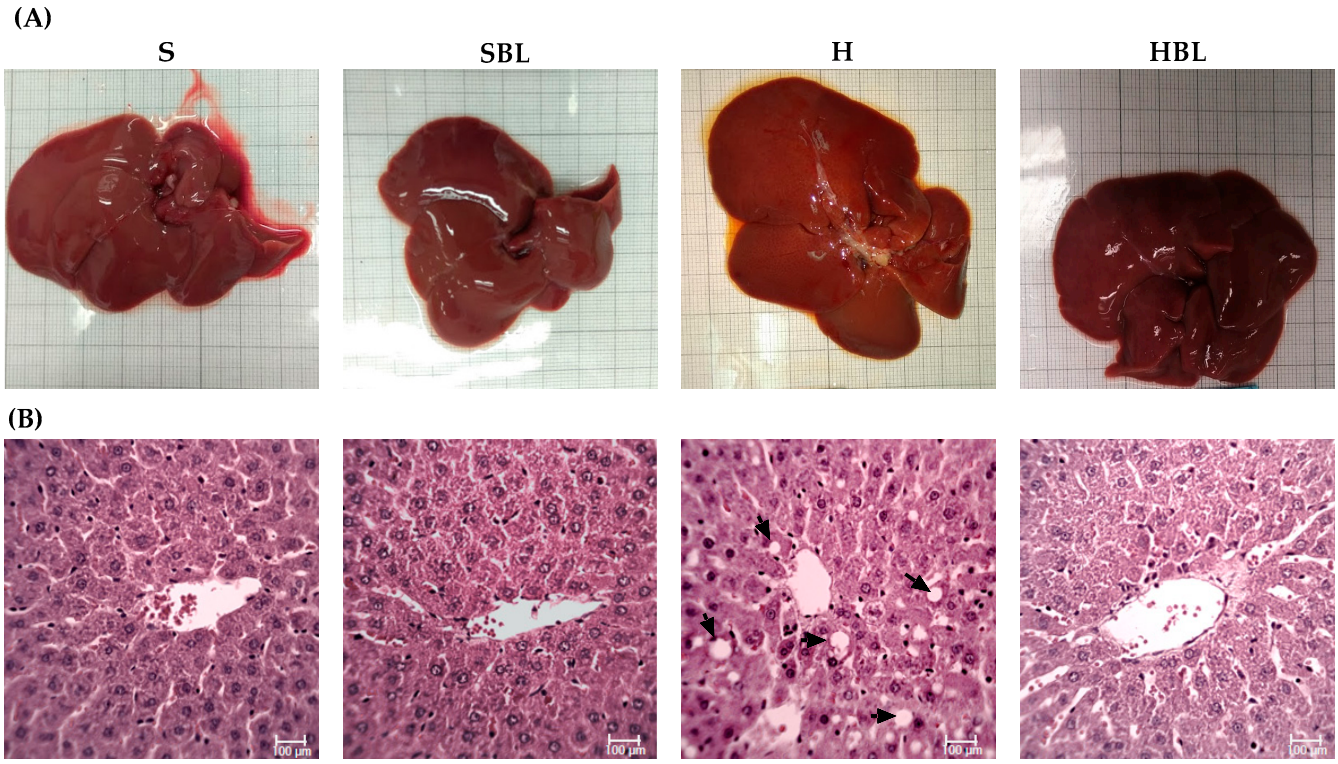

Figure 5. Effect of common bean leaves in (**A**) macroscopic appearance of liver and (**B**) histoarchitecture of hepatic tissue by H&E 400×. The arrows indicate lipid droplets inside the hepatocytes. S = standard diet, SBL = S + 10% bean leaves, H = high-fat/high-fructose diet, HBL = H + 10% bean leaves, H&E = hematoxylin and eosin.

Table 7. Prevention of liver steatosis in high-fat/fructose diet.

Group	Steatosis Grade		Findings
	0 (%)	I (%)	
S	100	0	Adequate histoarchitecture without damage
SBL	100	0	Adequate histoarchitecture without damage
H	0	100 *	Macrovesicular steatosis < 33%, centrilobular
HBL	100	0	Microvesicular steatosis < 5%, centrilobular

Scoring the steatosis grade according to Brunt. Statistical differences were analyzed by Chi-squared test, * $p < 0.05$. S = standard diet, SBL = S + 10% bean leaves, H = high-fat/high-fructose diet, HBL = H + 10% bean leaves.

Table 8. Effect of bean leaves (H + BL) in liver function.

	S	SBL	H	HBL
AST (U/L)	189.62 ± 11.89	180.57 ± 10.73	179.25 ± 12.50	185.36 ± 13.14
ALT (U/L)	87.62 ± 5.88	83.00 ± 4.59	73.06 ± 5.38	80.36 ± 3.78
AST/ALT ratio	4.34 ± 0.91	4.09 ± 0.82	5.79 ± 1.58	4.36 ± 0.91
Total protein (g/dL)	6.34 ± 0.08	6.29 ± 0.10	6.52 ± 0.08	6.59 ± 0.15
Albumin (g/dL)	4.23 ± 0.23	4.16 ± 0.24	4.28 ± 0.22	4.25 ± 0.24
Globulin (g/dL)	2.11 ± 0.26	2.13 ± 0.19	2.24 ± 0.20	2.34 ± 0.20
A/G ratio	9.09 ± 4.09	5.52 ± 1.61	6.19 ± 1.99	5.07 ± 1.53
CRP (mg/mL)	0.32 ± 0.05	0.30 ± 0.04	0.35 ± 0.02	0.34 ± 0.02

Values are mean ± SEM ($n = 9$), comparison against S was analyzed by ANOVA post hoc Dunnet ($p \leq 0.05$ and comparison between H and HBL was analyzed with Student's *t*-test ($p \leq 0.05$). Not statistically different. S = standard diet, SBL = S + 10% bean leaves, H = high-fat/high-fructose diet, HBL = H + 10% bean leaves, AST = aspartate aminotransferase, ALT = alanine aminotransferase, A = albumin, G = globulin, CRP = C-reactive protein.

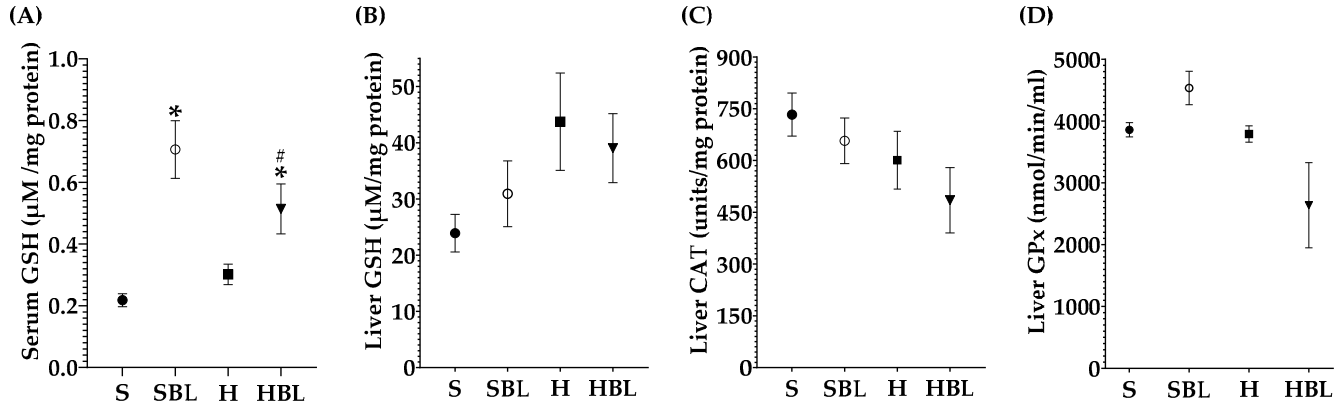

Figure 6. Effect of common bean leaves in antioxidant enzymes: (**A**) reduced glutathione (GSH) in serum, (**B**) GSH in hepatic tissue, (**C**) catalase (CAT) quantification in liver, and (**D**) glutathione peroxidase (GPx) in liver. Values are mean ± SEM (n = 9), comparison against S was analyzed by ANOVA post hoc Dunnet * $p \leq 0.05$, comparison between H and. HBL was analyzed with Student's t-test # $p \leq 0.05$. S = standard diet, SBL = S + 10% bean leaves, H = high-fat/high-fructose diet, HBL = H + 10% bean leaves.

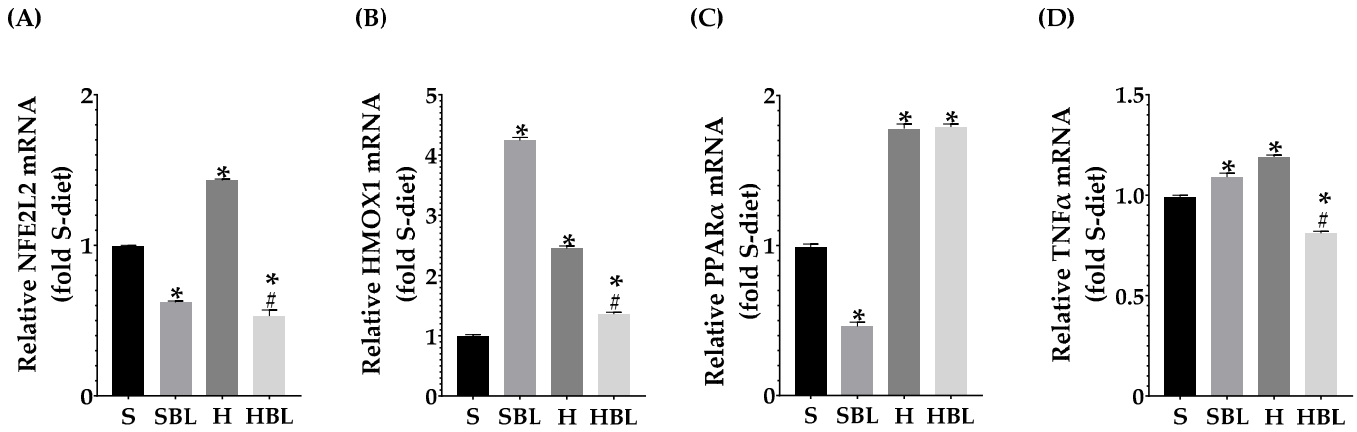

Figure 7. Effect of common bean leaves in relative mRNA expression of (**A**) *Nfe2l2*, (**B**) *Hmox1*, (**C**) *Ppara*, and (**D**) *Tnfa*. RT-qPCR analyzed by Livak's method; *Sod2* and *Ywhaz* were used for normalization as housekeeping genes. Values are mean ± SEM (n = 9), comparison against S was analyzed by ANOVA post hoc Dunnet * $p \leq 0.001$, comparison between H and. HBL was analyzed with Student's t-test # $p \leq 0.0001$. S = standard diet, SBL = S + 10% bean leaves, H = high-fat/high-fructose diet, HBL = H + 10% bean leaves, *Sod2* = Superoxide dismutase 2, *Ywhaz* = Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta, *Tnfa* = Tumor necrosis factor-alpha, *Nfe2l2* = Nuclear factor erythroid 2-related factor 2, *Ppara* = Peroxisome proliferator-activated receptor alpha, *Hmox1* = Heme oxygenase 1.

On the other hand, bean leaves supplementation (HBL) in a high-fat/high-fructose diet has shown a protective effect against impaired glucose tolerance, insulin resistance, and hypercholesterolemia. After 13 weeks, hepatic triglycerides were ↓51% lower in HBL rats than that of the H group (Figure 4), interestingly without any statistical difference against the S group. Hepatic triglycerides levels in HBL rats match not only with the histopathological analysis, macroscopically the liver had an intense red color without yellowish pots (Figure 5), and microscopically the liver had centrilobular microvesicular fat accumulation < 5%, without steatosis (Table 7), but also with the absence of liver function alterations (Table 8). Moreover, SBL animals did not exhibit any hepatic damage after thirteen weeks of supplementation with 10% of bean leaves.

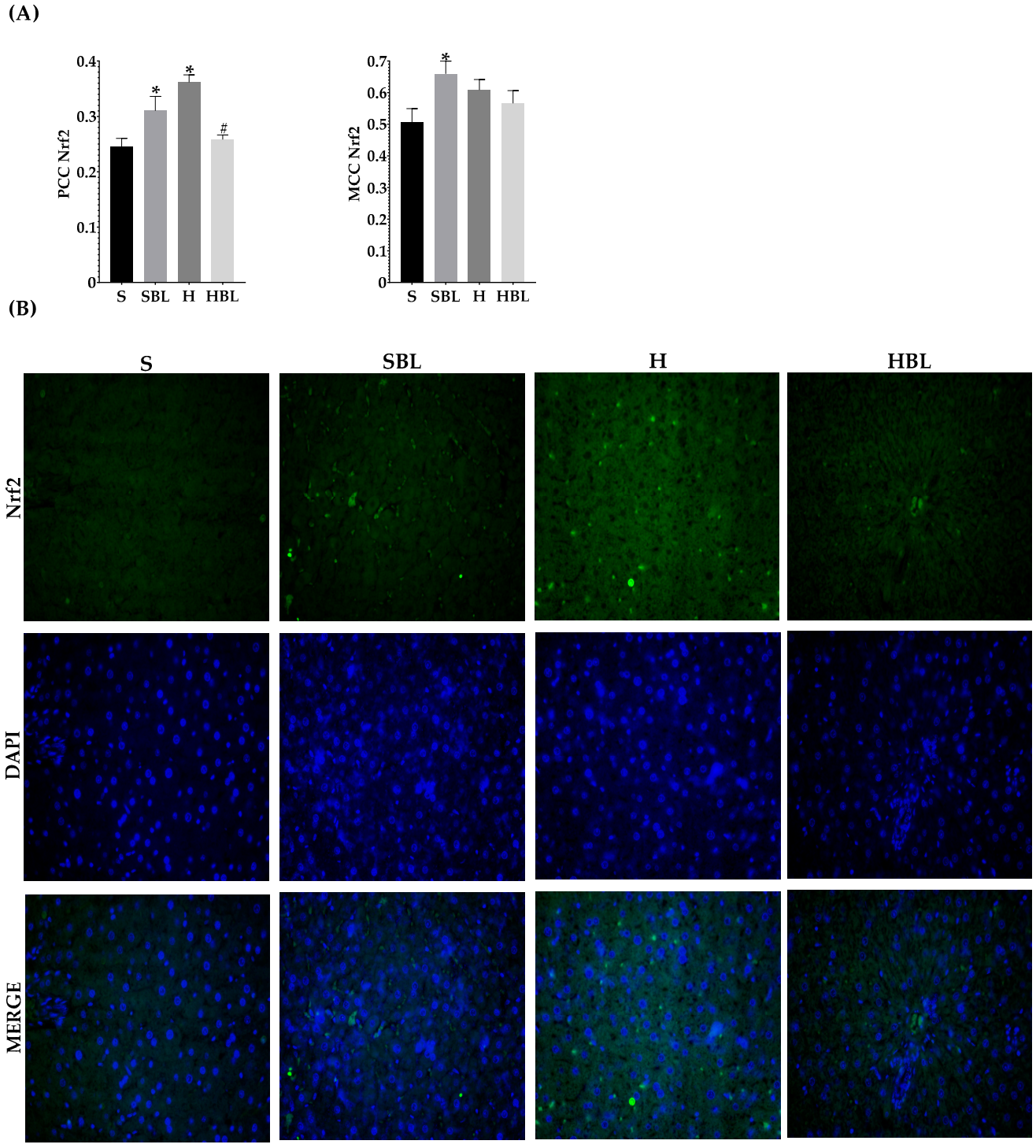

Figure 8. Effect of common bean leaves in Nrf2 nuclear translocation. (**A**) Graphs shows colocalization coefficients for Nrf2 in the nucleus: Pearson's correlation coefficient (PCC), Mander's correlation coefficient (MCC). (**B**) Immunofluorescence staining for Nrf2 (green Alexa488) and nucleus (blue, DAPI). Values are mean ± SEM (n = 5, 9 photos per slice), comparation against S was analyzed by ANOVA post hoc Dunnet * $p \leq 0.001$, comparison between H and. HBL was analyzed with Student's t-test # $p \leq 0.0001$. S = standard diet, SBL = S + 10% bean leaves, H = high-fat/high fructose diet, HBL = H + 10% bean leaves, Nrf2 = nuclear factor erythroid 2-related factor 2.

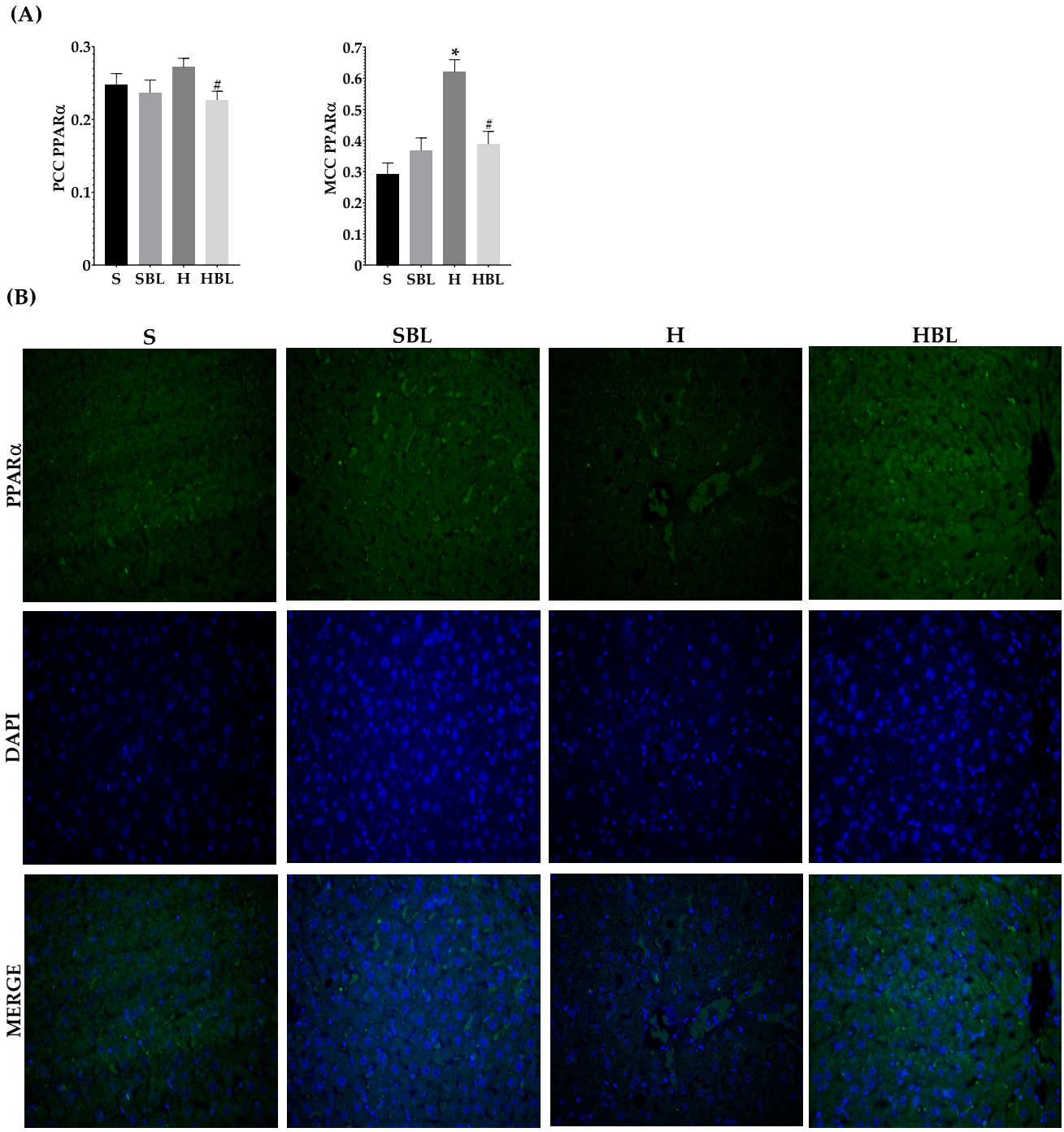

Figure 9. Effect of common bean leaves in PPARα nuclear translocation. (**A**) Graphs show colocalization coefficients for PPARα in the nucleus: Pearson's correlation coefficient (PCC), Mander's correlation coefficient (MCC) (**B**) Immunofluorescence staining for PPARα 2 (green Alexa488) and nucleus (blue, DAPI). Values are mean ± SEM (n = 5, 9 photos per slice), comparison against S was analyzed by ANOVA post hoc Dunnet * $p \leq 0.001$, comparison between H and. HBLwas analyzed with Student's t-test # $p \leq 0.0001$. S = standard diet, SBL = S + 10% bean leaves, H = high-fat/high-fructose diet, HBL = H + 10% bean leaves, PPARα = Peroxisome proliferator-activated receptor alpha.

Moreover, MDA hepatic levels in the HBL group decreased ↓66%, showing a protective effect against lipid peroxidation that was present in the H group. Even though liver CAT and GPx activity had not shown any statistical differences between groups (Figure 6), neither did hepatic GSH levels; physiologically, they had decreased. CAT activity (↓24%),

GPx activity (↓66%), and hepatic GSH (↓11%) were lower in HBL compared to H (Figure 6), probably because of less generation of hydroperoxides (↓MDA levels).

Interestingly, serum GSH levels in HBL animals increased ↑70% compared to that of the H animals and ↑136% compared to that of the S rats. In addition, serum GSH levels in SBL rats incremented ↑224% (Figure 6), compared to that of the S rats. Moreover, *Hmox1* mRNA expression drop ↓45% in HBL rats compared to H, possibly due to a lower fat accumulation and lipid peroxidation. However, *Hmox1* mRNA expression rose ↑37% in the HBL group and ↑328% times in the SBL group (Figure 7B), compared to that of the S group. These findings suggested that bean leaf supplementation enhanced antioxidant capabilities in the liver.

After 13 weeks (Figure 7A), possibly owing to the rise of GSH levels in serum, fat accumulation, and lower lipid peroxidation in the liver, HBL rats exhibited reduced expression of *Nfe2l2* mRNA ↓63% and Nrf2 nuclear intensity decreased ↓29% (PCC), compared to those of H rats. Meanwhile, *Nfe2l2* mRNA expression in HBL decreased ↓46% compared to S; but in the SBL group the intensity and translocation to the nucleus rose ↑27% for PCC and ↑27% for MCC (Figure 8), even though when the expression was ↓37% lower.

Regarding PPARα which is a main regulator of lipid metabolism, livers from HBL animals showed increased expression of Ppara mRNA ↑80% (Figure 7C) compared to that of S animals. The overexpression of PPARα could protect the liver against fat accumulation and lipid peroxidation [41]. However, the intensity and nuclear translocation in the HBL group diminished (Figure 9), ↓16% (PCC) and ↓37% (MCC) respectively, compared to the S group. Moreover, SBL rats showed reduced expression of Ppara mRNA ↓53% and ↓81% (MCC) lower translocation to the nucleus, compared to those of S rats. These lower expressions of PPARα in SBL could be related to a lower content of fatty acids in the liver. Appealingly, HBL rats exhibited reduced expression of Tnfa mRNA ↓ compared to H, and ↓ compared to S (Figure 7D).

Therefore, bean leaves showed ameliorated hepatic lipotoxicity derived from the consumption of a deleterious diet by a protective effect against inflammatory alterations and an antioxidant protector. Along with enhanced insulin resistance without increasing β pancreatic cells function, they enhanced impaired glucose tolerance.

4. Discussion

Bean leaves are a source of bioactive compounds like iron, protein, insoluble fiber, and polyphenols. Bioactive compounds of plant-based foods have been shown to play a role in the prevention of metabolic disorders related to high-fat/high-fructose diets [7,8]. The 10% of bean leaves supplementation that has shown a protective effect against lipotoxicity in fatty liver disease (12.6 g/kg body weight), extrapolated to humans the dose should be 2 g/kg of body weight [42]. This dose represents 30% of the daily recommended intake of fruits and vegetables (400 g per day) to prevent chronic diseases [43].

A positive energy balance coming from a high energy intake promotes excessive accumulation of visceral fat and obesity-related comorbidities [44,45]. As expected, the H group showed obesity (↑body weight gain and ↑body fat accumulation by MRI), as reported in high-fat/high-fructose diet models [45]. Meanwhile, HBL prevented obesity (↓body fat accumulation by MRI without changes in body weight gain). Rats had shown compensatory mechanisms in weight change, probably due to this HBL didn't show differences in weight gain against either S or H groups [46,47], further analysis is needed to evaluate the effect of bean leaves in the hypothalamic control center.

Previous data suggested that the decrease in body fat accumulation and weight gain after the supplementation with 10% of bean leaves in a high-fat/high-fructose diet, may not be due to alterations in fat absorption or fat fecal excretion [7]. Further studies are required to evaluate the role of bean leaves supplementation for longer time periods and to understand if bean leaves have a role in decreased fat absorption or increased thermogenesis, based on both high-fat/high-fructose groups (H and HBL) had similar energy intake but HBL showed less body fat accumulation.

High-fat/high-fructose diet intake drives insulin resistance adipocytes, leading to an increase in free fatty acids (FFAs) flux duet to increased lipolysis in adipose tissue [48,49]. Therefore, FFAS flux promotes deleterious effects in non-adipose tissue [3]. In the liver, FFAs raise the synthesis of triglyceride-enriched VLDL-c, which also generates LDL-c and HDL-c triglyceride-enriched. These HDL-c are easily cleared by the kidney, resulting in few HDL-c being able to accept cholesterol from the vasculature [49]. Additionally, patients with NAFLD had shown increased persistent VLDL secretion due to an overexpression of MTP (microsomal triglyceride transfer protein) [50]. Hence, the H group developed obesity and dyslipidemia by increased total cholesterol, triglycerides, VLDL-c, and HDL-c, in addition to impaired glucose tolerance, compensatory hyperinsulinemia, and insulin resistance. These common metabolic alterations induced by high-fat/high-fructose models are strongly related to nonalcoholic liver disease development [45].

Meanwhile, 10% bean leaves supplementation (HBL) prevented impaired glucose tolerance, hypercholesterolemia, and improved insulin resistance due to less body fat accumulation. HBL-diet had similar fasting glucose, HOMA-IR, and HOMA-β levels to the S-diet, but without differences with the H-diet; meanwhile, the postprandial glucose level in the HBL group was lower than that in the H group. Fasted glucose levels could be increased by the high-fructose diet [51]; this could be related to no differences between the H and the HBL groups. Additionally, the liver is mainly responsible for fasting glucose levels and the pancreas is responsible for postprandial glucose levels; therefore, the effect of bean leaves in β cell pancreatic function needs further investigation [51].

In a previous study, where bean leaves supplementation with 7-h daytime RFP was evaluated, fasting glucose levels and insulin resistance (HOMA-IR) were lower after 8 weeks of treatment [8]. Dietary fiber and phenolic compounds present in bean leaves had shown an increase in SCFA production on cecal content [7]. SCFA are synthesized by microbiota bacteria such as *Bifidobacterium* and *Lactobacillus*. Binding of SCFAs to their free fatty acid receptors (FFAR2/FFAR3) on enteroendocrine cells results in stimulated secretion of glucagon-like peptide 1 (GLP-1) that promotes insulin secretion and peptide YY (PYY) which reduces food intake. Meanwhile, in pancreatic β-cells, the interaction between SCFA and FFAR2/FFAR3 promotes insulin secretion [52]. Likewise, butyrate increases the expression of phosphoenolpyruvate carboxykinase-1 and glucose-6 phosphatase, key enzymes in intestinal gluconeogenesis sensed by the portal vein, improving insulin sensitivity [53]. This could be a possible mechanism of action of bean leaves on glucose metabolism that should be further explored.

Even when previous studies with bean leaves supplementation in high-fat/high-fructose diets had not shown decreases in circulating lipid levels [7,8,54], bean leaves supplementation with RFP downregulated *Scd1* (stearyl-coenzyme A desaturase1) expression in liver [8], leading to triglycerides exportation, keeping high levels of VLDL-c and triglycerides in circulation [54]. As with other plant-based diet interventions, bean leaf intake should be evaluated together with physical activity and changes in lifestyle [55].

Insulin resistance increases hepatic gluconeogenesis; likewise, compensatory hyperinsulinemia raises de novo lipogenesis [56,57]. The H animals not only showed compensatory hyperinsulinemia and insulin resistance, keeping similar glucose lower to the S group with higher levels of insulin, but also an increased β pancreatic function. Additionally, the increased FFAs flux downregulates β oxidation by the PPARα pathway (proliferator-activated receptor alpha) [58]. This disbalance between the synthesis of triglycerides and the capacity to utilize and export them (VLDL-c synthesis), saturates the liver capacity, leading to hepatic lipotoxicity [48,59]. Hepatic lipotoxicity has a key role in the progression of NAFLD, and it is present since its first stage (steatosis) [59]. As part of this process, H rats developed steatosis grade I, liver of H rats preserve protective mechanisms against fat accumulation, such as hepatic exportation of triglycerides (↑VLDL) and fatty acid oxidation facility (↑Ppara).

Steatosis is characterized by accumulating fat in up to 5% of the hepatocytes, and lipid peroxidation, without fibrosis, ballooning, inflammation, or changes in serum liver

function parameters [59,60]. High fat and fructose models induce alterations in glucose and lipid metabolism, such as insulin resistance and an increase in the circulating lipids, strongly associated with oxidative stress, inflammation, and liver fat accumulation (lipotoxicity) [3,61]. Therefore, reactive species rise and their contact with lipids, particularly polyunsaturated fatty acids, results in hydroperoxides (LOOH) and as a secondary product of MDA, this process is called lipoperoxidation [62]. Hence, MDA levels are a biomarker of a rise in hydroperoxides production, lipid peroxidation, and the rise of reactive species [62]. Once these hydroperoxides are generated, antioxidant enzymes such as GPx and CAT catalyze the reduction of H_2O_2 [62]. Accordingly, the livers of the H rats showed lipid peroxidation (↑MDA) and, consequently, the rise in reactive species switch on antioxidant mechanisms (↑GSH and *Hmox1*). HO-1 is an enzyme with antioxidant defense functions; it catalyzes heme to iron, carbon monoxide, and biliverdin [63]. HO-1 induction is regulated by different stimuli, its overexpression can be upregulated by the JNK pathway [64], related to insulin resistance and the overexpression of *Tnfa.* Meanwhile, HBL showed a lower expression of *Tnfa* than those in the S group. A decrease in TNFalpha has been related to lower activation of NFkB [65]. However, there are no reported studies where the decreased expression of TNFalpha has been related to a pathological condition or to a decrease in the immune system.

Nrf2 has an important dual role in NAFLD progression [50,66]. Under stress conditions, Nrf2 has canonical and non-canonical activation mechanisms [66]. In the canonical mechanism, Keap-1 senses the reactive species and releases Nrf2 in the cytoplasm, ready to translocate to activate the enzymatic (HO-1, GPx, CAT) and non-enzymatic (GSH) antioxidant defense systems by binding in the nucleus to ARE (antioxidant response element) [9,50]. The non-canonical pathway is p62-dependent; it is usually related to chronic Nrf2 activation and the development of chronic diseases such as diabetes, NAFLD, and cancer [66,67]. Then, the higher expression of *Nfe2l2* mRNA in the H group compared to that of S could be related to the activation of Nrf2 by the non-canonical pathway. Despite Nrf2 activation by the non-canonical pathway had been related to metabolic alterations development in their late stages, more studies are needed to explore the role of Nrf2 in early stages of NAFLD (steatosis) [66,67].

Plant extracts, probiotics, and prebiotics had been useful in NAFLD treatment by reducing inflammation and increasing the antioxidant defense system, particularly GSH levels [68]; and bean leaves had shown an important content of polyphenols and dietary fiber besides preventing obesity-related comorbidities [6–8]. This is the first study that evaluates the effect of bean leaves on NAFLD. HBL diet showed a protective effect against steatosis grade I. Formerly, the combination of bean leaves supplementation and RFP (7-h daytime-restricted-feeding protocol) had ameliorated insulin resistance and liver fat accumulation in a murine high-fat/high-fructose model [8]. Steatosis is asymptomatic and curable, so it is important to focus research on its prevention and treatment, considering local customs and habits [59,60].

PPARα which is a main regulator of lipid metabolism, regulates many genes involved in lipid metabolism, fatty acid uptake, oxidation (mitochondrial and peroxisomal), and triglyceride turnover [41]. PPARα also modulates inflammation mediated by direct binding to the p65 subunit of NFκB [41]. NFκB is a transcriptional regulator of TNFα [69], and PPARα is upregulated by Nrf2 [10,12]. *Ppara* expression in HBL rats increased compared to H rats. These findings suggest that bean leaves have a protective role against hepatic lipid accumulation. Previously, the supplementation with bean leaves for 6 weeks had a 54% increase in SCFA production due to the fermentation of the bioactive compounds present in bean leaves [7]. SCFA have positive effects in metabolic disease prevention and are able to interact with different tissues. Interestingly, some studies have suggested that SCFA can interact with hepatic PPARs; specifically, they can active PPARα expression reducing lipid accumulation by increasing lipid β-oxidation in the liver and adipose tissue [70], but the mechanism is not clearly elucidated [2]. Future research should evaluate the role of the

liver–gut axis in the prevention of NAFLD, highlighting the effect of bean leaves on SCFA production and their possible interaction with PPARα in the liver.

The liver is mainly responsible for GSH homeostasis in the body [68,71]. Glutathione, an important thiol redox agent, is mainly synthesized in macrophages in different tissues including blood, and the liver exports it into blood and bile. The increase in serum oxidized glutathione (GSSG) induces hepatic gamma-glutamyl transpeptidase (GGT), and GGT leads to the conversion of GSSG into GSH. Interestingly bean leaves supplementation increased serum GSH enhancing antioxidant capabilities in the body but without statistical changes in liver GSH. These could be related to less need for GSH in order to lower lipid peroxidation due to less reactive spices (↓MDA) in the liver due to the high capacity of the liver for GSH efflux through its basolateral and apical poles to maintain interorgan homeostasis of GSH by rising serum GSH [68].

Inducers of HO-1, such as curcumin, berberine, and resveratrol, had been studied as a possible treatment of NAFLD, because HO-1 overexpression had enhanced lipogenesis and collagen production [63,72]. Likewise, bean leaves induce a higher expression of Hmox1 mRNA in HBL and SBL groups. Additionally, this overexpression in bean leaves supplemented groups could be related to a higher intake of iron, based on the potential effect that bean leaves showed in a previous study, where bean leaves were used as a treatment for anemia rising up hemoglobin levels because of its iron bioavailability, [6]. Therefore, bean leaf intake could increase the requirement of HO-1 to catalyze heme [63].

GSH, HO-1, and other antioxidant enzymes are regulated by Nrf2. Nrf2 has shown potential as a therapeutical target in NAFLD progression [9]. Nrf2 activates PPARα, leading to β oxidation and ameliorated fat lipid accumulation in the liver [10,11].

Additionally, due to the interaction of dietary antioxidants as polyphenols with Keap-1 (canonical pathway), Nrf2 is able to induce antioxidant defense systems and delay inflammatory response mediated by NFκB [10,12]. Therefore, SBL rats showed a higher nuclear translocation; this suggested an antioxidant activation that needs to be further explored. However, HBL and SBL rats exhibited reduced expression of Nfe2l2 mRNA, possibly owing to the rise of GSH levels in serum, less hepatic fat accumulation, and lower lipid peroxidation. In light of these findings, bean leaves appear promising as a dietary alternative in the prevention of metabolic alterations, particularly steatosis and hepatic lipotoxicity (↓hepatic triglycerides and ↓MDA).

The expression of Ppara mRNA and the expression of Nfe2l2 mRNA did not seem to be regulated by bean leaves. Nrf2 is not the only regulator of PPARα, glucocorticoids, dietary fatty acids, eicosanoids, endocannabinoids, and (lyso)phospholipids [73]. Meanwhile, Nfe2l2 transcription is downregulated during oxidative stress in the liver by control nonderepressible 2 (GCN2) [74], further analyses are needed to determine if the decreased expression of Nfe2l2 in bean leaves supplementation is due to GCN2.

5. Conclusions

NAFLD is a worldwide public health problem, and understanding its complex pathology is key to the development of prevention strategies and treatments, particularly at the early stage of steatosis, which is reversible. Insulin resistance, hyperlipemia, and obesity play an important role in lipotoxicity in fatty liver development; prevention strategies should also approach them. Bean leaves supplementation such as plant-based foods interventions had shown beneficial effects in the treatment and prevention of metabolic disorders related to a long-term high-fat/high-fructose diet due to their content of bioactive compounds. Bean leaves are a source of bioactive compounds such as iron, dietary fiber, and polyphenols. As with other plant-based interventions, they are a low-cost alternative for nutritional interventions; moreover, beans are widely cultivated around the world. Supplementation with 10% of bean leaves in a high-fat/high-fructose diet ameliorated the insulin resistance and compensatory hyperinsulinemia; prevented the development of steatosis, and lipid peroxidation, activated the antioxidant defense system, and showed an anti-inflammatory effect, reducing hepatic lipotoxicity. Further studies are needed to deeply

understand the mechanism of action of bean leaves supplementation in the prevention of metabolic alterations.

Author Contributions: All the authors contribute to the conceptualization, methodology, and writing—review and editing; A.A.B.-C. performed formal analysis, visualization, and writing—original draft; A.A.B.-C., E.A.D.L.R.-A., P.V.O.-A. and S.M.A.-S. were in charge of the investigation, project administration, and funding acquisition; M.R.-G., Y.M., A.G.-G., T.G.-G. and S.M.A.-S. supervised and validated all the procedures. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Fondo de Proyectos Especiales de Rectoría-UAQ (FOPER: 2019–00798; 2020-FCN01886; and 2021-FCN02473); Programa para el Desarrollo del Profesorado-UAQ (PRODEP-UAQ-2018–2020-360); and Fondo para el Desarrollo del Conocimiento-UAQ (FONDEC–UAQ 2021). AABC was supported by Consejo Nacional de Ciencia y Tecnología (CONACYT: 734854).

Institutional Review Board Statement: All procedures performed in animals followed bioethical standards (NOM-062-Z00-1999 and ARRIVE guidelines) and were previously approved by the Ethics Committee of Faculty of Natural Sciences, Autonomous University of Queretaro (77FCN2019, 5 September 2019).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data will be available by contacting the corresponding author.

Acknowledgments: We are grateful to Leticia Castillo, Candelario Mondragón, Mariela Camacho, Verónica Andrade, Josué López, Nydia Hernández, Juan Ortiz, Luis Concha, and Andrea Herrera for their technical support. Moreover, we are grateful to the National Laboratory for Magnetic Resonance Imaging (LANIREM), Microscopy Unit, Proteogenomic Unit from INB/UNAM for the equipment, technical support, and infrastructure.

Conflicts of Interest: The authors declare that there are no conflict of interest regarding the publication of this paper. The funders had no role in any step of this research.

References

1. Younossi, Z.M.; Koenig, A.B.; Abdelatif, D.; Fazel, Y.; Henry, L.; Wymer, M. Global Epidemiology of Nonalcoholic Fatty Liver Disease—Meta-Analytic Assessment of Prevalence, Incidence, and Outcomes. *Hepatology* **2016**, *64*, 73–84. [CrossRef] [PubMed]
2. Wu, L.; Li, J.; Feng, J.; Ji, J.; Yu, Q.; Li, Y.; Zheng, Y.; Dai, W.; Wu, J.; Guo, C. Crosstalk between PPARs and Gut Microbiota in NAFLD. *Biomed. Pharmacother.* **2021**, *136*, 111255. [CrossRef] [PubMed]
3. Pola, A.; Sadananthan, S.A.; Gopalan, V.; Tan, M.-L.S.; Keong, T.Y.; Zhou, Z.; Ishino, S.; Nakano, Y.; Watanabe, M.; Horiguchi, T.; et al. Investigation of Fat Metabolism during Antiobesity Interventions by Magnetic Resonance Imaging and Spectroscopy. *Magn. Reson. Insights* **2014**, *7*, MRI.S19362. [CrossRef] [PubMed]
4. Li, H.-Y.; Gan, R.-Y.; Shang, A.; Mao, Q.-Q.; Sun, Q.-C.; Wu, D.-T.; Geng, F.; He, X.-Q.; Li, H.-B. Plant-Based Foods and Their Bioactive Compounds on Fatty Liver Disease: Effects, Mechanisms, and Clinical Application. *Oxid. Med. Cell. Longev.* **2021**, *2021*, 6621644. [CrossRef] [PubMed]
5. Jones, A. *PHASEOLUS BEAN: Post-Harvest Operations Organisation: Centro Internacional de Agricultura Tropical (CIAT)*; Montpellier, France. 1999. Available online: https://www.fao.org/documents/card/es/c/f961bcd6-85db-405e-af70-3ed044f1b1d7/ (accessed on 26 November 2022).
6. Martínez-Zavala, M.; Mora-Avilés, M.A.; Anaya-Loyola, M.A.; Guzmán-Maldonado, H.; Aguilera-Barreyro, A.; Blanco-Labra, A.; García-Gasca, T. Common Bean Leaves as a Source of Dietary Iron: Functional Test in an Iron-Deficient Rat Model. *Plant Foods Hum. Nutr.* **2016**, *71*, 259–264. [CrossRef]
7. Becerril-Campos, A.A.; Ocampo-Anguiano, P.V.; Mondragón-Jacobo, C.; Escobar-García, K.; Camacho-Barrón, M.; Anaya-Loyola, M.A.; Feregrino-Perez, A.A.; García-Gasca, T.; Ahumada-Solórzano, S.M. *Phaseolus vulgaris* L. Leaves Increase Short-Chain Fatty Acid (SCFA) Production, Ameliorating Early Metabolic Alterations. *Plant Foods Hum. Nutr.* **2022**, *77*, 421–426. [CrossRef]
8. Ramírez-Venegas, G.; De Ita-Pérez, D.L.; Díaz-Muñoz, M.; Méndez, I.; García-Gasca, T.; Ahumada-Solórzano, M.; Zambrano-Estrada, X.; Vázquez-Martínez, O.; Guzmán-Maldonado, H.; Luna-Moreno, D. Supplementation with Phaseolus Vulgaris Leaves Improves Metabolic Alterations Induced by High-Fat/Fructose Diet in Rats under Time-Restricted Feeding. *Plant Foods Hum. Nutr.* **2021**, *76*, 297–303. [CrossRef]
9. Zhou, J.; Zheng, Q.; Chen, Z. The Nrf2 Pathway in Liver Diseases. *Front. Cell Dev. Biol.* **2022**, *10*, 826204. [CrossRef]
10. Li, L.; Fu, J.; Sun, J.; Liu, D.; Chen, C.; Wang, H.; Hou, Y.; Xu, Y.; Pi, J. Is Nrf2-ARE a Potential Target in NAFLD Mitigation? *Curr. Opin. Toxicol.* **2019**, *13*, 35–44. [CrossRef]

11. Cave, M.C.; Clair, H.B.; Hardesty, J.E.; Falkner, K.C.; Feng, W.; Clark, B.J.; Sidey, J.; Shi, H.; Aqel, B.A.; McClain, C.J.; et al. Nuclear Receptors and Nonalcoholic Fatty Liver Disease. *Biochim. Biophys. Acta Gene Regul. Mech.* **2016**, *1859*, 1083–1099. [CrossRef]
12. Valenzuela, R.; Illesca, P.; Echeverría, F.; Espinosa, A.; Rincón-Cervera, M.Á.; Ortiz, M.; Hernandez-Rodas, M.C.; Valenzuela, A.; Videla, L.A. Molecular Adaptations Underlying the Beneficial Effects of Hydroxytyrosol in the Pathogenic Alterations Induced by a High-Fat Diet in Mouse Liver: PPAR-α and Nrf2 Activation, and NF-KB down-Regulation. *Food Funct.* **2017**, *8*, 1526–1537. [CrossRef]
13. National Centre for the Replacement Refinement & Reduction of Animal in Research. Training on the 3Rs Principles. Available online: https://www.nc3rs.org.uk/3rs-resources/training-3rs (accessed on 16 November 2022).
14. Novelli, E.L.B.; Diniz, Y.S.; Galhardi, C.M.; Ebaid, G.M.X.; Rodrigues, H.G.; Mani, F.; Fernandes, A.A.H.; Cicogna, A.C.; Novelli Filho, J.L.V.B. Anthropometrical Parameters and Markers of Obesity in Rats. *Lab. Anim.* **2007**, *41*, 111–119. [CrossRef]
15. Fedorov, A.; Beichel, R.; Kalpathy-Cramer, J.; Finet, J.; Fillion-Robin, J.C.; Pujol, S.; Bauer, C.; Jennings, D.; Fennessy, F.; Sonka, M.; et al. 3D Slicer as an Image Computing Platform for the Quantitative Imaging Network. *Magn. Reson. Imaging* **2012**, *30*, 1323–1341. [CrossRef]
16. 3D Slicer Image Computing Platform. Available online: https://www.slicer.org/ (accessed on 18 November 2022).
17. Lozano, I.; van der Werf, R.; Bietiger, W.; Seyfritz, E.; Peronet, C.; Pinget, M.; Jeandidier, N.; Maillard, E.; Marchioni, E.; Sigrist, S.; et al. High-Fructose and High-Fat Diet-Induced Disorders in Rats: Impact on Diabetes Risk, Hepatic and Vascular Complications. *Nutr. Metab.* **2016**, *13*, 15. [CrossRef]
18. Kuate, D.; Kengne, A.P.N.; Biapa, C.P.N.; Azantsa, B.G.K.; Abdul Manan Bin Wan Muda, W. Tetrapleura Tetraptera Spice Attenuates High-Carbohydrate, High-Fat Diet-Induced Obese and Type 2 Diabetic Rats with Metabolic Syndrome Features. *Lipids Health Dis.* **2015**, *14*, 50. [CrossRef]
19. Alnami, A.; Bima, A.; Alamoudi, A.; Eldakhakhny, B.; Sakr, H.; Elsamanoudy, A. Modulation of Dyslipidemia Markers Apo B/Apo A and Triglycerides/HDL-Cholesterol Ratios by Low-Carbohydrate High-Fat Diet in a Rat Model of Metabolic Syndrome. *Nutrients* **2022**, *14*, 1903. [CrossRef]
20. Yeh, M.M.; Brunt, E.M. Pathological Features of Fatty Liver Disease. *Gastroenterology* **2014**, *147*, 754–764. [CrossRef]
21. Brunt, E.M.; Janney, C.G.; Di Bisceglie, A.M.; Neuschwander-Tetri, B.A.; Bacon, B.R. Nonalcoholic Steatohepatitis: A Proposal for Grading and Staging the Histological Lesions. *Am. J. Gastroenterol.* **1999**, *94*, 2467–2474. [CrossRef]
22. Eggers, L.F.; Schwudke, D. Liquid Extraction: Folch. In *Encyclopedia of Lipidomics*; Springer: Dordrecht, The Netherlands, 2016; pp. 1–6, ISBN 978-94-007-7864-1.
23. Folch, J.; Lees, M.; Sloane Stanley, G. A Simple Method for the Isolation and Purification of Total Lipides From Animal Tissues—PubMed. *J. Biol. Chem.* **1957**, *226*, 497–509. [CrossRef]
24. Yagi, K. Simple Assay for the Level of Total Lipid Peroxides in Serum or Plasma. In *Free Radical and Antioxidant Protocols*; Armstrong, D., Ed.; Humana Press: Totowa, NJ, USA, 1998; pp. 101–106, ISBN 978-1-59259-254-8.
25. Ellman, G.L. Tissue Sulfhydryl Groups. *Arch. Biochem. Biophys.* **1959**, *82*, 70–77. [CrossRef]
26. Aebi, H. Catalase in Vitro. *Methods Enzymol.* **1984**, *105*, 121–126. [CrossRef] [PubMed]
27. Paglia, D.E.; Valentine, W.N. Studies on the Quantitative and Qualitative Characterization of Erythrocyte Glutathione Peroxidase. *J. Lab. Clin. Med.* **1967**, *70*, 158–169. [PubMed]
28. Chomczynski, P.; Sacchi, N. The Single-Step Method of RNA Isolation by Acid Guanidinium Thiocyanate–Phenol–Chloroform Extraction: Twenty-Something Years On. *Nat. Protoc.* **2006**, *1*, 581–585. [CrossRef] [PubMed]
29. Taylor, S.C.; Nadeau, K.; Abbasi, M.; Lachance, C.; Nguyen, M.; Fenrich, J. The Ultimate QPCR Experiment: Producing Publication Quality, Reproducible Data the First Time. *Trends Biotechnol.* **2019**, *37*, 761–774. [CrossRef]
30. Livak, K.J.; Schmittgen, T.D. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. *Methods* **2001**, *25*, 402–408. [CrossRef]
31. Zaqout, S.; Becker, L.L.; Kaindl, A.M. Immunofluorescence Staining of Paraffin Sections Step by Step. *Front. Neuroanat.* **2020**, *14*, 582218. [CrossRef]
32. Qin, W.; Luo, M.; Wang, K.; Yang, M.; Sheng, H.; He, G. A Combined Treatment with Erythrocyte Lysis Solution and Sudan Black B Reduces Tissue Autofluorescence in Double-Labeling Immunofluorescence. *Microscopy* **2018**, *67*, 345–355. [CrossRef]
33. Mcdonald, J.H.; Dunn, K.W. Statistical Tests for Measures of Colocalization in Biological Microscopy. *J. Microsc.* **2013**, *252*, 295. [CrossRef]
34. Schindelin, J.; Arganda-Carreras, I.; Frise, E.; Kaynig, V.; Longair, M.; Pietzsch, T.; Preibisch, S.; Rueden, C.; Saalfeld, S.; Schmid, B.; et al. Fiji: An Open-Source Platform for Biological-Image Analysis. *Nat. Methods* **2012**, *9*, 676–682. [CrossRef]
35. Harkness, J.E.; Turner, P.V.; VandeWoude, S.; Wheler, C.L. *Harkness and Wagner's Biology and Medicine of Rabbits and Rodents*, 5th ed.; Wiley-Blackwell: Hoboken, NJ, USA, 2009; ISBN 978-0-813-81531-2.
36. 12—Food and Water Intake. In *Techniques in the Behavioral and Neural Sciences*; Claassen, V. (Ed.) Elsevier: Amsterdam, The Netherlands, 1994; Volume 12, pp. 267–287, ISBN 0921-0709.
37. Dilek, Y.; Sezer, H. Insulin Resistance, Obesity and Lipotoxicity. In *Obesity and Lipotoxicity*; Engin, A.B., Engin, A., Eds.; Springer International Publishing: Cham, Switzerland, 2017; pp. 277–304, ISBN 978-3-319-48382-5.
38. Giknis, M.; Clifford, C. *Clinical Laboratory Parameters for Crl:WI (Han) Rats*; Charles River Laboratories International: Wilmington, MA, USA, 2008. Available online: http://www.criver.com/files/pdfs/rms/wistarhan/rm_rm_r_wistar_han_clin_lab_parameters_08.aspx (accessed on 19 November 2022).

39. De Andrade Mesquita, L.; Pavan Antoniolli, L.; Cittolin-Santos, G.F.; Gerchman, F. Distinct Metabolic Profile According to the Shape of the Oral Glucose Tolerance Test Curve Is Related to Whole Glucose Excursion: A Cross-Sectional Study. *BMC Endocr. Disord.* **2018**, *18*, 56. [CrossRef]
40. Pawlak, M.; Lefebvre, P.; Staels, B. Molecular Mechanism of PPARα Action and Its Impact on Lipid Metabolism, Inflammation and Fibrosis in Non-Alcoholic Fatty Liver Disease. *J. Hepatol.* **2015**, *62*, 720–733. [CrossRef]
41. Todisco, S.; Santarsiero, A.; Convertini, P.; De Stefano, G.; Gilio, M.; Iacobazzi, V.; Infantino, V. PPAR Alpha as a Metabolic Modulator of the Liver: Role in the Pathogenesis of Nonalcoholic Steatohepatitis (NASH). *Biology* **2022**, *11*, 792. [CrossRef]
42. Reagan-Shaw, S.; Nihal, M.; Ahmad, N. Dose Translation from Animal to Human Studies Revisited. *FASEB J.* **2008**, *22*, 659–661. [CrossRef]
43. World Health Organization. *Diet, Nutrition, and the Prevention of Chronic Diseases (Report of a Joint WHO and FAO Expert Consulation)*; WHO Technical Report Series; World Health Organization: Geneva, Switzerland, 2003; Volume 916, pp. 1–160.
44. You, Y.; Han, X.; Guo, J.; Guo, Y.; Yin, M.; Liu, G.; Huang, W.; Zhan, J. Cyanidin-3-Glucoside Attenuates High-Fat and High-Fructose Diet-Induced Obesity by Promoting the Thermogenic Capacity of Brown Adipose Tissue. *J. Funct. Foods* **2018**, *41*, 62–71. [CrossRef]
45. Songtrai, S.; Pratchayasakul, W.; Arunsak, B.; Chunchai, T.; Kongkaew, A.; Chattipakorn, N.; Chattipakorn, S.C.; Kaewsuwan, S. Cyclosorus Terminans Extract Ameliorates Insulin Resistance and Non-Alcoholic Fatty Liver Disease (NAFLD) in High-Fat Diet (HFD)-Induced Obese Rats. *Nutrients* **2022**, *14*, 4895. [CrossRef]
46. Jansson, J.O.; Palsdottir, V.; Hägg, D.A.; Schéle, E.; Dickson, S.L.; Anesten, F.; Bake, T.; Montelius, M.; Bellman, J.; Johansson, M.E.; et al. Body Weight Homeostat That Regulates Fat Mass Independently of Leptin in Rats and Mice. *Proc. Natl. Acad. Sci. USA* **2017**, *115*, 427–432. [CrossRef]
47. Yu, Y.H.; Vasselli, J.R.; Zhang, Y.; Mechanick, J.I.; Korner, J.; Peterli, R. Metabolic vs. Hedonic Obesity: A Conceptual Distinction and Its Clinical Implications. *Obes. Rev.* **2015**, *16*, 234–247. [CrossRef]
48. Wilcox, G. Insulin and Insulin Resistance. *Clin. Biochem. Rev.* **2005**, *26*, 19–39.
49. Ormazabal, V.; Nair, S.; Elfeky, O.; Aguayo, C.; Salomon, C.; Zuñiga, F.A. Association between Insulin Resistance and the Development of Cardiovascular Disease. *Cardiovasc. Diabetol.* **2018**, *17*, 122. [CrossRef]
50. Galicia-Moreno, M.; Lucano-Landeros, S.; Monroy-Ramirez, H.C.; Silva-Gomez, J.; Gutierrez-Cuevas, J.; Santos, A.; Armendariz-Borunda, J. Roles of Nrf2 in Liver Diseases: Molecular, Pharmacological, and Epigenetic Aspects. *Antioxidants* **2020**, *9*, 980. [CrossRef]
51. Pant, V.; Gautam, K.; Pradhan, S. Postprandial Blood Glucose Can Be Less than Fasting Blood Glucose and This Is Not a Laboratory Error. *JNMA J. Nepal. Med. Assoc.* **2019**, *57*, 67–68. [CrossRef] [PubMed]
52. Silva, Y.P.; Bernardi, A.; Frozza, R.L. The Role of Short-Chain Fatty Acids From Gut Microbiota in Gut-Brain Communication. *Front. Endocrinol.* **2020**, *11*, 508738. [CrossRef] [PubMed]
53. Coppola, S.; Avagliano, C.; Calignano, A.; Berni Canani, R. The Protective Role of Butyrate against Obesity and Obesity-Related Diseases. *Molecules* **2021**, *26*, 682. [CrossRef] [PubMed]
54. Adeli, K.; Xiao, C.; Higgins, V.; Taher, J.; Farr, S.; Lewis, G.F. Diabetic Dyslipidaemia. In *Biochemistry of Lipids, Lipoproteins and Membranes*; Ridgway, N.D., McLeod, R.S., Eds.; Elsevier: Amsterdam, The Netherlands, 2021; pp. 667–693, ISBN 978-0-12-824048-9.
55. Ferdowsian, H.R.; Barnard, N.D. Effects of Plant-Based Diets on Plasma Lipids. *Am. J. Cardiol.* **2009**, *104*, 947–956. [CrossRef] [PubMed]
56. Anderson, N.; Borlak, J. Molecular Mechanisms and Therapeutic Targets in Steatosis and Steatohepatitis. *Pharmacol. Rev.* **2008**, *60*, 311–357. [CrossRef]
57. Blüher, M. Adipose Tissue Inflammation: A Cause or Consequence of Obesity-Related Insulin Resistance? *Clin. Sci.* **2016**, *130*, 1603–1614. [CrossRef]
58. Qin, S.; Yin, J.; Huang, K. Free Fatty Acids Increase Intracellular Lipid Accumulation and Oxidative Stress by Modulating PPARα and SREBP-1c in L-02 Cells. *Lipids* **2016**, *51*, 797–805. [CrossRef]
59. Trauner, M.; Arrese, M.; Wagner, M. Fatty Liver and Lipotoxicity. *Biochim. Biophys. Acta* **2010**, *1801*, 299–310. [CrossRef]
60. Yan, T.; Yan, N.; Wang, P.; Xia, Y.; Hao, H.; Wang, G.; Gonzalez, F.J. Herbal Drug Discovery for the Treatment of Nonalcoholic Fatty Liver Disease. *Acta Pharm. Sin. B* **2020**, *10*, 3–18. [CrossRef]
61. Poloczek, J.; Kazura, W.; Chełmecka, E.; Michalczyk, K.; Jochem, J.; Gumprecht, J.; Stygar, D. Duodenojejunal Omega Switch Surgery Reduces Oxidative Stress Induced by Cafeteria Diet in Sprague-Dawley Rats. *Nutrients* **2022**, *14*, 4097. [CrossRef]
62. Ayala, A.; Muñoz, M.F.; Argüelles, S. Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal. *Oxid. Med. Cell. Longev.* **2014**, *2014*, 360438. [CrossRef]
63. Canesin, G.; Feldbrügge, L.; Wei, G.; Janovicova, L.; Janikova, M.; Csizmadia, E.; Ariffin, J.; Hedblom, A.; Herbert, Z.T.; Robson, S.C.; et al. Heme Oxygenase-1 Mitigates Liver Injury and Fibrosis via Modulation of LNX1/Notch1 Pathway in Myeloid Cells. *iScience* **2022**, *25*, 104983. [CrossRef]
64. Funes, S.C.; Rios, M.; Fernández-Fierro, A.; Covián, C.; Bueno, S.M.; Riedel, C.A.; Mackern-Oberti, J.P.; Kalergis, A.M. Naturally Derived Heme-Oxygenase 1 Inducers and Their Therapeutic Application to Immune-Mediated Diseases. *Front. Immunol.* **2020**, *11*, 1467. [CrossRef]

65. Diniz, T.A.; de Lima Junior, E.A.; Teixeira, A.A.; Biondo, L.A.; da Rocha, L.A.F.; Valadão, I.C.; Silveira, L.S.; Cabral-Santos, C.; de Souza, C.O.; Rosa Neto, J.C. Aerobic Training Improves NAFLD Markers and Insulin Resistance through AMPK-PPAR-α Signaling in Obese Mice. *Life Sci.* **2021**, *266*, 118868. [CrossRef]
66. Liu, P.; Dodson, M.; Li, H.; Schmidlin, C.J.; Shakya, A.; Wei, Y.; Garcia, J.G.N.; Chapman, E.; Kiela, P.R.; Zhang, Q.-Y.; et al. Non-Canonical NRF2 Activation Promotes a pro-Diabetic Shift in Hepatic Glucose Metabolism. *Mol. Metab.* **2021**, *51*, 101243. [CrossRef]
67. Dodson, M.; Zhang, D.D. Non-Canonical Activation of NRF2: New Insights and Its Relevance to Disease. *Curr. Pathobiol. Rep.* **2017**, *5*, 171–176. [CrossRef]
68. Vairetti, M.; Di Pasqua, L.G.; Cagna, M.; Richelmi, P.; Ferrigno, A.; Berardo, C. Changes in Glutathione Content in Liver Diseases: An Update. *Antioxidants* **2021**, *10*, 364. [CrossRef]
69. Liu, T.; Zhang, L.; Joo, D.; Sun, S.-C. NF-KB Signaling in Inflammation. *Signal Transduct. Target. Ther.* **2017**, *2*, 17023. [CrossRef]
70. Nie, Y.; Luo, F. Dietary Fiber: An Opportunity for a Global Control of Hyperlipidemia. *Oxid. Med. Cell. Longev.* **2021**, *2021*, 5542342. [CrossRef]
71. Irie, M.; Sohda, T.; Anan, A.; Fukunaga, A.; Takata, K.; Tanaka, T.; Yokoyama, K.; Morihara, D.; Takeyama, Y.; Shakado, S.; et al. Reduced Glutathione Suppresses Oxidative Stress in Nonalcoholic Fatty Liver Disease. *Euroasian J. Hepatogastroenterol.* **2016**, *6*, 13–18. [CrossRef]
72. Zhu, X.; Bian, H.; Wang, L.; Sun, X.; Xu, X.; Yan, H.; Xia, M.; Chang, X.; Lu, Y.; Li, Y.; et al. Berberine Attenuates Nonalcoholic Hepatic Steatosis through the AMPK-SREBP-1c-SCD1 Pathway. *Free Radic. Biol. Med.* **2019**, *141*, 192–204. [CrossRef] [PubMed]
73. Kersten, S.; Stienstra, R. The Role and Regulation of the Peroxisome Proliferator Activated Receptor Alpha in Human Liver. *Biochimie* **2017**, *136*, 75–84. [CrossRef] [PubMed]
74. Yuan, J.; Li, F.; Cui, B.; Gao, J.; Yu, Z.; Lu, Z. Inhibition of GCN2 Alleviates Cardiomyopathy in Type 2 Diabetic Mice via Attenuating Lipotoxicity and Oxidative Stress. *Antioxidants* **2022**, *11*, 1379. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

V. Conclusión

Los quelites son un alimento tradicional mexicano con potencial nutracéutico y funcional. Dentro del grupo de quelites se encuentra la hoja de frijol, la cual es fuente de hierro, proteína, fibra insoluble y polifenoles. El consumo y producción de alimentos tradicionales (locales o endémicos) ha disminuido en los últimos años, en México su cultivo es principalmente para autoconsumo de los productores. La globalización es uno de los factores que ha contribuido en mayor medida en los cambios en la elección y producción de alimentos, favoreciendo una dieta homogeneizada y disminuyendo el consumo de alimentos locales (Mateos-Maces et al., 2020). Alrededor del mundo, han surgido movimientos como Slow food que buscan incentivar el consumo de productos locales con preparaciones tradicionales (Slow Food, 2023). Lo anterior se alinea con uno de los objetivos del proyecto, al evaluar los efectos de la inclusión de hoja de frijol en la prevención de NAFLD, este proyecto contribuyó en la generación de conocimiento científico con el fin de incentivar y revalorizar el consumo de la hoja de frijol.

La fisiopatología de la NAFLD está estrechamente relacionada a la presencia de alteraciones metabólicas como a resistencia a la insulina, hiperlipemia y obesidad. Estas alteraciones metabólicas son un problema de salud mundial que debe atenderse de manera multidisciplinaria. Las intervenciones en el estilo de vida, como el incremento en la ingesta de compuestos bioactivos provenientes de una alimentación basada en plantas han mostrado efectos beneficiosos en el tratamiento y la prevención de trastornos metabólicos relacionados con una dieta rica en grasas/fructosa a largo plazo

La suplementación con 10% de hojas de frijol en una dieta alta en grasas/alta en fructosa mostró efectos preventivos contra la obesidad, las alteraciones en la glucemia en ayuno y postprandial. A nivel hepático, la suplementación con hoja de frijol evitó el desarrollo de esteatosis al incrementar la exportación de triglicéridos hepáticos al torrente sanguíneo (VLDL-c) y la expresión de PPARα (regulador positivo de β-oxidación). Dentro de los mecanismos involucrados en mantener el balance entre la producción, la acumulación y la utilización de ácidos grasos en el hígado (prevenir la lipotoxicidad hepática) se encuentran la exportación de triglicéridos, la lipogénesis y la β oxidación. Además, a nivel hepático la hoja de frijol evitó la formación de malonilaldehído (producto de

lipoperoxidación lipídica) al activar el sistema de defensa antioxidante (GSH sérico), y mostrando un efecto antiinflamatorio, reduciendo por tanto la lipotoxicidad hepática.

Los efectos antes mencionados podrían estar relacionados con un incremento en la producción de AGCC en contenido luminal mostrado en el grupo suplementado con hoja de frijol durante 6 semanas. Estudios previos han mostrado que los AGCC favorecen una modulación en la ingesta energética, disminuyendo la acumulación de lípidos y favoreciendo el control glucémico. Sin embargo, son necesarios más estudios que evalúen el efecto de la hoja de frijol en alteraciones metabólicas que permitan elucidar los mecanismos de acción involucrados, así como definir si la inclusión de la hoja de frijol en la alimentación solo tiene la capacidad de prevenir enfermedades o podría usarse también como parte del tratamiento de éstas.

VI. Referencias

Acosta Gallegos, J. A., Jiménez Hernández, Y., Sánchez García, B. M., Mendoza Hernández, F. M., Herrera Hernández, M. G., Salinas Pérez, R. A., y González Chavira, M. (2010). Flor de Mayo Eugenia, new bean cultivar for irrigated and rainfall conditions in Central Mexico. Revista Mexicana de Ciencias Agrícolas, 5(1).

Alcaraz, M. J., Vicente, A. M., Araico, A., Dominguez, J. N., Terencio, M. C., y Ferrándiz, M. L. (2004). Role of nuclear factor-κB and heme oxygenase-1 in the mechanism of action of an anti-inflammatory chalcone derivative in RAW 264.7 cells. British Journal of Pharmacology, 142(7), 1191–1199. https://doi.org/10.1038/SJ.BJP.0705821

Becerril Campos, A. A., Luna Moreno, A. D., García Gasca, M. T. de J., y Ahumada Solórzano, S. M. (2018). Prevención del riesgo metabólico en un modelo in vivo con el consumo de quínoa roja (Chenopodium Berlandieri Spp. Nuttalliae) y hoja de frijol (Phaseolus vulgaris). Revista de la Conferencia Científica Anual sobre Síndrome Metabólico, 4(1), 13–16. https://issuu.com/ccassindromemetabolico/docs/revista_conferencia_v4_n1

Becerril-Campos, A. A., Ocampo-Anguiano, P. V., Mondragón-Jacobo, C., Escobar-García, K., Camacho-Barrón, M., Anaya-Loyola, M. A., Feregrino-Perez, A. A., García-Gasca, T., y Ahumada-Solórzano, S. M. (2022). Phaseolus vulgaris L. leaves

increase short-chain fatty acid (SCFA) production, ameliorating early metabolic alterations. Plant Foods Human Nutrition, 77, 421–426. https://doi.org/ 10.1007/S11130-022-00992-1

Becerril-Campos, A. A., Ramos-Gómez, M., De Los Ríos-Arellano, E. A., Ocampo-Anguiano, P. V., González-Gallardo, A., Macotela, Y., García-Gasca, T., y Ahumada-Solórzano, S. M. (2023). Bean leaves ameliorate lipotoxicity in fatty liver disease. Nutrients, 15(13). https://doi.org/10.3390/nu15132928

Bernal-Reyes, R., Castro-Narro, G., Malé-Velázquez, R., Carmona-Sánchez, R., González-Huezo, M. S., García-Juárez, I., Chávez-Tapia, N., Aguilar-Salinas, C., Aiza-Haddad, I., Ballesteros-Amozurrutia, M. A., Bosques-Padilla, F., Castillo-Barradas, M., Chávez-Barrera, J. A., Cisneros-Garza, L., Flores-Calderón, J., García-Compeán, D., Gutiérrez-Grobe, Y., Higuera de la Tijera, M. F., Kershenobich-Stalnikowitz, D., Ladrón de Guevara-Cetina, L., Lizardi-Cervera, J., López-Cossio, J. A., Martínez-Vázquez, S., Márquez-Guillén, E., Méndez-Sánchez, N., Moreno-Alcantar, R., Poo-Ramírez, J. L., Ramos-Martínez, P., Rodríguez-Hernández, H., Sánchez-Ávila, J. F., Stoopen-Rometti, M., Torre-Delgadillo, A., Torres-Villalobos, G., Trejo-Estrada, R. Uribe-Esquivel, M. y Velarde-Ruiz Velasco, J. A. (2019). The mexican consensus on nonalcoholic fatty liver disease. Revista de Gastroenterología de México (English Edition), 84(1), 69–99. https://doi.org/10.1016/j.rgmxen.2019.02.003

Brooks, W. H., Li, J., Tiwari, R., Dadar, M., Weiskirchen, R., Lambertz, J., Weiskirchen, S., y Landert, S. (2017). Fructose: a dietary sugar in crosstalk with microbiota contributing to the development and progression of non-alcoholic liver disease. Frontiers in inmunology, 8, 1159. https://doi.org/10.3389/fimmu.2017.01159

Cave, M. C., Clair, H. B., Hardesty, J. E., Falkner, K. C., Feng, W., Clark, B. J., Sidey, J., Shi, H., Aqel, B. A., McClain, C. J., y Prough, R. A. (2016). Nuclear receptors and nonalcoholic fatty liver disease. Biochimica et Biophysica Acta - Gene Regulatory Mechanisms, 1859(9), 1083–1099. https://doi.org/10.1016/j.bbagrm.2016.03.002

Chalasani, N., Younossi, Z., Lavine, J. E., Charlton, M., Cusi, K., Rinella, M., Harrison, S. A., Brunt, E. M., y Sanyal, A. J. (2018). The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for

the Study of Liver Diseases. Hepatology, 67(1), 328–357. https://doi.org/ 10.1002/hep.29367

Dodson, M., y Zhang, D. D. (2017). Non-canonical activation of Nrf2: new insights and its relevance to disease. Current Pathobiology Reports, 5(2), 171–176. https://doi.org/10.1007/s40139-017-0131-0

Eslam, M., Sanyal, A. J., George, J., Sanyal, A., Neuschwander-Tetri, B., Tiribelli, C., Kleiner, D. E., Brunt, E., Bugianesi, E., Yki-Järvinen, H., Grønbæk, H., Cortez-Pinto, H., Fan, J., Valenti, L., Abdelmalek, M., Romero-Gomez, M., Rinella, M., Arrese, M., Bedossa, P., Newsome, P. N., Anstee, Q. M., Jalan, R., Bataller, R., Loomba, R., Sookoian, S., Sarin, S. K., Harrison, S., Kawaguchi, T., Wong, V., Yusuf Yilmaz, V. R., Younossi, Z. (2020). MAFLD: A Consensus-Driven Proposed Nomenclature for Metabolic Associated Fatty Liver Disease. Gastroenterology, 158(7), 1999-2014.e1. https://doi.org/10.1053/J.GASTRO.2019.11.312

Ferramosca, A., y Zara, V. (2014). Modulation of hepatic steatosis by dietary fatty acids. World Journal of Gastroenterology, 20(7), 1746–1755. https://doi.org/10.3748/wjg.v20.i7.1746

Galicia-Moreno, M., Lucano-Landeros, S., Monroy-Ramirez, H. C., Silva-Gomez, J., Gutierrez-Cuevas, J., Santos, A., y Armendariz-Borunda, J. (2020). Roles of Nrf2 in liver diseases: molecular, pharmacological, and epigenetic aspects. Antioxidants, 9(10). https://doi.org/10.3390/antiox9100980

Ganesh Yerra, V., Negi, G., Sharma, S. S., y Kumar, A. (2013). Potential therapeutic effects of the simultaneous targeting of the Nrf2 and NF-κB pathways in diabetic neuropathy. Redox Biology, 1(1), 394–397. https://doi.org/10.1016/j. redox.2013.07.005

Guzman Maldonado, S. (2017). Análisis químico proximal de la hoja de frijol variedad Flor de Mayo Eugenia. Proyectos de innovación y desarrollo tecnológico para regiones y prioritariamente a sistemas producto básicos y estratégicos agrícolas SAGARPA DF1600004770 (datos no publicados).

Hinds, T. D., Sodhi, K., Meadows, C., Fedorova, L., Puri, N., Kim, D. H., Peterson, S. J., Shapiro, J., Abraham, N. G., y Kappas, A. (2014). Increased HO-1 levels

ameliorate fatty liver development through a reduction of heme and recruitment of FGF21. Obesity, 22(3), 705–712. https://doi.org/10.1002/oby.20559

Jones, A. L., y Mejia, D. (1999). Phaseolus bean: post-harvest operations (D. Mejia y B. Lewis, Eds.). Centro Internacional de Agricultura Tropical (CIAT).

Keleku-Lukwete, N., Suzuki, M., y Yamamoto, M. (2018). An overview of the advantages of Keap1-Nrf2 system activation during inflammatory disease treatment. Antioxidants and Redox Signaling, 29(17), 1746–1755. https://doi.org/10.1089/ars.2017.7358

Letexier, D., Pinteur, C., Large, V., Fréring, V., y Beylot, M. (2003). Comparison of the expression and activity of the lipogenic pathway in human and rat adipose tissue. Journal of Lipid Research, 44(11), 2127–2134. https://doi.org/10.1194/jlr.M300235-JLR200

Li, L., Fu, J., Sun, J., Liu, D., Chen, C., Wang, H., Hou, Y., Xu, Y., y Pi, J. (2019). Is Nrf2-ARE a potential target in NAFLD mitigation? En Current Opinion in Toxicology (Vol. 13, pp. 35–44). Elsevier B.V. https://doi.org/10.1016/j.cotox.2018.12.005

Lian, C.-Y., Chu, B.-X., Xia, W.-H., Wang, Z.-Y., Fan, R.-F., y Wang, L. (2023). Persistent activation of Nrf2 in a p62-dependent non-canonical manner aggravates lead-induced kidney injury by promoting apoptosis and inhibiting autophagy. Journal of Advanced Research, 46, 87–100. https://doi.org/10.1016/j.jare.2022.04.016

Linares, E., Bye, R., Ortega, N., y Arce, A. E. (2017). Quelites: sabores y saberes, del sureste del Estado de México (Primera ed). Universidad Nacional Autonoma de Mexico. https://doi.org/10.22201/ib.9786073016667e.2019

Liu, P., Dodson, M., Li, H., Schmidlin, C. J., Shakya, A., Wei, Y., Garcia, J. G. N., Chapman, E., Kiela, P. R., Zhang, Q.-Y., White, E., Ding, X., Ooi, A., y Zhang, D. D. (2021). Non-canonical NRF2 activation promotes a pro-diabetic shift in hepatic glucose metabolism. Molecular Metabolism, 51, 101243. https://doi.org/10.1016/j.molmet.2021.101243

Ma, Q. (2013). Role of Nrf2 in oxidative stress and toxicity. Annual Review of Pharmacology and Toxicology, 53(1), 401–426. https://doi.org/10.1146/annurev-pharmtox-011112-140320

Martínez-Zavala, M. (2012). Estudio de la hoja de frijol Phaseolus vulgaris como alternativa de hierro biodisponible. Universidad Autónoma de Querétaro.

Martínez-Zavala, M., Mora-Avilés, M. A., Anaya-Loyola, M. A., Guzmán-Maldonado, H., Aguilera-Barreyro, A., Blanco-Labra, A., y García-Gasca, T. (2016). Common bean leaves as a source of dietary iron: Functional test in an iron-deficient rat model. Plant Foods for Human Nutrition, 71(3), 259–264. https://doi.org/10.1007/s11130-016-0554-5

Mateos-Maces, L., Chávez-Servia, J. L., Vera-Guzmán, A. M., Aquino-Bolaños, E. N., Alba-Jiménez, J. E., y Villagómez-González, B. B. (2020). Edible leafy plants from Mexico as sources of antioxidant compounds, and their nutritional, nutraceutical and antimicrobial potential: A review. Antioxidants, 9(6), 1–24. https://doi.org/ 10.3390/antiox9060541

Perez-Martinez, P., Perez-Jimenez, F., y Lopez-Miranda, J. (2010). n-3 PUFA and lipotoxicity. En Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids (Vol. 1801, Número 3, pp. 362–366). https://doi.org/10.1016/j.bbalip.2009. 09.010

Ramírez-Venegas, G., De Ita-Pérez, DL., Díaz-Muñoz, M., Méndez, I., García-Gasca, T., Ahumada-Solórzano, M., Zambrano-Estrada, X., Vázquez-Martínez, O., Guzmán-Maldonado, H., y Luna-Moreno, D. (2021). Supplementation with Phaseolus vulgaris leaves improves metabolic alterations induced by high-fat/fructose diet in rats under time-restricted feeding. Plant Foods for Human Nutrition, 76(3), 297–303. https://doi.org/10.1007/S11130-021-00904-9

Silva-Islas, C. A., y Maldonado, P. D. (2018). Canonical and non-canonical mechanisms of Nrf2 activation. Pharmacological Research, 134, 92–99. https://doi.org/10.1016/J.PHRS.2018.06.013

Slow Food. (2023). Slow Food in the UK - Celebrate what's on your plate! https://www.slowfood.org.uk/

Stefanson, A. L., y Bakovic, M. (2014). Dietary regulation of Keap1/Nrf2/ARE pathway: Focus on plant-derived compounds and trace minerals. Nutrients, 6(9), 3777–3801. https://doi.org/10.3390/nu6093777

Symons, J. D., y Dale Abel, E. (2013). Lipotoxicity contributes to endothelial dysfunction: a focus on the contribution from ceramide. Reviews in Endocrine and Metabolic Disorders, 14(1), 59–68. https://doi.org/10.1007/s11154-012-9235-3

Tebay, L. E., Robertson, H., Durant, S. T., Vitale, S. R., Penning, T. M., Dinkova-Kostova, A. T., y Hayes, J. D. (2015). Mechanisms of activation of the transcription factor Nrf2 by redox stressors, nutrient cues, and energy status and the pathways through which it attenuates degenerative disease. Free Radical Biology and Medicine, 88(Part B), 108–146. https://doi.org/10.1016/j.freeradbiomed.2015.06.021

Valenzuela, R., Illesca, P., Echeverría, F., Espinosa, A., Rincón-Cervera, M. Á., Ortiz, M., Hernandez-Rodas, M. C., Valenzuela, A., y Videla, L. A. (2017). Molecular adaptations underlying the beneficial effects of hydroxytyrosol in the pathogenic alterations induced by a high-fat diet in mouse liver: PPAR-α and Nrf2 activation, and NF-κB down-regulation. Food and Function, 8(4), 1526–1537. https://doi.org/10.1039/c7fo00090a

Vysakh, A., Suma, D., Syama, S., Raji, R. N., Mathew, J., y Latha, M. (2016). Role of NF-κB signaling pathway and oxidative stress in liver inflammation. Austin Hepatology, 1(1), 1004. https://austinpublishinggroup.com/austin-hepatology/fulltext/hepatology-v1-id1004.php

Wardyn, J. D., Ponsford, A. H., y Sanderson, C. M. (2015). Dissecting molecular cross-talk between Nrf2 and NF-κB response pathways. Biochemical Society Transactions, 43(4), 621–626. https://doi.org/10.1042/BST20150014

Yamamoto, M., Kensler, T. W., y Motohashi, H. (2018). The Keap1-Nrf2 system: A thiol-based sensor-effector apparatus for maintaining redox homeostasis. Physiological Reviews, 98(3), 1169–1203. https://doi.org/10.1152/physrev.00023.2017

Younossi, Z. M., Koenig, A. B., Abdelatif, D., Fazel, Y., Henry, L., y Wymer, M. (2016). Global epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology, 64(1), 73–84. https://doi.org/10.1002/hep.28431

Yu, J., Marsh, S., Hu, J., Feng, W., y Wu, C. (2016). The pathogenesis of nonalcoholic fatty liver disease: interplay between diet, gut microbiota, and genetic

background. Gastroenterology Research and Practice, 2016. https://doi.org/ 10.1155/2016/2862173

Zambon, A., Gervois, P., Pauletto, P., Fruchart, J. C., y Staels, B. (2006). Modulation of hepatic inflammatory risk markers of cardiovascular diseases by PPAR-α activators: Clinical and experimental evidence. Arteriosclerosis, Thrombosis, and Vascular Biology, 26(5), 977–986. https://doi.org/10.1161/01.ATV.0000204327. 96431.9a

Zhao, N., Guo, F.-F., Xie, K.-Q., y Zeng, T. (2018). Targeting Nrf2 is a promising intervention approach for the prevention of ethanol-induced liver disease. Cellular and Molecular Life Sciences, 75(17), 3143–3157. https://doi.org/10.1007/s00018-018-2852-6