UNIVERSIDADE FEDERAL DE MATO GROSSO DO SUL
PROGRAMA DE PÓS-GRADUAÇÃO EM SAÚDE E DESENVOLVIMENTO NA REGIÃO CENTRO-OESTE

ALINE CARLA INADA

AVALIAÇÃO DO EXTRATO AQUOSO DOS FRUTOS DE *Morinda citrifolia* Linn. (noni) NAS ALTERAÇÕES METABÓLICAS EM CAMUNDONGOS *SWISS* ALIMENTADOS COM DIETA RICA EM LIPÍDIOS E FRUTOSE

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Tese apresentada ao Programa de Pós-Graduação em Saúde e Desenvolvimento na Região Centro-Oeste da Universidade Federal de Mato Grosso do Sul, como requisito para obtenção do título de Doutor.

Orientadora: Profa. Dra. Priscila Aiko Hiane
Co-Orientadoras: Profa. Dra. Karine de Cássia Freitas e Profa. Dra. Rita de Cássia A. Guimarães

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DEDICATÓRIA

Ao meu eterno e querido pai, Kozo Inada (*in memoriam*), minha mãe amada (Midori), meu amado esposo (Daniel), minha filhinha (Olívia), meus queridos: irmã (Daniele), cunhado (Cristian), sobrinhos (Eduardo, Enzo e Emily) e meus sogros (Maisa e Luiz) por todo o carinho, amor e compreensão durante essa incrível caminhada.

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RESUMO

A síndrome metabólica (SM) engloba um conjunto de anormalidades metabólicas. Estudos demonstraram que *Morinda citrifolia* Linn. (noni) (*M. citrifolia*) apresenta ações anticancerígenas, antimicrobianas, antiulcerogênicas, antioxidativas e ações nas disfunções metabólicas envolvidas na SM. A desregulação na lipogênese *de novo* (DNL) hepática é umas das desordens metabólicas associadas a SM e possui relação direta com a doença hepática gordurosa não alcóolica (DHGNA). O objetivo deste estudo foi avaliar a ação oral de duas doses do extrato aquoso bruto de *M. citrifolia* (AE) dos frutos nos parâmetros bioquímicos, histopatológicos e na expressão de genes envolvidos no metabolismo lipídico e glicêmico em animais submetidos a dieta rica em lipídios e frutose. O AE foi analisado por cromatografia líquida ultra rápida acoplada a detector por arranjo de diodos e espectrômetro de massas (UFLC-DAD-MS). A toxicidade oral aguda foi realizada em camundongos *Swiss* fêmeas (n=10) divididos em grupo controle (CT) (n=5) e grupo extrato (n=5) 2000 mg/kg seguindo a metodologia conforme a OECD Guidelines 425 e o teste hipocrático foi realizado para avaliar as características morfológicas e comportamentais dos animais. Para avaliar o tratamento com o extrato, primeiramente, camundongos *Swiss* machos com 12 semanas de idade foram submetidos a dieta padrão - grupo controle (CT) (n=11) e dieta rica em lipídios e frutose (HFF) (n=31) durante o período de 9 semanas. A partir da 10ª semana, o tratamento com os extratos por via oral (gavagem) foi iniciado e os grupos foram divididos em grupos que foram submetidos a dieta padrão + água de beber (CTW) (n=11), dieta HFF + água de beber (HFFW) (n=10), dieta HFF + AE 250 mg/kg (HFF + AE 250) (n=11) e dieta HFF + AE 500 mg/kg (HFF + AE 500) (n=10) diariamente até a 16ª semana. Os animais foram submetidos a 8 horas de jejum para a realização do teste oral de tolerância à glicose (TOTG) sendo realizado três dias antes do tratamento e três dias antes da eutanásia. O consumo alimentar, massa corpórea, dosagem bioquímicas, dosagem dos níveis plasmáticos de insulina e as análises histológicas do fígado, tecido adiposo epididimal e pâncreas foram realizados para determinar os parâmetros bioquímicos e histológicos. A reação em cadeia de polimerase quantitativa em tempo real (qRT-PCR) foi utilizada para avaliar a expressão de alguns genes como receptores ativados por proliferadores de peroxissoma-α e -γ (PPAR-α e PPAR-γ), as enzimas ácido graxo sintase (FAS) e

glicose-6-fosfatase (G6P), proteína 1c de ligação ao elemento regulador de esterol (SREBP-1c), proteína de ligação ao elemento responsiva a carboidratos (ChREBP) e a hepatocina, fetuína-A. Dezessete compostos foram tentativamente identificados incluindo iridóides, noniosídeos e a rutina. Os animais que receberam extrato aquoso bruto não apresentaram sinais e sintomas de toxicidade aguda. O tratamento oral com AE 500 mg/kg/dia demonstrou reverter a tolerância a glicose induzida pela HFF, porém, ambas doses não mostraram efeitos em outros parâmetros bioquímicos e histológicos. AE 500 mg/kg diminuiu a expressão de RNAm de PPAR-γ, SREBP-1c e fetuína-A no fígado e aumentou a expressão de RNAm de PPAR-α no tecido adiposo branco, sugerindo que a ação hipoglicêmica poderia estar associada com a expressão de genes envolvidos no metabolismo de frutose e na DNL hepática.

Palavras-chave: Síndrome metabólica. Efeito sinérgico. Lipogênese *de novo*. Camundongos. Tolerância à glicose.

ABSTRACT

Metabolic syndrome (MS) encompasses a set of metabolic abnormalities. Studies have shown that *Morinda citrifolia* Linn. (noni) (*M. citrifolia*) has anticancer, antimicrobial, antiulcerogenic, antioxidative actions and actions on metabolic disorders involved in MS. Dysregulation in hepatic *de novo* lipogenesis (DNL) is one of metabolic disorders associated with MS and has a direct relationship with NAFLD. The aim of this study was to evaluate the oral action of two doses of a crude aqueous extract (AE) from fruits of *M. citrifolia* (AE) on biochemical and histopathological parameters and on the expression of genes involved in lipid and glycemic metabolism in animals fed a high-fat/high-fructose diet. AE was analyzed by ultra-fast liquid chromatography–diode array detector–tandem mass spectrometry (UFLC-DAD-MS). Acute oral toxicity was performed in female *Swiss* mice (n=10) divided in control group (CT) (n=5) and extract group (n=5) 2000 mg/kg according to OECD Guidelines 425 and the Hippocratic test was accomplished to evaluate morphological and behavioral feature from animals. To evaluate the extract treatment, 12-week-old male adult *Swiss* mice were submitted to a standard diet - control group (CT) (n=11) and a high-fat/high-fructose diet (HFF) (n=31) for 9 weeks. From the 10th week, treatment with extracts was started and the groups were divided into animals that were submitted to standard diet + drink water (CTW) (n=11), HFF diet + drink water (HFFW) (n=10), HFF diet + AE 250 mg/kg (HFF + AE 250) (n=11) and HFF diet + AE 500 mg/kg (HFF + AE 500) (n=10) daily until the 16th week. Animals were submitted for 8 hours of fasting to accomplish oral glucose tolerance test (OGTT) and was performed three days prior to the treatment and three days prior to euthanasia. Food intake, body mass, biochemical series, dosage of plasma insulin levels and histological analyzes of the liver, epididymal adipose tissue and pancreas were performed to determine biochemical and histological parameters. Quantitative real time - polymerase chain reaction (qRT-PCR) was used to evaluate the expression of some genes as peroxisome proliferator-activated receptors-α and -γ (PPAR-α and PPAR-γ), fatty acid enzymes synthase (FAS) and glucose-6-phosphatase (G6P), sterol regulatory element binding protein 1c (SREBP-1c), carbohydrate responsive element binding protein (ChREBP) and the hepatokine, fetuin-A. Seventeen compounds were tentatively identified including iridoids, noniosides and rutin. Animals that received the AE did not display signs and symptoms of acute toxicity.

AE 500 mg/kg/day oral treatment improved glucose tolerance induced by HFF diet, however, both doses showed no effects on other biochemical and histological parameters. AE 500 mg/kg downregulated PPAR-α, SREBP-1c and fetuin-A mRNA expression in liver and upregulated PPAR-α mRNA expression in white adipose tissue, suggesting that the hypoglycemic action could be associated with the expression of genes involved in fructose metabolism and hepatic DNL.

Keywords: Metabolic syndrome. Synergistic effect. *De novo* lipogenesis. Mice. Glucose tolerance.

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LISTA DE SIGLAS

ACC – Acetyl-CoA-Carboxylase (Acetil-CoA-Carboxilase)

ACS – Acetyl-CoA-Reductase (Acetil-CoA-Redutase)

AE – Extrato Aquoso Bruto de *M. citrifolia*

AE 250 mg/kg – Extrato Aquoso Bruto de *M. citrifolia* 250 mg/kg

AE 500 mg/kg – Extrato Aquoso Bruto de *M. citrifolia* 500 mg/kg

AGL – Ácidos Graxos Livres

ALT – Alanina Transaminase

ANVISA – Agência Nacional de Vigilância Sanitária

APOC3 – Apoliprotein C 3 (Apoliproteína C 3)

Caco-2 – Células do Intestino do tipo Caco-2

CAT – Catalase Enzyme (Catalase)

ChREBP – Carbohydrate Response Element Binding Protein (Proteína Ligada ao Elemento Responsivo a Carboidratos)

COX-2 – Cicloxigenase-2

CT - Grupo Controle

CTW - Animais que receberam ração comercial + água de beber

DCNTs – Doenças Crônicas Não – Transmissíveis

DCVs – Doenças Cardiovasculares

DHGNA – Doença Hepática Gordurosa Não-Alcóolica

DM2 – Diabetes Mellitus tipo 2

DNL – *De novo* Lipogenesis (Lipogênese *de novo*)

EHNA – Esteatose Hepática Não-Alcóolica

FABP – Fatty Acid Binding Protein (Proteína de Ligação de Ácidos Graxos)

FAO – Food and Agricultural Organization of the United Nations (FAO)

FAS – Fatty Acid Synthase (Ácido Graxo Sintase)

FAT – Fatty Acid Translocase (Ácido Graxo Translocase)

F-1-P – Fructose-1-Phosphate (Frutose-1-Fosfato)

FGF21 – Fibroblast Growth Factor 21 (Fator de Crescimento de Fibroblasto 21)

fNJ – Fermented Noni Juice (Suco Fermentado do Fruto do Noni)

FOXO1 – Forkhead Box O1 Protein (Fator de Transcrição 1)

GAP – Glyceraldehyde-3-Phosphate (Gliceraldeído-3-Fosfato)

G6P – Glucose-6-Phosphatase (Glicose-6-Fosfatase)

GLUT-2 – Glucose Transporter-2 (Transportador de Glicose-2)

GLUT-4 – Glucose Transporter-4 (Transportador de Glicose 4)

GLUT-5 – Glucose Transporter-5 (Transportador de Glicose-5)

GSH – Glutathione (Glutationa)

GSH-Px – Glutathione Peroxidase (Glutationa Peroxidase)

HbA1c – Hemoglobina Glicada

HDL – High Density Lipoprotein (Lipoproteínas de Alta Densidade)

HepG2 – Células de Hepatomas Humanos do tipo HepG2

HFD – High Fat Diet (Dieta Rica em Lipídios)

HFF – High-Fat High-Fructose (Dieta Rica em Lipídios e Frutose)

HFFW – Animais que receberam dieta HFF + água de beber

HFF + AE 250 – Animais que receberam dieta HFF + Extrato Aquoso Bruto 250 mg/kg

HFF + AE 500 – Animais que receberam dieta HFF + Extrato Aquoso Bruto 500 mg/kg

HIF-1α – Hypoxia Induction Factor-1α (Fator de Indução de Hipóxia-1α)

HIF-2α – Hypoxia Induction Factor-2α (Fator de Indução de Hipóxia-2α)

HOMA – β – Homeostasis Model Assessment - β (Modelo de Avaliação da Homeostase – Células β-Pancreáticas)

HOMA – IR – Homeostasis Model Assessment – Insulin Resistance (Modelo de Avaliação da Homeostase – Resistência à Insulina)

IL-6 – Interleukin-6 (Interleucina-6)

IL-10 – Interleukin-10 (Interleucina-10)

IMC – Índice de Massa Corpórea

iNOS – Induced Nitric Oxide Synthase (Óxido Nítrico Sintase Induzível)

KHKI – Ketohexoquinase (Cetohexoquinase)

MCP-1 – Monocytes Chemoattractant Protein-1 (Proteína Quimiotáxica de Monócitos)

MMP9 – Metalloproteinase 9 (Metaloproteinase 9)

mTOR - mammalian Target of Rapamycin (Alvo de Rapamicina em Mamíferos)

NJ – Noni Juice (Suco do Fruto do Noni)

PCR – Protein C Reactive (Proteína-C-Reativa)

PGC1β – Peroxisome Proliferator-Activated Receptor-1β Cofactor (Cofator 1-β de Receptores Ativados por Proliferadores de Peroxissoma-1β)

PKC – Protein Kinase C (Proteína Quinase C)

PEPCK – Phosphoenolpyruvate C Kinase (Fosfoenolpiruvato C Quinase)

PPAR-α – Peroxisome Proliferator-Activated Receptor-Alpha (Receptores Ativados por Proliferadores de Peroxissoma-α)

PPAR – γ – Peroxisome Proliferator-Activated Receptor-Gama (Receptores Ativados por Proliferadores de Peroxissoma-γ)

qRT-PCR – Quantitative Real Time Polymerase Chain Reaction (Reação em Cadeia de Polimerase Quantitativa em Tempo Real)

RNAm – Ribonucleic Acid Messenger (Ácido Ribonucleico Mensageiro)

SM – Síndrome Metabólica

SOD – Superoxide Dismutase (Superóxido Dismutase)

SREBP-1c - Sterol Regulatory Element-Binding Protein-1c (Proteína Ligada ao Elemento Regulado por Esterol 1c)

S6K – Serine/Threonine Kinase (Serina/Treonina Quinase)

TA – Tecido Adiposo

TAB – Tecido Adiposo Branco

TBARS – Thiobarbituric Acid Reactive Substances (Substância Reativa do Ácido Tiobarbitúrico)

TEAC – Trolox Equivalent Antioxidant Capacity (Capacidade Antioxidante Dependente de Trolox)

TG - Triglicerídeos

TGF-β – Tumoral Growth Factor -β (Fator de Crescimento Tumoral-β)

TNF–α Tumoral Necrosis Factor-α (Fator de Necrose Tumoral-α)

TOGG – Teste Oral de Tolerância à Glicose

UCP – Uncoupling Proteins (Proteínas Desacopladoras)

UFLC-DAD-MS - Ultra-Fast Liquid Chromatography–Diode Array Detector Mass Spectrometry (Cromatografia Líquida Ultra Rápida Acoplada a Detector por Arranjo de Diodos e Espectrômetro de Massas)

VEGF – Vascular Endothelial Growth Factor (Fator de Crescimento Vascular Derivado do Endotélio)

VLDL – Very Low Density Lipoprotein (Lipoproteína de Densidade Muito Baixa)

WHO – World Health Organization

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INTRODUÇÃO

As doenças crônicas não transmissíveis (DCNTs) são consideradas riscos à saúde na sociedade moderna aumentando a morbidade e mortalidade. A síndrome metabólica (SM) é um problema de saúde pública mundial e pode ser considerada uma DCNT (SAKLAYEN et al., 2018), visto que engloba um conjunto de distúrbios metabólicos como obesidade visceral, resistência à insulina/hiperglicemia, hipertensão arterial sistêmica, dislipidemia aterogênica e a doença hepática gordurosa não-alcóolica (DHGNA) (BUCKLEY et al., 2018).

Dentre os fatores responsáveis pelo aumento de casos de indivíduos com SM, pode-se destacar a adesão de um hábito alimentar não saudável da sociedade ocidental que é composto por alimentos com alto teor energético e baixo teor de fibras alimentares, como por exemplo, o consumo exacerbado de *fast-food*, associado a um estilo de vida sedentário (SAKLAYEN et al., 2018). A dieta com alto teor de frutose, sacarose e gordura saturada, pode ser considerada um exemplo de dieta ocidental (TASKINEN et al., 2019).

Devido ao aumento da prevalência da SM no mundo e pelo fato de que o estilo de vida e alimentação são fatores importantes para a ocorrência e prevalência da SM (ESPOSITO; CERIELLO; GIGLIANO, 2007), modelos animais que mimetizam e desenvolvem as anormalidades metabólicas características da SM em humanos foram e vem sendo desenvolvidos. Um exemplo inclui os modelos animais induzidos por dieta e, dentre estes, incluem os induzidos por dietas ricas em carboidratos, como frutose e sacarose, e lipídios, como gordura saturada, mais conhecida como dieta rica em lipídios e frutose (PANCHAL; BROWN, 2011).

A frutose, um monossacarídeo assim como a glicose, é encontrada na forma livre como hexose em frutas e mel sendo um dos carboidratos com sabor mais adocicado dentre os açúcares de origem natural (TAPPY et al., 2010). Por outro lado, o consumo de frutose na forma comercial é correspondente à adição artificial em alimentos, por exemplo em sucos artificiais, refrigerantes, xarope de milho (*corn syrup*) e produtos de confeitaria (TASKINEN et al., 2019). O aumento no consumo de frutose comercial adicionada às dietas tem resultado em implicações à saúde, uma vez que os estudos têm demonstrado que pode promover um desequilíbrio no processo da lipogênese *de novo* (DNL) hepática sendo um fator de risco para o aumento da adiposidade e desordens metabólicas (HORST; SERLIE, 2017).

A DNL hepática é um processo bioquímico fundamental no fígado contribuindo no estoque e secreção de lipídeos pelos hepatócitos (JENSEN-URSTAD; SEMENKOVICH, 2012) e consiste na síntese de ácidos graxos das subunidades de acetil-CoA que são produzidos por um número de diferentes vias dentro das células durante a glicólise, mais comumente no catabolismo de carboidratos (SANDERS; GRIFFIN, 2016). O aumento no consumo de frutose comercial, sendo esta um substrato lipogênico, na forma de xarope de milho e outros produtos, pode levar à desregulação das vias da DNL, e esta pode contribuir na patogênese da doença hepática gordurosa não-alcóolica (DHGNA), uma condição comumente associada com a SM e, consequente, resistência à insulina (SANDERS; GRIFFIN, 2016).

Estudos demonstraram que o uso de extratos aquosos de frutos de *Phyllanthus emblica*, mais conhecida como groselha indiana (USHARANI et al., 2019; KARCHEVA-BALCHEVANSKA et al., 2017), ou até mesmo suco de frutos naturais, como é o caso do suco de laranja e romã (SIMPSON et al., 2016; MOAZZEN; ALIZADEH, 2017), podem atuar como potenciais nutracêuticos para prevenir ou tratar os distúrbios metabólicos associados à SM. Produtos nutracêuticos podem ser considerados alimentos funcionais e são amplamente consumidos e possuem uma boa aceitação pela população devido aos benefícios à saúde. Na maioria das vezes, nutracêuticos não são considerados medicamentos pelo fato de que a regulação é complexa e por muitas vezes não se encaixarem em nenhuma destas regularizações de registro de produtos na Agência de Vigilância Sanitária (ANVISA) (DAUD et al., 2017).

Dentre estes nutracêuticos derivados de frutos inclui-se aqueles que contém *Morinda citrifolia* Linn., popularmente conhecido como noni, que é usado na medicina folclórica há 2000 anos pelos Polinésios (CHAN-BLANCO et al., 2006). Nos Estados Unidos da América, Ásia e Europa, a comercialização do suco de *M. citrifolia* (Tahitian Noni Juice®) e de produtos derivados de *M.citrifolia* é permitida pelos órgãos de vigilância sanitária locais (DIXON et al., 1999; SAMOYLENKO et al., 2006), porém, no Brasil, não existe legalização vigente para comercialização de produtos (ANVISA, 2007).

Embora não exista aprovação de comercialização de produtos de *M. citrifolia* no Brasil, estudos demonstram propriedades terapêuticas de *M. citrifolia*, desde

ações antiulcerogênicas (MAHATTANADUL et al., 2011), antiinflamatórias (AKIHISA et al., 2007), anticancerígenas (AKIHISA et al., 2007; BROWN, 2012) e, mais recentemente, na prevenção e tratamento de doenças metabólicas em modelos animais (MANDUKHAIL et al., 2010; NERURKAR et al., 2012; LIN et al., 2013; LEE et al., 2012; SHOEB et al., 2016; INADA et al., 2017) e em humanos (ALGENSTAEDT et al., 2018). Porém, ainda não existem estudos que demonstram as ações do uso oral do extrato aquoso bruto de *M.citrifolia* em camundongos *Swiss* alimentados com dieta rica em lipídeos e frutose, um modelo animal que mimetiza a SM induzida por dieta.

Desta forma, considerando os estudos publicados que utilizam *M. citrifolia* como um potencial terapêutico, o objetivo deste estudo foi avaliar os efeitos do extrato aquoso bruto dos frutos de *M. citrifolia* nos parâmetros bioquímicos e histológicos e na expressão de genes envolvidos no metabolismo lipídico e glicêmico no tecido adiposo branco (epididimal) e fígado em camundongos *Swiss* alimentados com dieta rica em lipídios e frutose.

OBJETIVOS

Objetivo geral

Avaliar os efeitos do tratamento oral do extrato aquoso bruto dos frutos de *Morinda citrifolia* (*M. citrifolia*) no metabolismo lipídico e glicêmico em camundongos *Swiss* que receberam dieta rica em lipídios e frutose. Além disso, analisar a composição química e a toxicidade aguda oral do extrato.

Objetivos específicos

- Elaborar o extrato aquoso bruto dos frutos de *M. citrifolia* e avaliar o perfil químico das substâncias ativas;
- Avaliar a toxicidade da administração oral aguda de extrato aquoso bruto dos frutos de *M. citrifolia* em camundongos Swiss fêmeas;
- Avaliar o efeito da administração oral de extrato aquoso bruto dos frutos de *M. citrifolia* na massa corpórea e consumo alimentar;
- Avaliar o efeito da administração oral de extrato aquoso bruto dos frutos de *M. citrifolia* na tolerância à glicose induzido por HFF;
- Avaliar o efeito da administração oral de extrato aquoso bruto dos frutos de *M. citrifolia* nos parâmetros bioquímicos: glicemia de jejum, colesterol total, triglicerídeos, colesterol HDL e não-HDL, insulina, índice HOMA-IR e índice HOMA-β;
- Avaliar o efeito da administração oral de extrato aquoso bruto dos frutos de *M. citrifolia* nos parâmetros histológicos do fígado, pâncreas e adipócitos;
- Avaliar o efeito da administração oral de extrato aquoso bruto dos frutos de *M. citrifolia* na expressão do conteúdo de RNA mensageiro (RNAm) de genes específicos envolvidos no metabolismo lipídico e glicêmico no tecido adiposo branco (epididimal) e fígado: peroxisome proliferator-activated receptor (receptores ativados por proliferadores de peroxissoma) -γ (PPAR-γ) e PPAR-α;

- Avaliar o efeito da administração oral de extrato aquoso bruto dos frutos de *M. citrifolia* na expressão do conteúdo de RNA mensageiro (RNAm) de genes específicos envolvidos no metabolismo lipídico e glicêmico no fígado: sterol regulatory element-binding protein 1c (proteína 1c de ligação ao elemento regulador de esterol) (SREBP-1c), carbohydrate sensitive response element binding protein (proteína de ligação ao elemento responsiva a carboidratos) (ChREBP), glucose-6-phosphate (glicose-6-fosfato) (G6P), fatty acid synthase (ácido graxo sintase) (FAS) e a hepatocina fetuína-A (fetuin-A).

CAPÍTULO 1: REVISÃO DE LITERATURA

1.1. A obesidade e a síndrome metabólica (SM)

As doenças crônicas não transmissíveis (DCNTs) são consideradas um grupo de desordens crônicas peristentes e de progressão lenta que envolve a combinação de fatores genéticos, ambientais, fisiológicos e comportamentais. Estima-se que 71% das mortes no mundo, ou seja, aproximadamente 41 milhões de pessoas a cada ano, são levadas ao óbito devido às DCNTs (WHO, 2018). As doenças cardiovasculares (DCVs), cânceres, doenças crônicas respiratórias e diabetes são classificadas dentre as DCNTs (WHO, 2018).

Embora estas condições de saúde estejam mais frequentemente associadas às populações de maior faixa etária (acima de 69 anos), estudos demonstram que as DCNTs acometem indivíduos mais jovens, dentre os 30 a 69 anos de idade, o que demonstra que as DCNTs estão entre as responsáveis pelas mortes prematuras no mundo (WHO, 2018). Alguns autores consideram a SM como a mais nova DCNT da atualidade, uma vez que a SM seria o conjunto de pelo menos três ou mais disfunções metabólicas, como a resistência à insulina/hiperglicemia, obesidade visceral/abdominal, hipertensão, dislipidemias. Estas condições que podem levar também ao desenvolvimento de DCVs e diabetes mellitus tipo 2 (DM2) (SAKLAYEN, 2018).

Segundo a *World Health Organization* (WHO), o diagnóstico de SM é realizado quando o indivíduo apresenta pelo menos três das seguintes características (ALBERTI et al., 2009): i) apresentar resistência à insulina (níveis de insulina > 6,1 mmol/L) e/ou hiperglicemia (110 mg/dL) ou após 2 horas do uso de glicose com valores de insulina superior a 7,8 mmol/L e níveis glicêmicos de 140 mg/dL, triglicerídeos acima de 150 mg/dL, colesterol HDL inferior a 35 mg/dL (homens) e 40mg/dL (mulheres), relação cintura/quadril superior a 0,9 (homens) e 0,85 (mulheres) ou índice de massa corpórea (IMC) superior a 30 kg/m^2 e pressão arterial sistêmica superior a 140/90 mmHg. A obesidade abdominal/visceral é característica importante em indivíduos com SM e é considerada um marcador de

um tecido adiposo disfuncional (DE LA IGLESIA et al., 2016). A maioria das pessoas que apresentam SM estão obesos ou em sobrepeso, consequentemente, o manejo do tratamento farmacológico e não farmacológico objetivando a diminuição de gordura visceral, é estratégia de importância para redução das disfunções metabólicas associadas ao aumento da adiposidade (DE LA IGLESIA et al., 2016).

Nos últimos 40 a 50 anos, o número de pessoas que apresentam SM tem tomado proporções epidêmicas (DE LA IGLESIA et al., 2016). É observado que a ocorrência desta síndrome atinge países desenvolvidos e, também, países em que a população possui um menor nível socioeconômico, e envolve o estilo de vida dos indivíduos, como o consumo de dietas não saudáveis (BERNARBÉ GARCÍA et al., 2013) e o sedentarismo (LEE et al., 2014).

O tratamento dos indivíduos com SM envolve medidas não farmacológicas como a mudança no estilo de vida com realização de exercícios físicos, consumo de dieta com baixo teor de carboidratos, calorias e gorduras. As estratégias não-farmacológicas são abordagens preventivas econômicas para pacientes intolerantes à terapia farmacológica e incluem a melhoria do estilo de vida do paciente. A adesão da atividade física como alteração do estilo de vida é importante no tratamento e prevenção da SM (MYERS; KOKKINOS, NYELLIN, 2019). Estudos demonstraram que a combinação de atividade física com o intuito de aumentar a capacidade cardiorrespiratória é inversamente relacionada com o desenvolvimento da SM (CHURCH, 2011; MYERS; KOKKINOS; NYELLIN, 2019).

Além da atividade física, estratégias nutricionais influenciam no controle do peso corporal possuindo papel importante na prevenção e no tratamento de diferentes implicações metabólicas associadas a SM (ESPOSITO; CERIELLO; GIUGLIANO, 2007). Um exemplo são as dietas de restrição calórica em que há um menor consumo de quantidade de calorias em relação ao total de energia gasta pelo indivíduo (BALES, KRAUS, 2013). Ainda, recomenda-se que as dietas sejam restritas em gorduras saturadas, gorduras trans, colesterol, sódio e açúcares simples (ESPOSITO; CERIELLO; GIUGLIANO, 2007).

Além da restrição de certos componentes da dieta, a inserção de outros também influenciam na prevenção e tratamento da SM, como as dietas ricas em ácidos graxos ômega 3 (WEN et al., 2014), ou aquelas com maior capacidade antioxidante total com a adição de substâncias ou vitaminas que combatem

disfunções metabólicas presentes na SM (BAHADORAN et al., 2012; DE LA IGLESIAS et al., 2017).

O Programa de Prevenção do Diabetes demonstrou que a mudança nutricional com dietas de baixas calorias e gorduras e, no mínimo, 150 minutos/semana de atividade física, reduziu a incidência de SM em 41% resultando na redução de 7% do peso corporal (MYERS; KOKKINOS; NYELLIN, 2019; ORCHARD et al., 2005). Estratégias farmacológicas são utilizadas em pacientes com SM que apresentam alto risco em desenvolver DCVs e DM2, um exemplo inclui o uso de substâncias ativas na prevenção e tratamento de indivíduos com parâmetros metabólicos limítrofes, o que pode ser útil para evitar a progressão da doença, bem como limitar os efeitos colaterais da administração de fármacos. Dessa forma, acredita-se que o uso de substâncias ativas na forma de nutracêuticos associado ao um estilo de vida saudável pode desempenhar papel benéfico na prevenção da SM (CICERO, COLLETTI, 2016).

1.2. Tecido adiposo branco e fígado: dois importantes órgãos metabólicos

A obesidade, ou seja, o aumento de massa de tecido adiposo possui papel importante no desenvolvimento da SM, resistência à insulina e na patogênese de DCVs e DM2. Esta associação depende não somente do equilíbrio entre o consumo calórico e o gasto energético, mas também do equilíbrio entre o tecido adiposo branco, que é o primeiro local de estoque energético, e os tecidos adiposos marrom e bege, que são os locais de gasto energético (KAHN; WANG; LEE, 2019).

O tecido adiposo (TA) é classicamente dividido baseado no local anatômico e na constituição da maioria dos tipos celulares. Em relação aos aspectos histopatológicos, existem três tipos de tecidos adiposos: i) o tecido adiposo branco (TAB), dividido em depósitos viscerais e subcutâneos representando mais de 95% da gordura em humanos. A gordura visceral está associada às doenças metabólicas e está localizada nas regiões: perigonadal, mesentérica, retroperitoneal, epididimal, entre outras (ROSEN; SPIEGELMAN, 2014); ii) o tecido adiposo marrom que representa 1 a 2% e está localizado nas regiões cervicais, axilares e paraespinhal e iii) tecido adiposo bege, o qual é difícil sua quantificação, representa células

intercaladas dentro do TAB que são capazes de se transformar em adipócitos parecidos com os marrons após a exposição ao frio ou estímulo adrenérgico (KAHN; WANG; LEE, 2019).

O TAB apresenta uma ampla heterogeneidade celular que incluem os pré-adipócitos, células-tronco mesenquimais, apresentam células vasculares o que o torna um órgão rico em vascularização e inervação, possuem ampla variedade de células imunológicas tais como macrófagos e linfócitos que secretam citocinas anti-inflamatórias e pró-inflamatórias e, ainda, adipócitos brancos. Estes, por sua vez, são predominantes no TAB e possuem uma grande gota lipídica unilocular e difere dos adipócitos marrom e bege que apresentam gotículas multiloculares e alta densidade mitocondrial que dissipa energia por meio de desacoplamento de prótons devido a ação de proteínas desacopladoras de mitocôndrias (UCPs) na respiração mitocondrial (CYPESS; KAHN, 2010; NEDERGAARD; BENGTSSON; CANNON, 2011).

Além do TAB ser um órgão de estoque energético, este pode ser considerado um órgão endócrino que secreta inúmeros fatores com propriedades hormonais, autócrinas e parácrinas. Entre os anos de 1980 a 1990, foi descoberto que o TA libera substâncias as quais denominaram adipsina (COOK et al., 1987), leptina (ZHANG et al., 1994) e fator de necrose tumoral-α (TNF-α) (HOTAMISGLIL et al., 1994), o que conferiu a este tecido, um papel fundamental na homeostasia energética.

Essas substâncias foram chamadas de adipocinas que incluem hormônios, citocinas e outras proteínas com funções biológicas específicas podendo atuar localmente, de forma parácrina ou autócrina, ou sistemicamente, de forma endócrina (KERSHAW; FLIER, 2004; ROSEN; SPIEGELMAN, 2014; VÁZQUEZ-VELA et al., 2008; LONGO et al., 2019). Além disso, com o passar dos anos foram descobertas outras proteínas secretadas pelo TAB, como a adiponectina, resistina; fatores de crescimento, como o fator de crescimento tumoral-β (TGF-β), fator de crescimento vascular derivado do endotélio (VEGF); mediadores inflamatórios: proteína-C-reativa (PCR), interleucinas-6 (IL-6), -10 (IL-10), proteína quimiotáxica de monócitos-1 (MCP-1), dentre outras (KAHN; WANG E LEE, 2019).

Em condições fisiológicas normais, os adipócitos brancos são os principais depósitos de armazenamento de triglicerídeos (TG) levando a eventual liberação de

ácidos graxos livres (AGL). Os AGL circulantes ou TG são hidrolisados via lipoproteínas lipases ligadas a membrana plasmática e são transportados para o citoplasma via proteínas de membranas, como a proteína de ligação de ácidos graxos (FABP) e ácido graxo translocase (FAT) (HENRY et al., 2012; CAROBBIO et al., 2017).

Entretanto, em condições de excesso energético, como na obesidade, ocorre a hidrólise de ácidos graxos. Uma vez dentro das células, os AGL são hidrolisados pela acil-CoA-redutase (ACS) para formar acil-Co-A que podem ser metabolizados pela via de síntese de ácidos graxos (HENRY et al., 2012). A lipólise basal está elevada em adipócitos hipertrofiados, o que leva ao aumento na dispersão de ácidos AGL. As funções endócrinas alteradas do TAB e as altas quantidades de AGL liberados do TAB irão sobrecarregar outros tecidos não-adiposos, como o fígado e o músculo esquelético, promovendo acúmulo de gordura ectópica e lipotoxicidade que pode resultar em inflamação de baixo grau e disfunções metabólicas nesses tecidos, ocasionando a resistência à insulina (RUTKOWSKI; STERN; SCHERER, 2015). Essa desregulação da lipólise e lipogênese possuem influência no DM2, na resistência à insulina e na doença hepática gordurosa não alcóolica (DHGNA) (HENRY et al., 2012; CAROBBIO et al., 2017).

Além disso, para compensar esse excesso de nutrientes associado com a obesidade, o TAB sofre um processo de remodelamento estrutural para otimizar sua expansão, e isso requer um coordenado aumento no tamanho (hipertrofia) e no número de adipócitos (hiperplasia) (WANG et al., 2013).

Esta hipertrofia dos adipócitos gera uma cascata de eventos levando a um remodelamento do TAB. Este remodelamento está associado a uma liberação desequilibrada de adipocinas produzindo uma inflamação crônica de baixo grau com aumento de adipocinas de caráter pró-inflamatório e diminuição das anti-inflamatórias. Além disso, com o aumento desse estado inflamatório, ocorre também, a infiltração de macrófagos M1, exacerbando ainda mais o processo inflamatório local (WEISBERG et al., 2003).

Este tecido adiposo disfuncional é caracterizado pela produção de altos níveis de citocinas pró-inflamatórias, capacidade de mediar a resistência a patógenos, fortes propriedades microbicidas, alta produção de nitrogênio reativo e oxigênio intermediários e promoção de respostas Th1 e linfócitos T no TAB, exacerbando

ainda mais o perfil inflamatório presente na obesidade (MAKKI; FROGEL; WOLLOWZUCK, 2013; WEISBERG et al., 2003). Ainda, a hipertrofia dos adipócitos induz a hipóxia que ocorre quando a disponibilidade do oxigênio não corresponde à demanda do tecido circulante, levando à diminuição de oxigênio disponível para o tecido. Existem dois fatores de transcrição de indução da hipóxia, o fator de indução de hipóxia (HIF)-1α, um fator de transcrição que medeia a resposta hipóxica, acelera a fibrose e induz inflamação no TAB (WARBRICK; RABKIN, 2019) e o HIF-2α que diminui a inflamação no TA no estado de obesidade (CHOE et al., 2014).

Toda essa cascata de eventos que ocorre no TAB prejudica ainda mais a entrega lipídica, a diferenciação dos precursores de adipócitos, promove fibrose e rigidez do TAB. A disfunção do TAB leva a resistência à insulina não somente no TAB, mas em importantes órgãos metabólicos periféricos, como o fígado e o músculo esquelético, através de mecanismos que envolvem a deposição de gordura e, ou nutrientes causando lipotoxicidade (CAROBBIO et al., 2017).

Assim como o TAB, o fígado é um órgão vital na homeostasia metabólica, tanto no processo de lipogênese, gliconeogênese e metabolismo do colesterol. Nas últimas décadas, uma variedade de condições patológicas ressalta a importância nos estudos das funções metabólicas do fígado, visto que como observado na sociedade ocidental, o aumento na prevalência de obesidade e SM promove alterações fisiopatológicas que podem acarretar no desenvolvimento da doença hepática gordurosa não-alcóolica (DHGNA) (BECHMANN et al., 2012).

O termo DHGNA é designado para descrever uma doença no fígado que pode evoluir de uma esteatose isolada, com ausência de inflamação e dano hepatocelular, para uma esteatose com inflamação lobular e com evidências de danos nas células hepáticas (hepatócitos), mais conhecida como a esteatose hepática não-alcóolica (EHNA). Pacientes com EHNA apresentam fibrose no fígado, o que pode levar à morte dos hepatócitos com consequente desenvolvimento de cirrose e carcinoma hepatocelular com grandes chances de transplante de fígado no futuro, se não for devidamente tratada. Ainda, a EHNA pode levar ao surgimento de doenças extra-hepáticas como DCVs e DM2 (HAAS; FRANCQUE; STAELS, 2016; WOO BAIDAL; LAVINE, 2016).

A resistência à insulina tem sido caracterizada como um fator crucial na fisiopatologia da DHGNA/EHNA. Contudo, além da resistência à insulina *per se*, o

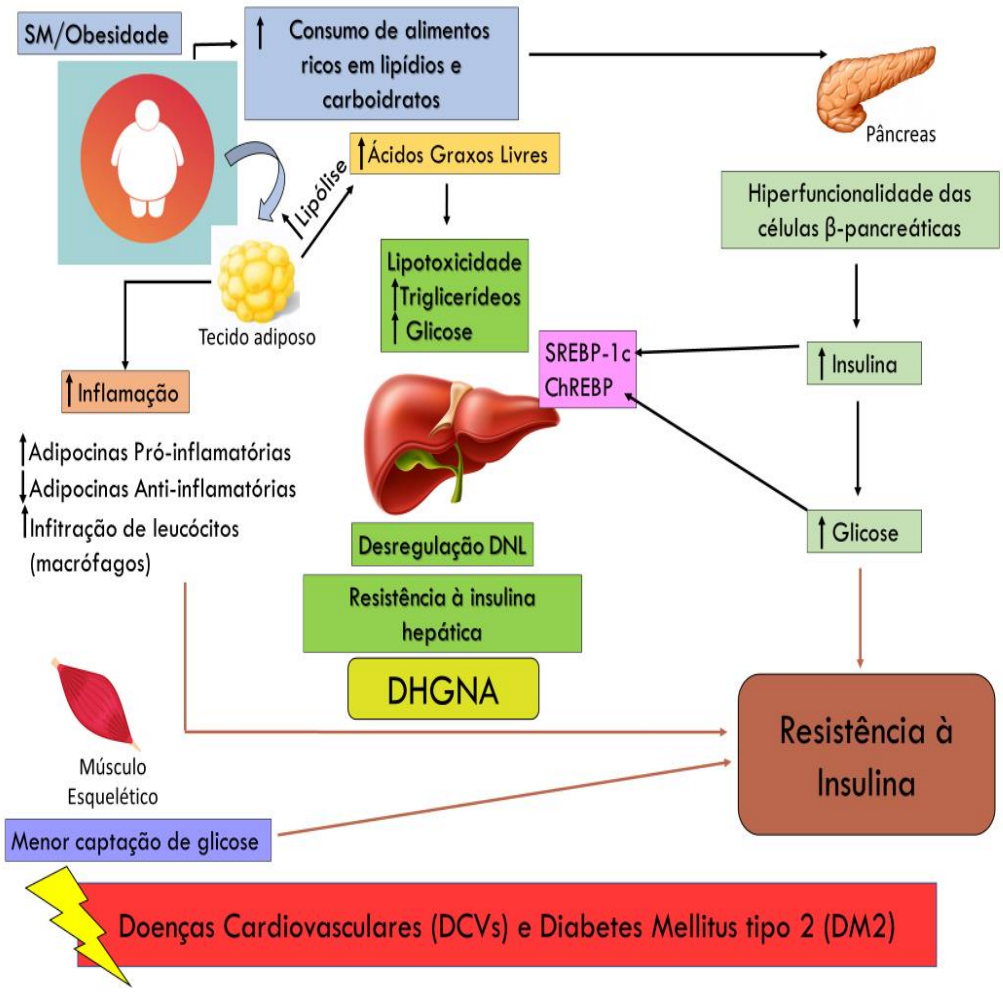
metabolismo lipídico, a função mitocondrial, a imunidade inata, a microbiota intestinal, os fatores genéticos e nutricionais, bem como o estilo de vida estão envolvidos no surgimento da DHGNA (MOSCHEN; KASER; TILG, 2013; HAAS; FRANCQUE; STAELS, 2016).

A resistência à insulina é definida como uma condição em que as células, tecidos e órgãos não respondem apropriadamente a uma determinada dose de insulina. A insulina tem a capacidade de interagir com o receptor de insulina presente no músculo esquelético, hepatócito e tecido adiposo promovendo a translocação do GLUT-4 que faz a captação de glicose nesses tecidos, reprimindo a produção de glicose no fígado (neoglicogênese) (KANG; TSAI; ROSEN, 2016).

Dessa forma, a resistência à insulina hepática é definida como um prejuízo na supressão da produção de glicose mediada por insulina, resultando em aumento na gliconeogênese e diminuição na síntese de glicogênio. A síntese de glicogênio hepática mediada por insulina correlaciona-se negativamente com o conteúdo de gordura no fígado. Um estudo demonstrou que em pacientes obesos com DM2, a presença de DHGNA está associada com severa hiperinsulinemia, dislipidemia e resistência à insulina hepática quando comparado com pacientes ausentes de DHGNA (LOMONACO et al., 2016).

Além da resistência à insulina hepática, nos estados de obesidade, SM e DHGNA, ocorre uma desregulação do processo de lipogênese *de novo* (DNL). Lambert e colaboradores demonstraram que em pacientes com DHGNA/EHNA, o processo de DNL é três vezes maior do que em pacientes fisiologicamente normais, representando uma característica importante de acúmulo de gordura hepática (LAMBERT et al., 2014). A DNL pode ser estimulada pela insulina, via proteína SREBP-1c (sterol regulatory element binding-protein 1c), e pela glicose, via proteína ChREBP (carbohydrate response element-binding protein). Assim, hiperinsulinemia e dietas ricas em gordura e carboidratos irão contribuir para a elevação do processo de DNL na obesidade/SM e DHGNA (RODEN, 2006; SAPONARO et al., 2015) (Figura 1).

Figura 1. Lipotoxicidade e doença hepática gordurosa não alcóolica (DHGNA)



Fonte: a autora (2021)

Além da desregulação da DNL que irá influenciar no desequilíbrio metabólico que ocorre na obesidade/SM e DHGNA, é importante ressaltar que o metabolismo de glicose e lipídios pode ser influenciado por proteínas que são produzidas no fígado, as quais são denominadas hepatocinas.

Assim como as adipocinas secretadas pelo TAB, as hepatocinas são produzidas exclusivamente ou predominantemente no fígado. Estudos demonstraram que estas proteínas afetam o metabolismo glicêmico e lipídico quando liberadas para a circulação sistêmica e que a DHGNA parece estar associada com a alteração na produção de hepatocinas (LEBENSZTEJN et al., 2016).

Dentre as hepatocinas identificadas, encontra-se a fetuína-A que é uma proteína descoberta em 1944 isolada do soro fetal de bovinos e secretada principalmente pelo fígado e em menor quantidade pelo TAB, sendo expressa nos rins e cérebro (TREPANOWSKI et al., 2015). Assim é denominada tanto de fetuína-A, glicoproteína α-2-HS ou glicoproteína α2-Heremans-Schmid. Possui peso molecular de 64 kDa e apresenta diversas funções nos processos fisiológicos e patológicos, que incluem a calcificação vascular, regulação do metabolismo ósseo, controle na atividade de proteases, na migração de queratinócitos, na sinalização das células proliferativas do câncer de mama, como biomarcadora de doenças neurodegenerativas e resistência à insulina (DOGRU et al., 2013; MORI; EMOTO; INABA, 2011).

A fetuína-A é importante promotora da resistência à insulina e é produzida principalmente no fígado e secretada para o soro. Altos níveis de fetuína-A plasmática estão associados com a DHGNA e as intervenções não farmacológicas, como dieta de curto prazo e exercícios, resultaram em diminuição dos níveis desta hepatocina, com melhora na progressão da DHGNA e redução do peso corporal. Stefan e colaboradores observaram que altos níveis de fetuína-A no plasma estão relacionados com o acúmulo de gordura no fígado e resistência à insulina em humanos, o que demonstra que esta hepatocina tem um papel potencial na associação da DHGNA com a resistência à insulina (REINEHR et al., 2006; STEFAN et al., 2006).

1.3. SM e DHGNA: o papel da lipogênese de novo (DNL)

Como descrito, a SM é o conjunto de três ou mais disfunções metabólicas que incluem hipertrigliceridemia, níveis abaixo dos de referência de HDL, hipertensão arterial sistêmica, elevada glicemia de jejum/resistência à insulina e aumento da circunferência abdominal. O conjunto destas disfunções metabólicas são fatores de risco para o desenvolvimento de DCVs e DM2 (SANDERS; GRIFFIN; 2016; SAKLAYEN, 2018). Por outro lado, a DHGNA é definida como uma desordem de excesso de gordura no fígado sem influência do álcool. Esta lipotoxicidade hepática faz com que a glicose e triglicerídeos, dois componentes importantes da SM, tenham suas produções aumentadas pelo fígado. Dessa forma, o fígado é um órgão determinante no controle metabólico e, uma vez que este apresenta excesso de gordura, pode levar às disfunções metabólicas. A prevalência de ambas SM e DHGNA é maior em pacientes obesos e, ambas as desordens, são preditoras das DCVs, DM2, EHNA e carcinoma hepatocelular (YKI-JARVINEN, 2014).

A DHGNA tem sido proposta como uma extensão da SM, visto que existe uma forte associação entre SM e DHGNA devido ao fato de que 90% dos indivíduos com DHGNA apresentam pelo menos uma e 33% apresentam três características da SM (SANDERS; GRIFFIN, 2016). Além disso, a prevalência de DHGNA em pacientes obesos com SM é de 86%, sendo significantemente maior do que em indivíduos sadios (MARCHESINI et al., 2003). Porém, em alguns casos a DHGNA pode ocorrer independente da presença de SM (VANNI et al., 2010; SANDERS, GRIFFIN, 2016).

Por muitos anos acreditava-se que o desenvolvimento da resistência à insulina estava relacionado apenas com o metabolismo de glicose, porém, foi observado que a patogênese da resistência à insulina também está associada com a desregulação do metabolismo lipídico (McGARRY, 1992). Os estudos demonstraram que a hiperinsulinemia leva a um aumento da DNL e aumento da resistência à insulina hepática levando a uma piora na supressão da gliconeogênese e sustentada hiperglicemia, estimulando a secreção de insulina das células β-pancreáticas (WILLIAMS et al., 2013; SANDERS, GRIFFIN, 2016).

A DNL é uma complexa via metabólica em que está ativa primeiramente no fígado e tecido adiposo. Em condições fisiológicas normais, a DNL converte o excesso de carboidratos em ácidos graxos, estes, por sua vez são esterificados em

triglicerídeos (TG) para estoque energético e depois para fornecimento de energia via β-oxidação (AMEER et al., 2014). Este processo de β-oxidação hepática está intimamente influenciado pelo fator de transcrição receptor-α ativado pelo proliferador de peroxissoma (PPAR-α), que regulam a expressão de genes envolvidos na oxidação de AG hepáticos. Dessa forma, estudos demonstram que existe uma baixa regulação na expressão gênica de PPAR-α na DHGNA que, consequentemente, regula o aumento no acúmulo de TG hepáticos (FON TACER; ROZMAN, 2011).

Estudos sugerem de que a DNL hepática possui importante contribuição no conteúdo de lipídios séricos em indivíduos que consomem dietas ricas em carboidratos (SCHWARZ et al., 2003; AMEER et al., 2014). A via lipogênica está ativa nos principais tecidos metabólicos, fígado e tecido adiposo, porém, é quantitativamente mais eficiente no fígado do que no tecido adiposo (DIRAISON et al., 2003; WALLACE; METALLO, 2020).

A DNL demonstrou ser altamente responsiva às mudanças dietéticas como demonstrado em dietas ricas em carboidratos que ativam a resposta lipogênica no fígado levando ao aumento na síntese e secreção de lipoproteínas de densidade muito baixa (VLDL) e induz o aumento da DNL hepática que por sua vez contribui para a hipertrigliceridemia (SCHWARZ et al., 2003).

Devido ao fato de que o fígado é o principal local da DNL no organismo, este órgão contribui a 10-37% de AG em VLDL no perfil pós prandial e possui importante influência pela dieta rica em carboidratos. Este aumento da DNL hepática foi demonstrado em pacientes com obesidade, DHGNA/EHNA e SM (SANDERS et al., 2018; MARQUES-LOPES et al., 2001; SMITH et al., 2020).

O mecanismo exato que explica esse aumento da DNL hepática na DHGNA é ainda desconhecido, porém, a primeira hipótese é que a desregulação da DNL hepática pode ter envolvimento com o aumento de insulina e glicose séricas e hepáticas, visto que a insulina e glicose ativam a proteína 1c de ligação ao elemento regulador de esterol (SREBP-1c) e proteína de ligação ao elemento responsiva a carboidratos (ChREBP), respectivamente (WALLACE; METALLO, 2020; SMITH et al., 2020).

Além disso, em indivíduos saudáveis, a insulina estimula a lipogênese e suprime a gliconeogênese, já em indivíduos resistentes à insulina ocorre a

desregulação na sinalização de insulina, esta ainda estimula a lipogênese, mas diminui a supressão da gliconeogênese. Anteriormente, acreditava-se que apenas a desregulação da via gliconeogênica, em que a insulina falha em suprimir o processo de gliconeogênese, poderia ser a principal responsável pela resistência à insulina hepática seletiva, porém, atualmente é demonstrado que a DNL é também responsiva à sinalização de insulina (BROWN; GODSTEIN, 2008). Dessa forma, uma outra hipótese de mecanismo que explica o aumento da DNL hepática seria a divergência na sinalização de insulina que regula os processos de gliconeogênese e DNL, porém, o aumento de AG e entrega de glicerol para o fígado devido a uma desregulação da lipólise no TAB pode também levar ao aumento da gliconeogênese (SAMUEL; SHULMAN, 2016; WALLACE; METALLO, 2020).

Uma terceira hipótese do aumento da DNL hepática seria a piora da sinalização de leptina no sistema nervoso central, visto que esta sinalização demostrou modular a DNL hepática (HACKL et al., 2019). Ainda, a sinalização inflamatória e o estresse do retículo endoplasmático são também propostos como moduladores da DNL hepática através da ativação de reguladores transcricionais que vão expressar enzimas da DNL (NEGRIN et al., 2014; LEBEAUPIN et al., 2018).

Assim, a DNL possui papel fundamental na comunicação lipídica entre tecido adiposo e fígado com o intuito de manter a homeostase metabólica. A disfunção da DNL nestes dois tecidos é uma característica comum associada com a SM e, por isso, o entendimento de como essa via contribui nas funções celulares é de extrema importância. Dessa forma, tendo como alvo a via da DNL demonstra ser uma abordagem clínica importante no tratamento da DHGNA associada com a SM (ESLER, BENCE, 2019). Porém, ainda existe uma escassez no entendimento dos mecanismos dessa desregulação da DNL na DHGNA (WALLACE; METALLO, 2020).

1.4. Lipogênese *de novo* (DNL) hepática: a influência da dieta rica em frutose e lipídios

O alto consumo de dieta rica em carboidratos, particularmente açúcares, está intimamente associada com o aumento de casos de obesidade, DM2 e DHGNA (CHIU et al., 2018), estas encontradas no conceito da SM (SANDERS; GRIFFIN, 2016; SAKLAYEN, 2018). A DNL é uma complexa via metabólica em que a

desregulação dessa via está intimamente associada com as desordens metabólicas associadas a SM e é uma anormalidade que possui relação direta com a DHGNA (WALLACE, METALLO, 2020). Um dos açúcares mais comumente usados para consumo humano seria a frutose (SOFTIC; COHEN; KAHN, 2016).

O consumo de frutose cresceu drasticamente nos últimos 40 anos e é usada também comercialmente na forma de refrigerantes, sucos, produtos de panificação e xarope de milho sendo que estes produtos compreendem uma grande proporção da dieta moderna particularmente em crianças, adolescentes e jovens adultos (SOFTIC; COHEN; KAHN, 2016).

Evidências demonstraram que o consumo de frutose e outros tipos de açúcares artificiais estão associados com o desenvolvimento da resistência à insulina sistêmica e hepática, acúmulo de lipídios hepáticos e hipertrigliceridemia (TASKINEN; PACKARD; BORÉN, 2019). A dieta rica em frutose pode aumentar os níveis de enzimas envolvidas na DNL de forma muito mais acentuada do que a dieta rica em lipídios. As propriedades do metabolismo de frutose a tornam particularmente um açúcar com propriedades lipogênicas (SOFTIC; COHEN; KAHN, 2016) demonstrando ainda ser mais lipogênica do que a sacarose o que aumenta o risco para o surgimento da DHGNA e dislipidemias (TASKINEN; PACKARD, BORÉN, 2019).

A glicose é a forma predominante de açúcar circulante no organismo, enquanto a sacarose é um dissacarídeo composto de porções iguais de glicose e frutose (HANNOU et al., 2018). Embora a frutose e glicose sejam monossacarídeos com fórmulas moleculares muito similares, as vias metabólicas são divergentes nos enterócitos, que seriam as células do intestino delgado e grosso, e nos hepatócitos, as células encontradas no fígado (FERRARIS CHOE; PATEL, 2018; HANNOU et al., 2018; TASKINEN; PACKARD; BORÉN, 2019).

Dessa forma, os órgãos que estão diretamente envolvidos no metabolismo de frutose seriam o intestino delgado e o fígado; porém, é notável que o metabolismo de frutose intestinal parece ser um processo que permite que altas doses de frutose sejam transportadas para o fígado (TASKINEN; PACKARD; BORÉN, 2019). O fígado metaboliza a maior parte da frutose ingerida e o intestino por si só pode metabolizar 30% do consumo de frutose (MAVRIAS; MAYER, 1973). É importante salientar que a dieta rica em frutose aumenta os níveis de enzimas envolvidas na

DNL e como a frutose é absorvida via veia porta, a entrega na concentração deste açúcar para o fígado é muito maior quando comparado a outros tecidos (TASKINEN; PACKARD, BORÉN, 2019).

Quando ocorre a ingestão de frutose em excesso, inicialmente, a absorção de frutose é mediada pelo transportador de glicose-5 (GLUT-5), que é um transportador de frutose expresso na borda apical dos enterócitos. Notavelmente, um grande fluxo de frutose para os enterócitos induz a expressão de GLUT-5, e este mecanismo pode responder ao excesso do consumo de frutose pelo aumento da capacidade do instestino para absorção da frutose e transporte para o fígado; por esta razão, a atividade de GLUT-5 é o regulador chave da concentração de frutose na veia porta. Além disso, o transporte da frutose dos enterócitos para a veia porta é, também, parcialmente mediada pelo transportador de glicose-2 (GLUT-2) (PATEL et al., 2015; HOFFMAN; ALVARES; ADELI, 2019).

No intestino delgado, a frutose é metabolizada pela cetohexoquinase (KHKI) em frutose-1-fosfato (F-1-P) (LEE et al., 2018) que é clivada pela aldolase em diidroxiacetona fosfato e gliceraldeído. O gliceraldeídio, por sua vez, é fosforilado pela trioquinase gerando gliceraldeído-3-fosfato (GAP) sendo que este e outras trioses fosfatos são ressintetizadas em glicose via gliconeogênese ou metabolizadas em lactato ou acetil-CoA, que são oxidados ou usados no processo de lipogênese (HANNOU et al., 2018).

Por outro lado, no fígado a frutose ativa os fatores de transcrição ChREBP e SREBP-1c resultando na suprarregulação das vias que estimulam frutólise, glicólise, lipogênese e produção de glicose. Isso resulta em aumento na produção da glicose hepática, na geração de lipídos que podem afetar na sensibilidade de insulina hepática, na expressão da apoliproteína C 3 (APOC3) e secreção de VLDL. Este aumento na expressão da proteína apoliproteína C3 (APOC3) induz o aumento de apoliproteína C-III (apoC-III) plasmático, um inibidor de lipoproteína lipase e depuração de lipoproteínas hepáticas remanescentes resultando em hipertrigliceridemia e acúmulo de lipoproteínas remanescentes ricas em triglicerídeos aterogênicos (TASKINEN; PACKARD, BOREN, 2019) (Figura 2).

O ponto chave da DHGNA é o acúmulo de triglicerídeos hepáticos quando há o desequilíbrio do influxo excedido de lipídios para o fígado, através de AG circulantes não esterificados, quilomícrons derivados da dieta e DNL hepática, em

relação à disposição de lipídios hepáticos excedidos via β-oxidação na mitocôndria e secreção de TG como partículas de lipoproteínas (STEFAN; KANTARTZIS, HARING, 2008).

Os estudos demonstraram que o aumento da DNL hepática contribui para o desenvolvimento da DHGNA e que as dietas ricas em carboidratos, particularmente a frutose, mostrou estimular a DNL e aumentar a gordura no fígado. Essa hipótese é observada em um estudo em que comparou a suplementação entre glicose e frutose em ratos fêmeas no período de dois meses e, embora, houvesse um aumento no consumo calórico nos ratos suplementados com glicose, a frutose causou uma grande piora nas respostas metabólicas quando comparada com a glicose (SANGUESA et al., 2017). Em contraste do metabolismo de glicose, a quebra de frutose leva a geração de metabólitos que estimulam a DNL hepática (TAPPY; LE, 2010).

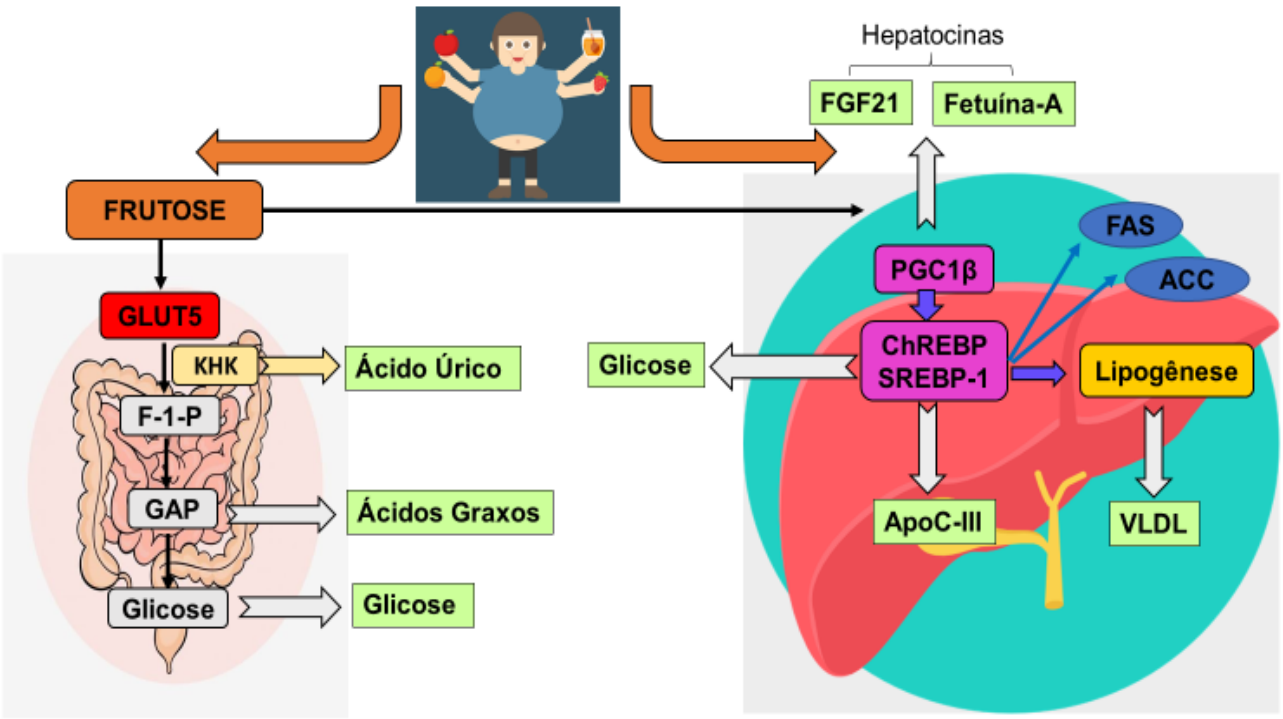
A frutose impulsiona a lipogênese no contexto da resistência à insulina, pois a frutose não requer insulina para seu metabolismo, e estimula diretamente a SREBP-1c, um importante regulador da transcrição da DNL (MALIK, HU, 2015). Além disso, a alimentação rica em frutose aumenta a expressão de ChREBP hepática, um fator de transcrição lipogênico do metabolismo de carboidrato e DNL. Este fator de transcrição regula a produção de glicose induzida por frutose independente da sinalização de insulina (KIM et al., 2016).

Como observado, o metabolismo de frutose possui aspectos únicos sendo que o mais importante destes é a habilidade deste açúcar em aumentar a DNL hepática. Os estudos tanto em roedores como em humanos indicam que a DNL hepática possui papel central no desenvolvimento da DHGNA. A frutose estimula a lipogênese de maneira independente de insulina e induz a resistência à insulina pela ativação de isoformas de proteínas quinases C (PKC) e gera lipídios que irão intermediar e promover a progressão da DHGNA (SOFTIC; COHEN; KAHN, 2016).

Assim, a DNL hepática, o tipo de ingestão dietética e a lipólise do TA são todos contribuidores para o surgimento da DHGNA. Porém, como as intervenções e adesões dietéticas dos indivíduos falham em alcançar respostas sustentadas e, também, a lipólise do TA como abordagem terapêutica seria mais desafiadora do ponto de vista técnico, a DNL hepática ou o metabolismo de frutose poderiam ser

duas abordagens promissoras do ponto de vista farmacológico para o manejo da SM e DHGNA (SOFTIC; COHEN; KAHN, 2016).

Figura 2. Esquema do metabolismo de frutose no intestino delgado e fígado



(Fonte: adaptado Taskinen, Packard, Borén, 2019; Jegatheesan, De Brandt, 2017)

1.5. *Morinda citrifolia* Linn (Noni)

Morinda citrifolia Linn, popularmente conhecida como noni ou “Indian Mulberry”, tem sido utilizada na medicina folclórica pelo polinésios há aproximadamente 2000 anos (DIXON et al., 1999). Esta planta pertence ao gênero *Morinda* (Rubiaceae) composta por aproximadamente 80 espécies. *M. citrifolia* é uma árvore perene com aproximadamente 3 a 10 metros de altura com abundantes folhas largas e elípticas (5 a 17 centímetros de comprimento e 10 a 40 centímetros de largura). As pequenas flores tubulares brancas são agrupadas juntas ou inseridas no pedúnculo (DIXON et al., 1999; CHAN-BLANCO et al., 2006) (Figura 3).

Figura 3. Foto das folhas, flores e fruto de *M.citrifolia*

Fonte: a autora (2021)

Os frutos de *M. citrifolia* podem ser coletados em diferentes estágios de maturação podendo ocorrer de forma natural. A evolução da coloração e firmeza das frutas no processo de maturação natural é relatada na Tabela 1. A mudança do

estágio 4 para o estágio 5 ocorre muito rapidamente, dependendo em horas, e a polpa praticamente se liquefaz mudando da cor verde para a branca e, ainda, há o surgimento de um cheiro adstringente e sabor amargo (MOTSHAKERI; MOHD GHAZALI, 2015).

Tabela 1 - Coloração e firmeza da casca de acordo com a evolução da maturação de *M. citrifolia* (noni)

Estágios de maturação	Coloração	Firmeza
1	Verde escuro	Muito duro
2	Verde-amarelo	Muito duro
3	Amarelo claro	Muito duro
4	Amarelo claro	Razoavelmente duro
5	Acinzentado translúcido	Macio

(Fonte: adaptado de CHAN-BLANCO et al., 2006)

Os frutos são individualmente selecionados e coletados à mão. No estágio branco (coloração) e duro (firmeza) de *M. citrifolia* , este é transportado em cestas ou containers e expostos à luz e em temperaturas mais altas não afetando a qualidade geral dos frutos. Antes do processamento de *M. citrifolia*, os frutos são amadurecidos naturalmente em temperatura ambiente por um dia ou mais e, assim, transformados em chás, sucos, produtos dietéticos na forma de pó e cápsulas, dentre outros (CHAN-BLANCO et al., 2006; MOTSHAKERI; MOHD GHAZALI, 2015).

O estágio em que os frutos de *M. citrifolia* apresentam maior concentração de substâncias ativas e melhor para o processamento industrial se encontram por volta dos 45 dias de maturação (EZHILARASAN; AHMED; RAJ, 2009). O processamento dos frutos de *M. citrifolia* Linn (noni) pode ser feito de várias maneiras sendo que o mais comum seria a fermentação do suco da fruta que são mantidos por 4 a 8 semanas em recipientes selados (CHAN-BLANCO et al., 2006; MOTSHAKERI; MOHD GHAZALI, 2015). Em 1996, o suco do fruto de *M. citrifolia* começou a ser comercializado nos Estados Unidos da América. Em 2003, houve a aprovação da Comissão Europeia do suco de *M. citrifolia* fermentado (Tahitian Noni Juice®) na Europa (DIXON et al., 1999).

A venda de produtos derivados dos frutos de *M. citrifolia* nos Estados Unidos da América, Europa e Ásia na forma de suco e pós encapsulados, além de permitida, é de comum acesso pelos consumidores (MOTSHAKERI; MOHD GHAZALI, 2015). Porém, no Brasil, a comercialização ainda não é legalizada devido às controvérsias que envolvem o consumo de forma empírica e toxicidade dos frutos de *M. citrifolia* na população. Há uma escassez de estudos que padronizam doses/concentração, tempo de tratamento, efeitos adversos, interação medicamentosa com outros fármacos e controvérsias em relação a toxicidade (ANVISA, 2007; STADLBAUER, 2005; MOHAMAD SHALAN; MUSTAPHA; MOHAMED, 2017).

Os estudos relacionados ao consumo de *M. citrifolia* não estão direcionados apenas nos frutos, mas também, nas folhas, raízes, casca e sementes (CHAN-BLANCO et al., 2006). Estima-se que *M. citrifolia* apresente em torno de 200 substâncias; estes são chamados de substâncias ativas e estão presentes em diferentes partes da planta, e incluem compostos fenólicos, antraquinonas, carboidratos, ácidos orgânicos, álcoois, vitaminas, flavonóides, iridóides, cetonas, lignanas, triterpenóides, nucleosídeos, esteróis, ácidos graxos, carotenóides e entre outros (CHAN-BLANCO et al., 2006; SAMOYLENKO et al., 2006; MOTSHAKERI; MOHD GHAZALI, 2015).

O conceito em se alimentar de forma saudável tem colocado em maior evidência os alimentos funcionais e nutracêuticos como abordagens terapêuticas devido aos benefícios à saúde (SINGH et al., 2012). Os fitoquímicos presentes na dieta não podem ser totalmente classificados como alimento e um novo termo entre nutrientes e farmacêutico, os nutracêuticos, é usado para designá-los e têm recebido grande atenção pela comunidade científica, consumidores e fabricantes de alimentos (GUL, SINGH, JABEEN, 2016).

Os termos alimentos funcionais e nutracêuticos se diferem entre si sendo que, segundo a *Food and Agricultural Organization of the United Nations* (FAO), alimentos funcionais são conceitualizados como alimentos similares em aparência aos alimentos convencionais, consumidos como parte de uma dieta usual, e contém componentes biologicamente ativos com benefícios fisiológicos e potencial em reduzir o risco de doenças crônicas através de funções nutricionais básicas (FAO, 2007). Enquanto, no Canadá, o conceito de nutracêuticos é designado como

produtos elaborados a partir de alimentos, mas vendidos como medicamentos na forma de cápsulas, extratos, tabletes, pós, soluções ou poções, não sendo geralmente associados com alimentos e demonstram apresentar benefícios fisiológicos e/ou fornecer proteção contra doenças crônicas, sendo atualmente definidos como produtos naturais para saúde (SHAHIDI, 2004; GUL; SINGH; JABEEN, 2016).

Devido à variedade de substâncias ativas presentes em diferentes partes de *M. citrifolia*, estudos demonstraram que esta planta pode ser usada como nutracêutico devido aos efeitos benéficos à saúde, dentre estes incluem ação anticancerígena (ANEKPANKUL et al., 2007), antimicrobiana (ZHANG et al., 2016), anti-leishmaniose (SIDDIQUI et al., 2014), antioxidante (WANG et al., 2012) e para o tratamento do refluxo esofágico e úlcera em animais (MAHATTANADUL et al., 2011). Por apresentar efeitos benéficos à saúde, estudos vieram com o intuito em avaliar os efeitos benéficos que *M. citrifolia* pode contribuir nas disfunções metabólicas, como atividades hipoglicemiantes, antidislipidêmicas, hepatoprotetoras, antihipertensivas e antiobesogênicas (INADA et al., 2017).

Estudos demonstraram que *M. citrifolia* possui propriedades importantes na dislipidemia, agindo como agente antidislipidêmico, inibindo a atividade da enzima conversora de angiotensina (ECA), com ação analgésica e hipoglicemiante (YAMAGUCHI et al., 2002; BASAR et al., 2010; WEST et al., 2011; WIGATI et al., 2017).

Além disso, Jambocus e colaboradores demonstraram a ação anti-obesogênica do extrato da folha de *M. citrifolia* em ratos obesos Sprague-Dawley submetidos à dieta hiperlipídica (HFD). Esse grupo observou que o extrato rico em rutina da folha de *M. citrifolia* preveniu o ganho de peso e o aumento de gordura corporal nesses animais submetidos à HFD com uma melhora nos perfis de insulina, lipídios e leptina plasmáticas e aumento na eliminação de gordura fecal. Nesse trabalho foi observado que o tratamento com o extrato da folha de *M. citrifolia* resultou em melhora no quesito dos prejuízos metabólicos presentes na obesidade (GOODA SAHIB JAMBOCUS, 2015).

Um outro estudo de Nerurkar e colaboradores avaliaram as ações do suco fermentado da fruta de *M. citrifolia* (fNJ) tanto em camundongos C57Bl/6 submetidos a HFD por 12 semanas juntamente com a administração do fNJ, como também, em

cultura de células de hepatocarcinoma humanos (HepG2). Esse trabalho comprovou que o fNJ apresentou melhora no metabolismo de glicose através da fosforilação do fator de transcrição 1 (FOXO1) bem como a diminuição do ganho de peso corporal; ainda, houve melhora nos parâmetros glicêmicos, como a intolerância à glicose, na glicemia de jejum e na resistência à insulina hepática. Esses efeitos hipoglicêmicos foram confirmados com a inibição de mRNA de FOXO1 hepático, com concomitante aumento na fosforilação de FOXO1 e liberação de proteínas nucleares. Genes relacionados com a gliconeogênese, fosfoenolpiruvato C quinase (PEPCK) e glicose-6-fosfatase (G6P) foram inibidos com a ação do fNJ. Além disso, nas células HepG2, houve 80% da inibição do mRNA dos genes G6P e PEPCK em células tratadas com siRNA FOXO1 e fNJ. Os efeitos podem ter sido atribuídos aos flavonóides quercetina e antocianinas, mais especificamente cianidina-3-O-rutinosídeo, que foram isolados do extrato metanólico do fNJ (NERURKAR et al., 2011).

Ainda, *M. citrifolia* apresentou efeito hepatoprotetor em hamsters alimentados com HFD. O suco da fruta de *M. citrifolia* (NJ) em diferentes doses suplementadas acompanhado de HFD demonstra benefícios tanto no dano hepático e na inflamação promovidos pela HFD, diminuindo os níveis dos biomarcadores relacionados ao dano hepático como a alanina transaminase (ALT), marcadores inflamatórios como TNF-α, IL-1β, óxido nítrico sintase induzível (iNOS), cicloxigenase-2 (COX-2), metaloproteinases 9 (MMP9) e melhorou as características morfofológicas da esteatose hepática. Além disso, a suplementação com NJ diminuiu os níveis séricos de colesterol total e TG, bem como aumentou a capacidade anti-oxidante hepática (catalase-CAT, superóxido dismutase-SOD, glutationa peroxidase-GSH-Px, glutationa-GSH, capacidade antioxidante dependente de trolox-TEAC) (LIN et al., 2013) e diminuiu a peroxidação lipídica hepática (substância reativa do ácido tiobarbitúrico-TBARS). O grupo demonstrou que todos esses efeitos possivelmente são provenientes da alta quantidade de ácidos fenólicos como ácido gentísico, ácido *p*-hidroxibenzóico e ácido clorogênico (LIN et al., 2013).

Os efeitos hipolipidêmicos foram observados por Shoeb e colaboradores em que duas doses de suplementação com fNJ em ratos hiperlipidêmicos induzidos por HFD e rica em colesterol, resulta na diminuição do colesterol total, TG e LDL em ambas as doses (SHOEB et al., 2016). Isso foi observado em um estudo de

Madukhail e colaboradores em que avaliaram a ação do extrato etanólico do fruto, folhas e raízes de *M. citrifolia* em ratos hiperlipidêmicos induzidos por tiloxapol (Triton WR-1339) e também em ratos hiperlipidêmicos induzidos por HFD. As maiores doses de todos os extratos diminuíram os níveis de colesterol total e TG no grupo WR 1339. Em contrapartida, no grupo hiperlipidêmico induzido por HFD os resultados se difereriram dependendo das maiores doses dos extratos. Ambos os extratos das folhas e frutos em maiores doses foram capazes de prevenir o aumento de colesterol total, LDL, a razão colesterol total/HDL, sem efeitos nos níveis de HDL. O extrato das raízes foi o que preveniu o aumento dos níveis lipídicos e glicêmicos com aumento nos níveis de HDL e preveniu o ganho de peso (MADHUKAIL et al., 2010).

Além disso, um estudo demonstrou os efeitos benéficos do extrato etanólico das folhas e frutos de *M. citrifolia* na pressão arterial de animais hipertensos. Wigati e colaboradores avaliaram em ratos *Wistar* hipertensos induzidos por dexametasona, a ação do extrato etanólico das folhas e frutos de *M. citrifolia* não apenas na pressão arterial, como também, na função renal. Nesse estudo foi observado que ratos hipertensos apresentaram diminuição na pressão arterial sistólica e diastólica, porém, em relação aos comprometimentos renais induzidos pela dexametasona, os extratos etanólicos não foram efetivos na reversão desses parâmetros (WIGATI et al., 2017). Ainda, evidências demonstraram que *M. citrifolia* apresentou efeitos na microbiota intestinal, o fNJ extraído com etanol promoveu ação probiótica promovendo o crescimento de espécies de bactérias *Bifidobacterium* e *Lactobacillus* e diminuiu o processo oxidativo intracelular e inflamação em células Caco-2, sugerindo que o fNJ pode ser uma alternativa na prevenção das doenças inflamatórias do cólon (HUANG et al., 2015).

Um estudo recente em humanos demonstrou que NJ (2 ml/kg de massa corpórea) por 2 meses em pacientes com DM2 reduziu os níveis glicêmicos em muitos, mas não em todos os pacientes, não causando hipoglicemia nos normoglicêmicos. Além disso, houve a redução nos níveis de hemoglobina glicada (HbA1c), diminuição nos níveis da proteína-C-reativa (PCR) ultrassensível de pacientes que iniciaram com níveis altos de PCR, sem alteração nos normoglicêmicos e, ainda, houve aumento dos níveis de peptídeo-C após 4 semanas de consumo de NJ em pacientes que iniciaram o estudo com os níveis

menores, mas não em pacientes com níveis maiores de peptídeo-C. Esses resultados demonstram que o NJ possui potencial em regular níveis glicêmicos elevados e outros parâmetros patológicos em pacientes que apresentam DM2 (ALGENSTAEDT; STUMPENHAGEM; WESTENDORF, 2018).

Diante de todas as evidências retratadas, a utilização das partes de *M. citrifolia* como frutos, folhas ou raízes pode ser uma alternativa terapêutica no tratamento ou prevenção de distúrbios metabólicos associados com a SM, como hiperglicemia/resistência à insulina, obesidade, hipertensão arterial sistêmica, dislipidemias e DHGNA/EHNA devido a ampla quantidade e variedade de compostos bioativos presentes na planta.

Dessa forma, para maior entendimento dos benefícios de *M. citrifolia* nas funções metabólicas, o capítulo 2 retrata uma revisão dos principais estudos publicados no decorrer dos anos demonstrando as ações terapêuticas de *M. citrifolia*, utilizando diferentes partes da planta e formas de processamento, nos distúrbios metabólicos associados ou não com a obesidade (Capítulo 2). Enquanto o capítulo 3 demonstra um estudo experimental em animais mimetizando um modelo de SM com o intuito de avaliar os efeitos terapêuticos de duas doses do extrato aquoso bruto dos frutos de *M. citrifolia* nos parâmetros bioquímicos, histológicos e na expressão de genes envolvidos no metabolismo lipídico e glicêmico neste modelo experimental (Capítulo 3).

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CAPÍTULO 2. *MORINDA CITRIFOLIA* LINN. (NONI) AND ITS POTENTIAL IN OBESITY-RELATED METABOLIC DYSFUNCTION

Aline Carla Inada[1], Priscila Silva Figueiredo[1], Rosângela Aparecida dos Santos-Eichler[2], Karine de Cássia Freitas[1], Priscila Aiko Hiane[1], Alinne Pereira de Castro[3] and Rita de Cássia Avellaneda Guimarães[1*]

1Post Graduate Program in Health and Development in the Central-West Region of Brazil, Federal University of Mato Grosso do Sul - UFMS, Campo Grande 79079-900, MS, Brazil; inada.aline@gmail.com (A.C.I.); pri.figueiredo92@gmail.com (P.S.F.); kcfreitas@gmail.com (K.d.C.F.); priscila.hiane@ufms.br (P.A.H.)

2Department of Pharmacology, Biomedical Science Institute, University of São Paulo, São Paulo 05508-900, SP, Brazil; reichler@icb.usp.br (R.A.S.E)

3Post-Graduate Program in Biotechnology, Catholic University Dom Bosco, Campo Grande 79117-900, MS, Brazil; p.alinne@gmail.com (A.P.C.)

* Correspondence: rita.guimaraes@ufms.br; Tel.:+55-67-3345-7445

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Review

Morinda citrifolia Linn. (Noni) and Its Potential in Obesity-Related Metabolic Dysfunction

Aline Carla Inada [1], Priscila Silva Figueiredo [1], Rosângela Aparecida dos Santos-Eichler [2], Karine de Cássia Freitas [1], Priscila Aiko Hiane [1], Alinne Pereira de Castro [3] and Rita de Cássia Avellaneda Guimarães [1,*]

1 Post Graduate Program in Health and Development in the Central-West Region of Brazil, Federal University of Mato Grosso do Sul-UFMS, Campo Grande 79079-900, MS, Brazil; inada.aline@gmail.com (A.C.I.); pri.figueiredo92@gmail.com (P.S.F.); kcfreitas@gmail.com (K.d.C.F.); priscila.hiane@ufms.br (P.A.H.)

2 Department of Pharmacology, Biomedical Science Institute, University of São Paulo, São Paulo 05508900, SP, Brazil; reichler@icb.usp.br

3 Post-Graduate Program in Biotechnology, Catholic University Dom Bosco, Campo Grande 79117-900, MS, Brazil; p.alinne@gmail.com

* Correspondence: rita.guimaraes@ufms.br; Tel.: +55-67-3345-7445

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Abstract: Cultural and economic shifts in the early 19th century led to the rapid development of companies that made good profits from technologically-produced commodities. In this way, some habits changed in society, such as the overconsumption of processed and micronutrient-poor foods and devices that gave rise to a sedentary lifestyle. These factors influenced host-microbiome interactions which, in turn, mediated the etiopathogenesis of "new-era" disorders and diseases, which are closely related, such as obesity, type 2 diabetes mellitus, non-alcoholic fatty liver disease, hypertension, and inflammatory bowel disease, which are characterized by chronic dysregulation of metabolic and immune processes. These pathological conditions require novel and effective therapeutic approaches. *Morinda citrifolia* (noni) is well known as a traditional healing plant due to its medicinal properties. Thus, many studies have been conducted to understand its bioactive compounds and their mechanisms of action. However, in obesity and obesity-related metabolic (dysfunction) syndrome, other studies are necessary to better elucidate noni's mechanisms of action, mainly due to the complexity of the pathophysiology of obesity and its metabolic dysfunction. In this review, we summarize not only the clinical effects, but also important cell signaling pathways in in vivo and in vitro assays of potent bioactive compounds present in the noni plant which have been reported in studies of obesity and obesity-associated metabolic dysfunction.

Keywords: *Morinda citrifolia* L.; obesity; obesity-related metabolic dysfunction; health

1. Introduction

The prevalence of obese individuals has doubled worldwide since the 1980s. In 2014, it was estimated that more than 1.9 billion adults were overweight, corresponding to 39% of all adults in the world. Of this latter group, 13% were already considered obese, i.e., 600 million [1]. In the United States (USA), obesity is a problem that increased by approximately 50% among adults throughout the 1980s and 1990s [2] and may result in a reduction in longevity of the North American population [3].

Obesity is prevalent in low-income groups who often live in urban areas in the USA and Europe. According to the World Health Organization (WHO), in 2015, more than 50% of adults were overweight or obese in 46 countries across Europe, especially in the eastern part of the region. Nowadays, overweight and obesity are estimated to result in the deaths of about 320,000 men and women in 20 countries in Western Europe every year [4]. In 2012, China's Minister of Health indicated that in the

country with 1.2 billion individuals, 300 million Chinese were obese [5]. In Brazil, more than a half of the Brazilian population was overweight (52.5%), of which 13.9% were considered obese in 2014 [6].

Although obesity is the result of an imbalance between energy intake and expenditure, it is likely that a disturbed metabolism due to inadequate nutrition contributes to an abnormal or excessive fat accumulation associated with impaired health. Central obesity is a sign of the most prevalent chronic metabolic disorder of our era with important global public health challenges. Genetic factors, together with inappropriate food supply, entertainment, and labor-saving devices, and the advertising of highly-appealing foodstuffs by the food industry, results in energy-dense diets and decreased physical activity. This constitutes a gene-environment interaction, where endocrine factors mediate and stimulate some of the pathways that lead to obesity [1,7,8]. Recently, many groups have been focusing their studies on the involvement of the gut microbiota in obesity and metabolic dysfunction. In 2006, one of the first studies that found a relationship between gut microbiota and weight gain reported that the latter was putatively caused by an increase in energy-harvesting capabilities of the microbiota in obese persons [9].

Central, as opposed to peripheral, obesity predisposes individuals to metabolic abnormalities, cardiometabolic complications, such as insulin resistance, type 2 diabetes mellitus (T2DM) dyslipidemia, hypertension, and non-alcoholic fatty liver disease (NAFLD), which are components of obesity-related metabolic syndrome (MetS) that put individuals at risk of developing cardiovascular disease (CVD) [10,11]. Nowadays, the term 'metabolic syndrome' is used widely and is defined as when an individual shows at least three of the following cardiovascular risk factors: central obesity (excessive upper body and visceral fat), dyslipidemia (high triglycerides or low high density lipoprotein cholesterol (HDL) levels), or hypertension or hyperglycemia (T2DM) [12].

There are various pharmacological and non-pharmacological options which are broadly accepted as treatment and prevention of obesity and obesity-related diseases, including dietary control, physical activity, lifestyle changes, weight-loss medications, weight-loss surgeries, or specific medications [13]. Various dietary patterns have been extensively studied for health promotion and to diminish the risks of chronic diseases. A "healthy eating concept" has been put forward in which functional foods and nutraceuticals are important parts [14].

In this context, according to Health Canada, the term nutraceutical is conceptualized as a product isolated or purified from foods that is generally sold in medicine formulations not usually associated with food. A nutraceutical has been shown to have a physiological benefit or provide protection against chronic diseases. The term 'functional food' is distinguished as a food that is similar in appearance to, or may be, a conventional food, that is consumed as part of a usual diet, and has demonstrated physiological benefits and/or to reduce the risk of chronic disease beyond basic nutritional functions [15].

Due to the complexity of obesity pathogenesis, the majority of the treatments are not related to obesity alone, seeing that it is associated with other metabolic disorders, such as oxidative stress and/or inflammatory alterations that can occur concomitantly in several tissues [11]. Recent studies have focused on the development of innovative therapeutic agents from natural sources as an alternative medicine. Nevertheless, the challenge of natural product studies is to evaluate, in a consistent way, the mechanisms of action and bioactive compounds which could assure the beneficial effects of the products [13,16].

Morinda citrifolia L. (noni) is an example of a plant used as a functional food and has been widely studied due to its apparent beneficial effects on human health. It has been investigated as an alternative in anticancer, antibacterial, and antimicrobial therapies, and in the treatment of esophageal reflux and ulcers in animals [17–21]. In humans, there are few studies showing the beneficial effects of noni. Sattar et al. [22] and Siddiqui et al. [23] demonstrated the effective benefits of a topical ointment prepared from noni stem extract against cutaneous leishmaniasis. Palu et al. [24] showed a 25% reduction in lipid peroxidation in the blood of athletes, after an endurance test, taking noni juice (NJ) compared to controls, thereby demonstrating an antioxidant effect. The antioxidant properties of noni

juice were also demonstrated by Wang et al. [25] in a study involving 132 heavy cigarette smokers. They reported reduced plasma levels of superoxide anion radicals and lipid hydroperoxide, which are considered biomarkers of degenerative diseases associated with cigarette smoking, in smokers who drank NJ compared to smokers who did not. Moreover, Issell et al. [26] reported less fatigue and maintenance of physical functions in patients with cancer.

Recently, more attention has been directed to the anti-obesity properties of the noni plant in animal models. However, studies are still scarce in humans and more investigations are needed.

Therefore, the aim of this review was to assess specific in vitro and in vivo studies related to the mechanisms of action and bioactive compounds that promote the benefits of *Morinda citrifolia* L. (noni) in the treatment of obesity and obesity-related metabolic dysfunction, such as insulin resistance/T2DM, dyslipidemia, hypertension, NAFLD [27,28], and its influence on the gut microbiota.

2. *Morinda citrifolia* Linn. (Noni)

The genus *Morinda* belongs to the Madder or Coffee family Rubiaceae and includes approximately 80 species, including *Morinda citrifolia* Linn (*Morinda citrifolia* L.), which is popularly called noni or Indian Mulberry [29]. Noni was used as a medicinal plant by the Polynesians 200 years ago, but it has never been a traditional food, although it has been called as a starvation fruit. Currently, noni is a typical plant found in tropical climate regions of the USA, such as Hawaii, to Brazil, reaching Tahitian, Malaysia, and Fiji Islands [30,31]. The first idea of potential benefits of noni fruit started with Heinicke [32] who demonstrated that noni contained the alkaloid xeronine. Even though noni fruits showed insignificant amounts of free xeronine, they contained considerable amounts of the precursor of xeronine, which was named proxeronine. One of the explanations for the medicinal action of noni fruits is that xeronine could modulate the conformation and stability of specific proteins. Heinicke described beneficial effects of noni fruits, such as in menstrual cramps, hypertension, burns, depression, atherosclerosis, digestion, relief for pain, and many others.

In 1996, because of the nutraceutical and therapeutic properties of noni, commercial NJ was marketed as a dietary supplement. Afterwards, in 2003, the European Commission approved Tahitian noni juice as a novel food by the Health and Consumer Protection Directorate General. Many investigators have studied the bioactive compounds present in noni fruits, as well as in other parts of the plant, such as leaves, roots, roots bark, seeds, stems, and flowers, because of their potential benefits to health [33–35].

On the other hand, noni fruit and juice display some challenging peculiarities, such as a bitter and astringent flavor, as well as a strong rancid odor, which prompts some companies to change these organoleptic properties to create a more palatable product. These companies have been producing flavored NJ with the addition of other fruit juices to create a better-tasting product [36]. Other issues concerning the noni plant are the toxicity, adverse side-effects, the safety of long-term consumption, bioactive compounds, and mechanisms of action, which are important factors to be elucidated in in vitro and in vivo studies to be totally acceptable for human consumption [37].

2.1. Nutritional Values of the Noni Plant

Relevant nutritional and chemical analyses have demonstrated that noni fruit contains 90% water and 10% dry matter. The dry matter is composed of soluble solids, dietary fibers, and proteins. Chunhieng et al. [38] reported that 5% of soluble solids are reducing sugars (glucose and fructose) and 1.3% is sucrose. Approximately 11.3% of the dry matter is protein and the main amino acids are glutamic acid, aspartic acid, and isoleucine. Moreover, 10–12% are minerals, which include calcium, sulfur, potassium, magnesium, sodium, phosphorus, and traces of selenium. The main vitamins reported in noni fruit puree are ascorbic acid (vitamin C), which corresponds to 250 mg ascorbic acid per 100 g fresh matter, niacin (vitamin B3), and vitamin A [33,39,40].

2.2. Chemical Composition of the Noni Plant

Despite the issues surrounding *Morinda citrifolia* fruit, especially its taste and odor, people still use the bottle-pasteurized juice, either in pure form or mixed with other juices, due to the various phytochemicals in the noni plant totaling approximately 200 compounds. These bioactive compounds are present in different parts of the plant. Noni fruit and other parts of the plant contain large amounts of phytochemicals, including phenolic compounds, anthraquinones, carbohydrates, organic acids, alcohols, vitamins, flavonoids, iridoids, ketones, lignans, triterpenoids, nucleosides, sterols, fatty acids, carotenoids, and many others [29,35,37].

The content of phenols, antioxidants, and ascorbic acid present in the noni fruit increase from the green to white hard stage, whereas they diminish from the white hard stage to the ripe/soft stage [36,41]. In the white hard stage, noni fruits have approximately two times more antioxidant activity, 1.5 times higher phenol content, and seven times higher ascorbic acid content, in comparison to immature green fruits, which have 1.1–1.5 times the antioxidant activity, 1.3 times the total phenols, and 1.3 times the content of ascorbic acid [36,42].

2.3. Important Phytochemicals of Morinda citrifolia on Obesity and Obesity-Related Metabolic Dysfunction

The high prevalence of obesity and obese-related metabolic dysfunction has led to extensive investigations to seek out alternative therapies because of several reports of side-effects that are promoted by synthetic drugs. Noni can provide important natural products that have been widely studied and may be considered an alternative therapy for many diseases [14]. Many scientific publications have shown that the noni plant contains a variety of nutritional and functional compounds. However, our current knowledge of these compounds is still not satisfactory. Some studies have demonstrated that the principal bioactive compounds from *Morinda citrifolia* have potentially beneficial effects in obesity and obesity-related metabolic dysfunction, and they are listed in Table 1.

Table 1. Principal phytochemicals from *Morinda citrifolia* as bioactive compounds against obesity and obesity-related metabolic dysfunction.

Part of Plant	Structural Class	Bioactive Compounds	Concentrations of Bioactive Compounds	References
Fruit	Phenolic acid	Chlorogenic acid	10.49 mg/100 mL [43]	[43–48]
		Gentisic acid	19.16 mg/100 mL [43]	[43–45,48]
		P-hydroxybenzoic acid	14.12 mg/100 mL [43]	[43–45,48]
	Flavonoids	Anthocyanin (cyanidin-3-*O*-rutinoside)	Data not shown	[49]
		Catechin	53.68 mg/g [50]	[50,51]
		Epicatechin	6.8 mg/g [51]	[51]
		Kaempferol	6.4 mg/g [51]	[52,53]
		Rutin	8.06 mg/g [50]	[45,54–56]
		Quercetin	7.4 mg/g [51]	[49,54]
	Iridoids	Asperulosidic acid	Data not shown	[48,57]
	Lignans	Americanin A	17.4 mg [58]	[58,59]
		Americanol A	21 mg 60]	[60]
		Isoprincepin	14 mg [60]	[60]
		Lirioresinol B	Data not shown	[61]
		Lirioresinol B dimethyl ether	Data not shown	[61]
		Morindolin	10 mg [60]	[60]
		3,3′-Bisdemethypinoresinol	69 mg [60]	[60]
	Coumarins	Scopoletin	46.1 mg [58]	[49,55,58,62]
	Minerals	Potassium	3900 mg/L [38]	[38,63–65]
	Triterpenoids/ terpenes	Ursolic acid	Data not shown	[61,66–68]
		Saponin	Data not shown	[69]
	Vitamins	Vitamin C	Data not shown	[70,71]
		Vitamin E	Data not shown	[70,71]

Table 1. *Cont.*

Part of Plant	Structural Class	Bioactive Compounds	Concentrations of Bioactive Compounds	References
Leaf	Flavonoids	Catechin	63.46 mg/g [50]	[50]
		Epicatechin	23.08 mg/g [50]	[50]
		Rutin	6.83 mg/g [50]	[45,50,55]
		Kaempferol	21–80 mg [52]	[52,66]
	Triterpenoids/terpenes	Ursolic acid	Data not shown	[61,68]
Root	Anthraquinones	Deacetylasperulosidic acid	Data not shown	[73]
		1,2-Dimethoxyanthraquinone	3.5 mg [72]	[72]
		Alizarin-2-methyl ether	11.3 mg [72]	[72]
		Rubiadin-1-methyl ether	15.5 mg [72]	[72]
		Lucidin 3-*O*-beta-D-primeveroside	Data not shown	[73]
		Damnacanthol-3-*O*-beta-D-primeveroside	Data not shown	[73]
		Morindone-6-*O*-beta-D-primeveroside	Data not shown	[73]
	Iridoid	Asperulosidic acid	Data not shown	[73]

Principal bioactive compounds from fruits, leaves, and roots of *Morinda citrifolia* used for obesity and obesity-related metabolic dysfunction.

These bioactive compounds, called phytochemicals, include phenolic acids, lignans, flavonoids, flavones, flavans-3-ol, anthocyanins, phytosterols, alkaloids, vitamins, and minerals. In recent decades, polyphenols have been the most important compounds shown to possess beneficial effects against obesity and metabolic dysfunction. Polyphenols include phenolic acids, flavonoids, and stilbenes, which are the most common compounds used in the development of natural products for metabolism-associated disorders/diseases [13,44,45].

Phenolic acids are divided into two classes: hydroxycinnamic and hydroxybenzoic acids. Hydroxycinnamic acids include *o*-coumaric acid, *m*-coumaric acid, caffeic acid, ferulic acid, and sinapic acid, and are present in the form of simple esters with glucose or quinic acid. Hydroxybenzoic acids include salicylic acid, gentisic acid, *p*-hydroxybenzoic acid, gallic acid, vanillic acid, and 3,4-dimethoxybenzoic acid. The most common acid derivative is chlorogenic acid [13,44,45].

Flavonoids are abundant compounds in nature and they are divided into six subgroups: flavonols, flavanones, isoflavanoids, flavones, flavand-3-ol, and anthocyanins. Flavonoids have been widely studied due to their therapeutic properties in the treatment of metabolic disorders due to their ability to modulate the numbers of cell signaling pathways that affect carbohydrate digestion, fat deposition, and the release rate of insulin or glucose uptake in insulin-responsive tissues [44,45,50,51].

Quinones are a class of organic compounds, where 9,10-anthraquinones (9,10 dioxoanthracenes) are an important subgroup [74]. Anthraquinones in *Morinda citrifolia* are found especially in the roots, and the main compounds with important effects on metabolism are alizarin, lucidin 3-*O*-β-D-primeveroside, damnacanthol-3-*O*-β-D-primeveroside, and rubiadin-1-methyl ether [72,73].

Coumarins are found in many edible plants and fruits. One of the most important coumarins found in noni plant is scopoletin (6-methoxy-7-hydroxycoumarin), which has shown a notable effect in the treatment of obesity and metabolic dysfunction [49,55]. Lignans and neolignans are present in many plants, being a large group of natural products derived from the oxidative coupling of two C6-C3 units [75]. The most important lignans isolated from noni fruit are americanin A, americanol A, episesamin 2,6-dicatechol, isoprincepin, lirioresinol, lirioresinol B dimethyl ether, morindolin, and 3,3′-bisdemethypinoresinol. These lignans improve parameters in obesity and associated disorders/diseases [58,59,61,76].

Triterpenoids are the largest class of secondary metabolites produced by plants. Ursolic acid and related triterpene compounds, such as oleanolic acid, betulinic acid, uvaol, and α- and β-amyrin are widespread in many plants [77,78]. The most abundant triterpenoid from noni is ursolic acid, which has been widely investigated because of its hypoglycemic property [61,67,68].

Iridoid is a monoterpene, differing from triterpenes, and it is derived from geraniol. Iridoid glucosides and glycosides, a subclass of iridoid, are terpenes bound to glucose, or any sugar,

respectively [79]. Asperulosidic acid is one of the most important iridoids isolated from *Morinda citrifolia*, and it has been shown to improve blood fluidity, which influences the health of obese patients and those with obesity-associated disorders, such as hypertension, diabetes and dyslipidemia [57,80,81]. Vitamins (C, ascorbic acid, and E, α-tocopherol) are two important non-enzymatic antioxidants that have important effects because of their free-radical scavenging property [70,71].

2.4. Toxicity of the Noni Plant

The toxicity and low palatability could explain why noni has never been a food plant; therefore, considerable effort is needed to extract or deactivate the toxins. Studies have reported human [41,82] and animal [83] toxicity. In some human cases reported, Millonig et al. [41] and Stadlbauer et al. [82] demonstrated that NJ led to signs of hepatoxicity. The first group observed that a 45-year-old man had elevated transaminases and lactate dehydrogenase. After stopping the ingestion of NJ, transaminase levels normalized. The second human clinical study reported that a 29-year-old man, who had previous toxic hepatitis after small doses of paracetamol, developed sub-acute hepatic failure following consumption of 1.5 L of NJ, while a 69-year-old woman without evidence of previous liver disease experienced an episode of self-limited acute hepatitis following consumption of two liters of NJ.

Recently, Shalan et al. [83] compared the chronic toxicity of NJ and noni leaf aqueous extracts (1 and 2 mg/mL, respectively) to drinking water for six months on the liver and kidneys in female mice. This study observed that none of the doses of noni leaf extracts showed toxic effects; however, mice that consumed noni fruit extract at 2 mg/mL showed toxicity symptoms, such as hypoactivity, excessive grooming, sunken eyes, and hunched posture, with 40% mortality after three months of use. The main cause of death was hepatoxicity with dose-dependent hepatocellular necrosis, though with no effects on kidney.

One possible explanation for noni fruit toxicity can be related to the large amount of anthraquinones. Inoue et al. [84] reported that madder dye, a food coloring extract from the roots of *Rubia tinctorium* L., containing large amounts of two anthraquinones, alizarin, and rubiadin (metabolite of lucidin-3-*O*-primeveroside), showed carcinogenicity in the kidney and liver of six-week-old male F344 rats, where rubiadin had higher carcinogenic potential than did alizarin. However, this study was not applied to noni fruit or leaves. West et al. [85] reported that NJ (Tahitian Noni Juice®, Tahitian Noni International, American Fork, UT, USA) (TNJ) was not hepatotoxic and demonstrated that anthraquinones did not possess the same universal biological effect and that it was not reasonable to assume that one category of anthraquinones would have the same exact toxic action as another. Westendorf et al. [86], using high-performance liquid chromatography (HPLC), did not detect genotoxic hydroxyanthraquinones (HAs), such as lucidin and rubiadin in NJ.

The same study demonstrated that the treatment of liver cells in vitro (primary rat hepatocytes and H4IIE rat hepatoma cells) with a common TNJ did not induce genotoxicity. To evaluate the possible genotoxicity of TNJ, they used the V79-HPRT assay and ex vivo hepatocyte UDS assay. V79 cells are Chinese hamster fibroblasts and are currently used as a mammalian cell model to determine the mutagenic effect of natural compounds. In V79 cells treated with TNJ no mutagenicity was observed. Moreover, when primary hepatocytes from rats treated with 10 g/kg body weight, about 700 mL of juice for an adult human, were tested, they showed no acute toxic effects, as seen by UDS (unscheduled DNA synthesis), which is a marker of DNA damage repair [86].

Although there are several controversial studies related to toxicity, some authors suggest a closer evaluation of noni products in general. At the time of NJ production, manufacturers must process the product to remove the toxic principles or deactivate them and test the resulting non-toxic preparations for beneficial activity. Additionally, hygienic measures must be taken to ensure safety for human consumption. Nonetheless, these toxicity studies will be irrelevant if NJ products are obtained using inappropriate processing methods, contain microbial contaminants, or have been adulterated with unsafe ingredients [87].

2.5. Therapeutical Use of the Noni Plant

Due to the potential bioactive compounds present in *Morinda citrifolia* fruit that are good for human health, some companies add other fruit juices to provide a flavorful and enhanced product [14,88]. However, not only the fruit, but also other parts of the plant, have phytonutrients with beneficial effects. Growing evidence suggests that noni has important antimicrobial and antibacterial activities. Extracts of noni leaves made with three different solvents, ethyl acetate extract, *n*-butanol, or water, showed a very strong antimicrobial and antibacterial activity against some microorganisms, including *Proteus vulgaris*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli* [17].

It has long been believed that noni leaves have a large number of phenolic compounds, especially coumarins and flavonoids, and also acubin, L-asperulose, alizarin, scopoletin, and other anthraquinones [29,89]. These phenolic compounds possess antimicrobial activity [18], which may, due to their antioxidant effects, possibly involve proton exchange processes [90]. The use of noni for esophageal reflux and gastric ulcerative disease has also produced good results, such as preventing the occurrence of esophagitis due to acid reflux, reducing the formation of acute gastric lesions induced by ethanol, suppressing the development of gastric lesions, and also significantly inhibiting gastric acid secretion and pepsin activity in the pylorus-ligated rat [19]. Scopoletin is the component of noni that is thought to have this valuable potential preventive and therapeutic action for gastro-esophageal inflammation [19].

Palu et al. [91] reported another anti-inflammatory action of NJ, where they demonstrated suppression of IL-4 in mice with NJ treatment in relation to water. NJ increased the production of IFN, which is correlated with the activation of macrophages. The suppressive effects of NJ on IL-4 production in splenocytes, concomitant with increased production of IFN, indicates that NJ modulates the immune system.

A recent study reported by Shalan et al. [92] demonstrated that noni leaf extract possessed ergogenic effects, helping delay fatigue by enhancing energy production, regulation, and efficiency in female mice after a swimming endurance test. Noni extract enhanced performance by improving angiogenesis in skeletal muscle and liver (via vascular endothelial growth factor A, VEGFA), showing antioxidant (superoxide dismutase–SOD2 and glutathione-GSH) and anti-inflammatory (IL-4 and IL-10) properties, and ameliorating mitochondrial biogenesis (via AMP-activated protein kinase (AMPK), uncoupling protein-3 (UCP-3), peroxisome transcriptional proliferator-activated receptor gamma, coactivator 1 alpha (PGC-1α), and nuclear respiratory factor-2 (NRF2)) and stress response (cortisol).

Another application of NJ was related to its anticancer properties since it reduces free radicals which are involved in oxidative damage and lipid peroxidation. One of the main components associated with this property is damnacanthal, a valuable anthraquinone found in the roots of the noni plant, which is widely used for the treatment of chronic diseases, such as cancer and heart disease. Damnacanthal is involved in the K-ras pathway, inducing actin fiber organization, which is affected in activated ras-expressing tumors [20,21]. The noni roots also show antispasmodic, vasodilator, and cardiodepressant activities [93].

A phase one clinical trial was conducted to determine the best dose of noni for capsule supplementation, in which a conventional dose escalation design was used to begin, where the subjects of the research were patients with some type of cancer. No adverse effects were found, and the only hurdle was the number of capsules to be ingested to complete the full dose of 14 g of encapsulated freeze-dried noni fruit per day. In addition, some quality of life factors, such as physical function and fatigue control, were improved in patients who took a mean dose of 8 g per day, compared with patients who took higher and lower doses [26].

3. Effects of Bioactive Compounds from the *Morinda citrifolia* L. Plant on Obesity and Obesity-Related Metabolic Dysfunction

3.1. Morinda citrifolia and Obesity

Obesity is characterized by the expansion of adipose tissue. This type of tissue can be classified as brown, beige, and white adipose tissue (WAT). WAT is considered not only as an energy reservoir, but also as an organ with endocrine functions. It is classified according to its localization as subcutaneous adipose tissue or visceral adipose tissue, the latter being one of the most important fat deposits associated with metabolic disease [94–99]. The endocrine functions that revolve around WAT are due to their capacity to maintain, under physiological conditions, lipid metabolism, such as lipogenesis, lipolysis, and adipogenesis processes [97–99], and releasing adipokines, which are substances with important biological and metabolic functions, such as adipsin, tumor necrosis factor-α (TNF-α), leptin, adiponectin, monocyte chemoattractant protein-1 (MCP-1), interleukins (IL-6, IL-10, IL-1β), plasminogen activator-1 (PAI-1), components of the renin-angiotensin-aldosterone system (RAAS), resistin, visfatin, omentin, and many others [100–105].

In this regard, natural products, such as plants, herbal supplements, and diet-based therapies have been widely studied because of their potential benefits in human health against obesity and its metabolic disorders [106–109]. Nishioka et al. [110] investigated the mechanisms underlying the beneficial effects of NJ with focus on glucose and lipid metabolism in high-fat diet (HFD) obese C57BL/6 mice (Table 2). The animals that consumed HFD + NJ showed decreased adipose tissue weights, plasma triglyceride levels, and improved glucose tolerance without toxicity and displayed a lower final body weight, compared to the HFD group. These benefits in the parameters and biomarkers of obesity demonstrated the anti-obesity effects of NJ.

Accordingly, the benefits of NJ in HFD mice have also been reported by others. The reduction of weight gain and improvement of metabolic parameters, such as total cholesterol, low-density lipoprotein-cholesterol, glucose and insulin tolerance, fasting glucose levels, and hepatic insulin resistance has been seen in rats [111], mice [49], and hamsters [43] (Table 2). No liver damage was observed. One explanation for the effectiveness of NJ is the large amount of phenolic acids present in its composition, including gentisic acid, *p*-hydroxybenzoic acid, and chlorogenic acid (Table 2).

A recent study demonstrated positive effects of *Morinda citrifolia* leaves (MLE) as dried plant material that were extracted with 60% ethanol (MLE 60) in HFD obese Sprague-Dawley male rats. They tested two different doses of MLE and compared those groups receiving MLE with the group receiving a synthetic anti-obesity drug (Orlistat 30 mg/kg). The parameters adiposity, fecal fat content and plasma lipids, insulin, and leptin with the higher dose of MLE (500 mg/kg) group were similar as that in the Orlistat group, except the ghrelin levels, which showed better results with the lower dose of MLE (250 mg/kg). Some metabolic pathways, including glucose metabolism and TCA (tricarboxylic acid) cycle, amino acid metabolism, choline metabolism, creatinine metabolism, and gut microbiome, were analyzed using a ^{1}H nuclear magnetic resonance (^{1}HNMR)-based metabolomics approaches. Both doses of the extract showed improvement in certain metabolic pathways that were impaired by HFD-induced obesity [112] (Table 2).

Under physiological conditions, lipogenesis and lipolysis are the two primary metabolic events in adipose tissue, and they are orchestrated to maintain lipid homeostasis. Non-esterified fatty acids accumulate in WAT and are esterified into triacylglycerol by lipoprotein lipase (LPL). This process of synthesis of esterified fatty acids (FAs) is called lipogenesis. On the other hand, lipolysis is the mobilization or hydrolysis of triglycerides. The availability of FAs and glycerol are necessary for energy storage. Glycerol is an important substrate for hepatic gluconeogenesis and FAs are important energy substrates for peripheral tissues [97–99]. Therefore, LPL is an important biomarker in obesity and it has been reported to be consistently augmented in the adipose tissue of obese subjects [50].

Table 2. The effects of administration of the *Morinda citrifolia* L. plant on obesity.

Host	Part of Plant	Dose/Time	Effects	Reference
Mice	Fruit Noni Juice	1.5 µL/g body weight (twice daily)/5 weeks	−Reduced body weight by 40% in mice fed control, while reduced body weight by 25% in HFD mice.	Nishioka et al. [110]
			−Reduced adipose tissue weights, plasma triglycerides and improved glucose tolerance.	
Rats	Fruit Noni Juice	50 mg/kg/day/30 days	−Reduced body weight (better at 50 mg/kg/day dose).	Shoeb et al. [111]
			−Reduced serum total cholesterol, triglycerides and lipid fractions: LDL and VLDL (all doses).	
		100 mg/kg/day/30 days	−Increased lipid fraction HDL (all doses).	
Mice	Fruit Fermented Noni Juice	1.5 µL/g body weight/twice daily/12 weeks	−Inhibited weight gain after 12 weeks.	Nerurkar et al. [49]
			−Improved glucose and insulin tolerance and fasting glucose in HFD-fed C57Bl/6 mice.	
			−Improved hepatic insulin resistance by FOXO-1 and inhibition of PEPCK and G6P (gluconeogenic enzymes).	
Hamster	Fruit Noni Juice	−3 mL (containing 64.23 mg crude polysaccharides/kg body weight/6 weeks.	−Decreased visceral fat in HFD-hamsters (all doses).	Lin et al. [43]
			−Decreased serum and liver lipids: total cholesterol and triglycerides in HFD hamsters (all doses).	
		−6 mL (containing 128.46 mg crude polysaccharides/kg body weight/6 weeks.	−Beneficial effects on liver and hepatic enzymes (ALT) in HFD hamsters (all doses).	
			−Increased antioxidant capacity in the liver in HFD hamsters (all doses).	
		−9 mL (containing 192.69 mg crude polysaccharides/kg body weight/6 weeks)	−Decreased inflammatory biomarkers in the liver (TNF-α, MCP-1, IL-1β) in HFD hamsters (all doses).	
			−Decreased gelatinolytic levels of MMP9 in HFD hamsters (all doses).	
Rats	Ethanolic Extract of Leaves	−250 mg/mL/9 weeks.	−Prevented weight gain, especially MLE 60 500 mg/kg.	Jambocus et al. [112]
			−Positive effects on adiposity, fecal fat content, plasm lipids, insulin and leptin levels, especially MLE 60 500 mg/kg.	
		−500 mg/mL/9 weeks.	−Improved ghreline levels, especially MLE 250 mg/kg.	
			−Improvement in metabolic perturbations caused by obesity, both concentrations of extract.	
In vitro	Ethanolic Extract of Fruit and Leaves	0.2 mg/mL in vitro	−Inhibited LPL activity.	Pak-Dek et al. [50]
In vitro	Ethanolic Extract of Fruit	1 mg/mL in vitro	−Inhibited LPL activity.	Sahib et al. [51]

Effects of administration of different doses and parts of the *Morinda citrifolia* plant on obesity in in vivo and in vitro studies.

The influence of *Morinda citrifolia* fruit (MFE) and leaf (MLE) extracts on LPL activity were evaluated in vitro by two independent research groups [50,51]. Pak-Dek et al. [50] studied MFE and MLE with green tea (GTE) and catechin extracts on the enzymatic activity of LPL. The data demonstrated that all extracts tested inhibited LPL activity substantially after 30 min of incubation. However, the greatest inhibition of LPL activity was seen with 0.2 mg/mL MLE in a dose-dependent manner when compared to MFE, GTE and catechin.

Sahib et al. [51] evaluated MFE, *Momordica charantia* (MCE) and *Centella asiatica* (CAE) extracts in LPL inhibition and the effects of the extracts in proliferation and differentiation of 3T3-L1 preadipocytes

(Table 2). The results showed that 1 mg/mL MFE exerted the most significant inhibitory effect on LPL, and in a dose-dependent manner. On the other hand, after 24, 48, and 72 hours of extract incubation, only MCE inhibited adipogenesis in the concentration range of 0–5 mg/mL and differentiation at the highest concentration of 0.5 mg/mL at 48 h. Interestingly, the data revealed that all of the extracts contained high concentrations of phenolic compounds, including catechin and epicatechin, which may be the responsible agents for these effects [51].

Several studies attributed these effects on lipid metabolism to the phenolic compounds, especially catechins present in the extracts [113,114]. However, due to the fact that catechins in MFE and MLE were lower than in GTE [50], one explanation about the inhibition of LPL may be the synergistic effect of catechin with other components present in the extracts since synergism between flavonoids is believed to be better than with one alone. In fact, low-processed whole plant extracts supply multiple chemicals, as much as food does, and depends on synergistic metabolic effects to confer health. In conclusion, the groups suggested that MLE and MFE may be used as anti-obesity agents [50,115].

Polyphenols have been intensively used in studies of obesity and weight management, as well as in other metabolic conditions [13,44,45]. The most used polyphenols include phenolic acids (gentisic acid, *p*-hydroxybenzoic acid, and the derivative chlorogenic acid) and flavonoids (epicatechin, catechin, rutin, quercetin, and kaempferol). Several transcriptional factors, such as proliferator-activated receptor (PPAR)-γ and CCAAT/enhancer-binding proteins (C/EBPs), are involved in the early stage of adipocyte differentiation [116]. PPAR-γ, for instance, influences glucose homeostasis and insulin sensitivity [117].

Flavonoids and phenol acids were able to inhibit adipogenesis in 3T3-L1 adipocytes [45]. The polyphenols rutin (flavonoid) and o-coumaric acid (phenol acid) showed the best results in the inhibition of differentiation with lower levels. Moreover, these compounds were able to inhibit the expression of PPAR-γ and C/EBPα protein levels, demonstrating that these polyphenols inhibit adipogenesis by affecting the transcriptional factor cascade upstream of PPAR-γ expression and also inhibiting the expression of leptin and upregulating adiponectin protein levels [45].

Chlorogenic acid has been claimed to modulate lipid and glucose metabolism in vivo in healthy, as well as in metabolic disorder conditions [46,47]. Eight weeks of treatment with chlorogenic acid exhibited important alterations in a model of HFD-obese male golden hamsters, decreasing body weight gain and visceral adiposity, and ameliorating several metabolic parameters. Furthermore, chlorogenic acid modified lipid and glucose metabolism due to (PPAR)-α action which, in turn, regulated binding, transport, oxidation, and synthesis of free fatty acids (FFAs) [46]. Thus, after activation of PPAR-α, the activity of FFA oxidation enzymes may increase elevating fat energy utilization in the liver and muscle, ameliorating insulin tolerance, and decreasing insulin resistance [118,119].

Additionally, another flavonoid that was isolated from the fruit and leaves of *Morinda citrifolia* is kaempferol [52,66,120]. This flavonoid is the major component of soy leaves (SLE), and a recent study evaluated the anti-obesity effects of SLE extracts in HFD-obese male C57BL/6 mice. Ten weeks of treatment suppressed body weight gain and fat accumulation of WAT. Furthermore, kaempferol supplementation (50 mg/kg/day) induced (i) a decrease in pro-inflammatory cytokine (TNFα and IL-6) gene expression; (ii) a downregulation of adipogenesis-related genes, such as C/EBP-α, sterol regulatory element-binding protein-1 (SREBP-1) and fatty acid synthase (FAS); and (iii) an upregulation of fat oxidation-related genes, such as hormone-sensitive lipase (HSL), carnitine palmitoyl transferase 1 (CPT-1), and uncoupling protein-2 (UCP-2), in WAT from HFD-obese mice. Similar results were observed in 3T3-L1 adipocytes, as well [121].

3.2. *Morinda citrifolia* L. and Insulin Resistance/Type 2 Diabetes Mellitus (T2DM)

Obesity can lead to insulin resistance, which is a condition in which a cell, tissue, or organism fails to respond appropriately to a given dose of insulin. To understand the mechanisms of insulin resistance, investigators have developed numerous models of insulin resistance using various chemicals, drugs,

and nutritional challenges [122]. T2DM is a difficult problem that has been increasing rapidly, and insulin resistance has an important role in the pathogenesis of T2DM. The reconstitution of insulin sensitivity is an important strategy for the treatment of T2DM. Thus, *Morinda citrifolia* has been widely studied as an alternative treatment for these complications.

Intensive research efforts have evaluated the positive effects of *Morinda citrifolia* on glucose homeostasis in models of T2DM [49,61,73,76]. Nguyen et al. [61] observed that methanolic *Morinda citrifolia* extract (part not identified) showed an anti-diabetic effect in vitro. The extract exhibited stimulatory effects on glucose uptake using a fluorescent-tagged glucose probe (2-NBDG) in 3T3-L1 adipocyte cells. The group identified two new lignans, three new neolignans, and 10 known compounds, where the lignans and ursolic acid were the bioactive compounds that confirmed the inhibitory effects on protein tyrosine phosphatase 1B-gene (PTP1B) and stimulatory effects on 2-NBDG. In this study, lignans, such as episesamin 2,6-dicatechol, lirioresinol B, lirioresinol B dimethyl ether, and ursolic acid, were considered the anti-diabetic effectors for the inhibition of PTP1B (Table 3). Protein tyrosine phosphatases (PTPs) are a group of proteins that participate in intracellular signaling and metabolism by dephosphorylating tyrosine residues. There are several PTPs, where PTP1b has important roles in insulin receptor signaling [123] and is a key regulator of the leptin signaling pathway [124].

Table 3. The effects of administration of the *Morinda citrifolia* L. plant on insulin resistance/type 2 diabetes mellitus (T2DM).

Host	Part of Plant	Dose/Time	Effects	Reference
In vitro	*Morinda citrifolia* powder (plant part not identified) Methanol extract.	−Isolation of compounds (two new lignans, three new neolignans, and 10 known acid compounds).	−Stimulatory effects on glucose uptake through a fluorescent-tagged glucose probe (2-NBDG) in 3T3-L1 adipocyte cells. −Inhibitory effects on protein tyrosine phosphatase 1B gene (PTP1B), which is overexpressed in insulin resistance.	Nguyen et al. [61]
Mice	Fruit (Fermented noni juice).	−1.5 µL/g body weight/twice daily/12 weeks	−Inhibited weight gain after 12 weeks. −Improved glucose and insulin tolerance and fasting glucose in HFD-fed C57Bl/6 mice. −Improved hepatic insulin resistance by FOXO-1 and inhibition of PEPCK and G6P (gluconeogenic enzymes)	Nerurkar et al. [49]
Mice	−Fruit (15 kg dried noni fruit powder fermented by Cheonggukjang and bacteria).	−fermented noni juice/90 days.	−Reduced fasting glucose levels, glycosylated hemoglobin (HbA1) in KK-Aγ diabetic-mice. −Improved insulin sensitivity in KK-Aγ diabetic-mice.	Lee et al. [76]
In vitro	−Fruit (70% methanol extract).	−200 and 400 µg/mL in vitro.	−Diminished lipid fraction LDL and triglycerides in KK-Aγ diabetic-mice. −70% methanol extract in culture cells (C2C12 cells) activated PPAR-γ and stimulated AMPK pathway.	
Mice	−Roots (methanol extract—soluble phases: $CHCl_3$, EtOAc, *n*-BuOH, H_2O).	−3 g/kg/single administration.	−*n*-BuOH fraction significantly reduced blood glucose levels after five hours of administration in streptozotocin-diabetic ddY mice.	Kamiya et al. [73]

Effects of administration of different doses and parts of the *Morinda citrifolia* plant on insulin resistance/T2DM in in vivo and in vitro studies.

In other studies, lignans from *Myristica fragrans* Houtt. (nutmeg) demonstrated strong stimulation AMPK activity in differentiated C2C12 cells. AMPK has been considered as a potential therapeutic target for the treatment of metabolic syndrome, including obesity and T2DM [125]. Ursolic acid is one of the most important triterpenoids isolated from various natural products, including *Morinda citrifolia*.

Jayaprakasam et al. [67] isolated ursolic acid, as well as anthocyanins from *Cornus mas* (cornelian cherry), and added them to the HFD for an additional eight weeks. The compounds diminished obesity and glucose intolerance in HFD-obese C57Bl/6 mice to some extent [67]. Indeed, the beneficial effects of acute (three days) and chronic (six weeks) treatment with ursolic acid was also reported by others. These treatments increased skeletal muscle and brown fat metabolism which, in turn, increased energy expenditure. These data were confirmed by the reduction of obesity, glucose intolerance, and fatty liver in HFD-obese C57Bl/6 mice [68].

Another important study demonstrated that fermented noni juice (fNJ) administered to HFD-fed C57Bl/6 male mice reduced body weight and improved glucose and insulin tolerance, as well as fasting blood glucose. These authors detected scopoletin, quercetin, and anthocyanin (cyanidin-3-*O*-rutinoside) in methanolic extracts of fNJ using HPLC. They suggested that the anti-diabetic effects of fNJ may be associated with quercetin and anthocyanin [49] (Table 3). Kampkotter et al. [54,126] demonstrated the properties of quercetin in resistance to oxidative stress in an established model of *Caenorhabditis elegans*, which is an in vivo model that has become increasingly popular to evaluate pharmacologically-active compounds of herbal origin. Quercetin not only had a strong antioxidant capacity, but also prolonged the lifespan of *Caenorhabditis elegans* and was considered a modulator of cell signaling processes to exert its protective properties.

Anthocyanins present in bilberry fruit extract ameliorated hyperglycemia and insulin sensitivity in male KK-Aγ mice, a genetic model of T2DM. Anthocyanins activate AMPK, a signaling pathway important because of its role in the control of hepatic glucose and lipid metabolism [127,128]. These data corroborated with another study [67] that isolated anthocyanins (cyanidin 3-*O*-galactoside, pelargonidin 3-*O*-galactoside, and delphinidin 3-*O*-galactoside) from *Cornus mas* (cornelian cherry). HFD-obese mice that received anthocyanins exhibited a non-obese pattern in the glucose tolerance test while HFD-obese mice showed substantial glucose intolerance [67]. Kaempferol and quercetin isolated from *Euonymus alatus* were shown to improve insulin-stimulated glucose uptake in 3T3-L1 mature adipocytes [53].

In addition, Zhang et al. [62] showed that scopoletin, a phenolic coumarin, had beneficial effects on insulin-resistant HepG2 cells. Insulin resistance was evaluated by measuring PI3K-linked protein kinase B/Akt (Akt/PKB). Thereafter, scopoletin was able to stimulate the reactivation of insulin-mediated Akt/PKB phosphorylation, which was greater compared to the positive control rosiglitazone, a thiazolidinedione and activator of PPARγ that markedly improves insulin and glucose parameters in T2DM patients [129]. In 3T3-L1 adipocytes, scopoletin upregulated the expression of PPARγ2, an isoform of PPARγ that has critical functions in adipocyte differentiation, lipid storage, and glucose metabolism [130].

Accordingly, Lee et al. [76] also reported the beneficial effects of noni fruit in diabetes. They used noni fruit powder fermented by Cheonggukjang, which is a fast-fermented soybean paste, and bacteria, such as *Bacillus subtilis* (KCTC11352BP), *Bacillus sonolensis* (KCTC11354BP), *Bacillus* sp. (KCTC 11351BP) and *Bacillus circulans* (KCTC 11355BP). The data showed that a fNJ (FMC)-based diet, for 90 days was effective in reducing fasting glucose and glycosylated hemoglobin (HBA1c), enhancing insulin sensitivity and decreasing LDL, triglycerides and cholesterol in KK-Aγ diabetic mice. These responses are believed to be due to the activation of PPAR-γ and AMPK phosphorylation (Table 3).

In fact, when HEK293 cells were transfected with a plasmid containing the PPAR-γ response-element-driven luciferase reporter gene, fermented noni extract activated the PPAR-γ-dependent luciferase activity. In addition, this compound stimulated glucose uptake in C2C12 culture cells via activation of the AMPK pathway. These effects could be due to the presence of anthraquinones, flavonoids and terpenoids [76].

The evaluation of dried roots of *Morinda citrifolia* were extracted with methanol, suspended in water (H_2O), and partitioned in different parts of chloroform ($CHCl_3$), ethyl acetate (EtOAc), and n-butanol (*n*-BuOH). Sequentially, the solvents were removed from these different parts in order to generate the soluble phases: $CHCl_3$, EtOAc, *n*-BuOH, and H_2O. Therefore, the fractions

of soluble phases of methanol extract from *Morinda citrifolia* roots (MRE) were administrated orally to streptozotocin-induced ddY diabetic male mice (single administration). Only *n*-BuOH exhibited a significant reduction of blood glucose levels after five hours of administration, whereas methanol extract and other soluble phases did not display any hypoglycemic effects. Hence, after isolation of compounds from the *n*-BuOH fraction, two iridoids and three anthraquinones were identified, where two anthraquinones, lucidin (lucidin 3-*O*-β-D-primeveroside), and damnacanthol-3-*O*-β-D-primeveroside, were responsible for the hypoglycemic effects [73] (Table 3).

Likewise, anthraquinones are important agents in the treatment of diabetes [72]. Three anthraquinones (1,2-dimethoxyanthraquinone, alizarin-2-methyl ether and rubiadin-1-methyl ether) were isolated from the *n*-hexane and $CHCl_3$ fractions of *Morinda officinalis* roots and used to investigate fat accumulation in 3T3-L1 pre-adipocytes using the oil red O staining method. Alizarin-2-methyl ether was the compound that produced the highest increase in adipocyte differentiation followed by rubiadin-1-methyl ether and 1,2-dimethoxyanthraquinone [72].

Morinda citrifolia also displayed positive effects in streptozotocin (STPZ)-diabetic rats [70,71,131,132]. In diabetic patients, wounds are very complex to manage due to impaired wound-healing. Nayak et al. [131] evaluated the wound-healing effects of NJ on an excision wound model in induced diabetic rats. These animals exhibited improvement in wound-healing after consuming NJ. The wound area was reduced earlier and had less dead tissue at the wound site in NJ-treated rats than in their respective controls. The authors correlated wound-healing improvement with low fasting glucose, which was also found to be reduced. Triterpenoids and tannins are bioactive compounds that promote wound-healing due to their astringent and antimicrobial properties, promoting wound contraction and increasing the rate of epithelialization. Furthermore, these substances, especially triterpenoids, may have hypoglycemic effects [133].

The anti-diabetic effects of fNJ could be seen in another STPZ-diabetic rat model [132]. A possible explanation for these effects was the presence of saponins, triterpenes, steroids, flavonoids (rutin), and cardiac glycosides in the extract. However, the group attributed these effects to triterpenes and saponins, the principal compounds that show the highest specific actions on glucose metabolism. Norberg et al. [69] reported that saponins may have a glucagon decreasing effect and may enhance glucose utilization, thereby lowering blood glucose. Moreover, saponins stimulate insulin release from the pancreas due to diminishing degradation of glucagon-like peptide (GLP). Triterpenoids have already been indicated as beneficial agents in diabetes mellitus, especially in alloxan-induced mice, improving symptoms of glycosuria and elevated blood sugar [134,135].

These data corroborate another study [70] in which aqueous and methanol MFE were administered to STPZ-diabetic rats for one week before diabetes induction, three days during induction, and five weeks afterwards. Both MFE reduced blood glucose, glycosylated hemoglobin, blood urea, and creatinine levels, which were explained by the possible prevalence of antioxidants (vitamin C, vitamin E, flavonoids, terpenoids, and anthraquinones) in these extracts. In the same animal model, an antihyperglycemic effect and antioxidant activity were observed for ethanolic MFE given for 30 days [71].

These antioxidant properties were demonstrated by thiobarbituric acid reactive substance (TBARS), hydroperoxidose, and enzymatic and non-enzymatic antioxidants, such as catalase (CAT), glutathione, superoxide dismutase (SOD), and vitamins C and E, respectively. It is believed that these beneficial effects of noni were due to the synergistic effect of several biologically-active ingredients in the extract, which provides for the antioxidant nature of the extract [71]. Another synergistic effect of components of NJ was observed in alloxan-diabetic Sprague-Dawley rats, whereas NJ given for four weeks combined with insulin was more effective in lowering fasting glucose levels compared to the use of NJ or insulin alone [136].

3.3. Morinda citrifolia and Non-Alcoholic Fatty Liver Disease (NAFLD)

The liver is an important organ that possesses a fundamental role in metabolic homeostasis, such as in the process of lipogenesis, gluconeogenesis, and cholesterol metabolism. In recent decades, a variety of pathological conditions emphasize the importance of metabolic functions that occur in the liver. The increased prevalence of obesity and metabolic syndrome lead to pathophysiological changes that may result in the development of non-alcoholic fatty liver disease (NAFLD) [137].

NAFLD is considered one of the modern diseases of the new era, being the major cause of mortality and morbimortality related to chronic liver diseases. Most of the time, this pathology occurs in 25% of the population, increasing to 70% in obese and T2DM patients [138–140]. NAFLD is a liver disease that may progress from hepatic steatosis alone, without inflammation and hepatocellular damage, to steatohepatitis with lobular inflammation, and with evidence of hepatocyte injury called non-alcoholic steatohepatitis (NASH). Many patients that have NASH develop liver fibrosis, which may result in hepatocyte death, cirrhosis, and hepatocellular carcinoma, with high chances for the need of liver transplantation [141].

Despite some studies having demonstrated hepatotoxic effects of noni in humans and animals [41,82,83], a few others have reported hepatoprotective effects of noni, but it has only been explored in obese animals [43,51]. The effects of *Morinda citrifolia* in NAFLD was performed by Lin et al. [43]. They reported that HFD-induced obese male Golden Syrian hamsters supplemented with different doses of NJ showed diminished biomarkers of liver damage, namely alanine transaminase (ALT), along with diminished TNF-α, IL-1β, inducible nitric oxide synthase (iNOS), cyclooxygenase 2 (COX-2), and metalloproteinase 9 (MMP9) levels, and improved morphological characteristics of hepatic steatosis, such as a decrease in microvesicular steatosis and blurred cellular boundaries. In addition, NJ supplementation in HFD-obese hamsters decreased serum and liver total cholesterol and triglycerides, improved liver antioxidative capacity (CAT, SOD, glutathione peroxidase (GSH-Px), GSH, trolox equivalent anti-oxidative capacity (TEAC)) and lowered liver lipid peroxidation (TBARS) (Table 4).

Table 4. Effects of the administration of the *Morinda citrifolia* L. plant on non-alcoholic fatty liver disease (NAFLD).

Host	Part of Plant	Dose/Time	Effects	Reference
Hamster	Fruit noni juice (2.14 g crude polysaccharides/100 mL)	−3 mL (including 64.23 mg crude polysaccharides/kg body weight/6 weeks.	−Diminished visceral fat in HFD-hamsters (all doses).	Lin et al. [43]
			−Lowered serum and liver lipids: total cholesterol and triglycerides in HFD hamsters (all doses).	
		−6 mL (including 128.46 mg crude polysaccharides/kg body weight/6 weeks.	−Beneficial effects on the liver and hepatic enzymes (ALT) in HFD hamsters (all doses).	
			−Increased antioxidant capacity in the liver in HFD hamsters (all doses).	
		−9 mL (including 192.69 mg crude polysaccharides/kg body weight/6 weeks).	−Diminished inflammatory biomarkers in the liver (TNF-α, MCP-1, IL-1β) in HFD hamsters (all doses).	
			−Lowered gelatinolytic levels of MMP9 in HFD hamsters (all doses).	
Mice	Fruit fermented noni juice	−1.5 µL/g body weight/twice daily/12 weeks.	−Inhibited weight gain after 12 weeks.	Nerurkar et al. [49]
			−Improved glucose and insulin tolerance and fasting glucose in HFD-fed C57Bl/6 mice.	
			−Improved hepatic insulin resistance by FOXO-1 and inhibition of PEPCK and G6P (gluconeogenic enzymes).	

Effects of administration of different doses and parts of the *Morinda citrifolia* plant on NAFLD in in vivo studies.

All of these beneficial effects are possibly due to the large amount of phenolic acids present in NJ. Large amounts of phenolic acids, such as gentisic acid, p-hydroxybenzoic acid, and chlorogenic acid, are the dominant compounds in this juice [43]. Many studies have demonstrated that phenolic compounds act as reactive oxygen species (ROS) scavengers, reducing lipid peroxidation, as well. In vitro studies conducted by Joshi et al. [142] pointed to gentisic acid as the antioxidative and ROS scavenging agent. If those effects are also beneficial to humans it remains unknown and further study is necessary.

The effects of noni juice compounds have been extensively studied in animals. Chlorogenic acid was able to enhance the activity of the important antioxidant enzymes SOD, CAT and GSH-Px in STPZ-nicotinamide-induced type 2 diabetic rats [143]. Complementary studies reported that aqueous extract of *Mesona procumbens*, which has chlorogenic acid as a major compound, has anti-inflammatory action via the upregulation of antioxidants and downregulation of pro-inflammatory biomarkers (TNF-α, iNOS and COX-2) [144], and it exhibits anti-obesity effects and improves lipid metabolism [145].

In corroboration of the hepatic benefits of fNJ supplementation in an obese model, Nerurkar et al. [49] demonstrated that fNJ produced positive effects on plasma glucose levels by modulating hepatic gene expression of phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase (G6P) and glucokinase (GCK). PEPCK and G6P are important gluconeogenic enzymes regulated by insulin. They were inhibited after fNJ supplementation, which was confirmed with HepG2 culture cells treated with FOXO1 siRNA and fNJ. GCK was upregulated by fNJ via forkhead box O1 (FOXO1) transcription factor phosphorylation. The hypoglycemic properties of fNJ were associated with the inhibition of hepatic FOXO1 mRNA with concomitant increase in FOXO1 phosphorylation. Consequently, fNJ improved hepatic insulin resistance indicated by homeostatic model assessment-insulin resistance (HOMA-IR) (Table 4).

Those effects may be attributed to flavonoids, quercetin, and anthocyanins, specifically cyanidin-3-*O*-rutinoside, which were isolated from methanolic extracts of fNJ. Some studies demonstrated the inhibitory effect of anthocyanins on oxidative stress via FOXO transcription factor regulation in *Caenorhabiditis elegans* [54,126]. In support of these studies, fNJ promoted the reduction of hepatocyte fatty degeneration (smaller fatty globules and less numerous) in a model of STPZ-diabetic rats. It was suggested that the hepatoprotective activity of *Morinda citrifolia* was due to the antioxidant activity of flavonoid constituents [132].

Anthocyanins (delphinidin and cyanidin) isolated from *Hibiscus sabdafera* extract (HSE) also showed positive effects against obesity and liver damage in HFD-obese hamsters. HSE and anthocyanins regulated total body weight and visceral fat, reduced serum cholesterol and triglyceride levels, protected against oxidation-associated damage in liver by regulating a liver antioxidant enzyme (paraoxonase 1), and also reduced liver damage biomarkers ALT and AST [146].

Another study evaluated the benefits of scopoletin in reducing obesity and liver damage by supplementing the diet with two doses of scopoletin in a HFD model of obese mice. Supplementation resulted in reduced body weight, visceral fat, pro-inflammatory adipokine serum levels (leptin, MCP-1, TNF-α, IL-6, IFNγ), insulin resistance, and hepatic lipid accumulation and, on the other hand, increased serum adiponectin and fecal lipid levels. Moreover, supplementation was able to downregulate genes, such as CIDEA (cell death-inducing DFFA-like effector A) and Apoa4 (apolipoprotein A-IV), which are known to be related to hepatic steatosis and inflammation [147].

3.4. Morinda citrifolia and Dyslipidemia/Hypertension

Atherosclerosis is the primary cause of heart disease and stroke. This problem is most common in obese, hypertensive, dyslipidemic, and diabetic patients leading to vascular damage [148]. Although hypertension and dyslipidemia are independent risk factors that lead to atherosclerosis, the latter is also a risk factor for CVDs, such as stroke and myocardial infarction and hypertension. In this way, both dyslipidemia and hypertension are important risk factors for the progression and development of

atherosclerosis [149–151]. Moreover, these factors are serious pathological conditions for endothelium damage, causing cell proliferation, vascular remodeling, apoptosis, and enhancement of cellular permeability with adhesion molecules that bind monocytes and T lymphocytes. The latter cells are redirected into the intima vasculature by pro-inflammatory and chemoattractant cytokines. Hence, monocytes differentiate into macrophages which overloaded excessive oxidized LDL, become foam cells, elaborate cytokines, and then form atherosclerotic plaques [148].

The atherogenic dyslipidemic phenotype is characterized by high plasma triglycerides, low levels of high-density lipoprotein cholesterol (HDL), and excessive LDL. Additionally, postprandial (non-fasting) triglycerides (postprandial hyperlipidemia) are also an important component of atherosclerosis [152]. The modern synthetic drugs that have been used as treatment for lipid abnormalities are effective at reversing the measured signs, such as decreased LDL levels, but are difficult to afford for many patients and are associated with several side-effects [153]. *Morinda citrifolia* has been demonstrated to be an alternative therapy for this problem. A current study evaluated the effects of NJ on serum lipid profiles in 132 heavy smokers (drinking 29.5 mL to 188 mL of NJ per day). Heavy smoker volunteers who drank NJ displayed a reduction in cholesterol levels, triglycerides, and high-sensitivity C-reactive protein (hs-CRP), a decrease in LDL and homocysteine, and an increase in HDL fraction [25].

However, the few human studies available did not address this issue. Thus, clinical trials are necessary to validate the beneficial qualities of *Morinda citrifolia* bioactive compounds in human metabolic diseases.

In animals, a recent study performed by Shoeb et al. [111] demonstrated that supplementation with two doses of fNJ in cholesterol-rich HFD-induced hyperlipidemia rats showed a significant decrease in total cholesterol, triglycerides, and LDL at both doses when compared to the hyperlipidemic group. The decrease in total cholesterol was observed with the lower dose of fNJ reaching similar values as the positive control atorvastatin (10 mg/kg). Furthermore, the lower dose reduced body weight compared to the hyperlipidemic group and it was also comparable to the hyperlipidemic group receiving atorvastatin (Table 5).

These data corroborate the hypolipidemic effects of noni in a work done by Mandukhail et al. [154]. These authors compared ethanolic extracts of different parts of *Morinda citrifolia* and evaluated different doses of MFE, MLE, and MRE extracts in tyloxapol (Triton WR 1339)-induced hyperlipidemia and HFD-induced dyslipidemia models, both in rats. The highest dose of all extracts produced a significant reduction in total cholesterol and triglyceride levels in the WR 1339 rat group. In contrast, the HFD-induced dyslipidemia group showed different results depending on the extracts at the highest dose of each. Both MFE and MLE prevented the rise in total cholesterol, LDL, total cholesterol/HDL ratio, and atherogenic index without significant effects on HDL. However, MLE prevented the increase in glucose levels and body weight in these animals, while MRE was the best extract in this study, which proved to prevent the rise in all lipid and glucose levels and, at the same time, increasing HDL and preventing weight gain, suggesting antidyslipidemic mechanisms for various parts of the noni plant (Table 5).

Hypolipidemic and other positive effects of *Morinda citrifolia* were suggested in two studies [111,154] that demonstrated the presence of strong antioxidant activity in the noni plant. According to previous studies, Kamiya et al. [60] demonstrated the effects of fruits of *Morinda citrifolia* in preventing atherosclerosis. MFE and its soluble phases ($CHCl_3$, EtOAc, n-BuOH, H_2O) inhibited copper-induced LDL oxidation according to the TBARS method. Lignans were isolated in the EtOAc-soluble phase, including 3,3′-bisdemethypinoresinol, americanol A, morindolin, and isoprincepin, which showed remarkable or strong antioxidant activity. Thus, lignan compounds of noni fruit are involved in the prevention of atherosclerosis, likely due to their numerous phenolic hydroxyl groups (Table 5).

Table 5. The effects of administration of the *Morinda citrifolia* L. plant on dyslipidemia.

Host	Part of Plant	Dose/Time	Effects	Reference
Rats	Fruit (Noni juice)	−50 mg/kg/day/ 30 days.	−Reduced body weight (better at 50 mg/kg/day dose).	Shoeb et al. [111]
		−100 mg/kg/day/ 30 days.	−Reduced serum total cholesterol, triglycerides, and lipids fractions: LDL and VLDL (all doses).	
			−Increased lipid fraction HDL (all doses).	
Rats Mice	Fruits, leaves and roots (70% ethanolic aqueous extract).	−Fruit ethanol extract (1000 and 500 mg/kg/day).	−All of the extracts on tyloxapol-induced hyperlipidemia: reduced total cholesterol and triglyceride levels.	Mandukhail et al. [154]
		−Leaf ethanol extract (1000 and 500 mg/kg/day).	−MFE in HFD-induced dyslipidemia (1000 mg/kg/day): prevented the rise of serum total cholesterol, LDL, total cholesterol/HDL ratio, and atherogenic index. No significant effects on HDL and glucose levels. No effect on body weight.	
		−Root ethanol extract (500 and 300 mg/kg/day)	−MLE in HFD-induced dyslipidemia (1000 mg/kg/day): prevented the rise in serum total cholesterol, LDL, total cholesterol/HDL ratio, atherogenic index, and glucose levels. No significant effects on HDL. Significantly prevented the gain in average body weight.	
			−MRE in HFD-induced dyslipidemia (500 mg/kg/day): prevented the rise in serum total cholesterol, LDL, total cholesterol/HDL ratio, atherogenic index, and glucose levels. Increased HDL. Significantly prevented the gain in average body weight.	
In vitro	Fruit (methanolic extract-soluble phases: $CHCl_3$, EtOAc, nBuOH, H_2O).	−Effective EtOAc purified and isolated lignans	−Lignans isolated from EtOAc fraction of methanol extract inhibited activity against copper-induced LDL oxidation by measuring the decrease in TBARS.	Kamiya et al. [60]
Hamster	Fruit (Fermented Noni Juice).	−3 mL NJ (containing 0.20 g solids/kg body weight)/day/6 weeks.	−Reduced sizes of heart, liver and visceral fat in HFD-cholesterol hamsters.	Lin et al. [48]
		−6 mL NJ (containing 0.40 g solids/kg body weight)/day/6 weeks	−Decreased serum triglycerides, total cholesterol, atherogenic index, malondialdehyde levels and hepatic lipids in HFD-cholesterol hamsters.	
		−9 mL NJ (containing 0.60 g solids/kg body weight) /day/6 weeks.	−Increased trolox equivalent antioxidant capacity (TEAC), glutathione (GSH), fecal lipids in HFD-cholesterol hamsters.	
			−Downregulated sterol regulator element binding protein-1c (SREBP-1c) and upregulated hepatic peroxisome proliferator-activated receptor-alpha (PPAR-α) and uncoupling protein 2 (UCP-2) mRNA in HFD-cholesterol hamsters.	

Effects of the administration of different doses and parts of the *Morinda citrifolia* plant on dyslipidemia in in vivo and in vitro studies.

Furthermore, Lin et al. [48] evaluated supplementation with fNJ at different concentrations in HFD-cholesterol hamsters. They found that the group supplemented with fNJ displayed hypolipidemic and antioxidative effects, demonstrated by decreases in serum triglyceride, total cholesterol, atherogenic index, malondialdehyde levels, and hepatic lipids, while antioxidant activity (TEAC and GSH) and fecal lipids were increased (Table 5).

To evaluate the effect of fNJ on lipid metabolism and mechanisms of actions, important genes related to lipid homeostasis were evaluated in the liver, which is the major organ in the regulation of lipid homeostasis. The data showed that SREBP-1c was upregulated after fNJ treatment; this is an important transcription factor that stimulates the expression of lipogenic genes, such as that of fatty acid synthase (FAS). This enzyme is responsible for the biosynthesis of FA. In energy expenditure,

peroxisome proliferator-activated receptor-alpha (PPAR-α) upregulates uncoupling protein 2 (UCP2), which increases thermogenesis, while reducing the efficiency of ATP synthesis. In this regard, the gene expression of PPAR-α, as well as UCP-2, was upregulated in the liver after fNJ supplementation. The bioactive compounds responsible for the effects of fNJ were gentisic acid, which was the phenolic in the highest amount, followed by *p*-hydroxybenzoic acid and chlorogenic acid [48].

Many alternatives of anti-hypertensive therapies have been widely studied. Accordingly, some authors have focused on *Morinda citrifolia* as an alternative therapy for hypertension. Using an animal model of hypertension, Wigati et al. [55] investigated the action of ethanolic MLE, MFE, and the combination of both on blood pressure in dexamethasone-induced hypertensive rats, where this model is characterized by nitric oxide deficiency and oxidative stress. All extracts decreased blood pressure, but were not able to repair or inhibit renal damage caused by dexamethasone induction. However, the combination of the two extracts had the highest hypotensive activity. According to the study, the phenolic compounds, such as rutin as a marker in MLE and scopoletin in MFE, were the agents responsible for the hypotensive effects (Table 6).

Table 6. The effects of the *Morinda citrifolia* plant on hypertension.

Host	Part of Plant	Doses/Time	Effects	Reference
Rats	Fruit and leaf (ethanolic extract)	−Ethanolic extract of leaves (500 mg/kg body weight)/14 days	−Reduced blood pressure in dexamethasone-induced hypertensive rats (all doses and extracts).	Wigati et al. [55]
		−Ethanolic extract of fruit (500 mg/kg body weight)/14 days	−The highest hypotensive effect was with the combination of the extracts.	
		−Ethanolic extract of leaves + fruit (1:1, 500 mg/kg body weight)/14 days		
Rabbit, Rat and Guinea-pig	Roots (70% ethanolic- aqueous extract)	−Different concentrations on rabbit jejunum, thoracic aorta of rats, guinea pig atria in vitro * study.	−Rabbit jejunum: produced concentration-dependent relaxation of spontaneous and high K^+-induced concentrations (antispasmodic effect).	Gilani et al. [93]
		−Positive control: verapamil (different concentrations in vitro * study).	−Guinea pig atria: caused inhibition of both atrial force and rate of spontaneous contractions (cardiopressant activity).	
		−* Concentration-response curve.	−Rabbit thoracic aorta: suppressed contractions induced by phenylephrine (1.0 μM) in normal –Ca^{+2} and Ca^{+2} - free Krebs solution- and by high K^+, like the positive control (verapamil).	
			−Rat thoracic aorta: also caused relaxation of the phenylephrine (1.0 μM)-induced contractions (vasodilatory activity).	
Rats	Fruit (noni fruit juice)	−5 mg/kg (single administration after 24 h).	−Increased urine volume in a dose-dependent manner (10 mg/kg higher than 5 mg/kg)	Shenoy et al. [63]
		−10 mg/kg (single administration after 24 h).	−Decreased ion excretion (sodium and potassium)	
			−Aquaretic action.	

Effects of administration of different doses and parts of *Morinda citrifolia* plant on hypertension in in vivo studies. * Studies involving animal tissues in an in vitro assay.

Previous studies have demonstrated that rutin and scopoletin are important phenolic compounds that affect the cardiovascular system, including blood pressure regulation. Rutin possesses renal-protective activity probably by inhibiting ROS production and through antioxidant activities, reducing elevated malondialdehyde levels and restoring depleted manganese-superoxide dismutase (MnSOD) and GSH, with positive effects on biochemical parameters, as well as on the histopathological morphology of the kidneys [56]. Scopoletin also demonstrated hypotensive effects and relaxation

of rat aorta. In addition, a possible inhibitory activity of angiotensin converting enzyme-1 (ACE1) was suggested as a property of this phenolic compound [155,156]. Asperulosidic acid, an iridoid glycoside present in MFE, showed substantial positive effects on blood fluidity and improved certain lifestyle-related diseases, such as hypertension, dyslipidemia, and diabetes [80].

MRE showed antispasmodic and vasodilator activities mediated through blockade of voltage-dependent calcium channels in isolated tissues of rats, guinea pigs, and rabbits [93] (Table 6). These effects were mediated by alkaloids, phenolic compounds, sterols, flavonoids, tannins, coumarins, and anthraquinones, which corroborate a study on *Zingiber officinale* Roscoe (ginger) that evaluated its hypotensive effects [157]. The same bioactive compounds that were found in the noni plant were also detected in ginger and could affect isolated tissues of rats, rabbits, and guinea pigs. The crude extract of ginger decreased blood pressure and exhibited a cardiodepressant activity, with the activity being mediated by Ca^{+2} channel-blocking, which was demonstrated when crude ginger extract shifted the Ca^{+2} dose-response curve to the right, mimicking the effect of the positive control verapamil.

Considering that diuretics are used in the treatment of hypertension, Shenoy et al. [63] evaluated the diuretic potential of NJ in normal rats. The effects observed were an increase in urine volume in a dose-dependent manner with augmentation of the diuretic index accompanied by a significant decrease in sodium and potassium ion excretion. The authors demonstrated that the noni plant had aquaretic, instead of diuretic, actions (Table 6). Some authors believe that herbs act only as aquaretic agents, which increase water excretion without affecting renal handling of electrolytes. In other words, aquaretics only increase urine output, acting on the glomerulus, unlike conventional diuretic drugs that act further along the nephron [63,64].

Herbs often contain large amounts of minerals (electrolytes) and noni fruit has a high content of potassium. Hook et al. [65] evaluated the diuretic effect of *Taraxacum officinale* Weber (dandelion) in normal mice and did not observe any significant variation in electrolytes (Na^+, K^+, Ca^{+2}), but the final volume of urine produced after five hours was greater than with the positive control, furosemide. That study concluded that the high potassium content of dandelion was responsible for any diuretic activity, where dandelion was similar in action to noni fruit and, thus, the increased urinary volume could be suggestive of an osmotic effect [63,65].

3.5. Morinda citrifolia and the Effect on Gut Microbiota

There is a vast community of gut microbes [158]. Much has been invested in the search for nutrients that are selective for a favorable modification of intestinal microbiota, especially those able to increase the amount of *Bifidobacterium* and *Lactobacillus*. The dysbiosis of the gut microbiota has been associated with the development and progression of many human diseases. The ratio of some microbiota species are greater in obese, rather than in lean, individuals [159,160]. Noni, and its juice, exhibit antimicrobial properties and high antioxidant activity, which would be beneficial for a healthy intestinal microbiota [161,162].

As observed in Table 7, fNJ showed a probiotic character by allowing a greater growth of *Lactobacillus*, as well as *Bifidobacterium*, species. This is possible because NJ (as a raw substrate) has a fermentative process with lactic acid bacteria (*Lactobacillus casei* and *Lactobacillus plantarum*) or *Bifidobacterium* (*Bifidobacterium longum*) [161,162].

Table 7. Effects of administration of *Morinda citrifolia* L. on gut microbiota.

Host	Methods	Effects	Reference
In vitro	Time courses of lactic acid fermentation of noni juice by *Lactobacillus casei*, *Bifidobacterium longum* and *Lactobacillus plantarum*	−All reached almost 10×10^8 CFU/mL after 48 h of fermentation at 30 °C	Wang et al. [161]
In vitro	Human stool sample from a healthy volunteer (10 g) with ethanolic extracts of fermented noni fruit	−*Bifidobacterium* and *Lactobacillus* species, presented growth (0.16–0.63 mg/mL for *Bifidobacterium* and *Lactobacillus* spp.) compared with negative control (0 mg/mL)	Huang et al. [162]
Hybrid duck	Supplementation with noni fruit powder using different concentrations	−Increased amount of lactic acid bacteria with supplementation with 2% noni fruit powder; −Decreased *Escherichia coli* with supplementation with 3% noni fruit powder.	Kurniawan, Widodo, Djunaidi [163]

Effects of administration of *Morinda citrifolia* on gut microbiota in in vivo and in vitro studies.

In addition, noni powder also has a prebiotic action. One of the reasons may be the high amount of polysaccharides in the fruit, since carbohydrates, except for starch, act as dietary fiber, coming intact with the gut and contacting the bacterial community present in the intestinal microbiota [164]. The high molecular weight fraction of NJ is mostly composed of pectic polysaccharides, including rhamnogalacturonan, homogalacturonan, and the neutral side chains of (arabino) galactan and arabinan [165].

Another reason may be the high phenolic composition of the fruit, which may also have a prebiotic function [162], such as quercetin and proanthocyanidin [166,167]. Previous studies have also indicated that phenolic compounds can inhibit the growth of pathogens such as *Escherichia coli* and *Helicobacter pylori* [166].

In addition to the microbiota, the size and height of the villi are important for intestinal function. Diet plays an important role in intestinal morphology [163]. The mucus layer in the small intestine protects the epithelial cells of the small intestine and mediates nutrient transport between the lumen and the membrane of the brush border. The ontogeny of the whole gut has extensive implications for intestinal function [168].

Supplementation with 1% noni fruit powder caused an increase in villus height, villus surface area, and crypt depth when compared with control [163]. Although few studies have evaluated noni fruit with regard to the microbiota and intestinal function, the in vitro and in vivo results presented in Table 7 show that noni fruit shows prebiotic activity, when administered alone, and probiotic activity, when used in fNJ, improving bacterial colonization and intestinal morphology.

4. Conclusions

Bioactive compounds from natural resources are a promising field of study for alternative medicines, where *Morinda citrifolia* Linn. (noni) is one of these important options. Noni has demonstrated positive effects in metabolic dysfunction, including the regulation of body weight and fat deposits, lipid and glucose metabolism and blood pressure, hepatoprotective effects, and improvement in bacterial colonization of the gut and intestinal morphology. However, *Morinda citrifolia* has never been an important food plant, probably due to its poor palatability and toxicity, in its natural homeland. This review reports the influence of the noni plant and its bioactive compounds, emphasizing the potential mechanisms of action and effects on cell signaling pathways involved in obesity and obesity-related metabolic dysfunction. The noni plant may be processed for some possible medicinal properties after the management of toxicity, or some parts of the plant may be mixed with

less nutritional, but appetizing, food for health benefits. Therefore, doses and the time of treatment or long-term supplementation, and also new alternatives in the near future to improve the bioavailability of the product, are very important issues to be evaluated. Finally, since *Morinda citrifolia* contains important bioactive compounds for health, it may be an alternative therapeutic resource with great potential in the treatment of obesity and obesity-related metabolic dysfunction.

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CAPÍTULO 3. THERAPEUTIC EFFECTS OF *MORINDA CITRIFOLIA* LINN. (NONI) AQUEOUS FRUIT EXTRACT ON THE GLUCOSE AND LIPID METABOLISM IN HIGH-FAT/HIGH-FRUCTOSE-FED SWISS MICE

Aline Carla Inada1*, Gabriela Torres Silva1, Laleska Pâmela Rodrigues da Silva1, Flávio Macedo Alves2 , Wander Fernando de Oliveira Filiú3, Marcel Arakaki Asato4, Wilson Hino Kato Junior5, Joaquim Corsino5, Patrícia de Oliveira Figueiredo5 , Fernanda Rodrigues Garcez5, Walmir Silva Garcez5, Renée de Nazaré Oliveira da Silva6 , Rosangela Aparecida dos Santos-Eichler6 , Rita de Cássia Avellaneda Guimarães1, Karine de Cássia Freitas1 and Priscila Aiko Hiane1

1Post Graduate Program in Health and Development in the Central-West Region of Brazil, Federal University of Mato Grosso do Sul, Campo Grande, MS 79070-900, Brazil; gabitorres483@gmail.com (G.T.S.); laleskaprodrigues@gmail.com (L.P.R.d.S.); rita.guimaraes@ufms.br (R.d.C.A.G.); kcfreitas@gmail.com (K.d.C.F.); priscila.hiane@ufms.br (P.A.H.)

2 Institute of Biosciences, Federal University of Mato Grosso do Sul-UFMS,

Campo Grande, MS 79070-900, Brazil; flaurace@yahoo.com.br (F.M.A.)

3Faculty of Pharmaceutical Science, Food and Nutrition, Federal University of Mato Grosso do Sul-UFMS, Campo Grande, MS 79070-900, Brazil; wander.filiu@gmail.com (W.F.O.F.)

4Faculty of Medicine, Federal University of Mato Grosso do Sul—UFMS, Campo Grande, MS 79070-900, Brazil; marcel_arakakiasato@hotmail.com (M.A.S.)

5Laboratory PRONABio (Laboratory of Bioactive Natural Products) — Chemistry Institute, Federal University of Mato Grosso do Sul-UFMS, Campo Grande, MS 79070-900, Brazil; hinokato@gmail.com (W.H.K.J.); corsinojoaquim@gmail.com (J.C.); patriciadeoliveirafigueiredo@gmail.com (P.d.O.F.); fernandagarcez@gmail.com (F.R.G.); walmir.garcez@ufms.br (W.S.G.)

6 Department of Pharmacology, Biomedical Sciences Institute, University of São Paulo, São Paulo, SP 05508-900, Brazil; oliveirarenee@gmail.com (R.d.N.O.d.S.); reichler@icb.usp.br (R.A.d.S.-E.)

* Correspondence: inada.aline@gmail.com; Tel.: +55-(67)-3345-7410

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Therapeutic Effects of *Morinda citrifolia* Linn. (Noni) Aqueous Fruit Extract on the Glucose and Lipid Metabolism in High-Fat/High-Fructose-Fed Swiss Mice

Aline Carla Inada [1,*], Gabriela Torres Silva [1], Laleska Pâmela Rodrigues da Silva [1], Flávio Macedo Alves [2], Wander Fernando de Oliveira Filiú [3], Marcel Arakaki Asato [4], Wilson Hino Kato Junior [5], Joaquim Corsino [5], Patrícia de Oliveira Figueiredo [5], Fernanda Rodrigues Garcez [5], Walmir Silva Garcez [5], Renée de Nazaré Oliveira da Silva [6], Rosangela Aparecida dos Santos-Eichler [6], Rita de Cássia Avellaneda Guimarães [1], Karine de Cássia Freitas [1] and Priscila Aiko Hiane [1]

1 Post Graduate Program in Health and Development in the Central-West Region of Brazil, Federal University of Mato Grosso do Sul, Campo Grande, MS 79070-900, Brazil; gabitorres483@gmail.com (G.T.S.); laleskaprodrigues@gmail.com (L.P.R.d.S.); rita.guimaraes@ufms.br (R.d.C.A.G.); kcfreitas@gmail.com (K.d.C.F.); priscila.hiane@ufms.br (P.A.H.)
2 Institute of Biosciences, Federal University of Mato Grosso do Sul-UFMS, Campo Grande, MS 79070-900, Brazil; flaurace@yahoo.com.br
3 Faculty of Pharmaceutical Science, Food and Nutrition, Federal University of Mato Grosso do Sul-UFMS, Campo Grande, MS 79070-900, Brazil; wander.filiu@gmail.com
4 Faculty of Medicine, Federal University of Mato Grosso do Sul—UFMS, Campo Grande, MS 79070-900, Brazil; marcel_arakakiasato@hotmail.com
5 Laboratory PRONABio (Laboratory of Bioactive Natural Products)—Chemistry Institute, Federal University of Mato Grosso do Sul-UFMS, Campo Grande, MS 79070-900, Brazil; hinokato@gmail.com (W.H.K.J.); corsinojoaquim@gmail.com (J.C.); patriciadeoliveirafigueiredo@gmail.com (P.d.O.F.); fernandagarcez@gmail.com (F.R.G.); walmir.garcez@ufms.br (W.S.G.)
6 Department of Pharmacology, Biomedical Sciences Institute, University of São Paulo, São Paulo, SP 05508-900, Brazil; oliveirarenee@gmail.com (R.d.N.O.d.S.); reichler@icb.usp.br (R.A.d.S.-E.)
* Correspondence: inada.aline@gmail.com; Tel.: +55-(67)-3345-7410

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Abstract: The aim of this study was to evaluate the therapeutic effects of two different doses (250 and 500 mg/kg) of *Morinda citrifolia* fruit aqueous extract (AE) in high-fat/high-fructose-fed Swiss mice. The food intake, body weight, serum biochemical, oral glucose tolerance test (OGTT), and enzyme-linked immunosorbent assay (ELISA), as well as histological analyses of the liver, pancreatic, and epididymal adipose tissue, were used to determine the biochemical and histological parameters. The chemical profile of the extract was determined by ultra-fast liquid chromatography–diode array detector–tandem mass spectrometry (UFLC–DAD–MS), and quantitative real-time PCR (qRT-PCR) was used to evaluate the gene expressions involved in the lipid and glucose metabolism, such as peroxisome proliferative-activated receptors-γ (PPAR-γ), -α (PPAR-α), fatty acid synthase (FAS), glucose-6-phosphatase (G6P), sterol regulatory binding protein-1c (SREBP-1c), carbohydrate-responsive element-binding protein (ChREBP), and fetuin-A. Seventeen compounds were tentatively identified, including iridoids, noniosides, and the flavonoid rutin. The higher dose of AE (AE 500 mg/kg) was demonstrated to improve the glucose tolerance; however, both doses did not have effects on the other metabolic and histological parameters. AE at 500 mg/kg downregulated the PPAR-γ, SREBP-1c, and fetuin-A mRNA in the liver and upregulated the PPAR-α mRNA in white adipose tissue, suggesting that the hypoglycemic effects could be associated with the expression of genes involved in de novo lipogenesis.

Keywords: metabolic syndrome; synergistic effect; de novo lipogenesis

1. Introduction

Noncommunicable diseases (NCDs) are considered one of the major health risks of modern society and are increasing in morbidity and mortality in developed and undeveloped countries [1]. Metabolic syndrome (MetS) is among these NCDs [1], and, although MetS started in the Western world, it has become a global problem. MetS is not considered a single pathological condition, but a cluster of metabolic abnormalities, including abdominal obesity, insulin resistance (or type 2 diabetes mellitus), systemic hypertension, and atherogenic dyslipidemia [2] and the hepatic component, such as non-alcoholic fatty liver disease (NAFLD) [3]. The two main factors that may be responsible for spreading MetS are the adherence to the Western lifestyle that is characterized by the consumption of non-healthy food known as the Western-type diet, which is composed of high-calorie/low-fiber fast food, in association with a sedentary lifestyle [1].

High-fat and high-carbohydrate diets, such as the high-fat/high-fructose (HFF) diet, are examples of a Western-type diet and generally contain fructose and sucrose, as well as saturated fat [2]. Fructose, which is considered the sweetest of all naturally occurring carbohydrates, is found as a hexose in fruits and honey [4] and is used commercially in juice, soft drinks, high-fructose corn syrup, and baked goods [5]; therefore, the consumption of fructose has increased considerably in everyday diets [5]. The fructose that is present in fruits and honey is considered a modest component of energy intake by individuals; unfortunately, the vast majority of fructose in the diet is due to added sugars, which refers to sugars not naturally occurring in foods. The two most important sources of added sugars are sucrose (containing 50% of fructose) and high-fructose corn syrup (containing 42–55% fructose) [4].

Evidence demonstrated the use of aqueous extracts from *Phyllanthus emblica* fruits [6] and natural juices, such as orange and pomegranate [7,8], as potential nutraceuticals to prevent or treat metabolic disturbances associated with MetS. Another example of fruits with nutraceutical properties may be attributed to *Morinda citrifolia* Linn. (*M. citrifolia*), popularly known as noni fruit and Indian mulberry, used in the folk medicine of Polynesians 200 years ago [9]. In 1996, noni fruit juice began to be commercialized in the USA [10,11], and, after that, in 2003, the European Commission approved the commercialized fermented *M. citrifolia* fruit juice (Tahitian Noni Juice®) as a novel food [10].

Although, in the USA, Europe, and Asia, the commercialization of *M. citrifolia* products, such as juice and encapsulated powder, is common [12], in Brazil, this market is not yet legalized, due to controversies that revolve around consuming *M. citrifolia* fruit [13]. Certain studies reported hepatotoxicity signs [14,15], whereas other studies did not demonstrate toxic effects [16,17]. Another issue is regarding the lack of pharmacological studies that standardize doses, times of treatment, adverse effects, and interactions of products derived from noni fruit with other conventional drugs [13]. The astringent odor and bitter taste of *M. citrifolia* fruit led companies to produce manufactured juice with the addition of other types of fruit juices, including grape, blueberry, and cranberry, in order to mask the taste [12,18], leading to the consideration that the actions of bioactive compounds from *M. citrifolia* fruit could not be attributed solely to themselves due to the addition of other bioactive components from the other fruits.

The *M. citrifolia* fruit processing should also be considered relevant, as it can be manufactured in many ways, including the fermentation of fruit juice kept in sealed containers for 4–8 weeks, which is one of the most common processing methods [9,12]. Food processing may activate some beneficial microorganisms to promote a probiotic effect or deactivate spoilage microorganisms, and enzymes may modify certain nutritional factors or the phytochemical composition and organoleptic properties of the products [12,19,20]. Nutraceutical products are functional foods consumed by the population and have recently gained attention due to their benefits on health; however, most nutraceuticals are

not considered pharmaceutical drugs. Nutraceutical regulation is complex due to the fact that they do not fall under the jurisdiction of pharmaceutical regulatory authorities [21].

Studies have demonstrated therapeutic properties from *M. citrifolia* fruit, such as antioxidant [22], antiulcerogenic [23], anti-inflammatory [24], and anticancer [24,25] properties. Current studies demonstrated noni fruit effects in the prevention or treatment of metabolic diseases in animal models [26–32]; and, more recently, an important study reported the role of noni fruit juice on type 2 diabetes in humans [33].

Thus, to assess whether *M. citrifolia* fruit displays nutraceutical properties to improve metabolic disturbances present in MetS, the aim of the present study was to evaluate the effects of a crude aqueous extract from *M. citrifolia* Linn. (noni) fruit in the biochemical and histological parameters and in the expression of genes involved in the lipid and glucose metabolism in white adipose tissue and liver in high-fat/high-fructose-fed Swiss mice.

2. Materials and Methods

2.1. Plant Material

Fruits of *M. citrifolia* were individually selected on the tree and harvested in the municipality of Campo Grande, Mato Grosso do Sul, Brazil (20°27′04.5′′ S and 54°36′04.3′′ W) in March 2017. The tree was properly identified by Flávio Macedo Alves, of the Federal University of Mato Grosso do Sul. A voucher specimen (number 75818) was deposited at the Herbarium Campo Grande/MS (CGMS) of the Federal University of Mato Grosso do Sul, Brazil. This study was approved for the acquisition of samples for genetic access of components and for the associated traditional knowledge (SISGEN—A26D547).

The fruit was harvested while still unripe (pale yellow/stage 3), washed with water to remove superficial dirt, and ripened at room temperature for a day or more, until they reached the normal size and became cream-colored (translucent-grayish/stage 5), indicating physiological maturity and used by the population in folk medicine according to Chan-Blanco et al. (2006) [9].

2.2. Preparation of M. citrifolia Fruit Aqueous Extract (AE)

M. citrifolia fruit aqueous extract (AE) was prepared in the Laboratory of Natural Products at the Institute of Chemistry in the Federal University of Mato Grosso do Sul, Brazil. Approximately 400 g of the fruit with pulp, seed, and peel [34] was chopped, ground with a blender (Britânia, Goias, Brazil) in 400 mL of deionized water, and filtered with a sieve. The procedure of grinding the content that remained in the sieve with 400 mL water and filtration was repeated three times, to extract the maximum number of present compounds. After that, the AE was lyophilized, fractionated in 1.5 mL cryogenic tubes, and maintained at −80 °C, until the treatments, to avoid oxidation.

2.3. Ultra-Fast Liquid Chromatography–Diode Array Detector–Tandem Mass Spectrometry (UFLC–DAD–MS) Analyses

The aqueous extract of *M. citrifolia* (AE) was analyzed through a UFLC LC-20AD coupled to a diode array detector (Shimadzu, Kyoto, Japan) and ESIqTOF microTOF-Q III (Bruker Daltonics, Billerica, MA, USA) mass spectrometer. The ultraviolet (UV) wavelength was monitored between 240 and 800 nm and the mass spectrometer operated in negative ion mode (*m/z* 120–1200). Nitrogen was applied as a nebulizer gas (4 Bar) and dry gas (9 L/min). The capillary voltage was 2.5 kV. The aqueous extract was dissolved in methanol–water (85:15 *v/v*), at a concentration of 1 mg/mL, and eluted through a C-18 solid phase cartridge, followed by filtration on a polytetrafluoroethylene (PTFE) membrane (0.22 μm × 3.0 mm, Millipore). Subsequently, 2 μL was injected into a Kinetex C-18 column (2.6 μm, 150 × 2.2 mm, Phenomenex) protected by a pre-column packed with the same material. The mobile phase was ultrapure water (solvent A) and acetonitrile (solvent B), both containing 0.1% of formic

acid (*v/v*), and the gradient elution profile was as follows: 0–2 min, 3% of B; 2–25 min, 3–25% of B; and 25–40 min, 25–80% of B at a flow rate of 0.3 mL/min.

2.4. Ethical Statement

All animal experiments were submitted and approved by the Ethics Committee on Animal Use, Federal University of Mato Grosso do Sul (Protocol n° 860/2017).

2.5. Acute Oral Toxicity

The acute oral toxicity test of AE was performed in female Swiss mice (*Mus musculus*) [15] based on the OECD (Organization for Economic Co-Operation and Development) Guidelines 425 [35] (OECD, 2008). The animals were divided into two groups (n = 10): a control group that received drinking water gavage (n = 5) and the treatment group that received AE orally by gavage at a dose of 2000 g/kg (n = 5). After treatment, the animals were observed at 30 min, 1 h, 2 h, 3 h, 4 h, 6 h, 12 h, 24 h, and then daily for 14 days. A Hippocratic screening test was conducted to qualify the effects of abnormal morphological and behavioral signs of toxicity. Changes in the body weight, water consumption, and food intake were evaluated on the 1st, 5th, 10th, and 14th days, adapted according to Malone, 1968 [36].

At the end of 14 days, the animals were euthanized (ketamine and xylazine), and the organs (heart lungs, kidneys, liver, spleen, and pancreas) were collected and weighed [37].

2.6. Animals and Experiment Design

Swiss adult male mice (n = 42, 12 weeks of age) were initially divided into two homogenous groups according to weight: The control group (CT) (n = 11) was fed a regular chow diet (70% carbohydrate, 20% protein, and 10% fat) (Nuvilab, Colombo, PR, Brazil), with a caloric content of 3.8 kcal/g [38]; and the high-fat/high-fructose group (HFF) (n = 31) was fed a high-fat/high-fructose diet (21.20% carbohydrate; 18.86% protein; 4% soybean oil, 31% lard, and 20% fructose), with a caloric content of 5.45 kcal/g [39] for 9 weeks. After this, the animals of the HFF groups were divided into three homogeneous groups according to weight and were concomitantly supplemented (oral gavage) with *M. citrifolia* aqueous fruit extract (AE) at two different doses: HFFW (HFF + drinking water) (n = 10), HFF + AE 250 (HFF + *M. citrifolia* aqueous fruit extract at 250 mg/kg of body weight) (n = 11), and HFF + AE 500 (HFF + *M. citrifolia* aqueous fruit extract at 500 mg/kg of body weight) (n = 10) (Figure 1). The CT group also received drinking water (CTW) at this stage of the study. The drinking water was used as a vehicle to dissolve the lyophilized AE during the supplementation, and the doses were chosen according to Jambocus et al. (2016) [40]. All groups had ad libitum access to water and food during the experimental period and were kept in an alternating 12 h light/dark cycle, in a temperature-controlled room (22 ± 2 °C).

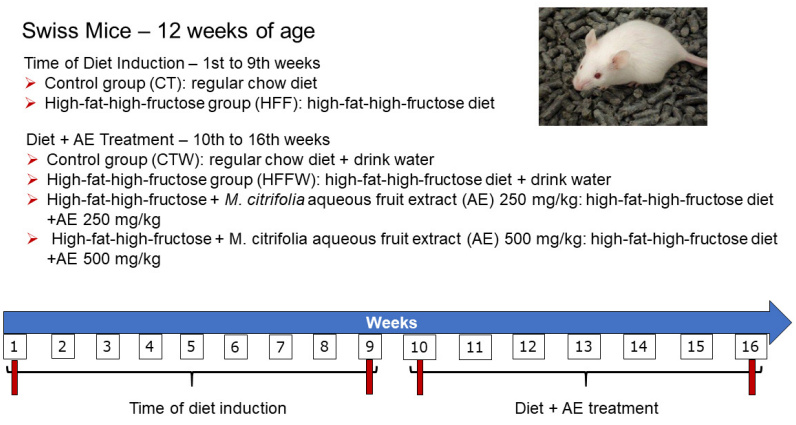

Figure 1. Schematic figure of the experimental design to assess the time of diet induction (from the 1st to 9th weeks) and diet + *Morinda citrifolia* aqueous fruit extract treatment (AE) (the 10th to 16th weeks).

After 16 weeks of the experiment, the animals were anesthetized with isoflurane (Isoforine, Cristália, Itapira, SP, Brazil) and euthanized by cardiac puncture [41]. The blood and organs were collected for subsequent analysis.

2.7. Assessment of Body Fat and Liver Weight

After euthanasia, the fat pads (retroperitoneal, epididymal, perirenal, omental, and mesenteric) and liver were dissected and weighed. The weight of each fat pad (mg) was normalized by the body weight (g) of the animal [38]. The adiposity index was calculated as the total sum of visceral white adipose tissue (g) divided by the final body weight of the animal ×100 and expressed as the percentage of adiposity [41–43].

2.8. Body Weight and Diet Intake

The mice were weighed two times per week, to evaluate weight changes up to the end of the experiment. The food intake was measured weekly and the feed efficiency index (FEI) [41,44], which refers to the amount of food consumed that can promote body weight gain, was calculated by using Equation (1), where FBW is the final body weight in grams, IBW is the initial body weight in grams, and TF is the total amount of food ingested in grams:

$$\text{FEI} = \text{FBW} - \text{IBW/TF} \quad (1)$$

2.9. Biochemical Analysis

Blood was collected from the inferior vena cava, during euthanasia, to evaluate the serum glucose, serum triglyceride, total cholesterol, high-density lipoprotein cholesterol (HDL-C), and non-HDL cholesterol (non-HDL-C), using the enzymatic colorimetric test (Labtest, Lagoa Santa, Minas Gerais, Brazil).

2.10. Histopathological Analysis

Samples of the liver, pancreas, and epididymal adipose tissue were fixed with 10% formalin solution. After fixation, the specimens were dehydrated, embedded in paraffin, cut in a microtome to a thickness of 5 mm each, and stained with hematoxylin and eosin [41,43]. An expert pathologist performed a blind histological analysis of the liver and pancreas and classified the samples according to a score system [45,46]. Pancreas analysis followed the architecture of pancreas evaluation, according to changes in the islets of Langerhans [41,43,47,48].

The adipocyte area of the epididymal adipose tissue was photographed by using a Leica DFC 495 digital camera system (Leica Microsystems, Wetzlar, Germany) integrated into a Leica DM 5500B microscope (Leica Microsystems, Wetzlar, Germany), with a magnification of 200x. The images were analyzed by using the Leica Application Suite software, version 4.0 (Leica Microsystems, Wetzlar, Germany), and a mean area of 100 adipocytes per sample was determined [49].

2.11. Oral Glucose Tolerance Test (OGTT)

An oral glucose tolerance test (OGTT) was performed three days prior to initiating treatment with the AE and three days prior to euthanasia. The animals were submitted for 8 h of fasting [41]. The fasting glucose was verified via the flow rate (time 0) using a G-tech glucometer (G-Tech Free, Infopia Co., Ltd., Anyang, Gyeonggi-do city, Korea) with the tail blood. After this, the animals received D-glucose (Vetec, Duque de Caxias, RJ, Brazil) at 2 g/kg of body weight by oral gavage, and the blood glucose was monitored at 15, 30, 60, and 120 min after glucose administration via the tail blood. The area under the curve (AUC) was calculated for each animal, and the mean was calculated for each experimental group [41,50].

2.12. Enzyme-Linked Immunosorbent Assay (ELISA)

The levels of plasma insulin were determined according to the Millipore ELISA (enzyme linked immunosorbent assay) Insulin EZRMI-13K kit and performed according to the manufacturer's instructions (Cat. #EZRMI-13K, EMD Millipore Corporation, St. Charles, MO, USA). Briefly, the blood samples were collected by cardiac puncture and centrifuged at 1900 G for 10 min at 4 °C. The serum was stored at −80 °C, until the day of the assay. The calculated values of the analyzed hormone were based on a standard curve and expressed as ng/mL.

2.13. Quantitative Real-Time PCR (qRT-PCR)

Messenger ribonucleic acid (mRNA) expressions were determined by using quantitative real-time PCR (qRT-PCR) in epididymal adipose tissue and liver tissue; briefly, we extracted the total RNA, using TRizol Reagent, according to the manufacturer's instructions (Thermo Fisher Scientific Inc., Waltham, MA, USA). Two micrograms of the total RNA were reverse transcribed, using a High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific Inc., Waltham, MA, USA). The relative quantification of the mRNA content was conducted by qRT-PCR, with specific primers and Sybr Green Master Mix (Thermo Fisher Scientific Inc., Waltham, MA, USA). The primers' sequences are shown in Table 1.

Table 1. Sequence of the oligonucleotides used in qRT-PCR.

Gene	Sequence	Amplicon (bp)	Accession Number
Peroxisome Proliferator Activated Receptor Gamma (PPAR-γ)	Fwd: ATCTTAACTGCCGGATCCAC Rev: CAAACCTGATGGCATTGTGAG	102	NM_001127330.2
Peroxisome Proliferator Activated Receptor Alpha (PPAR-α)	Fwd: TGCAATTCGCTTTGGAAGAA Rev: CTTGCCCAGAGATTTGAGGT	118	NM_011144.6
Fat Acid Synthase (FAS)	Fwd: GATTCGGTGTATCCTGCTGTC Rev: CATGCTTTAGCACCTGCTGT	95	NM_007988.3
Glucose-6-Phosphatase (G6P)	Fwd: CCGGATCTACCTTGCTGCTC Rev: GCATTGTAGATGCCCCGGAT	105	NM_008061.4
Fetuin-A	Fwd: GGAGATTTCCCGGGCTCAAA Rev: TGCAGTACAGTCAGTGGCAG	82	NM_001276450.1
Carbohydrate Response Element Binding Protein (ChREBP)	Fwd: CAGCATCGATCCGACACTCA Rev: GGCCTTTGAAGTTCTTCCACT	96	NM_021455.5
Sterol Regulatory Element Binding Transcription Factor 1-c (SREBP1-c)	Fwd: TGACGGAGACAGGGAGTTCT Rev: CAGAGAAACTGCAAGCAGGA	95	NM_001313979.1
Ribosomal Protein L19 (RPL19)	Fwd: CAATGCCAACTCCCGTCA Rev: GTGTTTTTCCGGCAACGAG	102	NM_009078.2

Sequence of the oligonucleotides: Fwd (Forward); Rev (Reverse).

We performed the relative quantification of mRNAs, using the 2-ΔΔCT method, and we analyzed the results by applying the Pfaffl equation [51]. We calculated the ΔΔCt = (Ct of the target gene in

the control group—Ct of the target gene in the diet group)/(Ct of housekeeping gene in the control group—Ct housekeeping gene in the diet group). The housekeeping used was ribosomal protein L19 (RPL19). Once the PCR products were produced exponentially, as verified with the primer efficiency curve, we transformed the Ct variation ratios into the fold change, using the formula 2-ΔΔCt [51].

2.14. Statistical Analysis

The data were expressed as the mean ± the standard error of the mean (SEM). The statistical differences were determined by using Student's unpaired *t*-test for independent samples or analysis of variance, followed by Tukey's post-test (for one-way analysis of variance (ANOVA) or Bonferroni's post-test (for two-way analysis of variance (ANOVA), to compare more than two groups, using GraphPad Prism, version 8.3.0 software (GraphPad Software, Inc., San Diego, CA, USA). Differences were considered statistically significant when $p \leq 0.05$.

3. Results

3.1. Chemical Profile of M. citrifolia Linn. (noni) Fruit Aqueous Extract (AE)

The identification of the components in the aqueous extract of the fruits of *M. citrifolia* was based on analyses of ultraviolet (UV), mass spectrometry (MS), and tandem mass spectrometry (MS^2) spectrometric data, which were compared with those published in the literature. Seventeen compounds were annotated (Figure 2 and Table 2).

Compound 1 showed an $(M\text{-}H)^-$ ion at *m/z* 341.1091, compatible with the molecular formula $C_{12}H_{22}O_{11}$, and was putatively identified as di-*O*-hexoside. Compound 2 was tentatively identified as tri-*O*-hexoside, based on the ions at *m/z* 503.1636 $(M - H)^-$ and 549.1679 $(M + HCOO)^-$, corresponding to the molecular formula $C_{18}H_{31}O_{16}$. Accordingly, the MS^2 spectrum of the formate adduct (*m/z* 549.1679) showed fragment ions at *m/z* 179 and *m/z* 161, attributed to a deprotonated monosaccharide and its dehydrated form, respectively. In addition, the fragment ion at *m/z* 221 agrees with a fragment of non-reducing monosaccharide linked to a 2-hydroxyacetaldehyde [52].

Compound 3, which was annotated as the iridoid deacetylasperulosidic acid, showed a deprotonated ion at *m/z* 389.1090. Aligned with this proposal are the MS^2 fragment ions depicted in the MS^2 spectrum [53], as well as the previous report on the isolation of this iridoid from the fruits of *M. citrifolia* [54,55].

Compound 10 was annotated as asperulosidic acid, an iridoid glucoside previously identified in *M. citrifolia* fruits [56], based on the molecular formula $C_{18}H_{24}O_{12}$, as indicated by the deprotonated ion at *m/z* 431.1217. The fragment ions in the MS^2 spectrum at *m/z* 165 and 147 agreed with this proposal [57].

Compounds 8, 11, 12, 13, 14, 16, and 17 were annotated as being noniosides (Table 2), considering that their respective accurate masses corresponded to molecular formulae compatible with these compounds, which had already been formerly obtained from *M. citrifolia* fruits [24,58]. Noniosides have been described as fatty acid, fatty alcohol, and hemiterpene glucosides [24,58]. No absorbances above 240 nm were observed in their UV spectra, due to the lack of a suitable chromophore. The only exception was compound 17, which showed a UV absorbance at λ 279 nm, which agrees with noniosides previously isolated from *M. citrifolia* bearing a conjugated fatty acid linked to the sugar moiety [59,60].

Compound 15 showed UV absorbances at λ 291 and 347 nm, and an accurate mass at *m/z* 609.1480, corresponding to the molecular formula $C_{27}H_{30}O_{16}$. These data are in accordance with those of a flavonol glycoside bearing a luteolin quercetin aglycone [61]. Thus, compound 15 was tentatively identified as rutin, which had been previously isolated and/or identified in *M. citrifolia* fruits [62–64].

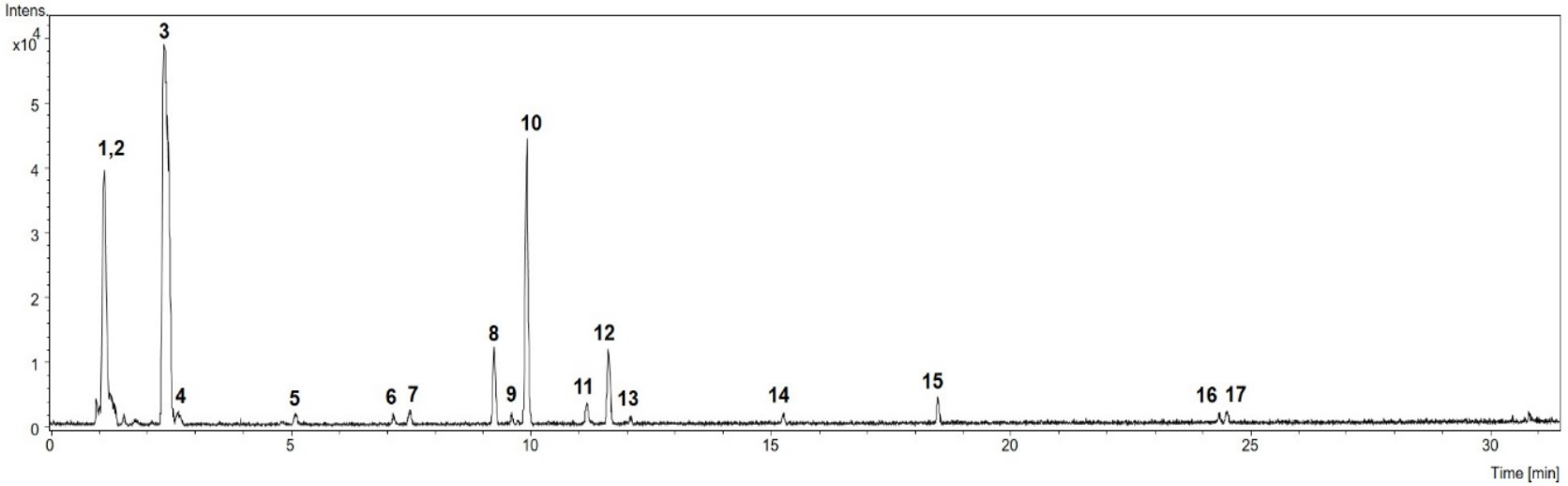

Figure 2. The base peak chromatogram (BPC) obtained by UFLC–DAD–MS of *M. citrifolia* aqueous fruit extract (AE), in negative mode. The identification of chromatographic peaks is described in Table 2.

Table 2. Compounds tentatively identified in *M. citrifolia* aqueous fruit extract (AE).

N°	Rt (min)	Compound	Molecular Formula *	MS (⁻)	MS ^{2}	UV λ_{max} (nm)
1	1.2	di-O-hexoside	$C_{12}H_{22}O_{11}$	341.1091 $(M-H)^-$	-	-
2	1.2	tri-O-hexoside	$C_{18}H_{32}O_{16}$	503,1636 $(M-H)^-$ 549.1679 $(M+HCOO)^-$	549.1679: 221, 179, 161	-
3	2.4	Deacetylasperulosidic acid	$C_{16}H_{22}O_{11}$	389.1090 $(M-H)^-$	389.1090: 209, 165	-
4	2.7	Unknown	$C_{17}H_{26}O_{12}$	421.1323 $(M-H)^-$	-	-
5	5.1	Unknown	$C_{16}H_{28}O_{12}$	411.1500 $(M-H)^-$	-	-
6	7.1	Unknown	$C_{15}H_{26}O_{11}$	381.1405 $(M-H)^-$	-	-
7	7.5	Unknown	$C_{15}H_{26}O_{11}$	381.1407 $(M-H)^-$	-	-
8	9.2	Nonioside (hemiterpene disaccharide)	$C_{17}H_{30}O_{11}$	409.1735 $(M-H)^-$	-	-
9	9.6	Unknown	$C_{16}H_{24}O_{10}$	375.1285 $(M-H)^-$	-	-
10	9.9	Asperulosidic acid	$C_{18}H_{24}O_{12}$	431.1217 $(M-H)^-$	431.1217: 165, 147	-
11	11.2	Nonioside	$C_{16}H_{28}O_{11}$	395.1593 $(M-H)^-$, 441.1623 $(M+HCOO)^-$	-	-
12	11.6	Nonioside (hemiterpene disaccharide)	$C_{16}H_{28}O_{10}$	379.1636 $(M-H)^-$, 425.1692 $(M+HCOO)$	-	-
13	12.1	Nonioside (hemiterpene disaccharide)	$C_{16}H_{28}O_{10}$	379.1647 $(M-H)^-$, 425.1679 $(M+HCOO)$	-	-
14	15.3	Nonioside (fatty acid ester disaccharide)	$C_{18}H_{32}O_{12}$	439.1831 $(M-H)^-$,	-	-
15	18.5	Rutin	$C_{27}H_{30}O_{16}$	609.1480 $(M-H)^-$	-	291, 347
16	24.3	Nonioside (fatty acid ester disaccharide)	$C_{20}H_{36}O_{12}$	467.2161 $(M-H)^-$	-	-
17	24.5	Nonioside (fatty acid ester disaccharide)	$C_{22}H_{34}O_{12}$	489.2001 $(M-H)^-$	-	279

Rt: retention time; UV: ultraviolet; MS: mass spectrometry; MS^2: tandem mass spectrometry. * All molecular formulas were determined from the accurate mass, considering a mass error lower than 8 ppm.

3.2. Acute Oral Toxicity of *M. citrifolia* (noni) Fruit Aqueous Extract (AE)

We performed the acute oral toxicity of the AE to evaluate whether the AE displayed any sign of toxicity. The results demonstrate no signs of systemic toxicity. There were no changes in the body weight (Figure S1), water consumption (Figure S1), food intake (Figure S1), or excretion of the urine and feces. No changes in the Hippocratic test were observed (Figure S2); for instance, no motor, sensory, and neurobiological changes were observed, as no animals died. The weight of the liver, pancreas, kidneys, lungs, spleen, and heart had no differences between the groups (Figure S1).

3.3. Effects of *M. citrifolia* (noni) Fruit Aqueous Extract (AE) on Body Weight and Food Intake

At the beginning of the experiment, the HFF groups did not display differences in body weight when compared to the CT group (Supplementary Materials Table S1). After nine weeks, the HFF group demonstrated increased final body weight and body weight gain compared to the CT. The HFF group presented decreased food and calorie intake and presented higher feed efficiency index when compared to the CT group. These data demonstrated that nine weeks of HFF-diet induction was able to promote body weight gain (Supplementary Materials Table S1).

During the ninth week of the diet, the HFF groups were divided, and we began the treatments with the two doses of the *M. citrifolia* (noni) fruit aqueous extract (AE). The effects of the high-fat/high-fructose diet and AE treatment on body weight are demonstrated in Figure 3A,B.

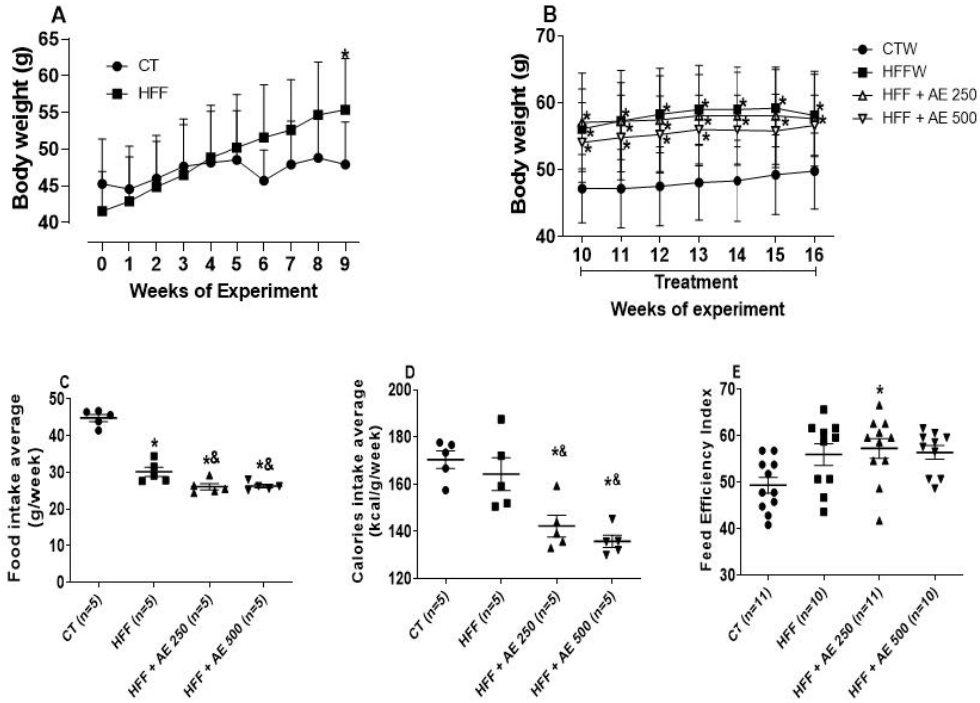

Figure 3. Effects of the high-fat/high-fructose diet and AE on body weight. (**A**) The body weight of animals fed on the regular chow diet (CT, n = 11) and on the high-fat/high-fructose diet (HFF, n = 31) for nine consecutive weeks (0: initial weight); (**B**) the body weight of animals of the full black circle: CTW diet (regular chow diet + drinking water, n = 11); full black quadrilateral: HFFW diet (high-fat/high-fructose diet + drinking water, n = 10); black up-pointing triangle: HFF + AE 250 diet (HFF + *M. citrifolia* fruit aqueous extract of 250 mg/kg of body weight, n = 11); and black down-pointing triangle: HFF + AE 500 diet (HFF + *M. citrifolia* fruit aqueous extract of 500 mg/kg of body weight, n = 10) for seven weeks (10th to 16th week); (**C**) the food intake average for 16 weeks; (**D**) the calorie intake average for 16 weeks; (**E**) the feed efficiency index for 16 weeks. The results are expressed as the mean ± standard error of the mean (SEM). Two-way ANOVA, followed by the Bonferroni post-test. * $p \leq 0.05$ vs. CTW. & = $p \leq 0.05$ vs. HFFW.

From the 10th to 16th week, the HFF, HFF + AE 250, and HFF + AE 500 groups showed lower food intake in comparison to the CTW group (Figure 3C); nonetheless, the treated groups had lower food and calorie intakes in comparison to the HFFW and CTW groups (Figure 3C,D). The feed efficiency index was increased only in the HFF + AE 250 group compared to CTW group (Figure 3E). The treatment with AE was not able to diminish body weight in mice that were fed with the HFF diet.

3.4. Effects of M. citrifolia (noni) Aqueous Extract (AE) on the Visceral Adiposity

We observed that the HFF diet was able to increase the adiposity index in all groups and the two doses of the AE were not able to decrease the adiposity index. The HFF + AE 500 group had higher epididymal fat content in comparison to the CTW group. The retroperitoneal, perirenal, and mesenteric fat contents were higher in HFFW, HFF + AE 250, and HFF + AE 500 in comparison to CTW; however, the HFF + AE 250 group showed increased perirenal fat when compared to HFFW. Our findings demonstrate that the AE treatments were not able to decrease the visceral adiposity. No differences were observed in the liver weight among the groups (Table 3).

Table 3. The effects of AE on the fat pads, adiposity index, and liver weight.

Parameter	Groups			
	CTW (*n* = 11)	**HFFW (*n* = 10)**	**HFF + AE 250 (*n* = 11)**	**HFF + AE 500 (*n* = 10)**
Epididymal fat (mg/g)	28.68 ± 2.69	42.10 ± 3.77	39.71 ± 3.95	48.67 ± 3.66 *
Retroperitoneal fat (mg/g)	8.13 ± 0.45	16.81 ± 1.96 *	16.09 ± 1.28 *	16.56 ± 1.00 *
Perirenal fat (mg/g)	6.79 ± 0.71	10.75 ± 1.14 *	15.90 ± 0.94 *&	14.27 ± 1.17 *
Omental fat (mg/g)	0.42 ± 0.07	1.04 ± 0.25	0.71 ± 0.17	0.62 ± 0.21
Mesenteric fat (mg/g)	18.47 ± 1.04	29.20 ± 2.86 *	34.14 ± 1.55 *	34.29 ± 1.3 4 *
Liver (mg/g)	38.11 ± 0.60	42.62 ± 3.87	42.83 ± 2.55	40.33 ± 2.92
Adiposity index (%)	6.25 ± 0.46	9.99 ± 0.62 *	10.65 ± 0.49 *	11.44 ± 0.61 *

CTW: regular chow diet + drinking water. HFFW: high-fat/high-fructose diet + drinking water. HFF + AE 250: HFF + *M. citrifolia* fruit aqueous extract of 250 mg/kg of body weight. HFF + AE 500: HFF + *M. citrifolia* fruit aqueous extract of 500 mg/kg of body weight for seven weeks (10th to 16th week). The results are expressed as the mean ± SEM. ANOVA followed by Tukey post-test. * $p \leq 0.05$ vs. CTW. & = $p \leq 0.05$ vs. HFFW.

3.5. Effects of M. citrifolia (noni) Aqueous Extract (AE) on Glucose Tolerance and Systemic Insulin Sensitivity

The groups were divided after 9 weeks of diet induction and the OGTT test was performed prior to the beginning of the treatment of AE (Figure S3). At this point, the HFF group displayed higher blood glucose values when compared to the group that received the regular chow diet (CT), demonstrating that the HFF diet was able to induce glucose intolerance in those animals (Figure S3).

The OGTT performed at the end of the experiment (16th week) indicated that the HFFW and HFF + AE 250 groups remained glucose intolerant when compared to CTW; however, the AE 500 treatment was able to diminish the blood glucose levels in comparison to HFFW, demonstrating that the higher dose of AE improved the glucose tolerance (Figure 4A,B).

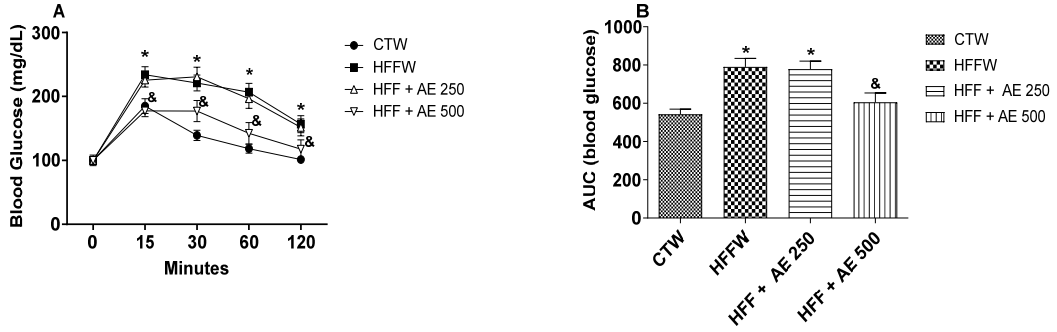

Figure 4. Evaluation of the glycemic profile at the end of the treatment with AE. (**A**) The oral glucose tolerance test at the end of the treatment (16th week); (**B**) the area under the curve (AUC) of the blood glucose of animals evaluated at the end of the treatment (16th week) of CTW (CT + drinking water, $n = 11$); HFFW (high-fat/high-fructose diet + drinking water, $n = 11$); HFF + AE 250 (HFF + *M. citrifolia* fruit aqueous extract of 250 mg/kg of body weight, $n = 11$); and HFF + AE 500 (HFF + *M. citrifolia* fruit aqueous extract of 500 mg/kg, $n = 10$) groups. The results are expressed as the mean ± SEM. * = $p \leq 0.05$ vs. CTW. & = $p \leq 0.05$ vs. HFFW. Two-way ANOVA, followed by the Bonferroni post-test for the oral glucose tolerance test. ANOVA, followed by the Tukey post-test for the AUC.

We observed that HFFW, HFF + AE 250, and HFF + AE 500 displayed higher insulin serum levels in comparison to CTW (Figure 5A). The same scenario was observed when we calculated the homeostatic model assessment for insulin resistance (HOMA-IR) index in which HFF diet and treatment with AE obtained higher values of HOMA-IR when compared to CTW (Figure 5B). The HFF and HFF + AE 250 groups presented increased values of the homeostatic model assessment for β cells (HOMA-β) index compared to CTW, while the HFF + AE 500 group had no differences among the groups (Figure 5C).

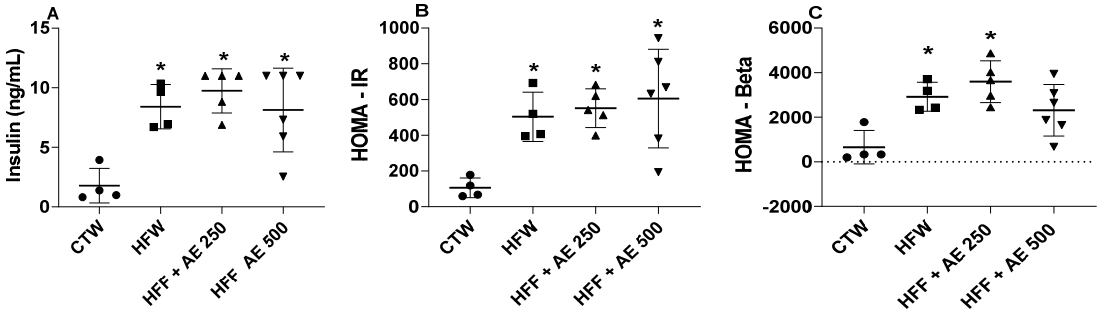

Figure 5. Evaluation of the insulin levels, homeostatic model assessment for insulin resistance (HOMA-IR), and homeostatic model assessment for β cells (HOMA-β). (**A**) The insulin serum levels (ng/mL). (**B**) HOMA-IR; (**C**) HOMA-β of animals evaluated at the end of the treatment (16th week) of full black circle: CTW (CT + drinking water, $n = 4$), full black quadrilateral: HFFW (high-fat/high-fructose diet + drinking water, $n = 4$); black up-pointing triangle: HFF + AE 250 (HFF + *M. citrifolia* fruit aqueous extract of 250 mg/kg of body weight, $n = 5$); and black down-pointing triangle: HFF + AE 500 (HFF + *M. citrifolia* fruit aqueous extract of 500 mg/kg, $n = 6$) groups. The results are expressed as the mean ± SEM. * = $p \leq 0.05$ vs. CTW. ANOVA, followed by the Tukey post-test.

3.6. *Effects of M. citrifolia (noni) Aqueous Extract on Serum Biochemical Parameters*

No differences were observed in the fasting blood glucose among the groups (Figure 6A).

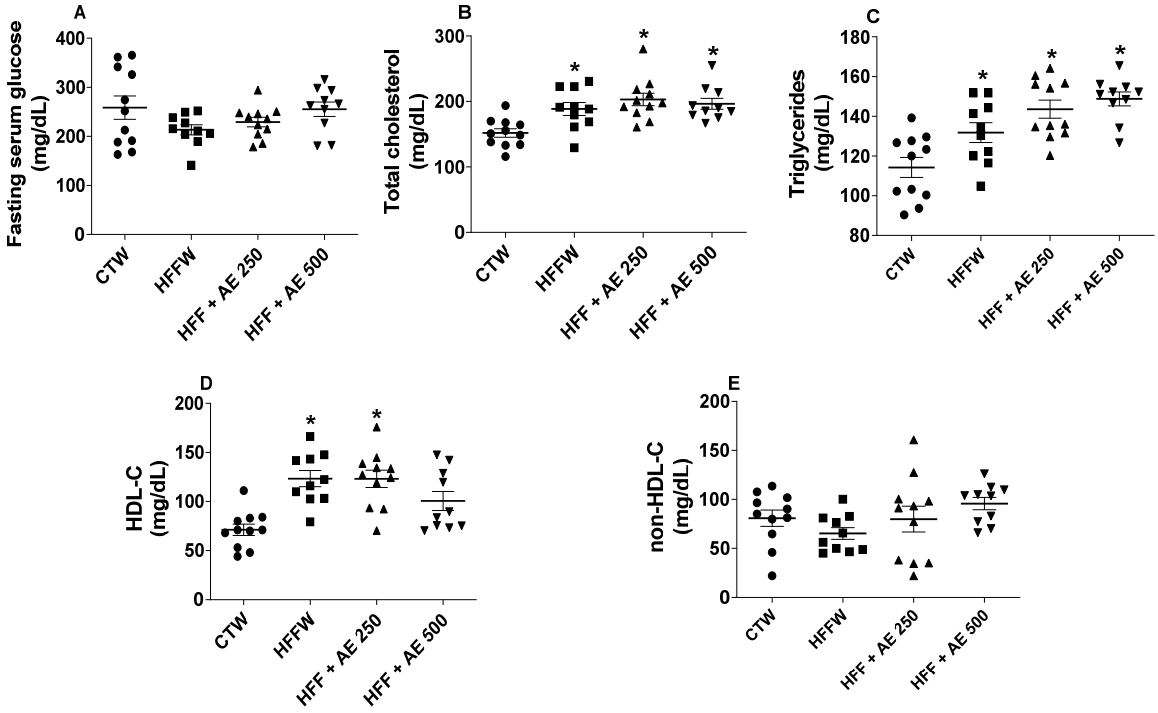

Figure 6. The effects of the high-fat/high-fructose diet and *M. citrifolia* (noni) aqueous extract (AE) on the serum biochemical parameters. (**A**) The fasting blood glucose (mg/dL); (**B**) total cholesterol (mg/dL); (**C**) triglycerides (mg/dL); (**D**) high-density lipoprotein cholesterol (HDL-C) (mg/dL); (**E**) non-HDL-C (mg/dL) of full black circle: CTW (CT + drinking water, n = 11), full black quadrilateral: HFFW (high-fat/high-fructose diet + drinking water, n = 10); black up-pointing triangle: HFF + AE 250 (HFF + *M. citrifolia* fruit aqueous extract of 250 mg/kg of body weight, n = 11); and black down-pointing triangle: HFF + AE 500 (HFF + *M. citrifolia* fruit aqueous extract of 500 mg/kg groups, n = 10) for seven weeks (10th to 16th weeks). The results are expressed as the mean ± SEM. * = $p \leq 0.05$ vs. CTW. ANOVA, followed by the Tukey post-test.

HFFW and the treated groups presented increased total cholesterol (Figure 6B) and triglycerides (Figure 6C) in comparison to CTW, while HFFW and HFF + AE 250 presented higher values of HDL-C in comparison to CTW (Figure 6D); no differences were observed in the non-HDL-C levels among the groups (Figure 6E). Our results demonstrate that the HFF diet was able to enhance the total cholesterol, triglycerides, and HDL-C levels; however, the AE was not able to influence the fasting blood glucose and lipid parameters after the treatment.

3.7. Histopathological Analysis of the Epididymal Adipose Tissue, Pancreas, and Liver

The histological analysis of the liver demonstrated a higher prevalence of hepatic steatosis > 5% in the HFW, HFF + AE 250, and HFF + AE 500 groups when compared to the CTW group (p = 0.0001). No significant differences were found among the groups that received the HFF diet and treatments (Table 4).

The histological analysis of the pancreas did not demonstrate differences in the variables of Langerhans islets (p = 0.29), pancreatic acini (p = 0.10), or inflammatory cells among the groups (Table 5). The histological analysis of the liver and pancreas is demonstrated in Figure 7.

The histological analysis of the epididymal adipose tissue demonstrated that the HFFW, HFF+ AE 250, and HFF+AE 500 groups displayed a higher adipocyte area in comparison to the CTW group, while the HFF+AE 500 group had an increased adipocyte area when compared to the HFF+AE 250 group (Figure 8E). Our results show that AE was not able to diminish the size of the adipocytes reaching the control values, and HFF+AE500 worsened the damage caused by the HFF diet.

Table 4. Results for the changes observed in the livers of animals from each experimental group.

Variable Liver Changes	Groups CTW (n = 10)	HFFW (n = 10)	HFF + AE 250 (n = 10)	HFF + AE 500 (n = 11)
Steatosis *($p < 0.0001$)				
Score: 0 = none; 1 = light, 2 = moderate; 3 = severe	0.0 ± 0.0	2.0 ± 0.15 *	1.9 ± 0.18 *	2.273 ± 0.14 *
Steatosis Localization	None	Zone 3	Zone 3	Zone 3
Microvesicular Steatosis ($p = 0.09$)				
0 = absent; 1 = present (number of mice)	0	0	1 (8 out of 10)	0
Lobular Inflammation*($p < 0.0001$)				
Score: 0 = Absent; 1 = < 2 focus/field; 2 = 2-4 focus/field	0.1 ± 0.1	0.9 ± 0.18 *	1.0 ± 0 *	1.182 ± 0.18 *
Ballooning *($p = 0.05$)				
Score: 0 = Absent; 1 = Few cells, 2 = Many cells	1.0 ± 0.15	1.7 ± 0.15 *	1.7 ± 0.15 *	1.64 ± 0.15 *
Mallory's Hyaline *($p = 0.07$)				
Score: 0 = Absent; 1 = Rare, 2 = Several	0.2 ± 0.13	0.3 ± 0.2	1.1 ± 0.28 *	1.1 ± 0.25 *
Apoptosis ($p = 0.45$)				
Score: 0 = Absent; 1 = Present	0.1 ± 0.1	0.6 ± 0.16	0.5 ± 0.17	0.36 ± 0.15
Glycogenate Nucleus ($p = 0.12$)				
Score: 0 = Absent/Rare; 1 = Some	0.6 ± 0.16	0.1 ± 0.1	0.5 ± 0.16	0.36 ± 0.15

CTW (CT + drinking water), HFFW (high-fat/high-fructose diet + drinking water), HFF + AE 250 (HFF + *M. citrifolia* fruit aqueous extract of 250 mg/kg of body weight), HFF + AE 500 (HFF + *M. citrifolia* fruit aqueous extract of 500 mg/kg). Inferential statistical analysis due to the absence of values in the analyzed categories. The data are expressed as the mean + SEM of the relative scores. Analysis of one-way variance (ANOVA) and Bonferroni post-test. * = $p \leq 0.05$ vs. CTW.

Table 5. Results for the changes observed in the pancreas of animals from each experimental group.

Variable Pancreas Changes	Groups CTW (n = 11)	HFFW (n = 10)	HFF + AE 250 (n = 11)	HFF + AE 500 (n = 10)
Islet of Langerhans ($p = 0.11$)				
Score: 0 = No change; 1 = Discrete Atrophy; 2 =Atrophy	0.3 ± 0.15	1.25 ± 0.3	0.62 ± 0.3	0.7 ± 0.29
Pancreatic acini ($p = 0.09$)	0.0 ± 0.0	0.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
Score: 0 = No change; 1 = Necrosis/Atrophy				
Inflammatory cells ($p = 0.33$)	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0
Score: 0 = No change; 1 = Perinsulitis				

CTW (CT + drinking water), HFFW (high-fat/high-fructose diet + drinking water), HFF + AE 250 (HFF + *M. citrifolia* fruit aqueous extract of 250 mg/kg of body weight), HFF + AE 500 (HFF + *M. citrifolia* fruit aqueous extract of 500 mg/kg). Inferential statistical analysis due to the absence of values in the analyzed categories. The data are expressed as the mean + SEM of the relative scores. Analysis of one-way variance (ANOVA) and Bonferroni post-test.

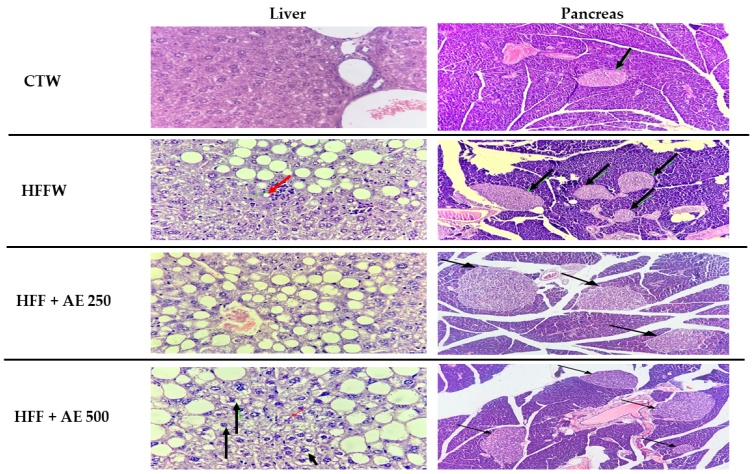

Figure 7. Histological analysis of the liver (red arrow indicates lobular inflammation, and black arrows indicate ballooning), 400× magnification, bar scale: 100 µm and pancreas (black arrows indicate Langerhans islets), 100× magnification, bar scale: 100 µm of CTW (CT + drinking water), HFFW (high-fat/high-fructose diet + drinking water), HFF + AE 250 (HFF + *M. citrifolia* fruit aqueous extract of 250 mg/kg of body weight), HFF + AE 500 (HFF + *M. citrifolia* fruit aqueous extract of 500 mg/kg).

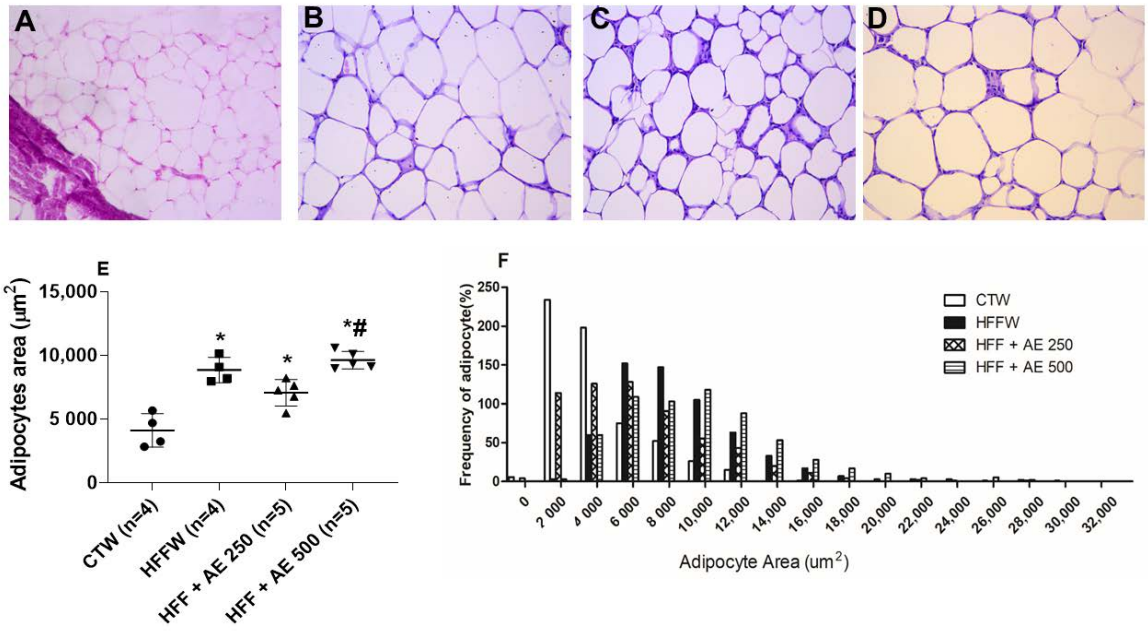

Figure 8. Histological analysis of the epididymal adipose tissue of each group. (**A**) CTW (CT + drinking water); (**B**) HFFW (high-fat/high-fructose diet + drinking water); (**C**) HFF + AE 250 (HFF + *M. citrifolia* fruit aqueous extract of 250 mg/kg of body weight); (**D**) HFF + AE 500 (HFF + *M. citrifolia* fruit aqueous extract of 500 mg/kg) 200 x magnification. Bar scale: 100 µm; (**E**) adipocyte area (µm^2) of full black circle: CTW (CT + drinking water); full black quadrilateral: HFFW (high-fat/high-fructose diet + drinking water); black up-pointing triangle: HFF + AE 250 (HFF + *M. citrifolia* fruit aqueous extract of 250 mg/kg of body weight); black down-pointing triangle: HFF + AE 500 (HFF + *M. citrifolia* fruit aqueous extract of 500 mg/kg); (**F**) graph of the frequency of distribution of adipocytes (%). The results are expressed as the mean ± SEM. * = $p \leq 0.05$ vs. CTW; $^{\#}$ = $p \leq 0.05$ vs. HFF + AE 250. ANOVA followed by the Tukey post-test.

3.8. Effects of M. citrifolia Fruit Aqueous Extract (AE) in the Expression of Genes Involved in Adipocyte Differentiation in White Adipose Tissue and the Lipid and Glycemic Metabolism in the Liver

We compared the mRNA content from genes involved in adipocyte differentiation in white adipose tissue, peroxisome proliferator-activated receptor-γ (PPAR-γ), and peroxisome proliferator-activated receptor-α (PPAR-α) (Figure 9A,B). The HFFW, HFF + AE 250, and HFF + AE 500 groups displayed decreased mRNA content of PPAR-γ compared to CTW (Figure 9A); on the other hand, PPAR-α displayed a lower expression in the HFFW and HFF + AE 250 groups in relation to the CTW group (Figure 9B), while HFF + AE 500 was able to upregulate the PPAR-α expression when compared to HFFW and HFF + AE 250 (Figure 9B) in white adipose tissue.

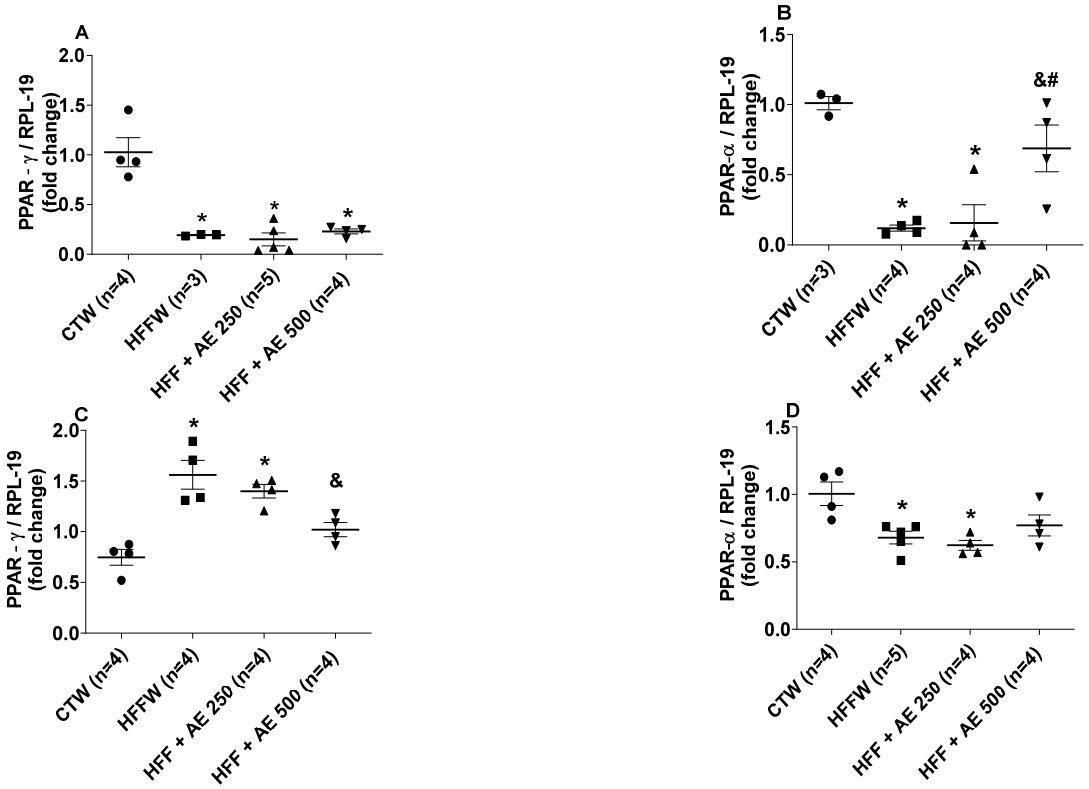

Figure 9. Effects of the HFF diet and *M. citrifolia* (noni) aqueous extract (AE) in the mRNA content of metabolic genes in the epididymal fat and liver. (**A**) Peroxisome proliferator-activated receptor-γ (PPAR-γ) in epididymal adipose tissue; (**B**) peroxisome proliferator-activated receptor-α (PPAR-α) in epididymal adipose tissue; (**C**) peroxisome proliferator-activated receptor-γ (PPAR-γ) in the liver; (**D**) peroxisome proliferator-activated receptor-α (PPAR-α) in the liver of full black circle: CTW (CT + drinking water), full black quadrilateral: HFFW (high-fat/high-fructose diet + drinking water); black up-pointing triangle: HFF + AE 250 (HFF + *M. citrifolia* fruit aqueous extract of 250 mg/kg); and black down-pointing triangle: HFF + AE 500 (HFF + *M. citrifolia* fruit aqueous extract of 500 mg/kg) groups. The results are expressed as the mean ± SEM. * = $p \leq 0.05$ vs. CTW; $^{\&}$ = $p \leq 0.05$ vs. HFFW; $^{\#}$ = $p \leq 0.05$ vs. HFF+AE 250. ANOVA followed by the Tukey post-test.

In the liver, the HFFW and HFF + AE 250 groups displayed increased mRNA content of PPAR-γ when compared to the CTW group; while the HFF + AE 500 group showed significant decreased expression of PPAR-γ in relation to HFFW but not significantly different from CTW (Figure 9C). On the contrary, HFFW and HFF + AE 250 presented decreased mRNA content of PPAR-α in relation to CTW, was only slightly decreased in HFF + AE 500, and was not significantly different from the CTW levels (Figure 9D).

The mRNA content of nuclear transcription factors, such as sterol regulatory element binding protein-1c (SREBP-1c) and carbohydrate response element binding protein (ChREBP),

the enzymes glucose-6-phosphatase (G6P) and fatty acid synthase (FAS), and the hepatokine, fetuin-A, were evaluated in the liver (Figure 10). The abundance of SREBP-1c mRNA was higher in HFFW compared to the CTW group (Figure 10A), whereas HFF + AE 500 was lower in relation to HFFW and not different from the CTW group (Figure 10A). No significant differences were observed in the ChREBP (Figure 10B) and FAS (Figure 10C) mRNA expressions among the groups. The G6P mRNA expression was lower in HFFW, HFF + AE 250, and HFF + AE 500 in comparison to the CTW group (Figure 10D). The hepatokine fetuin-A mRNA expression was reduced in the HFF + AE 500 group when compared to all groups (Figure 10E). Our results demonstrate that the AE 500 treatment influenced the regulation of the expression of the nuclear transcription factor, SREBP-1c, and the hepatokine, fetuin-A, in the liver.

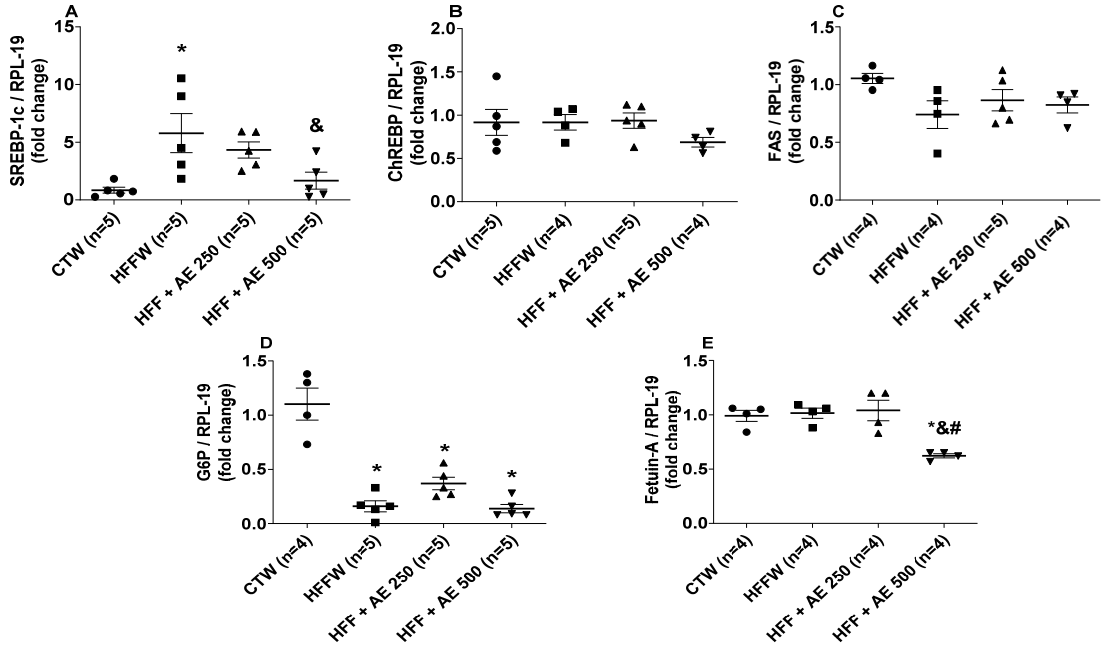

Figure 10. The effects of a high-fat/high-fructose diet and *M. citrifolia* (noni) aqueous extract (AE) in the expression of metabolic genes in the liver (**A**) sterol regulatory element binding protein-1c (SREBP-1c); (**B**) carbohydrate response element binding protein (ChREBP); (**C**) fatty acid synthase; (**D**) glucose-6-phosphatase (G6P); (**E**) fetuin-A of full black circle: CTW (CT + drinking water), full black quadrilateral: HFFW (high-fat/high-fructose diet + drinking water); black up-pointing triangle: HFF + AE 250 (HFF + *M. citrifolia* fruit aqueous extract of 250 mg/kg); and black down-pointing triangle: HFF + AE 500 (HFF + *M. citrifolia* fruit aqueous extract of 500 mg/kg) groups. The results are expressed as the mean ± SEM. * = $p \leq 0.05$ vs. CTW; $^{\&}$ = $p \leq 0.05$ vs. HFFW; $^{\#}$ = $p \leq 0.05$ vs. HFF + AE 250. ANOVA, followed by the Tukey post-test.

4. Discussion

The consumption of noni fruit has been discussed regarding its possible health benefits. Products derived from noni fruit are authorized to be commercialized in the form of juice and extracts as encapsulated powder by governmental authorities in European and Asian countries, as well as in the USA [10–12]. Even though the fruit is popularly consumed in folk medicine, noni product commercialization is not yet allowed in Brazil [13].

The majority of studies have focused on fermented noni juice or on adding cranberry, blueberry, and grape juice to mask the organoleptic features at the moment of consumption [9,12,18–20]. This processing may improve the quality and display a better acceptance from the final consumer [12,19,20]. However, one of the criticisms of consuming products derived from noni fruit is whether the effects of bioactive compounds should be attributed by bioactive compounds per se acting in a synergistic form, or if fermentation or adding other ingredients may alter the quality of the

final product. Thus, the use of a crude aqueous noni fruit extract, without fermentation processing or the addition of any type of product to mask noni's bitter taste, was chosen in our study.

Studies demonstrated that noni fruit juice promoted metabolic effects, such as hypoglycemic and hepatoprotective effects [28–30], the reduction in body weight gain [27,28], and antidislipidemic actions [27,29], in diet-induced metabolic dysfunction animal models.

To our knowledge, this is the first study to evaluate the effects of crude aqueous noni fruit extract in high-fat/high-fructose (HFF) fed mice, an animal model that mimics metabolic syndrome in humans [2,65,66].

The results demonstrate that no neurotoxic, behavioral, or mortality effects were produced by AE in the acute toxicity test after or during the post-treatment period (see Supplementary Materials). The HFF diet was able to promote the metabolic abnormalities observed in metabolic syndrome, such as body weight gain and increased visceral adiposity, hyperglycemia, systemic insulin resistance, hypercholesterolemia, hypertriglyceridemia, and hepatic steatosis. The two doses of crude aqueous noni fruit extract (AE) used in the study, AE 250 and AE 500, under the influence of the HFF diet were not able to diminish body weight or the visceral adiposity gain, total cholesterol, triglycerides, insulin levels, systemic insulin resistance, or hepatic steatosis. However, AE 500 was able to inhibit the impaired glucose tolerance promoted by the HFF diet.

The glucose tolerance improvement was also observed by others when fermented noni fruit juice was used to treat diet-induced type 2 diabetes in mice [28], genetic obese type 2 diabetes in mice [30], and type 1 diabetes in rats [67].

Our data demonstrated that AE presents iridoids, noniosides, and flavonoid (rutin); these compounds were previously observed in other studies [24,54,61,68]. Iridoids are found in combination with sugar in most plant species and are named as iridoid glycosides [69]. Iridoids derived from the *M. citrifolia* root extract were previously demonstrated to have hypoglycemic effects in streptozotocin-induced diabetic mice, a typical animal model of type 1 diabetes [54].

A total of 18 novel trisaccharide fatty acid esters (noniosides A-O and others) were isolated specifically from the fruits of *M. citrifolia* [69]; however, there were no studies in diabetes animal models that demonstrated hypoglycemic effects promoted by noniosides from noni fruit. On the other hand, rutin, an important flavonoid found in *M. citrifolia* fruit [68], demonstrated an influence on hypoglycemic parameters [70,71] and also improved dyslipidemia in streptozotocin-induced diabetic rats [70].

There is no evidence demonstrating the indirect influence of bioactive compounds derived from *M. citrifolia* fruit in genes related to lipogenesis. In the human body, the lipogenic pathway is active in at least two important lipogenic tissues, the liver and adipose tissue; however, lipogenesis was demonstrated to be more efficient in the liver compared with in adipose tissue [72]. Deregulations in the lipogenic pathway are associated with diverse pathological conditions. De novo lipogenesis was previously shown to be highly responsive to changes in the dietary regimen, as dietary carbohydrates, particularly fructose, are almost exclusively metabolized by the liver, and the excessive consumption of fructose has been shown to stimulate the deregulation of de novo lipogenesis leading to hepatic lipid accumulation. Hepatic de novo lipogenesis is a metabolic pathway that affects lipid and glucose regulation and plays a role in the development of diabetes, cardiovascular disease, and hepatic steatosis [73].

We evaluated the effects of an HFF diet on the genes involved in the lipid and glucose metabolism and the effect of AE treatment, and we sought for synergistic effects in both white adipose tissue and the liver. Our findings demonstrate that the animals responded differently under the influence of the HFF diet and two doses of AE treatment.

The HFF diet was able to decrease the PPAR-γ and PPAR-α mRNA expression in white adipose tissue. PPAR-γ is majorly expressed in white adipose tissue, is considered a regulator of lipogenesis, and plays a key role in glucose homeostasis and the adipocyte differentiation of fat cells [74]. PPAR-α is predominantly expressed in the liver and white adipose tissue, but not in skeletal muscle, and is a gene

involved in fatty acid oxidation that controls fatty acid transport and β-oxidation [75,76] and improves glucose metabolism defects, such as glucose intolerance, hyperglycemia, and insulin resistance [77].

Our results demonstrate that the AE 500 treatment was able to upregulate the PPAR-α mRNA expression but did not change the PPAR-γ mRNA isoform in white adipose tissue. In the liver, AE 500 treatment had a tendency to upregulate the PPAR-α mRNA and downregulate the PPAR-γ mRNA expression. As PPAR-α is able to increase the fatty acid transport and β-oxidation, these could be some of the responsible factors for the glucose tolerance improvement along with the increased lipid accumulation observed in the epididymal fat and liver observed in the AE 500-treated groups.

Shih et al. (2009) also demonstrated that a high-fructose diet promoted the downregulation of PPAR-γ mRNA in white adipose fat deposits and that *Momordica charantia* (bitter melon) extract treatment was able to upregulate the PPAR-γ mRNA expression. The bioactive components from bitter melon responsible for the promotion of hypoglycemic properties included glycosides, saponins, alkaloid, fixed oils, triterpenes, proteins, and steroids [78,79]. In another study, the PPAR-γ mRNA expression did not differ among the groups in white adipose tissue in mice that received a high-fat diet. Further elucidations regarding whether the polyphenols found in the study could activate PPAR-α in white adipose tissue were proposed [80].

Lipogenesis in adipose tissue is less responsive than hepatic de novo lipogenesis to acute or prolonged carbohydrate overfeeding, particularly with fructose. Lipogenesis is an insulin- and glucose-dependent process that is under the control of specific transcription factors, including sterol regulatory binding protein-1c (SREBP-1c), which is activated by insulin, and carbohydrate-responsive element-binding protein (ChREBP), which is activated by glucose in the liver. Insulin induces the maturation of SREBP-1c by a proteolytic mechanism initiated in the endoplasmic reticulum, and consequently, SREBP-1c activates glycolytic gene expression, promoting the glucose metabolism, as well as lipogenic genes in conjunction with ChREBP [81,82].

SREBP-1c and ChREBP respond differently under the influence of fructose or glucose supplementation in a high-fat diet. While SREBP-1c is upregulated after fructose stimulation, leading to hepatic lipogenesis and insulin resistance, ChREBP is upregulated and activated by glucose supplementation [83]. As observed in our data, an HFF diet upregulated the SREBP-1c mRNA expression and the AE 500 treatment downregulated its expression; however, no differences were observed in the ChREBP mRNA expression, indicating that the effects on inhibiting impaired glucose tolerance promoted by AE 500 treatment may be associated with the SREBP-1c pathway and not the ChREBP pathway. In the liver, research reported that ChREBP is under the influence of G6P [84,85]; hence, the lack of ChREBP activation in our experimental groups suggest that this was due to the low G6P expression.

On the contrary, SREBP-1c is primarily involved in the regulation of genes related to fatty acid synthesis, such as fatty acid synthase (FAS), a marker for lipogenesis and energy metabolism. However, the high-carbohydrate/high-fat diet did not display differences in the FAS mRNA expression in mice liver [86]. Huang and Lin (2012) observed that a high-fructose diet promoted a higher FAS gene expression and green, black, and pu-erh tea leaves, which contain catechins, caffeine, and theanine, led to a suppression of the FAS gene in the liver. We did not observe any differences in the hepatic FAS gene expression among the groups, suggesting no influence from the HFF diet or AE treatments [87].

The hepatic fetuin-A mRNA expression was shown to correlate with the hepatic mRNA levels of key enzymes in the lipid and glucose metabolism [88]. This hepatokine is a liver-derived protein and, together with free fatty acids, induces apoptotic signals in the beta islet cells of the pancreas, reducing the secretion of insulin and further exacerbating diabetes type 2 [89]. Jung et al., (2013) observed that the incubation of hepatocytes with palmitate, a model of NAFLD in vitro, induced upregulation of the fetuin-A expression in hepatocytes, accompanied by triglyceride accumulation and the induction of SREBP-1c. The knock-down of fetuin-A by silencing RNA (siRNA) restored these changes [88]. Another study observed that fetuin-A induced mammalian target of rapamycin (mTOR)

phosphorylation, which has been reported to induce the activation of SREBP-1c expression, thus, demonstrating the correlation of activating fetuin-A indirectly activating SREBP-1c [90].

Our findings demonstrate that the AE 500 treatment was able to reduce fetuin-A mRNA expression in relation to all groups, along with the downregulation of SREBP-1c mRNA, under the influence of the HFF diet. Although we observed that the AE treatments had no influence on most of the metabolic parameters, in our diet-induced metabolic syndrome animal model, the AE 500 treatment was shown to improve the glucose tolerance. This effect could be associated with the indirect influence of bioactive compounds found in noni extract, such as iridoids, noniosides, and rutin, acting in a synergistic manner in PPAR-α, PPAR-γ, SREBP-1c, and fetuin-A gene expression, which are involved in the lipid and glycemic metabolism in the liver and in white adipose tissue.

Certain considerations are important to be highlighted in our study, and one is that the introduction of an HFF diet at weaning could yield a more pronounced metabolic syndrome phenotype compared with the introduction of an HFF diet in young adults (12 weeks of age). The evaluation of an extended period of treatment or the use AE as a supplementation at the beginning of diet induction would be of value. Further studies are necessary to evaluate the role of the AE 500 treatment's influence in the SREBP-1c pathway, for instance, related to mammalian target of rapamycin (mTOR) phosphorylation and serine/threonine kinase (S6k), as well as to evaluate the influence of AE 500 on PPAR expression in white adipose tissue

5. Conclusions

Neither dose of AE exhibited neurotoxic, behavioral, nor mortality effects in the acute toxicity test, after or during the post-treatment period. Iridoids, noniosides, and the flavonoid rutin were tentatively identified in AE through analyses of the UFLC–DAD–MS data. Neither dose of AE influenced the majority of the metabolic indicators studied (including weight loss, the total cholesterol, triglycerides, non-HDL, HDL-C, and the insulin serum levels) or the histological parameters in the liver, pancreas, and epidydimal adipose tissue. However, the higher dose of AE (AE 500) was shown to be effective in improving the glucose tolerance in the OGTT test, which could be associated with the influence of the PPAR-α expression in adipose tissue and the PPAR-γ, PPAR-α, SREBP-1c, and fetuin-A expression in the liver. Further studies can explore how noni extract influences PPARs in adipose tissue and the SREPB-1c pathway in the liver.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-6643/12/11/3439/s1, Table S1: Initial and final body weight, weight gain, food and calories intake and feed efficiency index between the 1st and 9th weeks of CT (regular chow diet) and HFF (high-fat-high-fructose diet). Figure S1: Weight of organs of *M. citrifolia*, body weight gain, average food intake and average water intake of CT group and *M. citrifolia* aqueous fruit extract (AE) 2000 mg/kg during 14 days of acute oral toxicity test. Figure S2: Hippocratic Screening Test of AE 2000 mg/kg group during 14 days of acute oral toxicity test. Figure S3: Evaluation of the glycemic profile at the beginning of the treatment with AE (9th week) of CTW, HFFW, HFF + AE, HFF + AE 500 groups.

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Supplementary Material

Table S1. Initial and final body weight, weight gain, food and calories intake and feed efficiency index between the 1st and 9th weeks.

Parameter	Groups	
	CT (n=11)	**HFF (n=31)**
Initial Body Weight (g)	45.27±1.84	41.55±0.89
Final Body Weight (g)	47.91±1.75	54.97±1.25*
Weight Gain (g)	2.64±0.88	13.42±1.00*
Food intake (g/day)	45.36±1.71	27.89±0.28*
Calories intake (kcal)	172.4±6.49	149.1±1.70*
Feed Efficiency Index	47.80±1.75	54.80±1.25*

CT: regular chow diet. HFF: high-fat-high-fructose diet. Results are expressed as mean ± SEM. Student´s unpaired t-test. * $p \leq 0.05$ *vs* CT.

Figure S1. Acute oral toxicity

Figure S1.1. Weight of organs of CT group and *M. citrifolia* aqueous fruit extract (AE) 2000 mg/kg after 14 days of acute oral toxicity test.

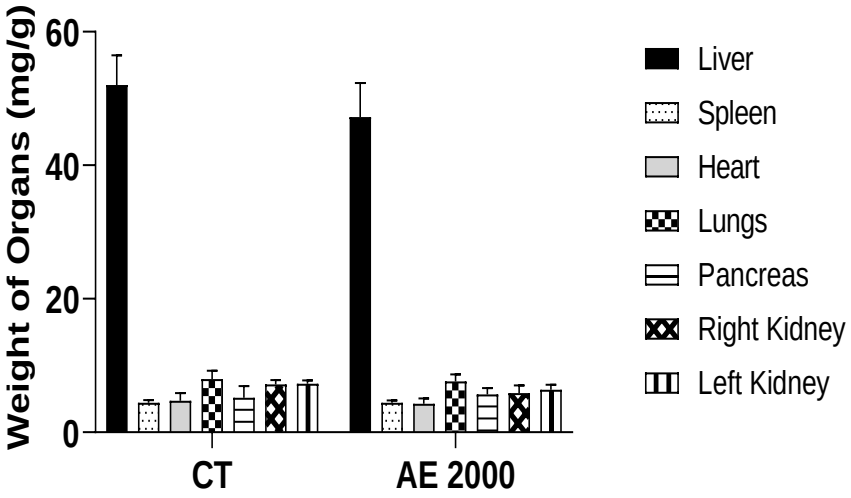


Figure S1.2. Body weight gain of CT group and *M. citrifolia* aqueous fruit extract (AE) 2000 mg/kg during 14 days of acute oral toxicity test (1st, 5th, 10th and 14th days).

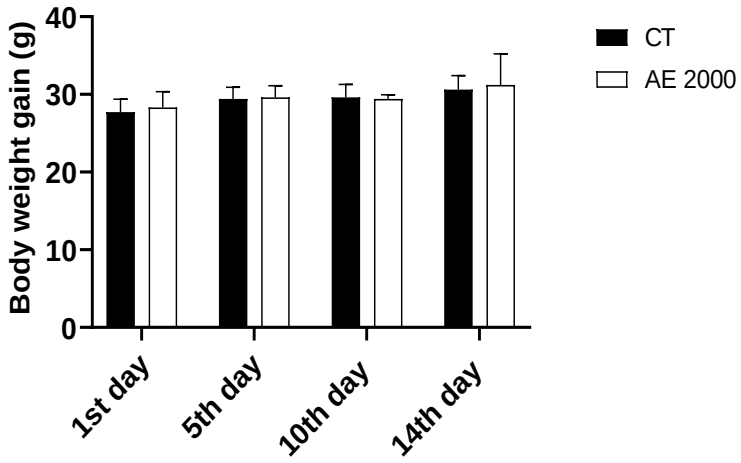
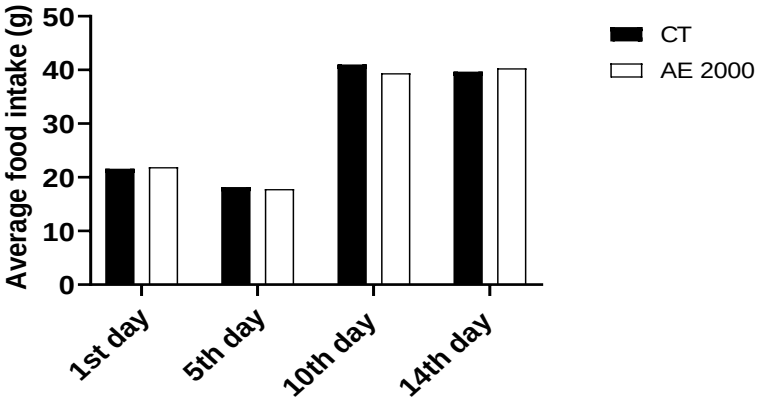


Figure S1.3. Average food and water intake of CT group and *M. citrifolia* aqueous fruit extract (AE) 2000 mg/kg during 14 days of acute oral toxicity test (1st, 5th, 10th and 14th days).



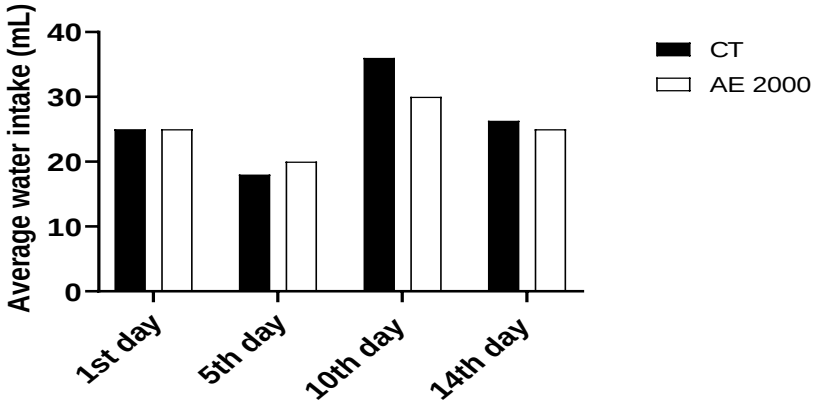


Figure S1.4. Hippocratic screening tests of *M. citrifolia* aqueous fruit extract (AE) 2000 mg/kg group (n=5).

HIPPOCRATIC SCREENING TEST

Drug: *Morinda citrifolia* aqueous fruit extract **Dose:** 2000 mg/kg (oral gavage)
Time of gavage: 6:37 AM Data: 27/10/2017 **Animal:** female Swiss mice number 1 **Weight:** 27.5 g

Symptoms	**Normal**	**Time**											
		0	30 m	1,0 h	2,0 h	3,0 h	4,0 h	6,0 h	12 h	24 h	2 days	3 days	4 days
Normal appearance	4	4	4	4	4	4	4	4	4	4	4	4	4
Vocal phrenic	0	0	0	0	0	0	0	0	0	0	0	0	0
Irritability	0	0	0	0	0	0	0	0	0	0	0	0	0
Touch response	4	4	4	4	4	4	4	4	4	4	4	4	4
Tail grip	4	4	4	4	4	4	4	4	4	4	4	4	4
Contortion	0	0	0	0	0	0	0	0	0	0	0	0	0
Muscle tonus	4	4	4	4	4	4	4	4	4	4	4	4	4
Grip strength	4	4	4	4	4	4	4	4	4	4	4	4	4
Ataxia	0	0	0	0	0	0	0	0	0	0	0	0	0
Tremors	0	0	0	0	0	0	0	0	0	0	0	0	0
Convulsions	0	0	0	0	0	0	0	0	0	0	0	0	0
Estimulations	4	4	4	4	4	4	4	4	4	4	4	4	4
Hypnosis	0	0	0	0	0	0	0	0	0	0	0	0	0
Anesthesia	0	0	0	0	0	0	0	0	0	0	0	0	0
Lacrimation	0	0	0	0	0	0	0	0	0	0	0	0	0
Urination	4	4	4	4	4	4	4	4	4	4	4	4	4
Defecation	4	4	4	4	4	4	4	4	4	4	4	4	4
Hypothermia	0	0	0	0	0	0	0	0	0	0	0	0	0
Respiration	4	4	4	4	4	4	4	4	4	4	4	4	4
Cyanosis	0	0	0	0	0	0	0	0	0	0	0	0	0
Death	-	-	-	-	-	-	-	-	-	-	-	-	-

- **Codes:**
Tests with normal annotation "0", the intensity of the effect varies on a scale from 1 to 4
Test with normal annotation "4", the intensity of the effect can vary from 0 to 3 when there is a decrease, 4 when equal to the control and from 5 to 8 when there is an increase

Symptoms	**Normal**	5 days	6 days	7 days	8 days	9 days	10 days	11 days	12 days	13 days	14 days
Normal appearance	4	4	4	4	4	4	4	4	4	4	4
Vocal phrenic	0	0	0	0	0	0	0	0	0	0	0
Irritability	0	0	0	0	0	0	0	0	0	0	0
Touch response	4	4	4	4	4	4	4	4	4	4	4
Tail grip	4	4	4	4	4	4	4	4	4	4	4
Contortion	0	0	0	0	0	0	0	0	0	0	0
Muscle tonus	4	4	4	4	4	4	4	4	4	4	4
Grip strength	4	4	4	4	4	4	4	4	4	4	4
Ataxia	0	0	0	0	0	0	0	0	0	0	0
Tremors	0	0	0	0	0	0	0	0	0	0	0
Convulsions	0	0	0	0	0	0	0	0	0	0	0
Estimulations	4	4	4	4	4	4	4	4	4	4	4
Hypnosis	0	0	0	0	0	0	0	0	0	0	0
Anesthesia	0	0	0	0	0	0	0	0	0	0	0
Lacrimation	0	0	0	0	0	0	0	0	0	0	0
Urination	4	4	4	4	4	4	4	4	4	4	4
Defecation	4	4	4	4	4	4	4	4	4	4	4
Hypothermia	0	0	0	0	0	0	0	0	0	0	0
Respiration	4	4	4	4	4	4	4	4	4	4	4
Cyanosia	0	0	0	0	0	0	0	0	0	0	0
Death	-	-	-	-	-	-	-	-	-	-	-

HIPPOCRATIC SCREENING TEST

Drug: *Morinda citrifolia* aqueous fruit extract **Dose:** 2000 mg/kg (oral gavage)
Time of gavage: 6:41 AM Data: 27/10/2017 **Animal:** female Swiss mice number 2 **Weight:** 30.4 g

Symptoms	**Normal**	**Time**											
		0	30 m	1,0 h	2,0 h	3,0 h	4,0 h	6,0 h	12 h	24 h	2 days	3 days	4 days
Normal appearance	4	4	4	4	4	4	4	4	4	4	4	4	4
Vocal phrenic	0	0	0	0	0	0	0	0	0	0	0	0	0
Irritability	0	0	0	0	0	0	0	0	0	0	0	0	0
Touch response	4	4	4	4	4	4	4	4	4	4	4	4	4
Tail grip	4	4	4	4	4	4	4	4	4	4	4	4	4
Contortion	0	0	0	0	0	0	0	0	0	0	0	0	0
Muscle tonus	4	4	4	4	4	4	4	4	4	4	4	4	4
Grip strength	4	4	4	4	4	4	4	4	4	4	4	4	4
Ataxia	0	0	0	0	0	0	0	0	0	0	0	0	0
Tremors	0	0	0	0	0	0	0	0	0	0	0	0	0
Convulsions	0	0	0	0	0	0	0	0	0	0	0	0	0
Estimulations	4	4	4	4	4	4	4	4	4	4	4	4	4
Hypnosis	0	0	0	0	0	0	0	0	0	0	0	0	0
Anesthesia	0	0	0	0	0	0	0	0	0	0	0	0	0
Lacrimation	0	0	0	0	0	0	0	0	0	0	0	0	0
Urination	4	4	4	4	4	4	4	4	4	4	4	4	4
Defecation	4	4	4	4	4	4	4	4	4	4	4	4	4
Hypothermia	0	0	0	0	0	0	0	0	0	0	0	0	0
Respiration	4	4	4	4	4	4	4	4	4	4	4	4	4
Cyanosis	0	0	0	0	0	0	0	0	0	0	0	0	0
Death	-	-	-	-	-	-	-	-	-	-	-	-	-

- **Codes:**

Tests with normal annotation "0", the intensity of the effect varies on a scale from 1 to 4

Test with normal annotation "4", the intensity of the effect can vary from 0 to 3 when there is a decrease, 4 when equal to the control and from 5 to 8 when there is an increase

Symptoms	**Normal**	5 days	6 days	7 days	8 days	9 days	10 days	11 days	12 days	13 days	14 days
Normal appearance	4	4	4	4	4	4	4	4	4	4	4
Vocal phrenic	0	0	0	0	0	0	0	0	0	0	0
Irritability	0	0	0	0	0	0	0	0	0	0	0
Touch response	4	4	4	4	4	4	4	4	4	4	4
Tail grip	4	4	4	4	4	4	4	4	4	4	4
Contortion	0	0	0	0	0	0	0	0	0	0	0
Muscle tonus	4	4	4	4	4	4	4	4	4	4	4
Grip strength	4	4	4	4	4	4	4	4	4	4	4
Ataxia	0	0	0	0	0	0	0	0	0	0	0
Tremors	0	0	0	0	0	0	0	0	0	0	0
Convulsions	0	0	0	0	0	0	0	0	0	0	0
Estimulations	4	4	4	4	4	4	4	4	4	4	4
Hypnosis	0	0	0	0	0	0	0	0	0	0	0
Anesthesia	0	0	0	0	0	0	0	0	0	0	0
Lacrimation	0	0	0	0	0	0	0	0	0	0	0
Urination	4	4	4	4	4	4	4	4	4	4	4
Defecation	4	4	4	4	4	4	4	4	4	4	4
Hypothermia	0	0	0	0	0	0	0	0	0	0	0
Respiration	4	4	4	4	4	4	4	4	4	4	4
Cyanosia	0	0	0	0	0	0	0	0	0	0	0
Death	-	-	-	-	-	-	-	-	-	-	-

HIPPOCRATIC SCREENING TEST

Drug: *Morinda citrifolia* aqueous fruit extract **Dose:** 2000 mg/kg (oral gavage)
Time of gavage: 6:44 AM Data: 27/10/2017 **Animal:** female Swiss mice number 3 **Weight:** 29.1 g

Symptoms	**Normal**	**Time**											
		0	30 m	1,0 h	2,0 h	3,0 h	4,0 h	6,0 h	12 h	24 h	2 days	3 days	4 days
Normal appearance	4	4	4	4	4	4	4	4	4	4	4	4	4
Vocal phrenic	0	0	0	0	0	0	0	0	0	0	0	0	0
Irritability	0	0	0	0	0	0	0	0	0	0	0	0	0
Touch response	4	4	4	4	4	4	4	4	4	4	4	4	4
Tail grip	4	4	4	4	4	4	4	4	4	4	4	4	4
Contortion	0	0	0	0	0	0	0	0	0	0	0	0	0
Muscle tonus	4	4	4	4	4	4	4	4	4	4	4	4	4
Grip strength	4	4	4	4	4	4	4	4	4	4	4	4	4
Ataxia	0	0	0	0	0	0	0	0	0	0	0	0	0
Tremors	0	0	0	0	0	0	0	0	0	0	0	0	0
Convulsions	0	0	0	0	0	0	0	0	0	0	0	0	0
Estimulations	4	4	4	4	4	4	4	4	4	4	4	4	4
Hypnosis	0	0	0	0	0	0	0	0	0	0	0	0	0
Anesthesia	0	0	0	0	0	0	0	0	0	0	0	0	0
Lacrimation	0	0	0	0	0	0	0	0	0	0	0	0	0
Urination	4	4	4	4	4	4	4	4	4	4	4	4	4
Defecation	4	4	4	4	4	4	4	4	4	4	4	4	4
Hypothermia	0	0	0	0	0	0	0	0	0	0	0	0	0
Respiration	4	4	4	4	4	4	4	4	4	4	4	4	4
Cyanosis	0	0	0	0	0	0	0	0	0	0	0	0	0
Death	-	-	-	-	-	-	-	-	-	-	-	-	-

- **Codes:**

Tests with normal annotation "0", the intensity of the effect varies on a scale from 1 to 4

Test with normal annotation "4", the intensity of the effect can vary from 0 to 3 when there is a decrease, 4 when equal to the control and from 5 to 8 when there is an increase

Symptoms	**Normal**	5 days	6 days	7 days	8 days	9 days	10 days	11 days	12 days	13 days	14 days
Normal appearance	4	4	4	4	4	4	4	4	4	4	4
Vocal phrenic	0	0	0	0	0	0	0	0	0	0	0
Irritability	0	0	0	0	0	0	0	0	0	0	0
Touch response	4	4	4	4	4	4	4	4	4	4	4
Tail grip	4	4	4	4	4	4	4	4	4	4	4
Contortion	0	0	0	0	0	0	0	0	0	0	0
Muscle tonus	4	4	4	4	4	4	4	4	4	4	4
Grip strength	4	4	4	4	4	4	4	4	4	4	4
Ataxia	0	0	0	0	0	0	0	0	0	0	0
Tremors	0	0	0	0	0	0	0	0	0	0	0
Convulsions	0	0	0	0	0	0	0	0	0	0	0
Estimulations	4	4	4	4	4	4	4	4	4	4	4
Hypnosis	0	0	0	0	0	0	0	0	0	0	0
Anesthesia	0	0	0	0	0	0	0	0	0	0	0
Lacrimation	0	0	0	0	0	0	0	0	0	0	0
Urination	4	4	4	4	4	4	4	4	4	4	4
Defecation	4	4	4	4	4	4	4	4	4	4	4
Hypothermia	0	0	0	0	0	0	0	0	0	0	0
Respiration	4	4	4	4	4	4	4	4	4	4	4
Cyanosia	0	0	0	0	0	0	0	0	0	0	0
Death	-	-	-	-	-	-	-	-	-	-	-

HIPPOCRATIC SCREENING TEST

Drug: *Morinda citrifolia* aqueous fruit extract **Dose:** 2000 mg/kg (oral gavage)
Time of gavage: 6:49 AM Data: 27/10/2017 **Animal:** female Swiss mice number 4 **Weight:** 25.2 g

Symptoms	**Normal**	**Time**											
		0	30 m	1,0 h	2,0 h	3,0 h	4,0 h	6,0 h	12 h	24 h	2 days	3 days	4 days
Normal appearance	4	4	4	4	4	4	4	4	4	4	4	4	4
Vocal phrenic	0	0	0	0	0	0	0	0	0	0	0	0	0
Irritability	0	0	0	0	0	0	0	0	0	0	0	0	0
Touch response	4	4	4	4	4	4	4	4	4	4	4	4	4
Tail grip	4	4	4	4	4	4	4	4	4	4	4	4	4
Contortion	0	0	0	0	0	0	0	0	0	0	0	0	0
Muscle tonus	4	4	4	4	4	4	4	4	4	4	4	4	4
Grip strength	4	4	4	4	4	4	4	4	4	4	4	4	4
Ataxia	0	0	0	0	0	0	0	0	0	0	0	0	0
Tremors	0	0	0	0	0	0	0	0	0	0	0	0	0
Convulsions	0	0	0	0	0	0	0	0	0	0	0	0	0
Estimulations	4	4	4	4	4	4	4	4	4	4	4	4	4
Hypnosis	0	0	0	0	0	0	0	0	0	0	0	0	0
Anesthesia	0	0	0	0	0	0	0	0	0	0	0	0	0
Lacrimation	0	0	0	0	0	0	0	0	0	0	0	0	0
Urination	4	4	4	4	4	4	4	4	4	4	4	4	4
Defecation	4	4	4	4	4	4	4	4	4	4	4	4	4
Hypothermia	0	0	0	0	0	0	0	0	0	0	0	0	0
Respiration	4	4	4	4	4	4	4	4	4	4	4	4	4
Cyanosis	0	0	0	0	0	0	0	0	0	0	0	0	0
Death	-	-	-	-	-	-	-	-	-	-	-	-	-

- **Codes:**
Tests with normal annotation "0", the intensity of the effect varies on a scale from 1 to 4
Test with normal annotation "4", the intensity of the effect can vary from 0 to 3 when there is a decrease, 4 when equal to the control and from 5 to 8 when there is an increase

Symptoms	**Normal**	5 days	6 days	7 days	8 days	9 days	10 days	11 days	12 days	13 days	14 days
Normal appearance	4	4	4	4	4	4	4	4	4	4	4
Vocal phrenic	0	0	0	0	0	0	0	0	0	0	0
Irritability	0	0	0	0	0	0	0	0	0	0	0
Touch response	4	4	4	4	4	4	4	4	4	4	4
Tail grip	4	4	4	4	4	4	4	4	4	4	4
Contortion	0	0	0	0	0	0	0	0	0	0	0
Muscle tonus	4	4	4	4	4	4	4	4	4	4	4
Grip strength	4	4	4	4	4	4	4	4	4	4	4
Ataxia	0	0	0	0	0	0	0	0	0	0	0
Tremors	0	0	0	0	0	0	0	0	0	0	0
Convulsions	0	0	0	0	0	0	0	0	0	0	0
Estimulations	4	4	4	4	4	4	4	4	4	4	4
Hypnosis	0	0	0	0	0	0	0	0	0	0	0
Anesthesia	0	0	0	0	0	0	0	0	0	0	0
Lacrimation	0	0	0	0	0	0	0	0	0	0	0
Urination	4	4	4	4	4	4	4	4	4	4	4
Defecation	4	4	4	4	4	4	4	4	4	4	4
Hypothermia	0	0	0	0	0	0	0	0	0	0	0
Respiration	4	4	4	4	4	4	4	4	4	4	4
Cyanosia	0	0	0	0	0	0	0	0	0	0	0
Death	-	-	-	-	-	-	-	-	-	-	-

HIPPOCRATIC SCREENING TEST

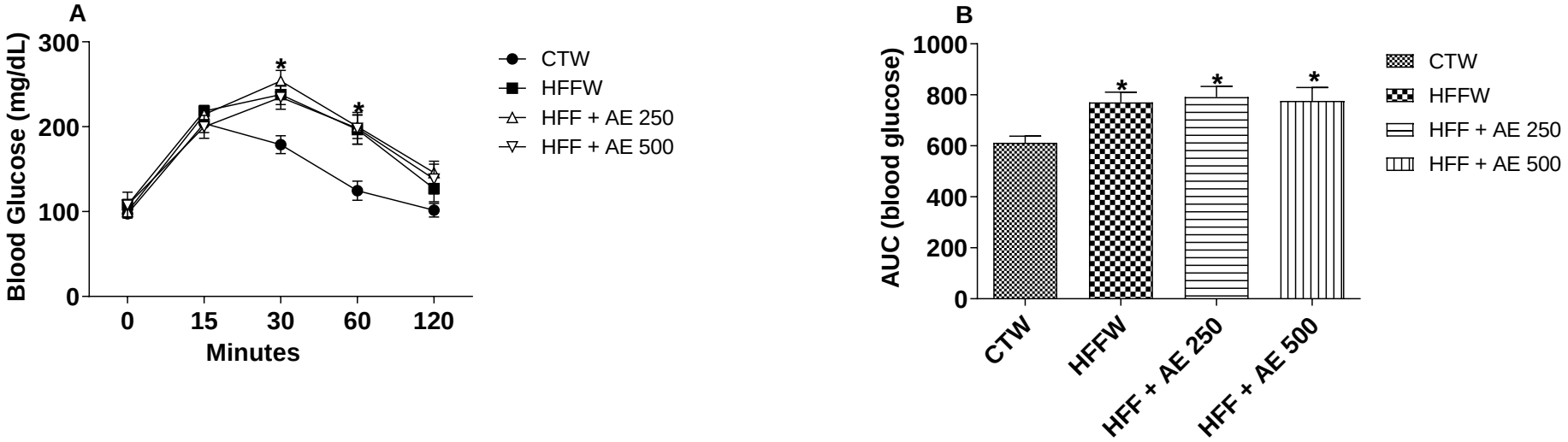
Drug: *Morinda citrifolia* aqueous fruit extract **Dose:** 2000 mg/kg (oral gavage)
Time of gavage: 6:53 AM PM Data: 27/10/2017 **Animal:** female Swiss mice number 5 **Weight:** 29.4 g

Symptoms	**Normal**	**Time**											
		0	30 m	1,0 h	2,0 h	3,0 h	4,0 h	6,0 h	12 h	24 h	2 days	3 days	4 days
Normal appearance	4	4	4	4	4	4	4	4	4	4	4	4	4
Vocal phrenic	0	0	0	0	0	0	0	0	0	0	0	0	0
Irritability	0	0	0	0	0	0	0	0	0	0	0	0	0
Touch response	4	4	4	4	4	4	4	4	4	4	4	4	4
Tail grip	4	4	4	4	4	4	4	4	4	4	4	4	4
Contortion	0	0	0	0	0	0	0	0	0	0	0	0	0
Muscle tonus	4	4	4	4	4	4	4	4	4	4	4	4	4
Grip strength	4	4	4	4	4	4	4	4	4	4	4	4	4
Ataxia	0	0	0	0	0	0	0	0	0	0	0	0	0
Tremors	0	0	0	0	0	0	0	0	0	0	0	0	0
Convulsions	0	0	0	0	0	0	0	0	0	0	0	0	0
Estimulations	4	4	4	4	4	4	4	4	4	4	4	4	4
Hypnosis	0	0	0	0	0	0	0	0	0	0	0	0	0
Anesthesia	0	0	0	0	0	0	0	0	0	0	0	0	0
Lacrimation	0	0	0	0	0	0	0	0	0	0	0	0	0
Urination	4	4	4	4	4	4	4	4	4	4	4	4	4
Defecation	4	4	4	4	4	4	4	4	4	4	4	4	4
Hypothermia	0	0	0	0	0	0	0	0	0	0	0	0	0
Respiration	4	4	4	4	4	4	4	4	4	4	4	4	4
Cyanosis	0	0	0	0	0	0	0	0	0	0	0	0	0
Death	-	-	-	-	-	-	-	-	-	-	-	-	-

- **Codes:**
Tests with normal annotation "0", the intensity of the effect varies on a scale from 1 to 4
Test with normal annotation "4", the intensity of the effect can vary from 0 to 3 when there is a decrease, 4 when equal to the control and from 5 to 8 when there is an increase

Symptoms	**Normal**	5 days	6 days	7 days	8 days	9 days	10 days	11 days	12 days	13 days	14 days
Normal appearance	4	4	4	4	4	4	4	4	4	4	4
Vocal phrenic	0	0	0	0	0	0	0	0	0	0	0
Irritability	0	0	0	0	0	0	0	0	0	0	0
Touch response	4	4	4	4	4	4	4	4	4	4	4
Tail grip	4	4	4	4	4	4	4	4	4	4	4
Contortion	0	0	0	0	0	0	0	0	0	0	0
Muscle tonus	4	4	4	4	4	4	4	4	4	4	4
Grip strength	4	4	4	4	4	4	4	4	4	4	4
Ataxia	0	0	0	0	0	0	0	0	0	0	0
Tremors	0	0	0	0	0	0	0	0	0	0	0
Convulsions	0	0	0	0	0	0	0	0	0	0	0
Estimulations	4	4	4	4	4	4	4	4	4	4	4
Hypnosis	0	0	0	0	0	0	0	0	0	0	0
Anesthesia	0	0	0	0	0	0	0	0	0	0	0
Lacrimation	0	0	0	0	0	0	0	0	0	0	0
Urination	4	4	4	4	4	4	4	4	4	4	4
Defecation	4	4	4	4	4	4	4	4	4	4	4
Hypothermia	0	0	0	0	0	0	0	0	0	0	0
Respiration	4	4	4	4	4	4	4	4	4	4	4
Cyanosia	0	0	0	0	0	0	0	0	0	0	0
Death	-	-	-	-	-	-	-	-	-	-	-

Figure S2. Evaluation of the glycemic profile at the beginning of the treatment with AE. (A) Oral glucose tolerance test at the end of the treatment (9th week); (B) Area under the curve (AUC) of blood glucose of animals evaluated at the end of the treatment (9th week) of CTW (CT + drink water, n=11); HFFW (high-fat-high-fructose diet + drink water, n=11); HFF + AE 250 (HFF + *M. citrifolia* fruit aqueous extract of 250 mg/kg of body weight, n=11). HFF + AE 500 (HFF + *M. citrifolia* fruit aqueous extract of 500 mg/kg, n=10) groups. Results are expressed as mean ± SEM. *p ≤ 0.05 vs. CTW. Two-way ANOVA followed by Bonferroni post-test for oral glucose tolerance test. ANOVA followed by Tukey post-test for AUC.



ANEXO A - CERTIFICADO DE APROVAÇÃO DA COMISSÃO DE ÉTICA EM USO DE ANIMAIS (CEUA)

Serviço Público Federal
Ministério da Educação
Fundação Universidade Federal de Mato Grosso do Sul

UFMS

C E R T I F I C A D O

Certificamos que a proposta intitulada "Efeitos metabólicos do extrato aquoso das folhas e frutos de *Morinda citrifolia Linn* (noni) em camundongos obesos/doença hepática gordurosa não alcoólica", registrada com o nº 860/2017, sob a responsabilidade de **Priscila Aiko Hiane** - que envolve a utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata, para fins de pesquisa científica – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovada pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS/CEUA DA UNIVERSIDADE FEDERAL DE MATO GROSSO DO SUL/UFMS, na 5ª reunião ordinária do dia 19/06/2017.

FINALIDADE	( ) Ensino (x) Pesquisa Científica
Vigência da autorização	20/03/2017 a 20/03/2020
Espécie/Linhagem/Raça	*Mus musculus* / Swiss
Nº de animais	252 15
Peso/Idade	40g / 60 dias
Sexo	Macho e Fêmea
Origem	Biotério Central/CCBS/UFMS

Joice Stein
Coordenadora da CEUA/UFMS
Campo Grande, 20 de junho de 2017.

Comissão de Ética no Uso de Animais/CEUA
http://www.propp.ufms.br/ceua
ceua.2000@gmail.com
fone (67) 3345-7925

CONSIDERAÇÕES FINAIS

Conforme apresentado no capítulo 2, diferentes partes de *M. citrifolia* apresentam ações terapêuticas em disfunções metabólicas que podem estar asssociadas ou não com a SM. Porém, as diferentes partes e o tipo de processamento de *M. citrifolia* Linn (noni) são requisitos importantes a serem levados em consideração para o direcionamento da abordagem terapêutica a ser realizada. No capítulo 3, foi observado que o extrato aquoso bruto dos frutos de *M. citrifolia* não apresentou efeitos tóxicos por não exibirem efeitos neurotóxicos, comportamentais e nenhum caso de mortalidade no teste de toxicidade aguda. Além disso, no perfil químico, foram observados 17 compostos, dos quais incluem iridódies, noniosídeos e o flavonóide, rutina e, ainda, 5 compostos dentre os 17 identificados, são desconhecidos na literatura. Nenhuma das doses do extrato aquoso bruto de *M. citrifolia* influenciou de forma proeminente nos indicadores metabólicos, como perda de peso, colesterol total, triglicerídeos, não-HDL, HDL e nos níveis séricos de insulina. Isso também foi observado nos parâmetros histológicos do fígado, pâncreas e tecido adiposo epididimal. Estes resultados sugerem que as duas doses do extrato aquoso bruto de *M. citrifolia* não influenciaram nos padrões metabólicos que qualificam o fenótipo de SM. Porém, a maior dose do extrato (AE 500 mg/kg/dia) demonstrou ser efetivo na melhora da tolerância à glicose no teste de TOGG e, este efeito pode estar associado com a influência na expressão de PPAR-α no tecido adiposo branco epididimal e na expressão de PPAR-γ, PPAR-α, SREBP-1c e fetuína-A no fígado. Certas considerações neste estudo devem ser salientadas e, uma delas, seria que a introdução da dieta HFF aos animais no desmame poderia produzir um fenótipo de SM muito mais pronunciado comparado a jovens adultos com 12 semanas. Além disso, a avaliação de um período mais prolongado no uso do AE 500 mg/kg/dia ou a utilização deste como suplementação logo no início da indução da dieta seria importante a ser avaliado. Pelo fato de que o AE 500 mg/kg/dia apresentou influência na expressão de SREBP-1c, estudos adicionais são relevantes para avaliar os efeitos de AE 500 mg/kg/dia nas vias de sinalização de SREBP-1c,

avaliando a expressão do conteúdo proteico de fetuína-A, a fosforilação do alvo de rapamicina em mamíferos (mTOR) e a serina/treonina quinase (S6K) (fetuin-A/mTOR/S6K), no fígado, como também, a expressão de PPAR-α e -γ no tecido adiposo branco epididimal. Ainda, estudos que avaliam os efeitos do extrato aquoso bruto dos frutos de *M. citrifolia* na microbiota intestinal serão de grande importância.