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EDILSON RODRIGUES ALBUQUERQUE

POTENCIAL FARMACOLÓGICO PRÉ-CLÍNICO DE *Petiveria alliacea* **E** *Pereskia grandifolia* **EM MODELO DE DOENÇA HEPÁTICA GORDUROSA ASSOCIADA AO METABOLISMO**

Umuarama
2025

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Orientação: Dra. Francislaine Aparecida dos Reis Lívero

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POTENCIAL FARMACOLÓGICO PRÉ-CLÍNICO DE *Petiveria alliacea* E *Pereskia grandifolia* EM MODELO DE DOENÇA HEPÁTICA GORDUROSA ASSOCIADA AO METABOLISMO

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EDILSON RODRIGUES ALBUQUERQUE

POTENCIAL FARMACOLÓGICO PRÉ-CLÍNICO DE *Petiveria alliacea* **E** *Pereskia grandifolia* **EM MODELO DE DOENÇA HEPÁTICA GORDUROSA ASSOCIADA AO METABOLISMO**

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"*Há verdadeiramente duas coisas diferentes: saber e crer que se sabe. A ciência consiste em saber; em crer que se sabe reside a ignorância.*"

(Hipócrates)

ALBUQUERQUE, Edilson Rodrigues. **Potencial farmacológico pré-clínico de** *Petiveria alliacea* **e** *Pereskia grandifolia* **em modelo de doença hepática gordurosa associada ao metabolismo.** Orientadora: Francislaine Aparecida dos Reis Lívero. 2025. 113 f. Tese (Doutorado em Ciência Animal com Ênfase em Produtos Bioativos) - Universidade Paranaense, Umuarama, 2025.

RESUMO

A doença hepática gordurosa associada ao metabolismo (DHGAM) é caracterizada pelo acúmulo de lipídios associado a distúrbios metabólicos sistêmicos. Fatores como diabetes, dislipidemia, tabagismo e alcoolismo intensificam sua progressão, e as terapias disponíveis ainda são limitadas. Nesse cenário, a etnofarmacologia surge como estratégia promissora para identificar novos agentes hepatoprotetores. Esta tese, composta por dois estudos, investigou a segurança e o potencial terapêutico de *Petiveria alliacea* e *Pereskia grandifolia* em modelos pré-clínicos de DHGAM. No primeiro estudo, avaliou-se a fração solúvel em etanol das folhas de *P. alliacea*. Ensaios de citotoxicidade em células HepG2 e teste de toxicidade aguda em ratos Wistar (n = 6) confirmaram sua segurança. A DHGAM foi induzida em ratos Wistar machos por diabetes (estreptozotocina, 60 mg/kg, i.p.), dislipidemia (dieta com 0,5% de colesterol) e consumo de álcool (5%) por cinco semanas. Nas duas últimas semanas, os animais foram distribuídos (n = 6–7) e tratados por gavagem com veículo (C-), *P. alliacea* (30, 100 ou 300 mg/kg) e sinvastatina (2,5 mg/kg) + insulina (6 UI, s.c.; SIM+INSU). Um grupo de ratos basal foi incluído. O modelo induziu hiperglicemia, aumento de colesterol e triglicerídeos plasmáticos e hepáticos, elevação de aspartato e alanina aminotransferase (AST e ALT, respectivamente), redução de defesas antioxidantes hepáticas, aumento da peroxidação lipídica e lesões histopatológicas, como esteatose, inflamação e degeneração hepatocelular. Houve dano renal com alterações glomerulares e túbulo-intersticiais. O tratamento com *P. alliacea* reduziu parcialmente a glicemia, diminuiu lipídios plasmáticos e hepáticos, normalizou AST e ALT e, na dose de 300 mg/kg, restaurou completamente os antioxidantes e reduziu lipoperoxidação, revertendo também a disfunção renal. Já SIM+INSU reduziu glicemia e lipídios, mas sem restaurar adequadamente enzimas hepáticas nem marcadores oxidativos. O segundo estudo avaliou a fração solúvel em etanol das folhas de *P. grandifolia*. Ensaios *in vitro* e teste de toxicidade aguda em ratos Wistar (n = 6) demonstraram segurança, e o extrato apresentou potencial anti-inflamatório *in vitro* ao inibir fagocitose e migração de macrófagos. Para avaliação terapêutica, a DHGAM foi induzida por diabetes e dislipidemia (mesmos métodos do estudo um) associadas à exposição à fumaça de cigarro por quatro semanas. Os animais foram distribuídos em seis grupos (n = 8) e tratados com veículo (C-), *P. grandifolia* (30, 100 ou 300

mg/kg) e SIM+INSU. Ratos não expostos aos fatores de risco compuseram o grupo basal. O modelo causou hiperglicemia, dislipidemia, aumento de AST e ALT, maior lipoperoxidação, redução de antioxidantes hepáticos e lesões histológicas, incluindo esteatose macro e microvesicular e infiltrado inflamatório. O tratamento com *P. grandifolia*, especialmente 100 mg/kg, reverteu essas alterações, normalizando enzimas hepáticas, restaurando defesas antioxidantes e reduzindo lipoperoxidação. O efeito hipolipemiante ocorreu tanto com o extrato quanto com SIM+INSU, embora o tratamento convencional não restaurasse plenamente enzimas hepáticas nem parâmetros oxidativos. Em conjunto, os resultados demonstram que *P. alliacea* e *P. grandifolia* são seguras e eficazes no tratamento pré-clínico da DHGAM, apresentando ação multialvo e atuando sobre hiperglicemia, dislipidemia, estresse oxidativo e danos hepáticos e renais, destacando-se como alternativas promissoras para doenças metabólicas complexas.

Palavras-chave: Anti-inflamatório. Antioxidante. Erva-de-alho. Hepatoprotetor. Nefroprotetor. Ora-pro-nóbis.

ALBUQUERQUE, Edilson Rodrigues. **Preclinical pharmacological potential of** *Petiveria alliacea* **and** *Pereskia grandifolia* **in a model of metabolically associated fatty liver disease.** Advisor: Francislaine Aparecida dos Reis Lívero. 2025. 112 p. Thesis (Doctorate degree in Animal Science with Emphasis on Bioactive Products) - Universidade Paranaense, Umuarama, 2025.

ABSTRACT

Metabolism-associated fatty liver disease (MAFLD) is characterized by the accumulation of lipids associated with systemic metabolic disorders. Factors such as diabetes, dyslipidemia, smoking, and alcoholism intensify their progression, and the available therapies are still limited. In this scenario, ethnopharmacology emerges as a promising strategy for identifying new hepatoprotective agents. This thesis, comprised of two studies, investigated the safety and therapeutic potential of *Petiveria alliacea* and *Pereskia grandifolia* in preclinical models of MAFLD. In the first study, the ethanol-soluble fraction of *P. alliacea* leaves was evaluated. Cytotoxicity assays in HepG2 cells and an acute toxicity test in Wistar rats (n = 6) confirmed their safety. MAFLD was induced in male Wistar rats by diabetes (streptozotocin, 60 mg/kg, i.p.), dyslipidemia (diet with 0.5% cholesterol), and alcohol consumption (5%) for five weeks. In the last two weeks, the animals were distributed (n = 6–7) and treated by gavage with vehicle (C-), *P. alliacea* (30, 100 or 300 mg/kg), and simvastatin (2.5 mg/kg) + insulin (6 UI, s.c.; SIM+INSU). A basal group of rats was included. The model induced hyperglycemia, increased plasma and hepatic cholesterol and triglycerides, elevation of aspartate and alanine aminotransferase (AST and ALT, respectively), reduction of hepatic antioxidant defenses, increased lipid peroxidation, and histopathological lesions, such as steatosis, inflammation, and hepatocellular degeneration. There was also renal damage with glomerular and tubulo-interstitial changes. Treatment with *P. alliacea* partially reduced glycemia, decreased plasma and hepatic lipids, normalized AST and ALT, and at the 300 mg/kg dose, completely restored antioxidants and reduced lipoperoxidation, also reversing renal dysfunction. SIM+INSU reduced glycemia and lipids, but without adequately restoring hepatic enzymes or oxidative markers. The second study evaluated the ethanol-soluble fraction of *P. grandifolia* leaves. *In vitro* assays and an acute toxicity test in Wistar rats (n = 6) demonstrated safety, and the extract showed anti-inflammatory potential *in vitro* by inhibiting macrophage phagocytosis and migration. For therapeutic evaluation, MAFLD was induced by diabetes and dyslipidemia (same methods as study one) associated with cigarette smoke exposure for four weeks. The animals were distributed into six groups (n = 8) and treated with vehicle (C-), *P. grandifolia* (30, 100 or 300 mg/kg), and SIM+INSU. Rats not exposed to risk factors comprised of the basal

group. The model caused hyperglycemia, dyslipidemia, increased AST and ALT, greater lipoperoxidation, reduced hepatic antioxidants, and histological lesions, including macro- and microvesicular steatosis and inflammatory infiltrate. Treatment with *P. grandifolia*, especially 100 mg/kg, reversed these changes, normalizing hepatic enzymes, restoring antioxidant defenses, and reducing lipoperoxidation. The hypolipidemic effect occurred with both the extract and SIM+INSU, although the conventional treatment did not fully restore hepatic enzymes or oxidative parameters. Collectively, the results demonstrate that *P. alliacea* and *P. grandifolia* are safe and effective in the preclinical treatment of MAFLD, showing a multi-target action and acting on hyperglycemia, dyslipidemia, oxidative stress, and hepatic and renal damage, standing out as promising alternatives for complex metabolic diseases.

Keywords: Anti-inflammatory. Antioxidant. Erva-de-alho. Hepatoprotective. Nephroprotective. Ora-pro-nóbis.

LISTA DE ILUSTRAÇÕES

Revisão bibliográfica – *Petiveria alliacea* **e** *Pereskia grandifolia***: uma revisão da literatura sobre os potenciais terapêuticos na doença hepática gordurosa associada ao metabolismo e fatores de risco**

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LISTA DE SIGLAS

CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
COPG	Coordenadoria de Pós-Graduação
CNPq	Conselho Nacional de Desenvolvimento Científico e Tecnológico
UNIPAR	Universidade Paranaense
DHGAM	Doença hepática gordurosa associada ao metabolismo
AST	Aspartato aminotransferase
ALT	Alanina aminotransferase
MASLD	*Metabolic Dysfunction-Associated Steatotic Liver Disease*
OECD	Organização para a Cooperação e Desenvolvimento Econômico
GSH	Glutationa reduzida
SOD	Superóxido dismutase
LPO	Lipoperoxidação
MASH	Esteato-Hepatite Associada à Disfunção Metabólica
DM2	Diabetes Mellitus Tipo 2
HDL	Lipoproteína de alta densidade, do inglês *High Density Lipoprotein*
LDL	Lipoproteína de baixa densidade, do inglês *Low-Density Lipoprotein*
HepG2	Células de hepatoblastoma
NAFLD	*Non-alcoholic Fatty Liver Disease*
UEPG	Universidade Estadual de Ponta Grossa
UFPR	Universidade Estadual do Paraná
ARRIVE	*Animal Research: Reporting of in vivo Experiments*
SOD	*Superoxide dismutase*
LPO	*Lipid peroxidation*
SIM	*Simvastatin*
INSU	*Insulin*

IC50	Concentração inibitória mediana
PC	*Positive control*
NC	*Negative control*
MTT	3-(4,5-dimetiltiazol-2yl)-2,5-di- fenil brometo de tetrazolina
C-	Grupo controle negativo

LISTA DE SÍMBOLOS

ºC	Graus Celsius
®	Marca registrada
±	Mais ou menos
M	Molaridade
%	Porcentagem
mL	Mililitro
g	Grama
µg	Micrograma
mg	Miligramas
dL	Decilitro
kg	Quilograma
n	Número
<	Menor que
>	Maior que
≥	Maior ou igual a
≤	Menor ou igual a

SUMÁRIO

CAPÍTULO 1

REVISÃO BIBLIOGRÁFICA

Petiveria alliacea L. **E** *Pereskia grandifolia* Haw.: **UMA REVISÃO DA LITERATURA SOBRE OS POTENCIAIS TERAPÊUTICOS NA DOENÇA HEPÁTICA GORDUROSA ASSOCIADA AO METABOLISMO E FATORES DE RISCO**

O capítulo 1 foi editado de acordo com as normas da Associação Brasileira de Normas Técnicas - ABNT.

1.1 Introdução

1.1.1 Caracterização da Doença Hepática Gordurosa Associada ao Metabolismo (DHGAM)

A DHGAM é definida por sua etiologia metabólica e caracterizada pelo acúmulo excessivo de lipídios no parênquima hepático, sem que a causa primária desta condição seja o consumo excessivo de álcool (Younossi *et al*., 2023). Em sua base fisiopatológica, a resistência insulínica e a consequente toxicidade lipídica promovem o dano celular, ativando mecanismos de inflamação e estresse oxidativo. Essa progressão pode desencadear a forma mais agressiva da doença, a Esteato-Hepatite Associada à Disfunção Metabólica (MASH), e o desenvolvimento de fibrose hepática (Allen *et al*., 2018). Embora a DHGAM seja reconhecida como a doença hepática crônica de maior prevalência mundial (Younossi *et al*., 2016; Estes *et al*., 2018), sua implicação clínica mais grave reside no fato de ser um preditor independente de Doença Cardiovascular, que representa a principal causa de morte em indivíduos com DHGAM. Portanto, a DHGAM é mais apropriadamente vista como um sinal orgânico da severidade da disfunção metabólica sistêmica, intensificando o risco cardiometabólico (Chalasani *et al*., 2018; Polyzos *et al*., 2019).

Por representar um desafio hepatológico crônico de magnitude global (Estes *et al*., 2018), a DHGAM sustenta a prevalência de esteatose hepática metabólica, que atinge aproximadamente 25% da população mundial, configurando uma verdadeira epidemia silenciosa (Younossi *et al*., 2016). Essa distribuição, contudo, não é uniforme, demonstrando uma forte correlação com a prevalência crescente da obesidade e Diabetes Mellitus Tipo 2 (DM2), que estão entre os principais fatores etiológicos da doença (Chalasani *et al*., 2018; Younossi *et al*., 2023). O aumento exponencial dessa condição impõe uma carga significativa sobre os sistemas de saúde, uma vez que a progressão para as formas mais avançadas, como a MASH e a fibrose hepática, está se tornando uma das causas primárias de cirrose com indicação de transplante hepático em todo o mundo (Estes *et al*., 2018). O reconhecimento dessa prevalência alarmante ressalta a urgência de estratégias eficazes de prevenção, rastreio e tratamento para amenizar seu impacto na saúde pública global (Younossi *et al*., 2016).

1.2 Revisão da literatura

1.2.1 Fatores de risco propulsores da DHGAM

A correlação acentuada entre a DHGAM e o risco elevado de complicações cardiovasculares e metabólicas é solidificada pela alta frequência de comorbidades interligadas, como o DM2, que manifesta uma forte associação com a DHGAM, acometendo até dois terços de indivíduos diabéticos, e favorecendo a progressão para a fibrose hepática avançada (Chalasani *et al*., 2018). Além disso, a dislipidemia com perfil aterogênico, caracterizada por níveis elevados de colesterol LDL (Lipoproteína de baixa densidade) e baixos de colesterol HDL (Lipoproteína de alta densidade), é um elemento metabólico relevante que contribui diretamente para a progressão simultânea da lesão hepática e da aterosclerose sistêmica (Bence *et al*., 2020). Outros hábitos de vida, como o tabagismo, que induz estresse oxidativo e prejudica a função endotelial, e o consumo alcoólico, mesmo que moderado, exacerbam o dano hepático e elevam o risco cardiometabólico (Eslam *et al*., 2020)

O DM2 não é apenas um fator de risco relevante para a DHGAM, como também um componente que estabelece com ela uma relação fisiopatológica bidirecional e sinérgica. A prevalência da DHGAM atinge uma proporção alarmante, ultrapassando 60% em indivíduos diagnosticados com DM2, sendo essa coexistência um preditor de prognósticos clínicos negativos (Younossi *et al*., 2019; Tomah *et al*., 2020). Essa associação é impulsionada primariamente pela resistência à insulina, um mecanismo central que promove tanto a hiperglicemia quanto o aumento da síntese hepática de lipídios, resultando no acúmulo de gordura e no consequente dano celular (Gastaldelli *et al*., 2019; Maldonado-Rojas *et al*., 2024). Além de ser um catalisador para o desenvolvimento da DHGAM, a presença do DM2 demonstrou ser um fator que acelera significativamente a progressão da fibrose hepática, aumentando o risco de cirrose e de suas complicações relacionadas, incluindo o carcinoma hepatocelular (Powell *et al*., 2021). Portanto, o rastreio e o manejo terapêutico otimizado da DHGAM em pacientes com DM2 são essenciais, visando interromper esse ciclo vicioso e reduzir o risco de morbimortalidade associada a ambas as condições (Tomah *et al*., 2020).

A dislipidemia, em particular o perfil aterogênico, é considerada um dos fatores metabólicos centrais que impulsionam tanto o surgimento quanto o agravamento da DHGAM. Esse desarranjo no metabolismo lipídico, tipificado pela hipertrigliceridemia, baixos níveis de colesterol HDL e a predominância de lipoproteínas LDL densas e pequenas, não é apenas um sinal da doença, mas um motor fisiopatológico ativo (Katsiki *et al*., 2016; Zhang *et al*., 2021). A relação com a DHGAM se dá pela excessiva oferta de ácidos graxos livres na circulação, que sobrecarregam a capacidade metabólica do fígado, resultando na toxicidade lipídica aos hepatócitos, iniciando uma cadeia de eventos que inclui disfunção mitocondrial e resistência à

insulina, o que leva à esteatose e, posteriormente, ao quadro inflamatório hepático (Ress *et al*., 2016).

A correlação entre a DHGAM e a dislipidemia é tão forte que a presença de perfis lipídicos alterados é observada em uma alta porcentagem de pacientes com Síndrome Metabólica e obesidade, reforçando a natureza sistêmica da DHGAM (Younossi *et al*., 2019). Além de ser um precursor da esteatose e acelerar a progressão da lesão hepática para estágios avançados, o padrão dislipidêmico intensifica drasticamente o risco de eventos cardiovasculares, considerados como principal causa de morte nesses pacientes, e contribuindo diretamente para a formação da placa aterosclerótica. Assim, o sucesso no tratamento da DHGAM exige uma estratégia que aborde simultaneamente a redução da gordura hepática e a normalização do perfil lipídico para diminuir a morbimortalidade (Katsiki *et al*., 2016; Rao *et al*., 2023).

O tabagismo representa um fator de risco modificável de grande relevância e intimamente associado à etiopatogenia e ao agravamento da DHGAM (Messner; Bernhard, 2014; Pan *et al*., 2015). Isso ocorre devido à múltipla toxicidade dos componentes da fumaça do cigarro, que atuam negativamente no metabolismo sistêmico, configurando um potente indutor da resistência à insulina. A diminuição na sensibilidade celular à insulina é um evento crucial que desregula o controle da glicose e dos lipídios, promovendo a hiperglicemia, que é um dos preditores da DHGAM (Sun; Liu; Ning, 2012; Liu *et al*., 2021). Adicionalmente, a exposição crônica ao tabaco compromete a integridade do sistema vascular, caracterizando-se pela disfunção endotelial e desequilíbrio no sistema nervoso autônomo, geralmente manifestado pelo aumento do tônus simpático (Berlin *et al*., 2012). No contexto da saúde hepática, esses mecanismos de resistência insulínica, inflamação sistêmica e lesão microvascular, se somam para intensificar o acúmulo de gordura e o subsequente dano hepático, solidificando o tabagismo como um catalisador da DHGAM (Zhang *et al*., 2023).

O risco para o desenvolvimento da DHGAM aumenta de forma diretamente proporcional à carga tabágica acumulada, usualmente medida em "*pack-years"*. Um estudo de análise populacional, observou que o risco de DHGAM é maior entre fumantes atuais e aqueles que cessaram o hábito recentemente, sendo que o risco aumenta com o número de anos-maço fumados, indicando uma relação dose-dependente (Jang *et al*., 2023; Joe *et al*., 2025). Essa associação epidemiológica é sustentada pela compreensão dos mecanismos fisiopatológicos: a fumaça do cigarro acelera a DHGAM por meio da ativação de vias metabólicas e vasculares, promovendo o estresse oxidativo, a inflamação crônica e, de forma central, a resistência à insulina, elementos-chave que levam à esteatose e à progressão da doença hepática (Jang *et al*., 2023).

Entende-se que o agravante dessa associação está no impacto do tabagismo sobre a mortalidade dos pacientes com DHGAM. Um estudo de coorte revelou um efeito patogênico significativo do tabagismo na sobrevida dos tabagistas, onde os resultados apontaram que o tabagismo atual aumentou o risco de mortalidade por todas as causas em 109% em mulheres com DHGAM, quando comparadas às não fumantes. Este risco foi ainda mais acentuado em mulheres com uma alta carga tabágica (acima de 10 *pack-years*), sugerindo uma interação particularmente perigosa entre o hábito e o sexo biológico na progressão da doença hepática para desfechos fatais (Charatcharoenwitthaya *et al*., 2020).

Considerando a relevância da cessação do tabagismo no contexto geral de saúde, é necessário compreender que seu efeito direto na prevalência de DHGAM é complexo. Uma meta-análise recente, ao comparar ex-fumantes com fumantes atuais, concluiu que a prevalência de DHGAM em ex-fumantes não era significativamente menor, e em alguns casos, era até ligeiramente superior, sugerindo que a cessação do fumo por si só pode não ser um fator de proteção imediato contra a prevalência da doença (Zhang *et al*., 2023). Essa ressalva pode ser parcialmente explicada pelo ganho de peso frequentemente associado à interrupção do tabaco, um fator que, por sua vez, é um forte preditor da DHGAM. Contudo, outros estudos reforçam que a prevalência de DHGAM é inversamente associada à duração da cessação, indicando que o tempo de abstinência é benéfico e diminui o risco metabólico (Joe *et al*., 2025). Coletivamente, esses dados enfatizam que o tabagismo é um fator de risco que atua primariamente na aceleração da fisiopatologia e dos desfechos de mortalidade, e seu controle é essencial no manejo da DHGAM.

O consumo de álcool também atua como agente de lesão hepática através de vias fisiopatológicas complexas, as quais contribuem para a exacerbação e o desenvolvimento da DHGAM. A toxicidade do álcool, primeiramente, deriva do seu metabolismo: após a ingestão, o etanol é majoritariamente processado no fígado pela enzima álcool desidrogenase (Cederbaum, 2012), resultando na produção de subprodutos tóxicos, como o acetaldeído. Este processo promove o estresse oxidativo decorrente da geração excessiva de espécies reativas de oxigênio, que excedem as capacidades de defesa celular (Liu, 2014). Adicionalmente, o álcool é um estímulo para a inflamação sistêmica e hepática, elevando os níveis de citocinas pró-inflamatórias, como o fator de necrose tumoral alfa (TNF-α), um fator que facilita o dano hepatocelular e a progressão da esteatohepatite (Szabo, 2015; Sun; Wang, 2021). Esse contexto prejudicial é ainda mais comprometido pela alteração na microbiota intestinal e pelo aumento da permeabilidade da barreira intestinal (Slevin *et al*., 2020), permitindo que endotoxinas atinjam o fígado e agravem as disfunções metabólicas desencadeadas por inflamação hepática e resistência à insulina (Jeon *et al*., 2020).

Ainda que a classificação da doença hepática alcoólica esteja relacionada ao uso crônico e elevado de álcool, abrangendo um espectro de lesões graves (Crabb *et al*., 2020), a ingestão alcoólica em qualquer nível está associada ao aumento da incidência e da taxa de progressão da DHGAM (Policarpo *et al*., 2022). Em uma sociedade onde tendências indicam um aumento no uso de álcool (Braun *et al*., 2021; Abrantes *et al*., 2022), muitas vezes ligadas a mecanismos cerebrais de reforço que podem evoluir para a dependência (Avena *et al*., 2006), a coexistência de fatores de risco metabólicos próprios da DHGAM e a ingestão alcoólica criam um impacto relativo negativo no tecido hepático (Volkow *et al*., 2007).

1.2.2 Abordagem diagnóstica da DHGAM

O processo diagnóstico da DHGAM, deve considerar não apenas a esteatose, mas também a concomitância de pelo menos um critério cardiometabólico, como a presença de obesidade, DM2, ou outras evidências de desregulação metabólica em indivíduos não obesos (Rinella; Sookoian, 2024, Nguyen; Vo, 2025). Embora a biópsia hepática ainda represente o padrão-ouro por sua capacidade insubstituível de diferenciar a esteatose simples da forma mais progressiva, e de mensurar o grau exato de fibrose (Eslam *et al*., 2020), sua natureza invasiva e os custos associados limitam sua aplicação como ferramenta de rastreamento populacional. Diante disso, a rotina clínica depende cada vez mais de métodos não invasivos para identificar indivíduos de maior risco e evitar biópsias desnecessárias. Assim, a detecção inicial da esteatose é frequentemente realizada pela ultrassonografia, por ser acessível, enquanto a estratificação do risco de doença avançada e fibrose utiliza ferramentas mais avançadas, incluindo a Elastografia de Transição (FibroScan) e escores séricos como o Índice de Fibrose-4 (FIB-4), que demonstraram utilidade clínica superior na classificação de pacientes com DHGAM (Rinella; Sookoian, 2024; Nguyen; Vo, 2025).

1.2.3 Estratégias terapêuticas convencionais para a DHGAM

O tratamento da DHGAM encontra na modificação intensiva do estilo de vida seu elemento central e mais eficaz. A estratégia não farmacológica sustenta o alicerce fundamental para a reversão da doença hepática (Lipscombe *et al*., 2018; Khatib *et al*., 2022), por ser uma abordagem que visa a redução do peso corporal e a melhoria do perfil metabólico, dois fatores etiológicos cruciais na patogênese da DHGAM (Eslam *et al*., 2020; Rinella *et al*., 2023). Estudos evidenciam que uma perda de peso sustentada de 5% a 7% do peso inicial é suficiente para promover uma redução significativa da esteatose hepática, enquanto perdas ≥10% frequentemente conduzem à resolução da esteato-hepatite e até mesmo à regressão da fibrose (Khatib *et al*., 2022; Sanyal *et al*., 2025). As intervenções mais recomendadas englobam a

adoção de padrões alimentares saudáveis e a prática regular de exercícios físicos, sendo a combinação dessas medidas a mais potente para a condição hepática e cardiovascular (Lipscombe *et al*., 2018).

A intervenção medicamentosa é reservada para pacientes com DHGAM que apresentam risco mais elevado de progressão, tipicamente aqueles com fibrose avançada ou a forma inflamatória, ou para aqueles em que as mudanças no estilo de vida não foram suficientes para controlar as comorbidades metabólicas (Godoy-Matos *et al*., 2023). Uma classe de medicamentos inicialmente desenvolvida para o diabetes, os agonistas do receptor do peptídeo-1 semelhante ao glucagon (AR GLP-1), surgiu como um tratamento promissor, demonstrando eficácia notável. A Semaglutida, por exemplo, alcançou a resolução histológica da esteato-hepatite sem piora da fibrose em ensaios de fase 3, além de induzir perda de peso significativa (Sanyal *et al*., 2025). Outra opção relevante para a terapia convencional é a Pioglitazona, um agente sensibilizador de insulina que se mostrou capaz de reduzir a gordura hepática e melhorar a inflamação e a fibrose, sendo recomendada para pacientes com quadro inflamatório hepático estabelecido e diabetes ou pré-diabetes (Godoy-Matos *et al*., 2023). Contudo, os custos elevados destes medicamentos bem como a especificidade que não incorpora todos os mecanismos envolvidos na DHGAM, além das alterações hepáticas, diarreia, cefaleia e náuseas decorrentes do uso, mantém os recursos farmacológicos disponíveis em um degrau de limitação.

A busca por terapias que atuem diretamente no fígado e nos processos de fibrose impulsionou o desenvolvimento de novas moléculas. Um avanço notável é o surgimento dos agonistas seletivos do receptor ß do hormônio tireoidiano (THR-ß), como o Resmetirom, que acelera o metabolismo hepático de lipídios e demonstrou impacto significativo no tratamento da MASH e na melhoria da fibrose (Harrison *et al*., 2024). Além disso, medicamentos que modulam as vias metabólicas e inflamatórias, como os inibidores do cotransportador de sódio-glicose 2 (iSGLT2) e outros agentes, indicam que o futuro do tratamento farmacológico da DHGAM será direcionado para a terapia combinada, buscando interferir na esteatose, inflamação, e na fibrose por meio de mecanismos de ação sinérgicos (Harrison *et al*., 2024; Rinella *et al*., 2023). A complexidade da DHGAM destaca a necessidade de tratamentos que controlem as comorbidades, especialmente a dislipidemia e a hipertensão, para reduzir o risco cardiovascular associado (Lipscombe *et al*., 2018).

1.2.4 Modelos experimentais para o estudo de DHGAM

Os ratos e camundongos são amplamente empregados na pesquisa translacional da DHGAM devido à sua alta similaridade fisiológica e genética com os humanos, o que os posiciona como modelos ideais para estudos comparativos (Lima *et al*., 2016; Guzzardi *et al*.,

2021). As vantagens de utilizar roedores transcendem as questões de manejo e custo, que são favoráveis, e se concentram principalmente em seu ciclo de vida curto e na facilidade de manipulação genética e ambiental, permitindo a observação da progressão da doença em um curto espaço temporal (Zamani-Garmsiri *et al*., 2021). A relevância desses modelos é evidenciada pela capacidade de reproduzir as características-chave da DHGAM humana, incluindo esteatose, inflamação hepática, fibrose e, crucialmente, as comorbidades metabólicas como obesidade, resistência à insulina e dislipidemia, que são essenciais para a definição da doença (Liou *et al*., 2019; Velázquez *et al*., 2019).

A DHGAM é classicamente induzida em roedores por meio de modificações dietéticas que simulam os fatores de risco nutricionais humanos, sendo o uso de dietas ricas em gordura, em frutose, ou a combinação de ambas as abordagens mais utilizadas para reproduzir o espectro da doença, da esteatose simples à MASH (Kawano *et al*., 1992, Kundu *et al*., 2020). Modelos de dieta de longa duração, que chegam a 16 a 20 semanas ou mais, permitem a progressão da esteatose simples para a MASH e, em algumas linhagens genéticas específicas, o desenvolvimento de fibrose avançada e até carcinoma hepatocelular, espelhando a evolução clínica observada em pacientes humanos (Karatzas *et al*., 2018; Cui *et al*., 2020; Vale *et al*., 2020). Essa indução, por meio de dieta controlada, é fundamental para o estudo detalhado da fisiopatologia, desde os mecanismos de lipogênese induzidos pela frutose, conforme descrito em estudos iniciais (Bray, 1977), até a avaliação da eficácia de novas terapias farmacológicas que visam interromper o ciclo de inflamação e a fibrose hepáticas (Guzzardi *et al*., 2021).

Apesar de grande parte dos estudos avaliarem os fatores de risco para DHGAM isoladamente, há uma tendência instalada em investigar múltiplos fatores associados para simular seu impacto global na progressão da doença de forma simultânea. Neste contexto, o desenvolvimento e validação de novos modelos *multi-hit* que associem sincronicamente os principais fatores de risco como dislipidemia, consumo de álcool, tabagismo e DM2, apresenta-se como um avanço essencial que torna os estudos de DHGAM cada vez mais robustos e aplicáveis na pesquisa translacional (Süntar, 2020) Assim, alguns protocolos experimentais comprometidos com esse avanço tão necessário, além de induzir o DM2 e associar uma dieta hipercalórica para a instalação da dislipidemia (Auth *et al*., 2022; Albuquerque *et al*., 2023), incorporam a indução do tabagismo em ratos, conforme demonstrado por Albuquerque *et al*. (2023), que utilizaram câmaras fechadas para exposição à fumaça do cigarro, simulando o tabagismo ativo. Essa metodologia de exposição à fumaça, permite aos pesquisadores avaliar os efeitos sistêmicos do tabagismo, como o aumento do estresse oxidativo e a amplificação de respostas inflamatórias, o que é extremamente relevante para o estudo da DHGAM, visto que o tabagismo é um conhecido fator de risco que acelera a progressão da fibrose hepática e agrava

as disfunções metabólicas, proporcionando assim um modelo mais complexo e representativo da doença humana (Lo *et al*., 2011; Barbosa *et al*., 2020; Dwivedi *et al*., 2020; Mendes *et al*., 2021).

A pesquisa translacional em DHGAM exige refinamento dos modelos experimentais para que os resultados pré-clínicos alcancem a aplicação clínica eficaz. Neste sentido, o aprimoramento contínuo na indução da doença em roedores, seja por meio de dietas específicas ou pela inclusão de cofatores de risco como o tabagismo, busca uma correspondência patológica cada vez mais próxima da DHGAM humana, da esteatose à fibrose. Essa precisão é crucial para fornecer dados robustos e fidedignos sobre a fisiopatologia e a eficácia de novas terapias a partir de modelos mais representativos, o que é fundamental para garantir a translação do conhecimento científico e o sucesso terapêutico (Süntar, 2020).

1.2.5 O perfil fitoquímico promissor de *Petiveria alliacea L.*: da etnofarmacologia à validação científica

Apesar de subestimada, a diversidade da flora brasileira ainda é considerada a maior do mundo (Forzza *et al*., 2018), com mais de 50.000 espécies reconhecidas (Brazil Flora Group, 2020). Neste contexto, a *P. alliacea* L., popularmente identificada em diversas regiões brasileiras pelos nomes de guiné, erva-de-alho, tipi ou amansa-senhor, é uma espécie herbácea perene pertencente à família *Phytolaccaceae* e ao gênero Petiveria (Cronquist, 1988; Neves *et al*., 2006). Esta família botânica engloba um número significativo de gêneros e espécies, amplamente distribuídos em zonas tropicais, com destaque para *P. alliacea* enquanto membro mais reconhecido de seu gênero (Duarte *et al*., 2005; Marchioretto *et al*., 2014). Morfologicamente, a planta é caracterizada como um subarbusto de crescimento ereto (Figura 1), que geralmente alcança de meio a um metro de altura, apresentando a marcante particularidade de exalar um odor semelhante ao do alho, apresentando folhas alternadas e simples (Duarte *et al*., 2005).

Figura 1. Subarbusto adulto de *Petiveria alliacea*.

Fonte: Giehl, 2025.

A ampla relevância desta espécie na medicinal tradicional está diretamente ligada à sua extensa distribuição geográfica, sendo considerada nativa de vastas áreas tropicais e subtropicais do continente americano (Marchioretto *et al*., 2014). Seu habitat natural se estende desde a Amazônia e o Caribe até a América Central e do Sul, abrangendo uma notável presença em diversos estados do Brasil. A capacidade adaptativa de *P. alliacea* em diferentes ambientes levou à sua naturalização e uso em regiões fora de sua área de origem, inclusive na África Subsaariana. Essa dispersão cultural impulsionou a consolidação da planta como um importante objeto de estudo etnofarmacológico, promovendo pesquisas em busca da validação científica de seus múltiplos usos terapêuticos (Neves *et al*., 2006).

Tradicionalmente, *P. alliacea* apresenta um rico histórico etnobotânico, com um repertório amplo na medicina tradicional (Taylor, 2002; Magalhães *et al*., 2022) seja através de suas raízes ou folhas, que constituem as estruturas de maior interesse popular, ou outras partes utilizadas na preparação de extratos (Paz Perafan; Montenegro, 2024; Santiago *et al*., 2025). O uso oral do infuso ou chá são indicados na medicina popular para o alívio de quadros sintomáticos inespecíficos, como febre, dor de cabeça, congestão e outras dores generalizadas, além de auxiliar no manejo de resfriados comuns (Taylor, 2002; Santiago *et al*., 2025). Já as preparações de uso tópico envolvem o macerado ou a infusão concentrada das folhas, que são aplicados externamente para amenizar dores musculares e tratar contusões, evidenciando a versatilidade da espécie principalmente no tratamento empírico de desordens agudas (Taylor, 2002).

Estudos farmacológicos têm fornecido evidências que confirmam as ações analgésica e anti-inflamatória de *P. alliacea*, corroborando seu uso tradicional para alívio da dor e contusões (Casagrande *et al*., 2023; Carbolim *et al*., 2025). Além disso, extratos da planta exibem uma

considerável capacidade antioxidante, justificando o uso popular como agente depurativo e protetor (Beltreschi *et al*., 2019; Bremm *et al*., 2020). Outras descobertas importantes sobre esta planta incluem o potencial antimicrobiano, a ação citotóxica contra células tumorais e a influência em mecanismos neurais, indicando que o arsenal terapêutico da espécie é vasto e merece aprofundamento (Guimarães *et al*., 2022; Carbolim *et al*., 2025).

O interesse científico em *P. alliacea* envolve sua rica composição fitoquímica, que abrange classes de metabólitos secundários como flavonoides, cumarinas, esteróis, triterpenos e alcaloides, reconhecidos por suas propriedades bioativas (Abdul Raheem *et al*., 2018; KAnmodi *et al*., 2022). No entanto, acredita-se que o diferencial químico mais marcante da espécie seja a presença de compostos organossulfurados voláteis, responsáveis pelo odor aliáceo característico da planta (Kim *et al*., 2005), com destaque para o dibenzil trissulfeto, um polissulfeto lipossolúvel que é frequentemente identificado como um dos principais responsáveis por atividades farmacológicas como a citotoxicidade e o potencial antiparasitário (Kim *et al*., 2005; Luz *et al*., 2016). Embora tanto as folhas quanto as raízes sejam ricas em constituintes ativos, pesquisas indicam que as raízes podem apresentar maior concentração de alguns desses organossulfurados, enquanto as folhas demonstram alta concentração de outros metabólitos como flavonoides, o que poderia justificar a extração de diferentes partes da planta para usos terapêuticos específicos (Luz *et al*., 2016; Arogbodo *et al*., 2021).

Para avaliar a atividade anti-inflamatória de *P. alliacea*, um estudo *in vivo* utilizou ratos Wistar tratados com o extrato etanólico bruto das raízes da planta, e como resultado obteve uma significativa inibição do processo inflamatório agudo em comparação com o ácido acetilsalicílico, agindo mais especificamente na redução do acúmulo de células de defesa, como neutrófilos e células mononucleares, na região da inflamação induzida por carragenina (Lopez-Martins *et al*., 2002). Em pesquisas mais recentes, o extrato aquoso das folhas de *P. alliacea* foi testado em camundongos para avaliar seu impacto na inflamação associada a distúrbios metabólicos, revelando um efeito promissor ao modular biomarcadores inflamatórios cruciais, o que indica uma possível aplicação no manejo de componentes crônicos da inflamação sistêmica (Olajubutu *et al*., 2022; Fatimah *et al*., 2024).

Além de suas propriedades anti-inflamatórias, *P. alliacea* tem sido estudada por seu potencial antimicrobiano e antifúngico, frequentemente atribuídos aos seus compostos organossulfurados e flavonoides, com eficácia comprovada *in vitro* contra diversos patógenos. É o que se observa no estudo de Machado e Ramos (2020), que utilizaram o extrato etanólico de folhas secas da planta para testar sua eficácia no combate a fungos de interesse agrícola e de saúde, como o *Aspergillus flavus*. Outro estudo *in vitro* destacou o potencial antibacteriano do extrato etanólico 80% das folhas e raízes de *P. alliacea*, revelando atividade contra

microrganismos clinicamente relevantes, incluindo *Staphylococcus aureus*, *Pseudomonas aeruginosa* e *Klebsiella pneumoniae* (Lozano *et al*., 2021). Em um estudo adicional, uma análise *in vitro* demonstrou que o tratamento com o extrato acetato de etila das folhas, caule e raízes de *P. alliacea* apresentou eficácia contra um espectro relevante de patógenos, incluindo bactérias Gram-positivas (*Staphylococcus aureus, Bacillus subtilis*), Gram-negativas (*Escherichia coli*) e fungos (*Candida albicans, Aspergillus fumigatus*). (Khadka *et al*., 2023).

P. alliacea também tem demonstrado potencial terapêutico relevante no âmbito neurológico através de estudos relacionados à função cognitiva. A partir do extrato aquoso das folhas utilizado para tratar ratos, foi possível observar efeito ansiolítico e antidepressivo, quando estes foram submetidos a testes cognitivo-comportamentais (Caicedo-Pinto *et al*., 2019). Outro estudo de relevância utilizou o extrato etanólico das folhas de *P. alliacea* para tratar camundongos com comprometimento significativo de memória e aprendizagem. Os resultados indicaram uma melhora aparente nos parâmetros de memória e aprendizado mediados pela redução do estresse oxidativo, além de promover a melhora cognitiva e de memória (Silva *et al*., 2015; Zavala-Ocampo *et al*., 2024).

Considerando a potencial ação do extrato de *P. alliacea* no metabolismo, Mustika *et al*. (2021) utilizaram uma nanoformulação do extrato das folhas da planta para tratar ratos Wistar com diabetes induzido por estreptozotocina. Os resultados apontam que o tratamento com o extrato nanoemulsionado não apenas promoveu a redução da resistência à insulina, conforme medido pelo índice HOMA-IR (*Homeostatic Model Assessment-Insulin Resistance*), mas também reduziu significativamente os níveis séricos de citocinas pró-inflamatórias, como a Interleucina 6 (IL-6) e o TNF-α.

1.2.6 Do uso popular à vitrine científica: aspectos botânicos e fitoquímicos e potencial medicinal de *Pereskia grandifolia* Haw.

No contexto da diversidade da flora brasileira, 483 espécies da família *Cactaceae* foram identificadas, das quais 206 são consideradas nativas do Brasil (Zappi; Taylor, 2020). *Pereskia grandifolia* Haw., popularmente conhecida como ora-pro-nóbis, tem sido uma das espécies mais utilizadas na medicina popular, especialmente nas regiões Sudeste e Nordeste do Brasil. Com suas folhas extremamente mucilaginosas, destaca-se por suas propriedades terapêuticas e alto valor nutricional. É utilizada principalmente como emoliente na medicina tradicional e na indústria farmacêutica (Farago *et al*., 2004).

Além de seu uso popular na medicina tradicional, a ora-pro-nóbis é considerada um vegetal não convencional que encontrou seu lugar na culinária regional brasileira (Souza *et al*., 2014). O uso da planta como suplemento alimentar natural ou componente de novos alimentos,

suplementos e formulações farmacêuticas impulsionou a exploração dessa espécie (Maciel *et al*., 2019), e tem despertado interesse na indústria alimentícia como suplemento devido ao seu alto potencial proteico (Mercê *et al*., 2001).

A família *Cactaceae* inclui o gênero *Pereskia* e suas 17 espécies, que se originam em regiões temperadas e tropicais e se distribuem principalmente em áreas de climas secos e quentes. No Brasil, seu domínio fitogeográfico se concentra na Caatinga, Mata Atlântica, Cerrado, Pampa e Pantanal (Santos *et al*., 2021; Torres *et al*., 2022). As plantas deste gênero têm folhas grandes, verde-brilhantes, semelhantes às da ligustrina. São fáceis de cultivar, têm crescimento rápido e floração abundante. Podem ser classificadas como arbustos (até três metros de altura), trepadeiras ou mesmo árvores suculentas (atingindo 5-20 m de altura). Suas flores podem aparecer isoladas ou em cachos, geralmente semelhantes às flores da roseira (Sim *et al*., 2010a).

Uma espécie do gênero é *P. grandifolia*, nativa da flora brasileira, variando em tamanho de arbustivo a arbóreo, de 3 a 6 m de altura, cujas folhas são espessas, simples, ovais, suculentas, mucilaginosas e ligeiramente carnudas (Farago *et al*., 2004). Seu caule é lenhoso e bem desenvolvido e possui flores terminais dispostas em ápices. Seu tronco possui muitos espinhos e pode medir até 20 cm de diâmetro. A epiderme do limbo é uniestratificada, coberta por uma cutícula lisa e fina que possui estômatos paracíticos em ambos os lados. Em vista frontal, as células apresentam uma parede anticlinal em formato poligonal. As folhas são caracteristicamente anfiestomáticas, apresentando estômatos predominantemente do tipo paracítico, inseridos no mesmo nível das demais células epidérmicas. O mesófilo está posicionado dorsoventralmente e consiste em parênquima paliçádico atípico, cujas células são relativamente grandes e não muito alongadas, correspondendo a cerca de dois terços do mesófilo. Este conjunto de tecidos multiestratificados também apresenta espaços intercelulares reduzidos com pequenos feixes vasculares colaterais que são circundados por uma bainha parenquimática e distribuídos por todo o mesófilo (Manfron, 2004).

A nervura principal de *P. grandifolia* tem formato plano-convexo. Subjacente à epiderme, o clorênquima é interrompido e substituído pelo parênquima fundamental, que é acompanhado por três grandes feixes vasculares colaterais e geralmente dois feixes menores que estão dispostos em um arco aberto. Na região laminar, são evidentes grandes células com mucilagem, além de idioblastos que consistem em células parenquimáticas que contêm drusas de oxalato de cálcio em vacúolos que ocupam grande parte do protoplasto (Manfron, 2004). As principais características botânicas de *P. grandifolia* são apresentadas na Figura 2.

Figura 2. Inflorescência e folhas de *P. grandifolia* arbustiva jovem

Fonte: Taylor *et al*., 2015.

P. grandifolia é uma planta perene cujas flores têm pigmentação rosa-arroxeada (Sim *et al*., 2010b). Ela também produz pequenos frutos que compõem a dieta de algumas espécies de aves, que também são responsáveis por sua dispersão biológica (Souza *et al*., 2016). De acordo com Massocatto *et al*. (2022), a espécie que é nativa do Brasil, também é amplamente cultivada em países tropicais e pode ser conhecida por outros nomes populares menos frequentes que ora-pro-nóbis, como groselha barbados e cacto rosa.

Com base no crescente uso de plantas alimentícias não convencionais como componentes incomuns dos cardápios diários, a ora-pro-nóbis está presente em uma lista diversificada de plantas que compõem esse grupo. No entanto, essa espécie vegetal ainda é relativamente pouco explorada em relação ao seu uso popular, com uma escassez de estudos pelas comunidades técnico-científicas. Assim, a disseminação de suas possíveis aplicações nutracêuticas tem sido difícil, embora tenha sido comprovado que ela é uma excelente fonte de nutrição adequada para o consumo humano (Liberato *et al*., 2019).

No Brasil, as folhas de *P. grandifolia* são consideradas alimentos vegetarianos não-convencionais, com ênfase em certas regiões, como Diamantina, Minas Gerais, onde 77% da população inclui esse vegetal rico em suas principais refeições diárias (Dias *et al*., 2005). O uso convencional de *P. grandifolia* tem sido especialmente relacionado à prevenção da deficiência de ferro, anemia, osteoporose, câncer e constipação. Além de ser uma rica fonte de nutrientes

para a dieta humana, ela também pode melhorar parâmetros biológicos, como triglicerídeos e glicose sérica (Almeida; Corrêa, 2012).

Na medicina tradicional, as folhas de *P. grandifolia*, devido ao seu conteúdo mucilaginoso, têm potencial uso na indústria alimentícia como agente emulsificante e estabilizante (Lima Junior *et al*., 2013). As folhas também são utilizadas no tratamento de doenças associadas à inflamação, câncer, hipertensão, diabetes mellitus e reumatismo. Além de aliviar dores de estômago, úlceras, dores de cabeça, hemorroidas e dermatite atópica. Considerando as ações potenciais de *P. grandifolia* que fundamentam seu uso popular, acredita-se que a planta carregue consigo efeitos anticancerígenos, antitumorais, antirreumáticos, antiulcerogênicos e antiinflamatórios. As formas de uso relatadas variam desde o consumo de folhas cruas e refogadas até preparações mais elaboradas, como sopas feitas com as folhas cozidas, às vezes acompanhadas de carne (Nurestri *et al*.,2009; Sim *et al*., 2010a; Sim *et al*., 2010b).

Os primeiros estudos fitoquímicos identificados de *P. grandifolia* destacaram frações de saponinas que, após hidrólise, produziram ácido oleanólico (Sahu *et al*., 1974). Posteriormente, um estudo isolou da mesma planta três alcaloides (p-metoxi-β-hidroxi-β-feniletilamina, 3-metoxitiramina, e tiramina), além de saponinas e compostos fenólicos (Doestsch *et al*., 1980). Pesquisas posteriores destacaram 2,4-di-terc-butilfenol, β-sitosterol, vitamina E, fitona e uma mistura que continha palmitato de metila, oleato de metila e estearato de metila. Um extrato hexânico de *P. grandifolia* consistia em palmitato de metila, linoleato de metila, α-linolenato de metila e estearato de metila (Nurestri *et al*., 2009; Sim *et al*., 2010a; Kazama *et al*., 2012).

Um estudo fitoquímico realizado por Souza *et al*. (2016) aplicou hidrodestilação para avaliar a composição química de óleos essenciais extraídos de folhas de *P. grandifolia*, produzindo 0,09% de óleo essencial. Entre os componentes mais abundantes estavam diterpenos oxigenados (55,5%), óxido de manol (30,1%), ftal (25,1%), N-octadecano (9,2%) e cis-tujopseno (6,7%). A composição de folhas frescas de *P. grandifolia* foi avaliada para quantificar e identificar seu perfil de carotenoides. Os resultados mostraram níveis variáveis de 22,7 a 31,0 μg/g de β-caroteno e de 6,1 a 8,4 μg/g de α-caroteno, sugerindo que a espécie é promissora como fonte de pró-vitamina A (Agostini-Costa *et al*., 2014). Outro estudo fitoquímico destacou compostos fenólicos ativos, flavonas, flavonóis, e antioxidantes em extratos aquosos e hidroalcoólicos obtidos por refluxo e sonicação da mesma planta (Vicente *et al*., 2020; Torres *et al*., 2022).

O óleo essencial de *P. grandifolia* foi analisado por Souza *et al*. (2014). Os fitoquímicos predominantes foram os diterpenos oxigenados fitol (25,1%) e óxido de manool (30,1%), que

foram os dois componentes com as maiores concentrações. Outros compostos que foram identificados no óleo essencial da planta foram acetato de 9-decenila (0,9%), desmetil isotorquatona (0,8%), éter 10-epi-italileno (0,8%), n-heneicosano (4,4%), heptadecano (4,2%), n-hexadecano (4,2%), nonadecano (2,5%), n-octadecano (9,2%), óxido de 13-epi-manool (0,3%), n-pentadecano (1,7%), tetraidrorimueno (1,7%) e cis-tujopsenal (6,7%) (De Moraes *et al*., 2020).

Sim *et al*. (2010b) investigaram o potencial antioxidante de um extrato metanólico de *P. grandifolia* e suas frações (metanol, hexano, acetato de etila e água) usando diferentes testes *in vitro*, como a atividade sequestradora de 2,2-difenil-1-picrilhidrazil (DPPH), o ensaio de poder redutor e o método do β-caroteno. Usando o método de Folin-Ciocalteu, as concentrações de compostos fenólicos (4, 8, 12, 16 e 20 mg/mL) em um extrato metanólico de *P. grandifolia* e suas frações mencionadas foram determinadas. Em segundo lugar, depois do extrato metanólico, a fração de acetato de etila apresentou a maior quantidade de compostos fenólicos, além de 2,4-di-terc- butilfenol e α-tocoferol, que podem contribuir para sua propriedade antioxidante. Estudos também indicaram que a fração de acetato de etila de *P. grandifolia* tinha a capacidade de sequestrar DPPH, reforçando seu potencial atividade antioxidante na prevenção e reparação de lesões oxidativas. O estudo concluiu que a fração de acetato de etila de *P. grandifolia* possui considerável potencial antioxidante.

Um estudo de Philip *et al*. (2009) investigou os efeitos antibacterianos *in vitro* de um extrato metanólico e frações solúveis em hexano e acetato de etila de folhas de *P. grandifolia* contra *E. coli*, *P. aeruginosa*, *S. aureus* e *B. subtilis*. Os resultados mostraram que a fração de acetato de etila (500 mg/mL) exerceu atividade antimicrobiana contra *P. aeruginosa*, *S. aureus* e *B. subtilis*, mas não inibiu o crescimento da bactéria Gram-negativa *E. coli*.

Outro estudo realizou um ensaio bioautográfico, que foi conduzido para avaliar a atividade anticolinesterásica de um extrato hidroetanólico de folhas e frutos de *P. grandifolia* em diferentes concentrações de massa seca (600, 400, 200, 150, 100, 50 e 25 μg) do extrato que foi eluído em uma solução de diclorometano-metanol (9:1, v/v). Os compostos mais polares que foram obtidos dos extratos das folhas da planta mostraram maior capacidade de inibir a acetilcolinesterase, sugerindo uma atividade neuroprotetora promissora (Massocatto *et al*., 2022).

Um estudo de Nurestri *et al*. (2009) investigou a atividade citotóxica de um extrato metanólico de frações foliares de *P. grandifolia* contra cinco linhagens de células de carcinoma humano. A fração hexânica apresentou atividade contra células de carcinoma espinocelular nasofaríngeo (KB), e a fração de acetato de etila promoveu forte citotoxicidade contra células KB e células de adenocarcinoma de mama humano (MCF-7), além disso, não foi identificada

citotoxicidade em células de fibroblastos humanos normais (MRC-5). As propriedades citotóxicas foram atribuídas principalmente ao antioxidante 2,4-di-terc-butilfenol, que é amplamente utilizado na indústria de plásticos, mas ainda precisa ser analisado biogeneticamente.

A atividade diurética e hipotensora de uma infusão e extrato etanólico de folhas de *P. grandifolia* foi analisada em ratos Wistar normotensos. Para avaliar o efeito diurético, os animais foram tratados oralmente com 3, 10, 30 e 300 mg/kg dos extratos em dose única (diurese aguda) ou doses diárias durante sete dias (diurese prolongada). Os resultados foram comparados com animais tratados com hidroclorotiazida, um diurético tiazídico. Os diferentes tratamentos não alteraram os níveis de ureia, creatinina ou aldosterona, nem a atividade da enzima conversora de angiotensina. O tratamento agudo e prolongado com 30 mg/kg do extrato etanólico de *P. grandifolia* reduziu a excreção de potássio e cloro, sem influenciar os níveis de sódio, enquanto o tratamento prolongado com a mesma dose promoveu diurese. Reduções significativas nos níveis de vasopressina e na pressão arterial média também foram observadas, indicando que a atividade diurética do extrato pode estar relacionada à liberação de arginina-vasopressina (Kazama *et al*., 2012).

Os efeitos antiobesidade de *P. grandifolia* foram investigados em um estudo com ratos Wistar que foram submetidos a dietas hipercalóricas compostas por ração comercial (Biobase Bio- tec Rat and Mice, 46%), leite condensado adoçado (46%) e óleo de milho (8%) durante 10 semanas, e em seguida, tratados durante quatro semanas com uma dieta fracionada com porções de farinha produzida a partir das folhas secas de *P. grandifolia* em proporções de 5% e 10%. Após o tratamento, tecidos hepáticos foram coletados e avaliados quanto à presença de esteatose, que foi classificada como ausente, leve, moderada e grave quando o comprometimento do órgão excedeu 75%. A análise dos dados indicou uma ação três vezes mais eficaz na redução do peso corporal para a dieta fracionada com 10% de farinha de folhas em comparação com a fração de 5%, além de apresentar o menor índice de massa corporal. A análise histológica do tecido hepático mostrou que ambos os grupos tratados com *P. grandifolia* nas proporções de 5% e 10% apresentaram gotículas lipídicas no citoplasma classificadas como leves e sem características de esteatose, enquanto o grupo controle apresentou um índice muito alto de gotículas lipídicas no citoplasma, caracterizando esteatose grave. Os resultados indicaram a eficácia da farinha de *P. grandifolia* na redução do peso corporal e dos níveis de lipídios no fígado, sugerindo seu potencial para o tratamento da obesidade e de doenças hepáticas (Almeida *et al*., 2016).

Diante do exposto, apesar do histórico etnofarmacológico e dos achados pré-clínicos que evidenciam ações anti-inflamatórias, antioxidantes, protetoras e metabólicas de *Petiveria*

alliacea e *Pereskia grandifolia*, ainda há uma lacuna significativa na validação científica da segurança toxicológica da terapêutica clínica e de seu potencial no manejo da DHGAM. A complexidade e a natureza multifatorial da DHGAM, impulsionada pela associação de comorbidades como diabetes, dislipidemia, tabagismo e consumo de álcool, destacam a necessidade de modelos experimentais *multi-hit* que mimetizem essa interação de risco na progressão da doença. Portanto, o presente estudo buscou avaliar a segurança toxicológica e caracterizar os efeitos farmacoterapêuticos de extratos de *P. alliacea* e *P. grandifolia* em modelos experimentais de DHGAM associando múltiplos fatores de risco, fornecendo uma base robusta que contribua para o avanço de alternativas mais eficientes no tratamento desta condição.

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1.4 Objetivo

Investigar o potencial terapêutico e os efeitos farmacológicos de *Petiveria alliacea* e *Pereskia grandifolia* em modelos experimentais de doença hepática associada a múltiplos fatores de risco metabólicos, como dislipidemia, diabetes e consumo de álcool ou tabagismo, bem como avaliar a segurança toxicológica aguda de ambas as espécies por meio de testes *in vivo* e *in vitro*.

CAPÍTULO 2

ARTIGOS

ARTIGO 1 - *Petiveria alliacea L.* MITIGATES HEPATORENAL DYSFUNCTION IN AN INNOVATIVE MULTI-HIT PRECLINICAL MODEL OF METABOLIC DYSFUNCTION-ASSOCIATED STEATOTIC LIVER DISEASE

Artigo editado de acordo com as normas de publicação do Periódico *Naunyn-Schmiedeberg's Archives of Pharmacology* – ISSN 1432-1912

Petiveria alliacea L. ***mitigates hepatorenal dysfunction in an innovative multi-hit preclinical model of Metabolic Dysfunction-Associated Steatotic Liver Disease***

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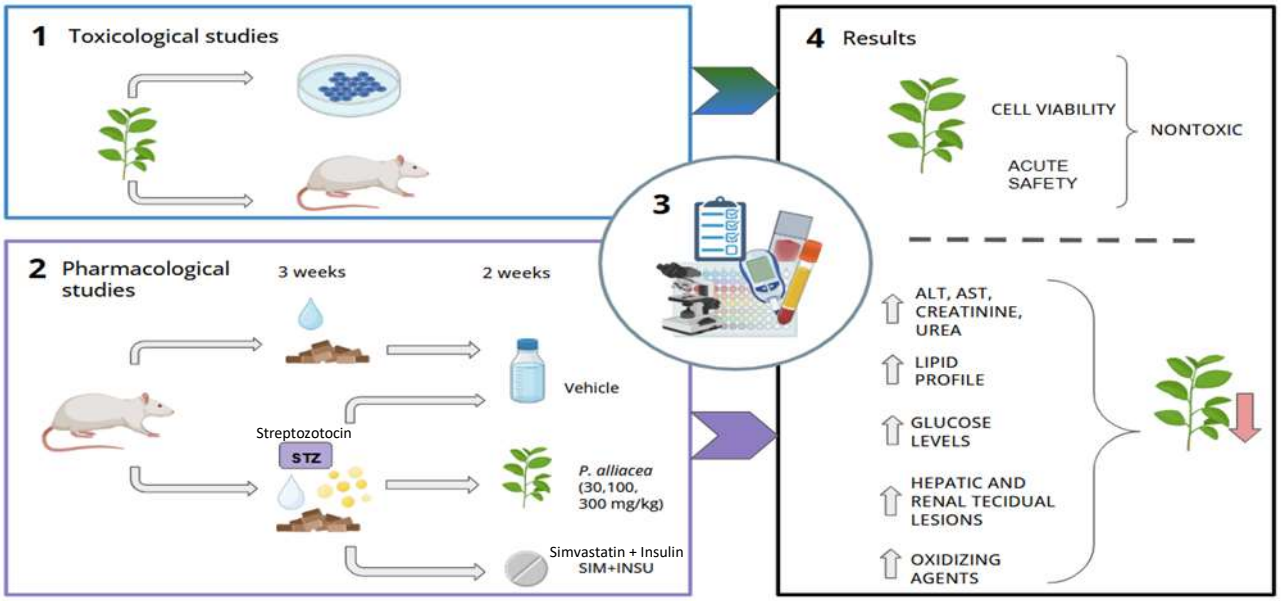
Abstract

Purpose: Metabolic dysfunction-associated steatotic liver disease (MASLD) is a prevalent chronic liver disorder often accompanied by metabolic and extrahepatic complications, including renal dysfunction. Effective therapies are limited. *Petiveria alliacea* L., a medicinal plant traditionally used for diabetes and liver disorders, has not been systematically evaluated in MASLD-related syndromes. Here, we assessed the toxicological safety and therapeutic efficacy of *P. alliacea* in a novel preclinical multi-hit model of MASLD-associated hepatorenal dysfunction, combining diabetes, dyslipidemia, and ethanol exposure. **Methods:** *In vitro* cytotoxicity was evaluated in HepG2 cells, and acute oral toxicity was assessed in male Wistar rats. MASLD hepatorenal syndrome was induced by combined diabetes, dyslipidemia, and ethanol exposure over five weeks. Animals received vehicle, *P. alliacea* (30, 100, or 300 mg/kg), or simvastatin plus insulin during the last three weeks. Serum biochemical parameters, hepatic and renal histology, and hepatic oxidative stress markers were analyzed. **Results:** *P. alliacea* extract maintained HepG2 cell viability and caused no behavioral, hematological, biochemical, or histopathological alterations, confirming safety. In MASLD rats, the extract partially reduced hyperglycemia and dose-dependently lowered plasma and hepatic cholesterol and triglycerides while normalizing liver enzymes. The 300 mg/kg dose fully restored hepatic antioxidant defenses, reduced lipid peroxidation, and reversed renal dysfunction. **Conclusion:** *P. alliacea* exerts hepatoprotective and nephroprotective effects in MASLD, supporting its potential as a therapeutic agent for complex metabolic disorders.

Keywords: Alcohol; Diabetes; Dyslipidemia; Guiné; Hepatoprotection; Nephroprotection.

Graphical abstract



1. Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD), formerly known as non-alcoholic fatty liver disease (NAFLD), is a multifactorial condition characterized by hepatic steatosis (≥5% fat accumulation in hepatocytes), often accompanied by obesity, insulin resistance, dyslipidemia, and chronic inflammation (Chalasani *et al*. 2018; Polyzos *et al*. 2019; Eslam *et al*. 2020). MASLD has emerged as the most prevalent chronic liver disorder worldwide, affecting approximately 25–30% of the adult population, and is projected to increase by over 30% by 2030 due to the rising incidence of diabetes mellitus and obesity (Estes *et al*. 2018; Younossi *et al*. 2023).

Recent evidence indicates that MASLD is not limited to hepatic impairment but rather represents a multisystemic disorder strongly associated with the development of extrahepatic complications, particularly cardiovascular disease and chronic kidney disease (Sinn *et al*. 2020; Jung *et al*. 2022; Park *et al*. 2025). These comorbidities frequently coexist in clinical settings and have contributed to the conceptualization of hepatorenal syndrome, a condition in which hepatic metabolic dysfunction exacerbates renal injury. This interplay is mediated by shared pathophysiological mechanisms, including insulin resistance (Alkerwi *et al*. 2017; Duan *et al*. 2019; Younossi *et al*. 2019; Thongnak *et al*. 2020; Krishnan *et al*. 2024), lipid accumulation (Hsu *et al*. 2021; Liang *et al*. 2020; Chen *et al*. 2023), endothelial dysfunction (Stinghen *et al*. 2009; Recio-Mayoral *et al*. 2011; Yilmaz *et al*. 2011; Pasarín *et al*. 2012; Chen *et al*., 2015; Wang *et al*. 2019; Jiang *et al*. 2020), oxidative stress (Kim and Vaziri 2010; Li and Liu 2024), chronic inflammation (Tonelli *et al*. 2005; Amdur *et al*. 2016; Monseu *et al*. 2016), and gut microbiota dysbiosis (Tang *et al*. 2015; Jäckel *et al*. 2017; Nanto-Hara *et al*. 2020). Current therapeutic options for MASLD and associated hepatorenal dysfunction are limited, costly, and often associated with adverse effects, especially when multiple comorbidities are present.

Preclinical models are essential for understanding MASLD pathophysiology and testing potential therapies. Traditional single-hit models, such as high-fat diet or chemical induction, often fail to reproduce the complex metabolic and extrahepatic complications observed in patients. In contrast, multi-hit models, which combine factors such as dyslipidemia, hyperglycemia, and ethanol exposure, more accurately mimic the multifactorial nature of MASLD and its progression to hepatorenal dysfunction. These models allow evaluation of therapeutic interventions in a context that closely reflects the clinical scenario, providing a robust platform to assess hepatoprotective and nephroprotective agents (Cui *et al*., 2024; Fu *et al*., 2024; Dua *et al*., 2025).

Natural products have emerged as promising alternatives for managing complex metabolic syndromes (Rodrigues *et al*. 2020; Süntar 2020; Lopes *et al*. 2023; Silva *et al*. 2024). *Petiveria alliacea* L. (Phytolaccaceae), Guinea henweed, commonly known in Brazil as “guiné”, “tipi”, or “erva-de-alho”, is a medicinal plant widely distributed across Latin America (Cronquist 1988; Duarte *et al*. 2005; Neves *et al*. 2006). It has been traditionally used in folk medicine for a variety of ailments, including pain, inflammation, infections, diabetes, liver congestion, and urinary disorders (Taylor 2002; Beltreschi *et al*. 2019; Bremm *et al*. 2020; Guimarães *et al*. 2022; Magalhães *et al*. 2022; Casagrande *et al*. 2023; Paz Perafan and Montenegro Paz 2024; Carbolim *et al*. 2025; Santiago *et al*. 2025). Preclinical studies have demonstrated a broad pharmacological spectrum, including antimicrobial (Guedes *et al*. 2009; Oyeleke *et al*. 2021; Khadka *et al*. 2023), anti-inflammatory (Lopez-Martins *et al*. 2002; Cruz Salomón *et al*. 2022; Olajubutu *et al*. 2022; Fatimah *et al*. 2024), mnemonic (Silva *et al*. 2015; Zavala-Ocampo *et al*. 2024), anxiolytic and antidepressant (Caicedo-Pinto *et al*. 2019), antifungal (Machado and Ramos, 2020; Khadka *et al*. 2023), antioxidant (Zavala-Ocampo *et al*. 2022; Carlosama *et al*. 2024), and antitumoral effects (Murillo *et al*. 2023; Prada *et al*. 2023; Rojas *et al*. 2023; Carlosama *et al*. 2024). More recently, ethnobotanical surveys and

experimental evidence have highlighted its potential hepatoprotective, hypoglycemic, and nephroprotective properties (Randle *et al*. 2018; Mustika *et al*. 2021; Conceição *et al*. 2023). However, its therapeutic efficacy in complex metabolic conditions such as MASLD-associated hepatorenal syndrome remains largely unexplored (Urueña *et al*. 2008; Silva *et al*. 2024).

Considering the traditional use of *P. alliacea* for liver and kidney-related ailments, and the growing interest in natural therapies for multisystemic metabolic disorders, here, we evaluated the toxicological safety and therapeutic potential of *P. alliacea* in a novel multi-hit preclinical model combining diabetes, dyslipidemia, and ethanol exposure. We hypothesized that *P. alliacea* extract exerts hepatoprotective and nephroprotective effects in MASLD by modulating oxidative stress and lipid metabolism. The study aimed to: (i) assess acute toxicological safety; (ii) evaluate hepatoprotective and nephroprotective effects; and (iii) provide preclinical evidence supporting its ethnopharmacological use in chronic metabolic disorders.

2. Material and Methods

2.1. Reagents

Ammonium ferric sulfate (Êxodo Científica®, Sumaré, Brazil), dipotassium phosphate (Êxodo Científica®, Sumaré, Brazil), ethanol (Êxodo Científica®, Sumaré, Brazil), eosin (Êxodo Científica®, Sumaré, Brazil), formalin (Êxodo Científica®, Sumaré, Brazil), Hayem's solution (Êxodo Científica®, Sumaré, Brazil), Harris hematoxylin (Êxodo Científica®, Sumaré, Brazil), hydrochloric acid (Êxodo Científica®, Sumaré, Brazil), monopotassium phospate (Êxodo Científica®, Sumaré, Brazil), pyrogallol (Êxodo Científica®, Sumaré, Brazil), sodium citrate dihydrate (Êxodo Científica®, Sumaré, Brazil), sulfuric acid (Êxodo Científica®, Sumaré, Brazil), butylated hydroxytoluene (Synth®, Diadema, Brazil), chloroform (Biotec®, Curitiba, Brazil), isopropanol (Biotec®, Curitiba, Brazil), Doxorubicin (Sigma-Aldrich®, Missouri, USA), Ellman's reagent - DTNB (Sigma-Aldrich®, Saint Louis, USA), MTT (Sigma-Aldrich®, Missouri, USA), saline solution (Sigma-Aldrich®, Saint Louis, USA), streptozotocin (Sigma-Aldrich®, Missouri, USA), xylenol orang'e (Sigma-Aldrich®, Saint Louis, USA), EDTA (Neon Reagentes®, Suzano, Brazil), methanol (Neon Reagentes®, Suzano, Brazil), glutathione-reduced (USB®, Cleveland, USA), hexane (Dinâmica®, Indaiatuba, Brazil), isoflurane (Isoforine®, Cristália, Itapira, Brazil), paraffin (Allkimia®, Campinas, Brazil), xylene (Allkimia®, Campinas, Brazil), simvastatin (Novartis®, Basel, Switzerland), tris (Invitrogen™, Carlsbad, USA), Turk's solution (NewProv®, Pinhais, Brazil), trichloroacetic acid (Proquímios®, Bangu, Brazil).

*2.2. **Preparation of** Petiveria alliacea*

The aerial parts of *P. alliacea* were collected in April 2022 in Dourados, Mato Grosso do Sul, Brazil (22°11'43.8"S, 54°56'06.4"W). A voucher specimen (no. 1651) was authenticated and deposited at the Herbarium of the Federal University of Grande Dourados (UFGD). The botanical identification was confirmed using the *World Flora Online* (WFO) Plant List online database, accessed on June 1st, 2022. To obtain the purified aqueous extract, the aerial parts were washed and dried in a forced-air circulation oven at 35°C for three days. The dried material was ground into powder and subjected to infusion following the method described by Souza *et al*. (2020). Specifically, 100 g of the powdered material was infused in 1 L of boiling chlorinated water. The extraction process lasted 5 to 6 hours, allowing the mixture to cool naturally at room temperature (25°C). Subsequently, three volumes of ethanol were added to the infusion to precipitate insoluble components. The resulting precipitate was removed

by filtration, and the ethanol-soluble fraction was lyophilized and stored at –20°C. The final yield of the extract was 9.31%.

*2.3. **Toxicological studies of** Petiveria alliacea*

2.3.1. In vitro assessment of cell viability

The impact of *P. alliacea* extract on cell viability was evaluated using the 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazoline bromide (MTT) colorimetric assay. HepG2 cells were seeded in 96-well flat-bottom plates (Corning® Costar® 3596, Corning Inc., NY, USA) at a density of 2×10^4 cells per well in complete Dulbecco's Modified Eagle Medium (DMEM) and allowed to adhere for 24 h at 37 °C in a humidified atmosphere with 5% CO_2. Cells were subsequently treated with increasing concentrations of *P. alliacea* extract (10–900 μg/mL) for 24, 48, or 72 h. Doxorubicin (1 μM; D1515, Sigma-Aldrich, St. Louis, MO, USA) served as a positive control, and cells treated with vehicle (filtered water) were used as the negative control. At the end of each exposure period, 10 μL of MTT solution (0.5 mg/mL; CT02, Sigma-Aldrich) was added to each well and incubated for 4 h. The supernatant was then carefully removed, and the formazan crystals formed were dissolved in 100 μL of dimethyl sulfoxide (DMSO; D2650, Sigma-Aldrich). Absorbance was measured at 570 nm using a microplate spectrophotometer (Multiskan™ FC, Thermo Fisher Scientific). Cell viability was expressed as the percentage of absorbance relative to untreated control cells. All experiments were performed in independent triplicates.

2.3.2. Acute toxicity evaluation

2.3.2.1. Animals

The toxicological evaluation of *P. alliacea* extract was conducted using male Wistar rats (200–250 g) obtained from the State University of Ponta Grossa (UEPG, Brazil). Animals were housed under controlled laboratory conditions at the Laboratory of Cardiometabolic Pharmacology, Federal University of Paraná (UFPR), with *ad libitum* access to food and water. Environmental parameters were maintained at a temperature of 22 ± 2 °C, relative humidity of 50 ± 10%, and a 12-hour light/dark cycle. Environmental enrichment was provided to promote animal welfare. All procedures were performed in accordance with the current guidelines of the Brazilian National Council for the Control of Animal Experimentation (CONCEA) and the ARRIVE (Animal Research: Reporting of *In vivo* Experiments) guidelines for ethical animal research, ensuring minimization of animal use and adherence to best practices (Percie du Sert *et al*. 2020). The experimental protocol was approved by the Institutional Animal Care and Use Committee of UFPR (approval number: 1536).

2.3.2.2. Experimental design

Acute toxicity was assessed according to guideline 423 of the Organization for Economic Cooperation and Development (OECD, 2001). After an 8-hour fasting period, male Wistar rats ($n = 6$) received a single oral dose of *P. alliacea* extract. Due to the absence of prior toxicological studies on this extract, an initial dose of 300 mg/kg was selected. Subsequent doses were determined based on the presence or absence of clinical signs of toxicity or mortality. The acute toxicity evaluation continued until one of the following endpoints was reached: (i) a dose produced relevant toxic effects; (ii) mortality occurred; (iii) no adverse effects were observed at the highest dose; or (iv) deaths occurred at the lowest dose. A basal control group ($n = 5$) received the vehicle (filtered water) under the same experimental conditions, and all the data were included in the statistics.

2.3.2.3. Assessment of clinical signs

Following oral administration of the *P. alliacea* extract, animals were observed at predetermined time points: within the first 30 minutes, and then at 1, 2, 3, and 4 hours post-treatment. Behavioral and clinical signs were monitored, including grooming behavior, piloerection, dyspnea, abdominal constriction, diarrhea, prostration, ataxia, sedation, coma, and mortality. After the initial 4-hour observation period, animals were allowed free access to food and water. Subsequently, they were monitored daily for 14 days for the presence of clinical signs or death (Silva *et al*. 2022).

2.3.2.4. Euthanasia, material collection and analysis

On day 15, following a 12-hour fasting period, animals were euthanized by anesthetic overdose using isoflurane (20%) in a saturated chamber. Blood samples were collected via decapitation for hematological and biochemical analyses. Serum glucose levels were measured using a glucometer (Accu-Chek Active®, Roche, Mannheim, Germany). Hematological parameters, including erythrocyte count, total leukocyte count, and differential leukocyte count, were determined. Serum levels of cholesterol, triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, and creatinine were measured using a semi-automatic biochemical analyzer and commercial diagnostic kits. The liver, spleen, heart, and kidneys were excised, rinsed in saline solution, and weighed using an analytical balance. Relative organ weights (%) were calculated as (organ weight/body weight) × 100. Representative tissue samples were fixed in 10% buffered formalin, processed using standard histological procedures, embedded in paraffin, and sectioned. Histological sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope (Leica DM 2500®) by a veterinary pathologist to assess morphological alterations potentially associated with the treatment.

2.4. Pharmacological studies

2.4.1. Animals

Male Wistar rats (200–250 g) were obtained from the Central Animal Facility of the Federal University of Paraná (UFPR) and housed in the animal facility of the Laboratory of Cardiometabolic Pharmacology. Animals were maintained under standard environmental conditions (temperature 22 ± 2 °C; relative humidity 50 ± 10%; 12-hour light/dark cycle), with environmental enrichment, and had free access to food and water. All experiments were conducted during the light phase of the cycle. Animals were randomly assigned to experimental groups (n = 6–7). Sample size was determined based on previous studies employing similar experimental designs and in accordance with the principles of the 3Rs (Replacement, Reduction, and Refinement) (Percie du Sert *et al*. 2020; Auth *et al*. 2022; Albuquerque *et al*. 2023; Amaral *et al*. 2023; Silva *et al*. 2024). Body weight was measured weekly using an analytical balance. The experimental protocol was approved by the Institutional Animal Care and Use Committee of UFPR (approval number: 1536). All procedures complied with relevant national and international regulations and adhered to the ARRIVE guidelines (Percie du Sert *et al*. 2020).

2.4.2. Experimental design and treatments

The MASLD model used in this study was established by combining multiple risk factors, including diabetes, dyslipidemia, and ethanol intake. Diabetes was induced via a single intraperitoneal injection of streptozotocin (60 mg/kg) dissolved in 10 mM citrate buffer (pH 4.5) following a 12-hour fasting period (Vit *et al*. 2002; Souza *et al*. 2020). Blood glucose levels were measured from tail vein samples using a glucometer (Accu-

Chek Active®, Roche, Mannheim, Germany) three days after streptozotocin administration. Animals presenting glycemia ≥ 250 mg/dL were classified as diabetic. Dyslipidemia was induced by feeding a cholesterol-enriched diet (0.5%) for five weeks, as described by Silva *et al*. (2024). This diet was prepared by mixing 150 g of standard chow with one egg yolk, 13.5 mL of corn oil, and water. The mixture was dried in a laboratory oven at 50°C for 36 hours and stored in vacuum-sealed bags. Each 150 g portion contained approximately 225 mg of cholesterol, 1.8 g of saturated fat, 2.16 g of monounsaturated fatty acids, and 0.72 g of polyunsaturated fatty acids. Additionally, animals received free access to a liquid diet containing 5% ethanol, following the protocol by Lívero *et al*. (2016). During the final two weeks of the experiment, animals were treated by oral gavage with either filtered water (negative control group [C–]), *P. alliacea* extract at doses of 30, 100, or 300 mg/kg, or a combination of simvastatin (2.5 mg/kg) and insulin (6 IU) administered subcutaneously (SIM+INSU group). A basal control group consisting of normoglycemic, normolipidemic rats not exposed to ethanol received only filtered water. The experimental design is present in **Figure 1**.

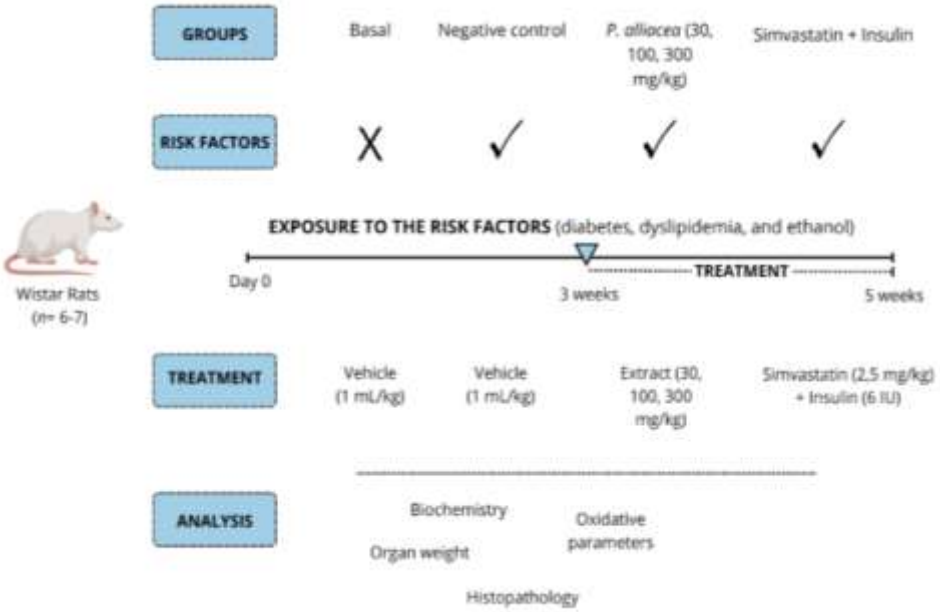


Fig 1. Experimental design. The model of metabolic dysfunction-associated steatotic liver disease was induced with diabetes, dyslipidemia, and ethanol in male Wistar rats during five weeks. In the last three weeks of the experiment the baseline group and the negative control group were treated with vehicle (filtered water, 1 mL/kg). Meanwhile the other three groups were treated with the *P. alliacea* extract (30, 100 and 300 mg/kg) and one group received the standard treatment with simvastatin + insulin. After the treatment the therapeutic effects of *P. alliacea* were evaluated. Visual elements created using Canva and Biorender.

2.4.3. Euthanasia, sample collection and biochemical analysis

At the end of the treatment period, and following a 12-hour fast, animals were euthanized by anesthetic overdose using isoflurane (20%) in a saturated chamber. Blood samples were collected via decapitation. Serum glucose levels were determined using a glucometer (Accu-Chek Active®, Roche, Mannheim, Germany). Plasma levels of ALT, AST, total cholesterol, triglycerides, urea, and creatinine were measured using colorimetric enzymatic assays in a semi-automatic analyzer (Global Analyzer®, model GTA-300, Calgary, AB, Canada). The

liver and kidneys were excised, weighed using an analytical balance, and dissected. One portion of each organ was fixed for histological evaluation, while the remaining tissue was stored at –20°C for subsequent analyses of oxidative stress parameters and lipid content.

2.4.4. Measurement of hepatic cholesterol and triglycerides

Liver samples were lyophilized and processed for lipid extraction using the gravimetric method (Lívero *et al*. 2016). For this analysis, samples were mixed with hexane at a ratio of 1:10 (sample:solvent) and incubated at 80°C for 2 hours. The supernatant was transferred to a vial and allowed to evaporate at room temperature. This extraction procedure was repeated three times to ensure maximal lipid recovery. Lipid content was expressed as a percentage and calculated using the following formula:

$$Lipid\,(\%) = 100 \times (final\ weight\ of\ vial\ /\ initial\ weight\ of\ vial)\ /\ 0.1\ g$$

The extracted lipids were weighed and resuspended in a solution of 1 mL chloroform and 2 mL isopropanol. Cholesterol and triglyceride concentrations were quantified using enzymatic colorimetric assays on an automated analyzer.

2.4.5. Histopathological analysis

Liver and kidney samples were collected immediately after necropsy, rinsed in saline solution to remove residual blood, fixed in 10% neutral-buffered formalin for at least 48 hours, dehydrated through graded ethanol and xylene series, embedded in paraffin, and sectioned at 6 µm (liver) and 4 µm (kidney). Sections were stained with hematoxylin and eosin (H&E) to assess cellular architecture and tissue alterations and examined under a Leica DM 2500® light microscope by a board-certified veterinary pathologist blinded to the treatment groups. Hepatic lesions were semi-quantitatively scored according to the classification by Kleiner *et al*. (2005), which evaluates ballooning and microvesicular and macrovesicular steatosis based on the percentage of affected parenchyma, graded on a scale from 0 to 3: 0 (no lesions), 1 (mild, 5–33%), 2 (moderate, 34–66%), and 3 (severe, 67–100%). Renal evaluation considered three main lesion categories: tubular (degeneration, necrosis, edema), glomerular (degeneration, necrosis, glomerulonephritis), and inflammatory infiltrates (presence of polymorphonuclear or mononuclear cells in the interstitium). Each parameter was scored on a semi-quantitative scale from 0 to 3, where 0 indicates absence of lesions, 1 mild lesions (focal, <25% of parenchyma), 2 moderate lesions (multifocal, 25–50%), and 3 severe lesions (diffuse, >50%). Scores were assigned individually to each kidney section, and group medians were calculated for statistical comparison. Representative photomicrographs were obtained to illustrate typical findings in each experimental group.

2.4.6. Investigation of hepatic antioxidant system

To assess the antioxidant status of hepatic tissues, liver samples were homogenized at a ratio of 1:10 (w/v) using potassium phosphate buffer (0.1 M, pH 6.5). For the determination of superoxide dismutase (SOD) activity and lipid peroxidation (LPO) levels, the homogenates were centrifuged at 9,700 rpm for 20 minutes at 4°C. Quantification of SOD and LPO was performed following the methodologies described by Gao *et al*. (1998) and Jiang *et al*. (1992), respectively. For reduced glutathione (GSH) quantification, 100 µL of the homogenate was mixed with 80 µL of 12.5% trichloroacetic acid, vortexed, and centrifuged at 3,000 rpm for 15 minutes at 4°C. GSH levels were determined in the supernatant according to the protocol established by Sedlak and Lindsay (1968).

2.5. Statistical analysis

Statistical analyses were performed following an initial assessment of data distribution and homogeneity of variance. One-way analysis of variance (ANOVA) was conducted to compare means among groups, followed by the Tukey post hoc test. The significance level of $p < 0.05$ was established. Data are presented as mean ± standard error of the mean (S.E.M.). All analyses were carried out using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Safety assessment of Petiveria alliacea extract

3.1.1. Impact of P. alliacea on HepG2 cell viability

Treatment of HepG2 cells with *P. alliacea* extract demonstrated that low to intermediate concentrations had minimal impact on cell viability, whereas higher concentrations and prolonged exposure resulted in measurable reductions in metabolic activity (**Figure 2**). At 24 h, viability remained above 70% at concentrations up to 300 µg/mL, with significant decreases observed only at 900 µg/mL. The median inhibitory concentration (IC_{50}) at this time point was relatively high (1297 µg/mL), indicating that short-term exposure to moderate doses does not compromise cellular viability. After 48 h, viability remained above 70% for concentrations up to 100 µg/mL, with notable reductions appearing at higher doses. The IC_{50} decreased to 583.3 µg/mL, consistent with a moderate, time-dependent effect becoming relevant primarily at concentrations ≥ 300 µg/mL. At 72 h, the impact was more pronounced, though cells maintained approximately 70–80% viability at concentrations ≤ 100 µg/mL. A steep decline was observed from 300 µg/mL onwards, with an IC_{50} of 309.5 µg/mL. Overall, this temporal profile indicates that the extract exerts a progressive effect at higher doses and longer exposures, while lower concentrations are well tolerated even after 72 h. Doxorubicin (1 µM), used as a positive control, caused a sharp decrease in viability under all conditions, confirming assay sensitivity. Statistical analysis revealed significant differences ($p < 0.001$) primarily at higher concentrations (≥ 100 µg/mL after 48 h and ≥ 300 µg/mL after 72 h) compared with untreated controls.

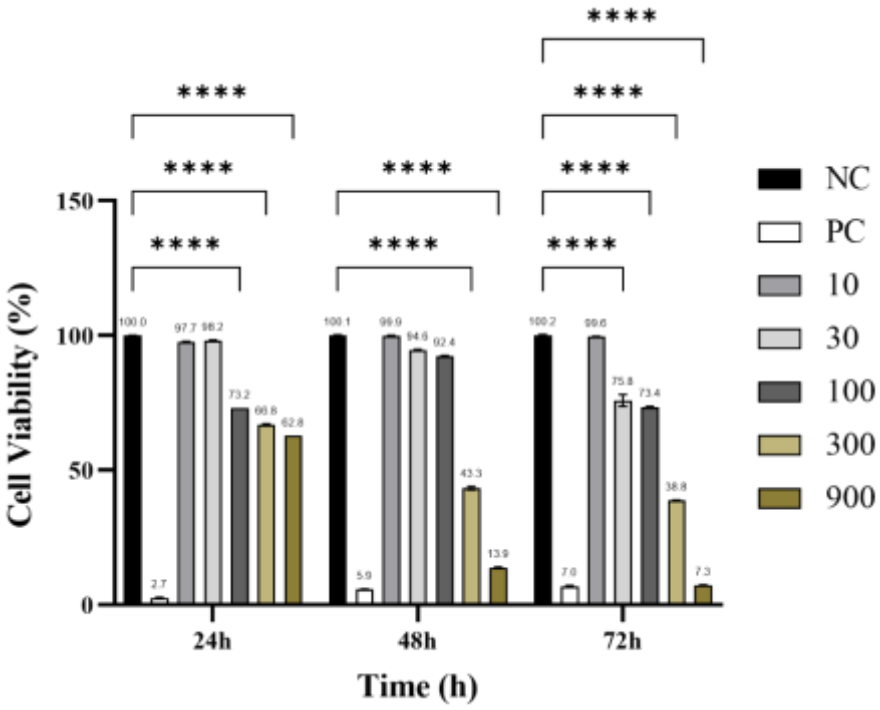


Fig 2. Effect of *P. alliacea* **on cell viability.** HepG2 cells after 24, 48, and 72 h of treatment with *Petiveria alliacea* extract at different concentrations (10, 30, 100, 300, and 900 µg/mL). Saline solution was used as the negative control (NC), and doxorubicin (200 µg/mL; 2%) as the positive control (PC). Statistical analysis was performed by one-way ANOVA followed by Tukey's post hoc test. *$p < 0.001$ *vs* NC.

3.1.2. Evaluation of clinical signs, relative organs and body weight of rats

Animals orally treated with a single dose of *P. alliacea* extract at dose of 300 mg/kg and 2000 mg/kg exhibited no behavioral or physiological alterations during the entire observation period (from 30 minutes up to 14 days), when compared to the untreated basal group (**Table 1**).

Table 1. Assessment of clinical signs in Wistar rats treated with vehicle or *Petiveria alliacea* extract (300 or 2000 mg/kg), orally, in a single dose

		Clinical sign	**Time**					
			30 min	1h	2h	3h	4h	14 days
Basal (vehicle)		*Self-cleaning absence*	0/5	0/5	0/5	0/5	0/5	0/5
		Piloerection	0/5	0/5	0/5	0/5	0/5	0/5
		Dyspnea	0/5	0/5	0/5	0/5	0/5	0/5
		Abdominal constriction	0/5	0/5	0/5	0/5	0/5	0/5
		Diarrhea	0/5	0/5	0/5	0/5	0/5	0/5
		Prostration	0/5	0/5	0/5	0/5	0/5	0/5
		Ataxia	0/5	0/5	0/5	0/5	0/5	0/5
		Sedation	0/5	0/5	0/5	0/5	0/5	0/5
		Coma	0/5	0/5	0/5	0/5	0/5	0/5
		Death	0/5	0/5	0/5	0/5	0/5	0/5
Extract of *Petiveria alliacea*	**300 mg/kg**	*Self-cleaning absence*	0/6	0/6	0/6	0/6	0/6	0/6
		Piloerection	0/6	0/6	0/6	0/6	0/6	0/6
		Dyspnea	0/6	0/6	0/6	0/6	0/6	0/6
		Abdominal constriction	0/6	0/6	0/6	0/6	0/6	0/6
		Diarrhea	0/6	0/6	0/6	0/6	0/6	0/6
		Prostration	0/6	0/6	0/6	0/6	0/6	0/6
		Ataxia	0/6	0/6	0/6	0/6	0/6	0/6
		Sedation	0/6	0/6	0/6	0/6	0/6	0/6
		Coma	0/6	0/6	0/6	0/6	0/6	0/6
		Death	0/6	0/6	0/6	0/6	0/6	0/6
	2000 mg/kg	*Self-cleaning absence*	0/6	0/6	0/6	0/6	0/6	0/6
		Piloerection	0/6	0/6	0/6	0/6	0/6	0/6
		Dyspnea	0/6	0/6	0/6	0/6	0/6	0/6
		Abdominal constriction	0/6	0/6	0/6	0/6	0/6	0/6
		Diarrhea	0/6	0/6	0/6	0/6	0/6	0/6
		Prostration	0/6	0/6	0/6	0/6	0/6	0/6
		Ataxia	0/6	0/6	0/6	0/6	0/6	0/6
		Sedation	0/6	0/6	0/6	0/6	0/6	0/6
		Coma	0/6	0/6	0/6	0/6	0/6	0/6
		Death	0/6	0/6	0/6	0/6	0/6	0/6

Body and organ weight data for all groups are presented in **Table 2**. Throughout the 14-day experimental period, a progressive increase in body weight was observed in all groups. The basal group showed consistent weight gain, reaching a final average of 328.60 ± 13.57 g on day 14. Similarly, rats treated with 300 mg/kg and 2000 mg/kg of the extract also demonstrated a steady increase in weight, with final body weights of 328.70 ± 8.00 g and 333.70 ± 11.73 g, respectively. The pattern of weight gain in treated groups closely mirrored that of the basal group, indicating that administration of *P. alliacea* extract did not adversely affect normal body weight progression. No statistically significant differences were found in weight gain between control and treated animals. Additionally, the relative weights of vital organs—including liver, spleen, heart, and kidneys—were evaluated, and no significant differences were observed between the extract-treated groups and the basal group.

Table 2. The body weight and relative organ weight of Wistar rats treated with vehicle or *Petiveria alliacea* extract (300 or 2000 mg/kg), orally, in a single dose, after the 14-day experimental period

	Basal	**Extract of** *Petiveria alliacea*	
		300 mg/kg	**2000 mg/kg**
		Body weight (g)	
14-day	328.60 ± 13.57	328.70 ± 8.00	333.70 ± 11.73
		Relative organ weight (%)	
Liver	3.31 ± 0.097	3.07 ± 0.052	3.01 ± 0.143
Spleen	0.18 ± 0.009	0.18 ± 0.007	0.17 ± 0.013
Heart	0.36 ± 0.009	0.37 ± 0.009	0.31 ± 0.017
Kidneys	0.77 ± 0.032	0.74 ± 0.030	0.66 ± 0.030

Values are expressed as mean ± S.E.M., n = 5-6. Data are presented as mean ± S.E.M., n = 5-6. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test.

3.1.3. Biochemical serum parameters in Wistar rats treated with Petiveria alliacea extract

The biochemical evaluation of serum parameters in Wistar rats treated with *P. alliacea* extract demonstrated no statistically significant alterations across the assessed markers (**Figure 3**). Total cholesterol showed a non-significant increase, with the control group presenting 47.60 ± 6.03 mg/dL (**Figure 3a**). Triglyceride levels remained unchanged, with the basal group showing 54.20 ± 3.14 mg/dL and no differences detected among the treated groups (**Figure 3b**). Similarly, the hepatic enzymes AST and ALT did not show significant changes. ALT levels were 66.23 ± 2.59 U/L (**Figure 3c**), while AST levels in the control group were 130.90 ± 14.74 U/L (**Figure 3d**). Renal function markers also remained stable, with urea concentrations at 31.10 ± 0.86 mg/dL in the basal group (**Figure 3e**). Serum creatinine levels were comparable across groups: 0.47 ± 0.04 mg/dL in the basal group, 0.46 ± 0.03 mg/dL in the 300 mg/kg group, and 0.46 ± 0.03 mg/dL in the 2000 mg/kg group (**Figure 3f**).

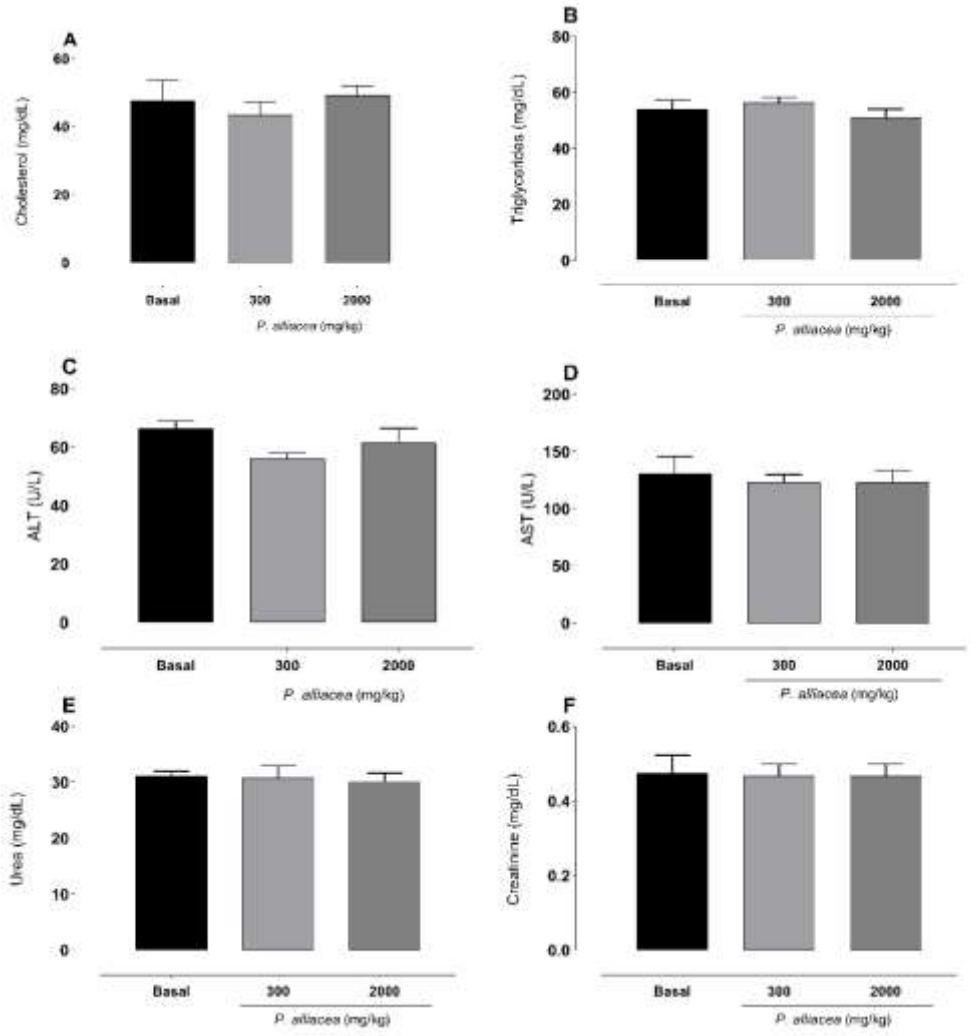


Fig 3. Serum biochemical parameters of Wistar rats treated orally with a single dose of *Petiveria alliacea* extract (300 or 2000 mg/kg) or vehicle (filtered water). Measured parameters include **(a)** total cholesterol, **(b)** triglycerides, **(c)** alanine aminotransferase (ALT), **(d)** aspartate aminotransferase (AST), **(e)** urea, and **(f)** creatinine. n = 5-6 per group. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test.

3.1.4. Hematological profile of rats treated with Petiveria alliacea extract

Hematological parameters did not show significant alterations among the groups treated with vehicle or *P. alliacea*. Regarding red and white blood cell counts, no significant differences were observed between the extract-treated groups and the basal group. Similarly, the analysis of leukocyte subtypes revealed no significant changes in basophils, neutrophils, or lymphocytes. A slight but statistically significant difference was observed in monocyte levels in the 300 mg/kg ($0.01 \pm 0.01 \times 10^3$ cells/µL) and 2000 mg/kg ($0.02 \pm 0.03 \times 10^3$ cells/µL) groups compared to the basal group (**Table 3**).

Table 3. Hematological parameters of Wistar rats treated with vehicle or *Petiveria alliacea* extract (300 or 2000 mg/kg), orally, single dose

Parameter	**Basal**	**Extract of** *Petiveria alliacea*	
		300 mg/kg	**2000 mg/kg**
Red blood cells (10^6 mm/µL)	8.14 ± 0.49	9.33 ± 0.13	6.14 ± 0.16

Leukocyte (10^3 mm/µL)	3.98 ± 0.56	3.35 ± 0.20	3.87 ± 0.40
Rods (10^3 mm/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Segmented (10^3 mm/µL)	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Lymphocyte (10^3 mm/µL)	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00
Monocyte (10^3 mm/µL)	0.00 ± 0.00	0.01 ± 0.00[a]	0.02 ± 0.00[a]
Eosinophil (10^5 mm /µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Values are expressed as mean ± S.E.M., *n*= 5-6. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. [a] $p < 0.05$ *vs*. Basal.

3.2.5. Petiveria alliacea does not induce hepatic, cardiac and renal cellular alterations

Histopathological analysis of liver, spleen, heart, and kidney tissues from rats treated with both doses (300 mg/kg and 2000 mg/kg) of *P. alliacea* extract revealed no significant tissue alterations when compared to the basal group. The microscopic evaluation demonstrated preservation of normal histoarchitecture across all organs examined. In hepatic tissue, hepatocytes were arranged in regular cords with well-preserved cytoplasm and nuclei, without evidence of steatosis, inflammatory infiltrates, ballooning degeneration, or necrosis. Similarly, the spleen exhibited intact white and red pulp regions, with no signs of lymphoid depletion or architectural disruption. Cardiac tissue analysis revealed normal myocardial fibers, with no signs of cellular disorganization, inflammatory cell infiltration, or interstitial fibrosis. Renal tissue sections maintained preserved glomerular and tubular morphology, with no indications of tubular necrosis, interstitial edema, or inflammatory changes. No morphological differences were detected between the extract-treated groups and the basal control, supporting the histological safety of *P. alliacea* at the tested doses (**Figure 4**).

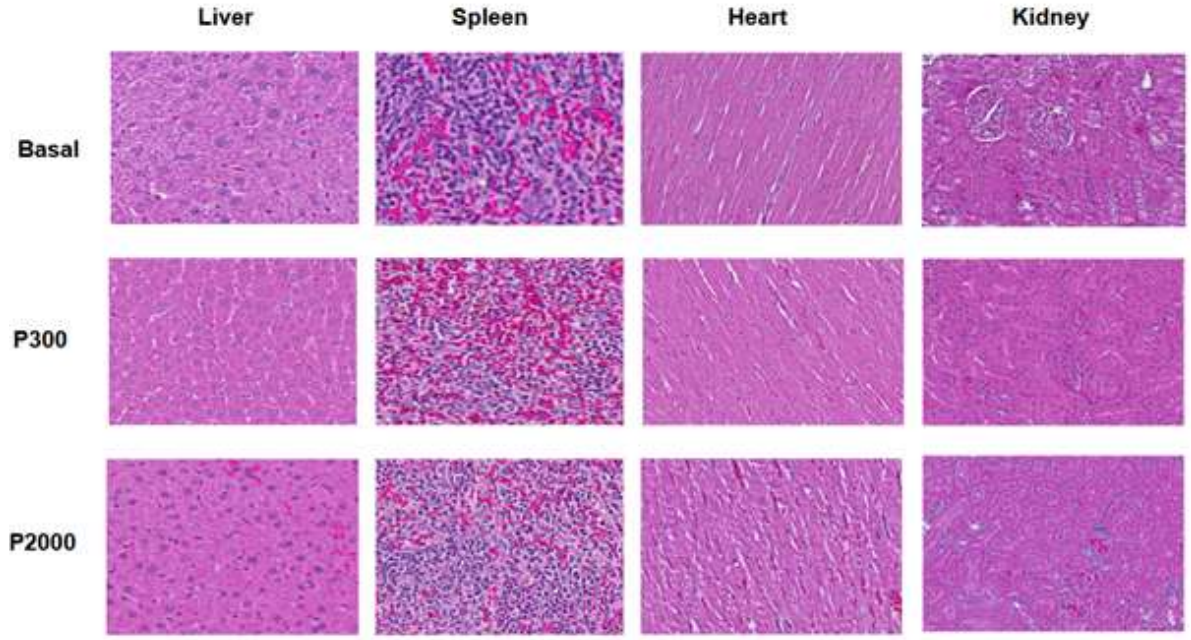


Fig 4. Histopathological analysis of the liver, spleen, heart, and kidney from Wistar rats treated with *Petiveria alliacea* (P) extract (300 mg/kg and 2000 mg/kg), compared to the basal (vehicle) group. Representative H&E-stained tissue sections show no significant morphological alterations in the liver, heart, or kidney at both dosages of the extract. Magnification: 20 *x*.

3.2. Pharmacological effects of *Petiveria alliacea* extract

3.2.1. Petiveria alliacea exhibits a median hypoglycemic effect, and demonstrates significant hepatic protective properties

After diabetes induction, a marked increase in serum glucose levels was observed in the C− group (448.60 ± 18.31 mg/dL) compared to the basal group, which remained normoglycemic (115.30 ± 3.75 mg/dL). Co-administration of SIM+INSU significantly reduced blood glucose levels (165.30 ± 16.21 mg/dL), while treatment with *P. alliacea* partially reversed hyperglycemia (**Figure 5a**). The combined effects of diabetes, dyslipidemia, and alcohol consumption led to considerable hepatic alterations, as evidenced by elevated levels of the liver enzymes ALT (107.00 ± 3.81 U/L) and AST (155.90 ± 6.18 U/L), compared to the basal group (48.00 ± 3.81 U/L). Notably, administration of *P. alliacea* at a dose of 300 mg/kg completely reversed the elevated ALT and AST levels (**Figure 5b** and **5c**, respectively). Treatment with lower doses (30 mg/kg and 100 mg/kg) partially reversed hepatic alterations, while the SIM+INSU combination was not effective.

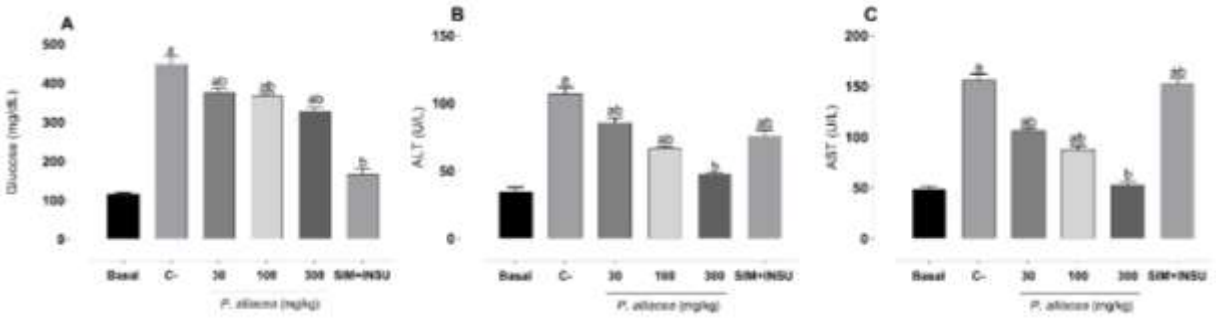


Fig 5. Serum levels of **(a)** glucose, **(b)** alanine aminotransferase (ALT), and **(c)** aspartate aminotransferase (AST). Basal: healthy rats, not subjected to the disease model, treated with vehicle. C–: diabetic, dyslipidemic rats exposed to ethanol, treated with vehicle. *P. alliacea*: diabetic, dyslipidemic rats exposed to ethanol, treated with *P. alliacea* extract (30, 100, or 300 mg/kg). SIM+INSU: diabetic, dyslipidemic rats exposed to ethanol, treated with simvastatin (2.5 mg/kg) plus insulin (6 IU). n = 6-7 per group. Data are presented as mean ± SEM. [a] $p < 0.05$ *vs*. basal; [b] $p < 0.05$ *vs*. C– (one-way ANOVA followed by Tukey's post hoc test).

3.3.2. Petiveria alliacea extract exerted median lipid-lowering effects

The concomitant induction of diabetes, dyslipidemia, and alcohol intake resulted in a marked elevation of plasma lipid levels. Total cholesterol reached 610.30 ± 22.29 mg/dL, and triglycerides reached 537.30 ± 8.78 mg/dL in the untreated control group exposed to all risk factors (C– group), compared to 58.71 ± 4.25 mg/dL observed in the basal group. Treatment with *P. alliacea* extract at doses of 30, 100, and 300 mg/kg led to a reduction in both triglycerides and cholesterol levels in all treated groups (**Figure 6a** and **6b**, respectively). While the effect was partial at all tested doses, a progressive decrease in lipid levels was observed across increasing doses. In contrast, animals treated with the combination of SIM+INSU exhibited plasma lipid values comparable to those of the basal group, indicating complete reversal of the induced dyslipidemia (**Figure 6a** and **6b**). The disease model group (C–), exposed to diabetes, dyslipidemia, and ethanol intake, exhibited a marked increase in hepatic triglyceride levels (211.70 ± 20.72 mg/dL) compared to the basal group (27.90 ± 3.50 mg/dL). Treatment with *P. alliacea* extract at doses of 30 and 100 mg/kg resulted in a dose-dependent reduction in triglyceride content, reaching 126.60 ± 6.44 and 83.88 ± 4.52 mg/dL, respectively. Administration of the 300 mg/kg dose led to a statistically significant reduction (51.89 ± 3.59 mg/dL), as did the combination therapy with simvastatin and insulin

(34.16 ± 2.80 mg/dL), with both treatments restoring triglyceride levels to values close to those observed in healthy controls (**Figure 6c**). A comparable trend was observed in hepatic cholesterol levels. In the C– group, cholesterol increased significantly to 108.80 ± 4.53 mg/dL, compared to 29.57 ± 3.26 mg/dL in the basal group. Treatment with *P. alliacea* extract at doses of 30 and 100 mg/kg led to a partial reduction, with levels decreasing to 71.65 ± 9.32 and 58.54 ± 3.50 mg/dL, respectively. Administration of the 300 mg/kg dose resulted in a statistically significant reduction to 38.96 ± 1.88 mg/dL. Similarly, the SIM+INSU group exhibited a significant decrease in hepatic cholesterol to 32.29 ± 2.78 mg/dL, with values approaching those observed in the basal group (**Figure 6d**).

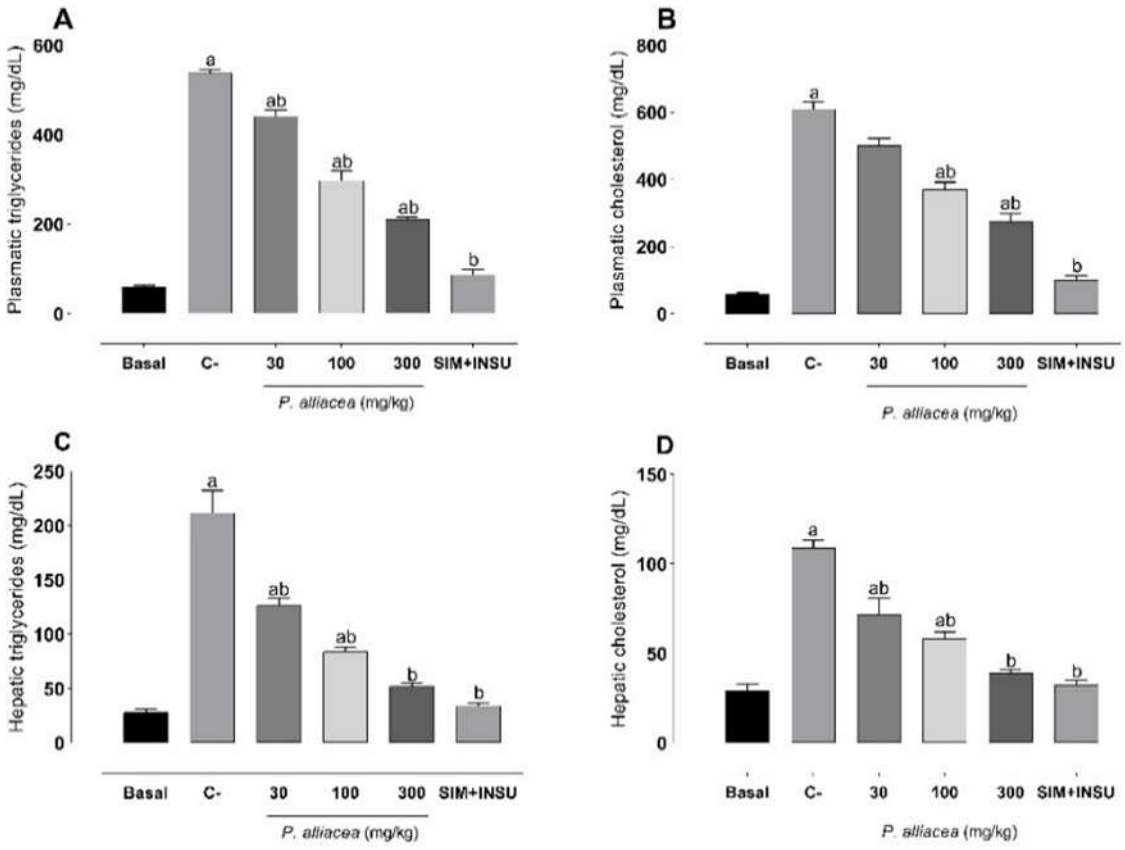


Fig 6. Serum levels of **(a)** triglycerides and **(b)** total cholesterol, and hepatic levels of **(c)** triglycerides and **(d)** cholesterol. Basal: healthy rats, not subjected to the disease model, treated with vehicle. C–: diabetic, dyslipidemic rats exposed to ethanol, treated with vehicle. *P. alliacea*: diabetic, dyslipidemic rats exposed to ethanol, treated with *P. alliacea* extract (30, 100, or 300 mg/kg). SIM+INSU: diabetic, dyslipidemic rats exposed to ethanol, treated with simvastatin (2.5 mg/kg) plus insulin (6 IU). n = 6-7 per group. Data are presented as mean ± SEM. [a] $p < 0.05$ *vs*. basal; [b] $p < 0.05$ *vs*. C– (one-way ANOVA followed by Tukey's post hoc test)

3.3.3. Petiveria alliacea reversed hepatocellular alterations

A significant increase in relative liver weight was observed in the group exposed to multiple risk factors (C– group), with values reaching 5.62 ± 0.121 g, compared to 3.00 ± 0.05 g in the basal group. Treatment with *P. alliacea* extract at a dose of 300 mg/kg resulted in an intermediate reduction of this parameter. Animals treated with the combination of SIM+INSU showed a partial normalization of liver weight, with a value of 5.00 ± 0.06 g (**Figure 7a**). Moreover, the presence of risk factors led to a marked increase of 230.63% in hepatic lipid content in the C– group relative to the basal group (13.63 ± 0.77%). In contrast, administration of *P. alliacea* extract at

300 mg/kg effectively normalized lipid accumulation, while the 30 mg/kg and 100 mg/kg doses resulted in partial attenuation of hepatic lipid levels (**Figure 7b**). Histological analysis of liver tissue revealed no evidence of steatosis in the basal group. In contrast, the C– group exhibited marked hepatic alterations, with ballooning degeneration graded as 3, microvesicular steatosis as grade 3, and macrovesicular steatosis as grade 2. Treatment with *P. alliacea* extract resulted in dose-dependent attenuation of steatotic features. Animals treated with 30 mg/kg displayed ballooning grade 2, microvesicular steatosis grade 2, and macrovesicular steatosis grade 2. At the 100 mg/kg dose, findings included ballooning grade 2, microvesicular steatosis grade 2, and macrovesicular steatosis grade 1. The highest dose, 300 mg/kg, resulted in ballooning grade 1, microvesicular steatosis grade 1, and macrovesicular steatosis grade 2. The SIM+INSU group exhibited minimal histological changes, with ballooning grade 1, and no evidence of microvesicular or macrovesicular steatosis, with grade 0 for both (**Figure 7c** and **7d**).

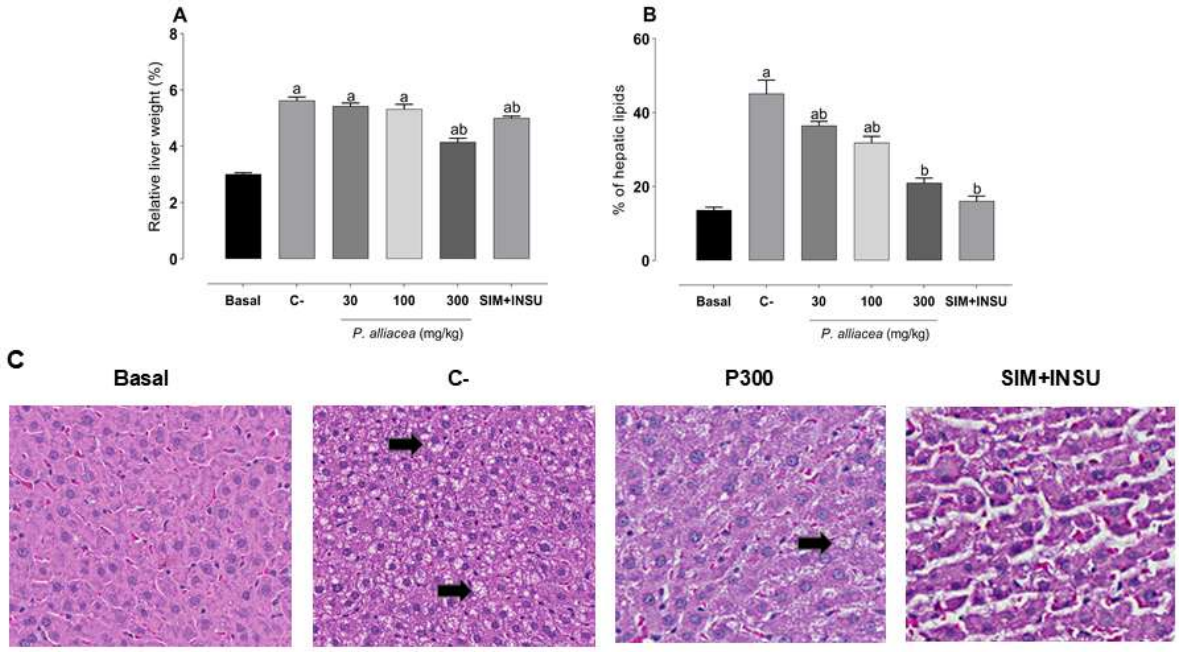


Fig 7. Relative liver weight **(a)**, percentage of hepatic lipid content **(b)**, and representative histological sections of the liver stained with hematoxylin and eosin (H&E) **(c)**. Basal: healthy rats, not subjected to the disease model, treated with vehicle. C–: diabetic, dyslipidemic rats exposed to ethanol, treated with vehicle. *P. alliacea*: diabetic, dyslipidemic rats exposed to ethanol, treated with *P. alliacea* extract (30, 100, or 300 mg/kg). SIM+INSU: diabetic, dyslipidemic rats exposed to ethanol, treated with simvastatin (2.5 mg/kg) plus insulin (6 IU). n = 6-7 per group. Data are presented as mean ± SEM. [a] $p < 0.05$ *vs.* basal; [b] $p < 0.05$ *vs.* C– (one-way ANOVA followed by Tukey's post hoc test). In the histological sections, black arrows highlight areas of hepatic steatosis (fatty degeneration). Images captured at 20× magnification.

3.3.4. Petiveria alliacea restores hepatic antioxidant defenses and reduces lipid peroxidation

Evaluation of oxidative stress markers in the liver revealed significant disturbances in animals subjected to the combination of risk factors (**Table 4**). SOD activity was significantly reduced in the C– group (25.14 ± 1.06) compared to the basal group (39.12 ± 0.97). Treatment with *P. alliacea* at 30 mg/kg (32.36 ± 1.42) and 100 mg/kg (34.77 ± 1.12), as well as SIM+INSU (31.56 ± 0.65), partially restored SOD activity, whereas the 300 mg/kg dose normalized enzyme activity (41.40 ± 0.54), with values not statistically different from the basal group. Similarly, GSH levels were markedly depleted in the C– group (10.48 ± 2.25) relative to basal animals (58.46 ± 2.14). Partial

recovery of GSH was observed with *P. alliacea* at 30 mg/kg (26.39 ± 2.93), 100 mg/kg (36.48 ± 4.02), and with SIM+INSU (33.15 ± 3.10). Full restoration was achieved with the 300 mg/kg dose (61.36 ± 1.36), which was not statistically different from basal values. In parallel, LPO was significantly increased in the C– group (155.20 ± 10.16) compared to the basal group (56.63 ± 3.66). Treatment with *P. alliacea* at 30 mg/kg (95.25 ± 4.42), 100 mg/kg (79.93 ± 3.05), and SIM+INSU (82.89 ± 3.30) partially reversed this increase. Complete normalization was observed with the 300 mg/kg dose (61.60 ± 2.99), which showed no statistical difference from basal values.

Table 4. Hepatic oxidative stress markers in Wistar rats (n=6-7) subjected to the Metabolic dysfunction-associated steatotic liver disease (MASLD) model and treated with *Petiveria alliacea* extract or simvastatin + insulin.

			Extract of *Petiveria alliacea* (mg/kg)			
	Basal	**C–**	**30**	**100**	**300**	**SIM+INS**
SOD	39.12 ± 0.97	25.14 ± 1.06[a]	32.36 ± 1.42[ab]	34.77 ± 1.12[ab]	41.40 ± 0.54[b]	31.56 ± 0.65[ab]
GSH	58.46 ± 2.14	10.48 ± 2.25[a]	26.39 ± 2.93[ab]	36.48 ± 4.02[ab]	61.36 ± 1.36[b]	33.15 ± 3.10[ab]
LPO	56.63 ± 3.66	155.20 ± 10.16[a]	95.25 ± 4.42[ab]	79.93 ± 3.05[ab]	61.60 ± 2.99[b]	82.89 ± 3.30[ab]

C-, negative control; SIM+INS, simvastatin + insulin; GSH, reduced glutathione (µg GSH/g of tissue); LPO, lipoperoxidation (nmol LPO/g of tissue); SOD, superoxide dismutase (U SOD/g of tissue). Values are expressed as mean ± S.E.M.. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. [a] $p < 0.05$ *vs*. basal; [b] $p < 0.05$ *vs*. C–.

3.3.5. Petiveria alliacea ameliorates renal dysfunction and histopathological damage

A significant increase in relative kidney weight was observed in the group exposed to the combination of risk factors (C– group), with a mean value of 1.17 ± 0.04 g, compared to 0.71 ± 0.01 g in the basal group. Treatment with *P. alliacea* extract at a dose of 300 mg/kg effectively normalized kidney weight (0.82 ± 0.01 g). In contrast, the SIM+INSU group exhibited a partial reduction in this parameter (1.01 ± 0.02 g) (**Figure 8a**). In parallel, renal function markers were markedly elevated in the C– group, as evidenced by increased plasma levels of urea (69.86 ± 3.48 mg/dL) and creatinine (74.17 ± 1.51 mg/dL), indicating renal impairment associated with the metabolic disorder. Treatment with *P. alliacea* extract at 300 mg/kg resulted in complete reversal of these elevations, with urea and creatinine levels reduced to 26.57 ± 1.77 mg/dL and 32.67 ± 2.80 mg/dL, respectively. The lower doses of *P. alliacea* (30 and 100 mg/kg) produced partial nephroprotective effects, with intermediate reductions in both renal markers. The SIM+INSU group showed modest improvements in urea and creatinine levels, but the reductions were less pronounced than those observed with the 300 mg/kg dose of *P. alliacea* (**Figure 8b** and **8c**, respectively).

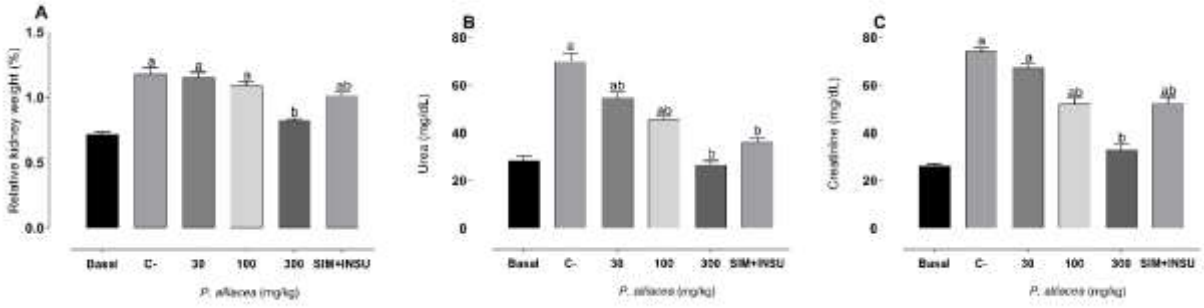


Fig 8. Relative kidney weight **(a)**; plasma urea levels **(b)**; and plasma creatinine levels **(c)**. Basal: healthy rats, not subjected to the disease model, treated with vehicle. C–: diabetic, dyslipidemic rats exposed to ethanol, treated with vehicle. *P. alliacea*: diabetic, dyslipidemic rats exposed to ethanol, treated with *P. alliacea* extract (30, 100, or 300 mg/kg). SIM+INSU: diabetic, dyslipidemic rats exposed to ethanol, treated with simvastatin (2.5 mg/kg) plus insulin (6 IU). *n* = 6-7 per group. Data are presented as mean ± SEM. [a] $p < 0.05$ *vs*. basal; [b] $p < 0.05$ *vs*. C– (one-way ANOVA followed by Newman-Keuls's post hoc test).

Histological evaluation revealed preserved renal architecture in the basal group (tubular score: 0; glomerular score: 0; inflammation score: 0). In contrast, the C– group exhibited marked pathological alterations, with severe multifocal to diffuse tubular necrosis and degeneration (tubular score: 3), occasional moderate glomerular involvement (glomerular score: 2), and severe multifocal inflammatory infiltrates (inflammation score: 3), frequently associated with proteinuria. In the *P. alliacea* 30 mg/kg group, acute tubular necrosis was the predominant finding (tubular score: 2), accompanied by mild to moderate glomerular alterations (glomerular score: 1), and mild inflammation (inflammation score: 1). Two animals in this group displayed normal histology. The 100 mg/kg group showed a higher incidence of glomerular lesions (glomerular score: 2), including acute multifocal nephritis, chronic nephritis with moderate multifocal necrosis, membranous glomerulonephritis, and mild glomerulonephritis. Tubular damage remained moderate (tubular score: 2), and inflammation was generally mild to moderate (inflammation score: 1). One animal in this group presented normal renal morphology. Administration of *P. alliacea* at 300 mg/kg was associated with improved renal histology, with most animals showing normal tissue (tubular score: 0; glomerular score: 0–1; inflammation score: 0). Mild acute glomerulonephritis (score 1) was observed in two animals, and one case of mild glomerular degeneration with necrosis was recorded. The SIM+INSU group presented heterogeneous findings, including chronic pyelonephritis, chronic proliferative multifocal glomerulonephritis, and myeloid substance accumulation with tubular degeneration (tubular score: 1; glomerular score: 2; inflammation score: 1), while two animals exhibited normal histology (**Figure 9**).

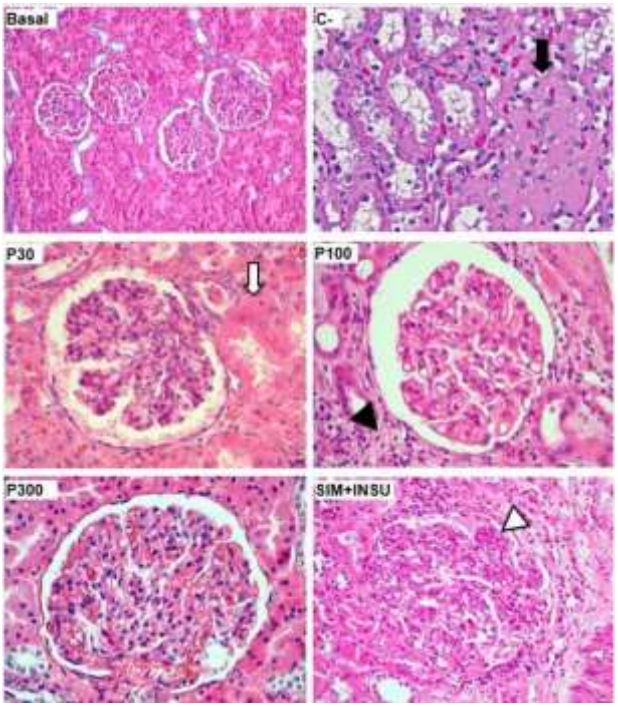


Fig 9. Representative histological sections of the kidneys stained with hematoxylin and eosin (H&E). Basal: healthy rats, not subjected to the disease model, treated with vehicle. C–: diabetic, dyslipidemic rats exposed to ethanol, treated with vehicle. *P. alliacea*: diabetic, dyslipidemic rats exposed to ethanol, treated with *P. alliacea* extract (30, 100, or 300 mg/kg). *n* = 6-7. SIM+INSU: diabetic, dyslipidemic rats exposed to ethanol, treated with simvastatin (2.5 mg/kg) plus insulin (6 IU). Symbols: large black arrow, marked necrosis and multifocal severe tubular degeneration; large white arrow outlined in black, acute tubular necrosis; black-headed arrow, mild glomerulonephritis; white-headed arrow outlined in black, chronic pyelonephritis. Images captured at 40× magnification

4. Discussion

The present study provides the first integrated evaluation of the safety and therapeutic potential of *Petiveria alliacea* in a multifactorial rat model of metabolic dysfunction-associated steatotic liver disease (MASLD), induced by the concomitant exposure to diabetes, dyslipidemia, and ethanol intake. This experimental approach mimics the complex clinical scenario in which multiple risk factors act synergistically to accelerate liver and kidney injury, thereby offering a relevant platform for assessing both the safety and efficacy of the extract.

An additional innovative aspect of this study is the implementation of a multi-hit preclinical model that combines diabetes, dyslipidemia, and ethanol exposure to emulate the multifactorial pathophysiology of MASLD. Unlike conventional single-hit models, which typically reproduce isolated metabolic alterations, this approach induces simultaneous hepatic and renal dysfunction, better reflecting the complexity observed in patients with advanced disease. Importantly, the inclusion of chronic low-dose ethanol exposure acts as a metabolic aggravator, exacerbating oxidative stress and lipid peroxidation. This factor amplifies the severity of steatosis and accelerates the onset of renal injury, thereby producing a more clinically relevant and translational phenotype (Acierno *et al*., 2025). The concurrent metabolic insults act synergistically to reproduce the cascade of cellular and systemic disturbances typical of MASLD progression, strengthening the predictive validity of the model for testing hepatoprotective and nephroprotective interventions.

Our *in vitro* results demonstrated that *P. alliacea* extract was non-cytotoxic to HepG2 cells at all tested concentrations, indicating a high degree of biocompatibility with hepatic cells. This absence of cytotoxicity suggests that the extract preserves fundamental cellular functions, supporting its potential safety for further pharmacological or nutraceutical applications. Given that HepG2 cells are a well-established model of human liver, these findings provide meaningful insights into the hepatic tolerability of the extract and highlight its translational relevance for future preclinical studies (Gómez-Lechón *et al*. 2014).

Acute oral toxicity testing in rodents (limit dose: 2000 mg/kg) revealed no signs of behavioral alterations, physiological distress, or mortality during the 14-day observation period. These results are consistent with ethnobotanical reports describing the traditional use of *P. alliacea* without immediate harmful effects (Lawal *et al*. 2024; Cruz-Salomón *et al*. 2022). The lack of adverse effects at this dose places the extract within the least toxic category according to OECD guideline 423, indicating low acute toxicity potential. Taken together, these preliminary safety data support the potential of *P. alliacea* extract for further investigation, including subchronic toxicity testing and pharmacological efficacy studies. Although limited to acute exposure, the present results provide an initial scientific basis for its safe use in future preclinical applications. Notably, relative organ weights, clinical signs, hematological parameters, and histopathological analyses remained unchanged across treatment groups, confirming the absence of systemic or organ-specific toxicity. Importantly, the absence of hepatotoxic or nephrotoxic histological findings provides strong support for the extract's safety. These findings fill a significant gap in the toxicological validation of *P. alliacea*, which until now had limited preclinical toxicodynamic documentation, particularly under standardized laboratory conditions.

In the MASLD model, untreated animals displayed clear signs of metabolic and hepatic dysfunction, including elevated plasma cholesterol and triglycerides, increased hepatic lipid accumulation, and marked histopathological alterations characterized by steatosis, ballooning, and inflammation. Treatment with *P. alliacea* extract ameliorated these alterations in a dose-dependent manner. The 300 mg/kg dose significantly reduced hepatic cholesterol and triglyceride content, restoring them to levels approaching those of basal animals, and substantially improved histological architecture, with reductions in steatosis and ballooning. These hepatoprotective effects were comparable to those achieved with simvastatin combined with insulin, underscoring the pharmacological relevance of the extract. Notably, serum AST and ALT levels remained unchanged across groups, suggesting that the extract exerted protective rather than hepatotoxic actions.

Oxidative stress is a central mechanism in MASLD progression (Karkucinska-Wieckowska *et al*. 2021), and this was corroborated by the observed reductions in antioxidant defenses and the increase in lipid peroxidation in untreated animals. *P. alliacea* treatment restored the antioxidant balance, with the 300 mg/kg dose completely normalizing SOD and GSH levels while reducing lipid peroxidation to basal values. Lower doses and the pharmacological control produced only partial improvements. These results strongly suggest that the protective effects of *P. alliacea* are mediated by its antioxidant properties.

Renal impairment was also evident in the C– group, with increased relative kidney weight, elevated plasma urea and creatinine levels, and histopathological evidence of tubular necrosis, glomerular injury, and inflammatory infiltrates. Treatment with *P. alliacea* at 300 mg/kg completely reversed these alterations, normalizing renal function markers and restoring tissue integrity, while lower doses produced partial effects. The nephroprotective efficacy of the extract at the highest dose surpassed that of the pharmacological control, reinforcing its potential in conditions of metabolic stress. These findings resonate with ethnopharmacological uses of *P. alliacea* in kidney-related disorders, now supported by experimental validation.

Collectively, the data presented here demonstrate that *P. alliacea* is safe and exerts significant hepatoprotective, nephroprotective, and antioxidant effects in a complex and clinically relevant model of MASLD. The extract not only attenuated biochemical and histological markers of disease but also restored redox homeostasis, with effects that in several parameters were comparable or superior to those of conventional pharmacological treatment. These results underscore the ethnopharmacological relevance of *P. alliacea* and highlight its potential as a complementary therapeutic resource for metabolic and chronic liver diseases. However, caution is warranted when extrapolating to humans. The identification of active compounds, elucidation of molecular mechanisms, and evaluation of long-term safety and pharmacokinetics remain essential steps before clinical translation.

Despite only partial correction of hyperglycemia and dyslipidemia, *P. alliacea* displayed comparable or superior tissue-protective effects relative to simvastatin plus insulin. The extract notably reversed elevated renal markers and normalized kidney weight, highlighting its multi-organ protective profile. These results expand the pharmacodynamic spectrum of *P. alliacea*, previously explored primarily for antioxidant, immunomodulatory, or anti-inflammatory properties (Mustika *et al*. 2021; Cruz-Salomón *et al*. 2022; Zavala-Ocampo *et al*. 2022).

This study contributes to the growing body of evidence supporting the repositioning of medicinal plants as nutraceuticals or phytotherapeutics for chronic disease management (Sarkar *et al*. 2024; Siddiqui *et al*. 2024). The broad-spectrum safety and organ-protective effects demonstrated by *P. alliacea* in this study provide a promising foundation for future clinical trials or functional food development. Importantly, the extract could represent a cost-effective alternative or adjuvant in public health systems targeting populations with limited access to conventional polypharmacy. Additionally, our approach integrates biochemical, histological, and functional data, aligning with recent regulatory frameworks emphasizing comprehensive safety and efficacy profiling of natural products. The data also contribute toward standardizing *P. alliacea* as a potential candidate for inclusion in official pharmacopoeias or public health lists such as RENISUS (Brazil's National List of Medicinal Plants of Interest to SUS).

Some limitations should be acknowledged. Mechanistic studies were not performed, leaving the molecular pathways underlying the extract's effects to be elucidated. The MASLD model was assessed under acute treatment conditions; long-term studies are needed to evaluate sustained efficacy. Furthermore, while the extract exerted moderate glucose-lowering effects, its lipid-lowering and tissue-protective actions were more pronounced, suggesting that *P. alliacea* may act primarily as a tissue-protective agent rather than a potent metabolic modulator, a distinction with potential clinical implications for combinatory therapies.

Conclusion

Petiveria alliacea is a safe and promising botanical candidate with multi-organ protective effects, particularly in the context of hepatic and renal complications associated with metabolic syndrome. These results warrant further exploration into its mechanisms of action, chronic effects, and clinical applicability. Future studies should also investigate the potential synergistic actions of *P. alliacea* when combined with conventional treatments, as well as its incorporation into functional foods or phytopharmaceutical formulations.

Declarations

Ethical Approval

All applicable international, national, and institutional guidelines for the care and use of animals were followed. The experimental protocol was approved by the Institutional Animal Care and Use Committee of the Federal University of Paraná (approval number: 1536).

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Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Authors Contributions

AJK: Conceptualization, Methodology, Formal Analysis, Investigation, Writing—Original Draft Preparation, Writing—Review & Editing. ERA, ANH, RCSCA, MAA, CSB, GRS: Methodology, Investigation. LPG, JARF, DLF, LNB: Methodology, Formal Analysis, Investigation. JTRP and AGJ: Methodology, Formal Analysis, Writing—Review & Editing. FARL: Conceptualization, Methodology, Formal Analysis, Writing—Original Draft Preparation, Writing—Review & Editing, Supervision, Project Administration, Funding Acquisition. All authors read and approved the manuscript.

Conflict of Interest Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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List of Abbreviations

ALT: alanine aminotransferase, ANOVA: One-way analysis of variance, AST: aspartate aminotransferase, GSH: reduced glutathione, H&E: hematoxylin and eosin, LPO: lipid peroxidation, MASLD: metabolic dysfunction-associated steatotic liver disease, NAFLD: non-alcoholic fatty liver disease, S.E.M.: standard error of mean, SIM+INSU: simvastatin and insulin, SOD: superoxide dismutase.

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ARTIGO 2 - BRIDGING THE GAP: EXPLORING THE PRECLINICAL POTENTIAL OF *Pereskia grandifolia* IN METABOLIC-ASSOCIATED FATTY LIVER DISEASE

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Research Article

Bridging the Gap: Exploring the Preclinical Potential of *Pereskia grandifolia* in Metabolic-Associated Fatty Liver Disease

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Metabolic-associated fatty liver disease (MAFLD) is a complex condition characterized by steatosis and metabolic disturbances. Risk factors such as diabetes, cigarette smoking, and dyslipidaemia contribute to its development and progression. Effective and safe therapies for MAFLD are urgently needed. *Pereskia grandifolia* has shown potential as an alternative treatment, but its effectiveness against liver disease remains unexplored. This research aims to determine the hepatoprotective properties of *P. grandifolia* using a model of MAFLD. The study was carried out through various phases to assess the safety and efficacy of the ethanol-soluble fraction of *P. grandifolia*. Initially, an *in vitro* assay was performed to assess cell viability. This was followed by an acute toxicity test conducted in rats to determine the safety profile of the extract. Subsequently, the anti-inflammatory properties of *P. grandifolia* were examined in macrophages. For the MAFLD study, diabetic Wistar rats were made diabetic and exposed to a high fat diet and cigarette smoke, for 4 weeks. During the last 2 weeks, the rats were orally given either the vehicle (negative control group; C-), *P. grandifolia* (30, 100, and 300 mg/kg), or insulin in addition to simvastatin. A basal group of rats not exposed to these risk factors was also assessed. Blood samples were collected to measure cholesterol, triglycerides, glucose, ALT, and AST levels. Liver was assessed for lipid and oxidative markers, and liver histopathology was examined. *P. grandifolia* showed no signs of toxicity. It demonstrated anti-inflammatory effects by inhibiting phagocytosis and macrophage spreading. The MAFLD model induced liver abnormalities, including increased AST, ALT, disrupted lipid profile, oxidative stress, and significant hepatic damage. However, *P. grandifolia* effectively reversed these changes, highlighting its potential as a therapeutic agent. These findings emphasize the significance of *P. grandifolia* in mitigating hepatic consequences associated with various risk factors.

1. Introduction

Metabolic-associated fatty liver disease (MAFLD) is an increasingly prevalent health concern globally [1]. It entails the buildup of fatty deposits within the liver, causing inflammation, fibrosis, and harm to the liver. MAFLD has now become the prevailing chronic liver ailment worldwide and is influenced by a combination of genetic, environmental, and behavioral factors [2]. Obesity, insulin resistance, type 2 diabetes, dyslipidaemia, tabagism, and sedentary lifestyle are some of the risk factors associated with MAFLD. Therefore, effectively tackling this condition necessitates a comprehensive approach that considers all these aspects [3].

Diabetes mellitus significantly increases the risk of MAFLD development and progression. Insulin resistance, a hallmark of diabetes, promotes hepatic lipid accumulation by altering fatty acid oxidation and increasing de novo lipogenesis. Hyperglycemia and chronic inflammation also contribute to liver injury, fibrosis, and the progression of MAFLD [4, 5]. Cigarette smoking has been recognized as an independent risk element for MAFLD particularly in individuals with diabetes. Smoking-induced oxidative stress and systemic inflammation exacerbate insulin resistance, promote hepatic lipid accumulation, and increase the risk of liver injury and inflammation [6, 7]. Finally, dyslipidaemia, characterized by elevated low density lipoprotein and triglycerides levels, frequently coexists with MAFLD and contributes to hepatic steatosis by increasing lipid delivery to the liver and impairing hepatic lipid metabolism. It promotes inflammation, oxidative stress, and fibrosis, exacerbating MAFLD progression [8].

It is important to note that the combination of diabetes, cigarette smoking, and dyslipidaemia can have a synergistic impact on MAFLD development and progression. These factors interact through various pathways, including oxidative stress, chronic inflammation, impaired lipid metabolism, and insulin resistance, resulting in amplified liver damage and fibrosis. Their combined effects can accelerate disease progression and increase the risk of complications [9, 10]. In this way, understanding the influence of diabetes mellitus, cigarette smoking, and dyslipidaemia on MAFLD is essential for comprehensive disease management strategies.

One of the main challenges in the treatment of MAFLD is its multifactorial nature, and unfortunately, there is no specific approved pharmacological treatment for the condition. Given the interplay between diabetes, cigarette smoking, dyslipidaemia, and MAFLD, comprehensive management strategies are crucial. Multidisciplinary approaches that target glycemic control, smoking cessation, and dyslipidaemia management are essential in MAFLD treatment. However, the drugs currently available for the treatment of the disease have limited efficacy and exert significant adverse effects [11].

In this line, animal models with multiple risk factors associated with MAFLD can provide valuable information on the complex pathogenesis of the disease and potential therapeutic targets. The proposition of these animal models is justified by the need to address the complex nature of MAFLD and the limited effectiveness of current treatments [12–15]. By utilizing these models, it is possible to enhance the likelihood of identifying promising therapeutic candidates for further development and clinical trials [16].

Under these circumstances, medicinal plants are acknowledged as a valuable reservoir for the exploration and advancement of novel pharmaceuticals. For centuries, traditional communities have taken advantage of the therapeutic properties of various plant species to address a broad spectrum of illnesses. Today, scientific research is shedding light on the vast potential of medicinal plants as a valuable reservoir of phytochemicals constituents. Medicinal plants offer a vast repertoire of bioactive compounds that have the potential to act as a reservoir for the identification of novel drugs [17].

Pereskia grandifolia Haw. (Cactaceae), commonly known as "ora-pro-nóbis," has a variety of medicinal properties that have been recognised and utilized for centuries. This tropical plant is a valuable resource in traditional medicine due to its numerous therapeutic benefits. Its leaves are traditionally used for dietary supplementation (due to their rich protein content) and for their antioxidant, antitumor, anti-inflammatory, lipid-lowering, antidiabetic, and hypotensive effects [18–22]. However, pharmacological research into its effectiveness in treating liver disorders remains lacking. Considering the extensive traditional use of *P. grandifolia* for addressing liver conditions and its promising medicinal properties, it is plausible to explore this species as a potential hepatoprotective agent, particularly for liver diseases associated with multiple risk factors. Hence, this study aims to establish a preclinical model of MAFLD incorporating dyslipidaemia, diabetes, and smoking, with the objective of assessing the hepatoprotective actions of *P. grandifolia*.

2. Materials and Methods

2.1. Preparation of Pereskia grandifolia Extract. The leaves of *P. grandifolia* were gathered in June 2021 at the Medicinal Plants Garden of Paranaense University (UNIPAR), situated at an elevation of 430 meters above sea level (S23°46′11.3″–W53°16′412″). A voucher sample (number 142) was archived in the UNIPAR herbarium. The plant underwent a drying process in an oven and subsequent spraying. The infusion extract was prepared using the approach detailed in Barbosa et al. [12]. The crushed leaves (100 g) were utilized for the infusion extraction, employing 1 L of boiling water. The resultant infusion was stored in an amber flask for 5 hours. Following filtration, 95% ethanol was introduced to the infusion at a 1 : 3 (v/v) ratio to precipitate proteins and polysaccharides, resulting in a separated heterogeneous phase, which was then filtered out. The ethanol-soluble fraction underwent concentration using a rotary evaporator and subsequent lyophilization. The accuracy of the plant name was verified on https://www.theplantlist.org and was confirmed to be accurate.

2.2. Toxicological Studies

2.2.1. In Vitro Cytotoxicity of Pereskia grandifolia. The 3-(4,5-dimethylthiazol-2,5-diphenyl) tetrazolium bromide (MTT) cytotoxicity assay was conducted according to the method previously described by Tsuboy et al. [23]. Mouse dermal fibroblasts (NIH/3T3, ATCC® CRL-1658™) and hepatocellular carcinoma HUH7 cells were seeded into 96-well microtiter plates and incubated in culture medium for 24 hours at 37°C with 5% CO_2. When the cells reached approximately 75% confluence after 24 hours, they were exposed to five different concentrations of *P. grandifolia* (100, 200, 400, 800, and 1,600 μg/mL). For the negative control (NC), the extract was substituted with a physiological solution, while for the positive control (PC), it was replaced with 2% (v/v) Tween 80. The treatments were conducted for 24, 48, and 72 hours.

2.2.2. Acute Toxicity Evaluation of Pereskia grandifolia

(1) Animals. The toxic impacts of the *P. grandifolia* extract were investigated in adult male Wistar rats weighing between 300 and 400 g, sourced from Maringá State University. These rats were accommodated in the vivarium of the laboratory for preclinical research of UNIPAR, with unrestricted access to both liquid and solid diets, while maintaining precise environmental conditions (temperature: 22°C ± 2°C; relative humidity: 50% ± 10%; and 12-hour light/dark cycle). Ethical clearance for the animal study protocols was granted by the Ethics Committee in Research involving Animal Experimentation of UNIPAR (protocol no. SPP2020031000167). The animals were provided with environmental enrichment to enhance their overall well-being. All guidelines and recommendations pertaining to animal welfare, including minimizing the number of animals used and transparent reporting of animal research, were diligently followed [24].

(2) Experimental Design. The assessment of acute toxicity followed the guidelines outlined by the Organization for Economic Cooperation and Development (OECD) guideline 423. The initial dose administered was 300 mg/kg due to the absence of prior information on *P. grandifolia* extract. Subsequent animal groups received either higher or lower fixed doses based on the presence or absence of signs of toxicity or mortality. This iterative process continued until the dose causing evident toxicity was identified or no more than one death occurred, when no effects were observed at the highest dose, or when deaths were noted at the lowest dose. The *P. grandifolia* extract was administered orally via gavage in a single dose after an 8-hour fast. Following treatment, the rats underwent an additional 4-hour fasting period. A control group of animals ($n = 6$), treated with the vehicle (filtered water), was included for comparison.

(3) Assessment of Clinical Signs. After the administration of *P. grandifolia*, the animals were carefully observed at designated time intervals, which included the initial 30 minutes, as well as 1, 2, 3, and 4 hours post-treatment. A range of behavioral parameters and clinical signs were recorded, encompassing self-grooming, piloerection, labored breathing (dyspnea), abdominal constriction, diarrhea, weakness (prostration), lack of coordination (ataxia), drowsiness (sedation), unconsciousness (coma), and mortality. After the initial 4-hour observation period, the animals were provided with water and food and observed daily for a total of 14 days to document any clinical changes and occurrences of mortality, according to proposed by Silva et al. [25].

(4) Euthanasia, Material Collection, and Analysis. On the 15th day, following a 12-hour fasting period, the rats were euthanized using deep anesthesia with isoflurane in a saturation chamber (maintaining a concentration of 1–3%). Blood samples were collected via decapitation for subsequent analysis. Levels of cholesterol, triglycerides, aspartate aminotransferase, alanine aminotransferase, urea, and creatinine were measured using commercially available kits within an automated system. Several blood parameters were recorded, encompassing red blood cell counts, hemoglobin levels, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, leukocyte counts, rod-shaped cells, lymphocytes, and platelet counts. Furthermore, the liver, spleen, heart, and kidneys were meticulously dissected and weighed using an analytical balance. Regarding kidney mass, the average mass between the right and left sides was taken into account. To calculate the relative organ mass (%), the mass of each organ was multiplied by 100 and divided by the body weight of the animal before euthanasia. Small sections of the liver, spleen, heart, kidney, and brain were carefully excised, rinsed with chilled saline solution, and then preserved in 10% buffered formalin. Following established histological procedures, the tissue sections were stained with hematoxylin and eosin and subsequently examined by a veterinarian pathologist for histopathological analysis. The slides containing the tissue samples were observed using a Leica DM 2500 optical microscope to identify any cellular alterations resulting from the administered treatments.

2.3. Pharmacological Studies

2.3.1. In Vitro Anti-Inflammatory Activity of Pereskia grandifolia

(1) Treatments of Macrophage Cells. In the entirety of the anti-inflammatory experiments, the dry extract from *P. grandifolia* underwent dissolution in distilled water at the subsequent concentrations: 200 μg/mL, 400 μg/mL, and 600 μg/mL. Within the positive control (PC) group, the extract doses were replaced with dexamethasone (40 μg/mL), whereas the negative control (NC) group received a substitution of a saline solution.

(2) Culture and Selection of Macrophages. Raw 264.7 murine macrophages of the ATCC TIB-71 strain were thawed and cultured in a cell culture flask using Dulbecco's modified Eagle medium (DMEM) Ham's F-12 culture medium, maintained at 37°C with 5% CO_2. The cells were allowed to

grow until reached a confluence of 70–80%. Afterward, cell harvesting was performed using a cell scraper, followed by cell counting in a Neubauer chamber and centrifugation at 1500 rpm for 5 minutes. The supernatant was removed, and the cells were resuspended in the culture medium to attain the desired concentration for the assessment of spreading and phagocytosis.

(3) Macrophage Spreading. The methodology employed in this study was adapted from Fracasso et al. [26]. Prepared slides were examined using an optical microscope at 400× magnification, with a total count of 100 cells. The calculation of the inhibition of spreading (%) was performed using the following formula: (Inhibition of spreading (%) = $(E0 - ET)/E0 \times 100$). In this equation, $E0$ represents the average number of cells spread in the NC group, while ET represents the average number of spread cells in the treated groups.

(4) Inhibition of Phagocytosis. Prepared slides were examined using an optical microscope at 400× magnification, and a total of 100 cells were counted, following the protocol proposed by Fracasso et al. [26]. This experiment was conducted in triplicate. The inhibition of phagocytosis (IP) was calculated using the following formula: (IP (%) = $(E0 - ET)/E0 \times 100$). In this formula, $E0$ denotes the average number of cells in the NC group that phagocytosed zymosan particles, and ET represents the average number of cells in the treated groups that phagocytosed these particles.

2.3.2. Animals. The research model involved the development of male Wistar rats with a weight range of 200–250 g. These rats were obtained from the central vivarium of the State University of Maringá and then transferred to the vivarium of the laboratory for preclinical research of UNIPAR. They were maintained under carefully regulated environmental conditions, which included a temperature within the range of 22°C ± 2°C, a relative humidity maintained at 50 ± 10%, and a consistent 12-hour light/dark cycle. The rats had free access to food and water and were provided with environmental enrichment. The experiments were conducted during the light phase. Animals were randomly divided into experimental groups, with 8 rats per group. The selection of this group size was based on previous results from similar experimental protocols and adhered to the 3R principles (reduce, refine, and replace) [12–14, 27–29]. Weights were assessed on a weekly basis using an analytical balance. The experimental plan obtained clearance from the Ethics Committee on Animal Use at Paranaense University, identified by protocol number SPP202 0031000167. All applicable national and international guidelines were strictly adhered to throughout the study. The animal research findings were reported and analyzed in compliance with the Animal Research Reporting of in vivo Experiments (ARRIVE) guidelines [24].

2.3.3. Experimental Design and Treatments. The experimental model for metabolically associated fatty liver disease (MAFLD) in this study encompassed a combination of various risk factors such as diabetes, dyslipidemia, and tobacco smoking. At the onset of the treatment, the animals were allocated randomly into respective groups. Induction of diabetes was achieved by subjecting the animals to a 12-hour fasting period, succeeded by intraperitoneal injection of streptozotocin (60 mg/kg) diluted in citrate buffer (10 mM, pH 4.5), following the procedure outlined by Souza et al. [27]. Three days postdrug administration, peripheral blood samples were gathered from the lateral tail vein, and blood glucose levels were determined using an AccuCheck Active glucose meter (Roche®, São Paulo, Brazil). Rats exhibiting glycemia levels ≥250 mg/dL were categorized as diabetic. To induce dyslipidemia, the animals were provided with a standard commercial diet enriched with 0.5% cholesterol for a duration of 6 weeks. This diet comprised 150 g of standard rodent feed combined with one egg yolk and 13.5 mL of corn oil. The mixture was moistened with water, baked in an oven at 50°C for 36 hours, and subsequently vacuum-packed. Each 150 g of this diet contained 225 mg of cholesterol, 1.8 g of saturated fat, 2.16 g of monounsaturated fatty acids, and 0.72 g of polyunsaturated fatty acids. In addition, the rats were exposed to the smoke from nine commercial cigarettes (containing 0.8 mg nicotine, 10 mg tar, and 10 mg carbon monoxide) for 1 hour daily, 5 days a week. Diabetic animals were subjected to the specified risk factors (dyslipidemia and smoking) for a period of 6 weeks, following the protocol proposed by Souza et al. [27]. In the final 3 weeks of the experiment, the animals received daily oral treatment via gavage with either the vehicle (filtered water, serving as the negative control (C-) group), *P. grandifolia* extract (at doses of 30, 100, and 300 mg/kg), or a combination of simvastatin (2.5 mg/kg) and insulin (6 IU, administered subcutaneously; SIM + INS group). Concurrently, a baseline group comprising of normoglycemic, nondyslipidemic, and nonsmoke-exposed rats was treated solely with the vehicle. The experimental setup is depicted in Figure 1.

2.3.4. Euthanasia, Sample Collection, and Biochemical Analysis. At the conclusion of the four-week experimental period, subsequent to a 12-hour fasting period, the animals were humanely euthanized through the administration of deep isoflurane anesthesia within a saturation chamber (20%). Blood samples were obtained through decapitation, and plasma was isolated by centrifugation at 1,500 × g for 10 minutes, then stored at −80°C for subsequent analysis of alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol, and triglycerides utilizing the colorimetric enzymatic method on an automated analyzer (Quick Lab, Drake®, São Paulo, Brazil). The liver was then collected, weighed using an analytical balance, and promptly sectioned into samples. One of these samples was rapidly separated and frozen in liquid nitrogen to evaluate oxidative stress and conduct biochemical analyses. Another sample was preserved by submersion in a 10% formalin solution, intended for subsequent histological analysis. In addition, feces accumulated over a two-day period were directly gathered from the animal cages on the final day of the experiment. These fecal samples were preserved at −20°C until processing for the assessment of cholesterol and triglyceride levels.

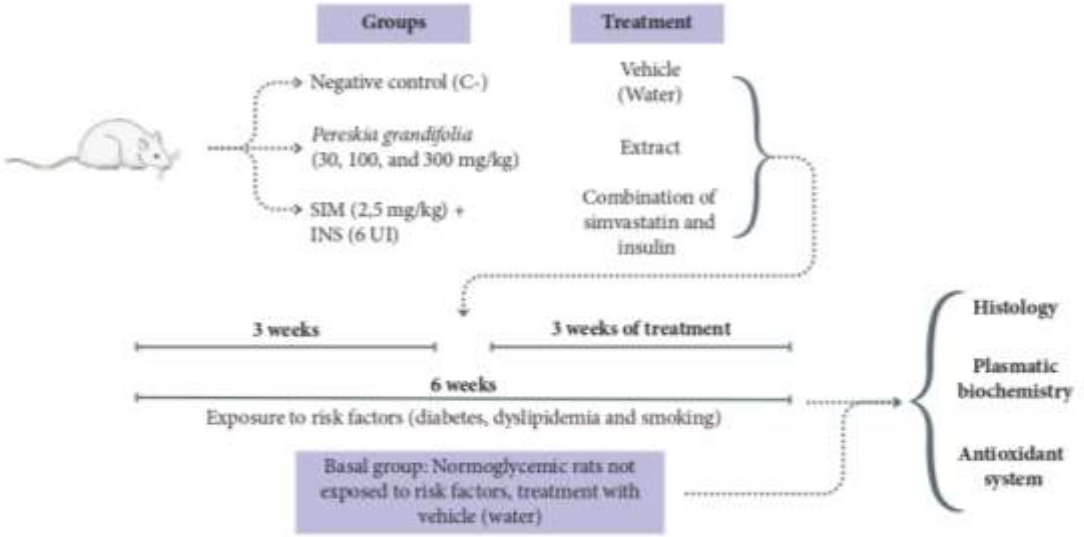

FIGURE 1: Experimental design: the model of fatty liver disease associated with metabolism was induced with three risk factors: diabetes, dyslipidemia, and smoking. The therapeutic effect of *Pereskia grandifolia* extract (30, 100, and 300 mg/kg) and standard treatment with simvastatin + insulin was evaluated. The baseline group of rats not exposed to risk factors was included as the control group.

2.3.5. Determination of Hepatic and Fecal Cholesterol and Triglyceride Levels. The freeze-dried liver and fecal specimens underwent lipid extraction using the gravimetric method outlined by Livero et al. [30]. In this approach, liver and fecal samples were combined with hexane, serving as the solvent, at a ratio of 1 : 10 (feces: solvent) and were heated to 80°C. Following a 12-hour period, the resulting supernatant was transferred to a second flask and allowed to evaporate naturally. This extraction process was repeated three times. The resulting lipid content was then measured and suspended in a mixture of 1 mL chloroform and 2 mL isopropanol. The levels of triglycerides and cholesterol in both liver and fecal samples were determined using the colorimetric enzymatic technique in an automated analyzer. The lipid percentage in the liver was computed using the formula: (lipids [%] = 100 × [final flask weight/initial flask weight]/0.1 g).

2.3.6. Examination of Hepatic Antioxidant System. To explore the hepatic antioxidant system, the liver samples were initially homogenized using a 1 : 10 dilution of potassium phosphate buffer (0.1 M, pH 6.5). Subsequently, 100 μL of the homogenate was isolated and combined with 80 μL of trichloroacetic acid (12.5%). The resulting mixture was vortexed and then centrifuged at 6,000 rotations per minute (rpm) for 15 minutes at 4°C to assess the levels of reduced glutathione (GSH), following the protocol outlined by Sedlak and Lindsay [31]. The residual homogenate underwent another centrifugation at 9,700 rpm for 20 minutes at 4°C. This additional step was designed to ascertain the superoxide dismutase (SOD) activity and lipoperoxidation (LPO) levels, based on the methodologies established by Gao et al. [32] and Jiang et al. [33], respectively.

2.3.7. Hepatic Histopathological Study. A section of the liver sample was preserved through immersion in a buffered 10% formalin solution, comprising distilled water, 35–40% formaldehyde, and monobasic and dibasic sodium phosphate. Postfixation, the liver sample underwent a dehydration process using alcohol and xylene, and subsequently, it was embedded in paraffin for ease of handling and sectioning. The sections were cut at a thickness of 6 μm and stained with hematoxylin and eosin to visualize cellular structures and any alterations [13]. Another liver sample followed a distinct procedure: it was soaked in sucrose solutions at increasing concentrations (10%, 20%, and 30%) for 24 hours at each level. Following saturation, the liver sample was stored in a medium known as Tissue Tek (O.C.T. Sakura) and quickly frozen using liquid nitrogen. The frozen sample was then sectioned at a thickness of 6 μm and underwent Nile Blue staining, as detailed in the study by Livero et al. [30]. The stained slides were examined using an optical microscope (Leica DM 2500) to assess cellular abnormalities. The observed hepatic lesions were categorized on a scale from 0 to 3 based on the percentage of affected tissue: 0 (0%; no lesions), 0.5 (1–5%; minor lesions), 1 (6–33%; moderate lesions), 2 (34–66%; marked lesions), and 3 (67–100%; extensive lesions) as per the classification by Mendes et al. [13].

2.4. Statistical Analysis. The collected data underwent evaluation for homogeneity of variance and adherence to normal distribution. For comparing means across different groups, a one-way analysis of variance (ANOVA) was executed. In instances where the data did not meet the assumptions of normality or homogeneity of variance, the Kruskal–Wallis test was employed as an alternative. Following this, post hoc tests, such as the Newman–Keuls or Dunnett post hoc test, were utilized to discern specific

differences among the means. The threshold for statistical significance was set at 95% confidence ($p < 0.05$). Results are presented as the mean ± standard error of the mean (SEM). The statistical analysis was conducted using GraphPad Prism 9.0 software.

3. Results

3.1. Impact of Pereskia grandifolia on Fibroblast Cell Viability. Table 1 presents the outcomes of an MTT cytotoxicity assay conducted on NIH/3T3 mouse dermal fibroblast cells, aiming to assess the impact of *P. grandifolia* on cell viability at different concentrations and time intervals. The groups treated with varying concentrations of *P. grandifolia* exhibited diverse effects on cell viability. Notably, at a concentration of 100 μg/mL of *P. grandifolia*, the cell viability remained relatively high. These results suggest that *P. grandifolia* at this concentration did not induce a significant cytotoxic effect on the NIH/3T3 cell lineage. At higher concentrations of *P. grandifolia* (200 μg/mL and 400 μg/mL), the cell viability exhibited a noticeable decrease. Moreover, at 400 μg/mL, the viability further declined at 24, 48, and 72 hours. These results suggest that higher concentrations of *P. grandifolia* had a moderate cytotoxic effect on the NIH/3T3 cells. Interestingly, at 800 μg/mL of *P. grandifolia*, the cell viability exhibited a significant decrease at 24 and 48 hours. However, the viability then increased at 72 hours. This indicates that *P. grandifolia* at this particular concentration may have initially induced cytotoxicity, but some cells were able to recover or adapt over time. At the highest concentration of *P. grandifolia* tested (1600 μg/mL), the cell viability was significantly reduced, indicating a strong cytotoxic effect of *P. grandifolia* on the NIH/3T3 cells. The statistical analysis conducted demonstrated that all treatment groups, except for the negative control (NC) group, exhibited significant differences compared to the negative control. The IC50% for *P. grandifolia* in NIH/3T3 cells lineage was determined to be 598.6 μg/mL, 567 μg/mL, and 540.4 μg/mL at 24, 48, and 72 hours. The IC50% values decrease over time, suggesting that the inhibitory potency of *P. grandifolia* increases with longer exposure periods.

3.2. Impact of Pereskia grandifolia on Carcinoma Cell Type. Table 2 depict the results of an MTT cytotoxicity assay conducted on HUH7 cells to assess the impact of *P. grandifolia* on cell viability. The experiment examined different concentrations of the extract and measured viability at various time points in a carcinoma cell type. The treated groups exhibited varying effects on cell viability when compared to both the negative control (NC) and positive control (PC) groups. At a concentration of 100 μg/mL of *P. grandifolia*, cell viability decreased. These findings indicate a cytotoxic effect of *P. grandifolia* on HUH7 cells at this concentration. Likewise, at concentrations of 200 μg/mL and 400 μg/mL, cell viability continued to decrease. At 200 μg/mL, viability was measured at 41.79 ± 0.09%, 37.61 ± 0.11%, and 17.74 ± 0.28% at 24, 48, and 72 hours. Similarly, at 400 μg/mL, viability was determined to be 35.99 ± 0.08%, 35.35 ± 0.22%, and 15.62 ± 0.41% at the same time intervals. These results suggest a dose-dependent cytotoxic effect of *P. grandifolia* on HUH7 cells. At a concentration of 800 μg/mL of *P. grandifolia*, an interesting observation was made as cell viability increased. This suggests that *P. grandifolia* may have had a different effect on HUH7 cells at this concentration, potentially promoting cell growth or survival. Lastly, at the highest tested concentration of the extract (1600 μg/mL), cell viability decreased once again, indicating a significant cytotoxic effect of *P. grandifolia* on HUH7 cells.

The IC50% value of 183.3 μg/mL at 72 hours indicates that a lower concentration of *P. grandifolia* is required to achieve the desired inhibitory effect compared to the values at 24 and 48 hours. This suggests that longer treatment durations result in increased potency of *P. grandifolia* in inhibiting HUH7 cell growth or viability.

3.3. Pereskia grandifolia Exhibit Anti-Inflammatory Effects. In phagocytosis, all treatments had significantly different effects compared to the NC group, which received saline (Figure 2(a)). *P. grandifolia* exhibited inhibitory effects on phagocytosis at concentrations of 200 μg/mL, 400 μg/mL, and 600 μg/mL, reducing it by 43.18 ± 1.6%, 47.66 ± 0.99%, and 56.66 ± 1.80%, respectively. The PC group, represented by dexamethasone, inhibited phagocytosis by 70.0%. Spreading is typically defined as an unsuccessful attempt at phagocytosis. In cases where no substance or microorganism is present to be phagocytosed, spreading refers to the action of a responsive cell that adheres to the slide and produces microvilli [34]. All treatments administered in this assay exhibited significantly distinct effects compared to the NC group (Figure 2(b)). *P. grandifolia* exhibited the potential to inhibit spreading, manifesting in the following proportions: 46.99 ± 2.40% at 200 μg/mL, 62.05 ± 1.6% at 400 μg/mL, and 66.87 ± 1.06% at 600 μg/mL. This assay substantiates the findings of the phagocytosis assay, suggesting that *P. grandifolia* holds promise in alleviating inflammation symptoms in macrophages. This potential is comparable to that of 40 μg/mL dexamethasone, which reduced spreading by 70.00%.

3.4. Pereskia grandifolia Does Not Induce Any Toxic Effects in Rats. During the animal acute toxicity assessment, administering oral doses of *P. grandifolia* did not lead to significant alterations. Specifically, the rats subjected to oral doses of 300 and 2000 mg/kg of *P. grandifolia* did not display any discernible modifications concerning clinical signs, body weight, or relative mass of essential organs such as the liver, spleen, kidneys, or heart, in comparison to the control group. Moreover, there were no notable changes in the biochemical and hematological profiles, and no cellular alterations were identified in the liver, spleen, heart, or kidneys when comparing the experimental groups to the control group (data were not shown).

3.5. Pereskia grandifolia Did Not Exhibit a Hypoglycemic Effect But It Demonstrated Significant Hepatoprotective Properties. Following the induction of diabetes, a significant rise in blood glucose levels was observed in the C-group (566.9 ± 16.5 mg/dL) compared to the basal group

Table 1: Effect of *Pereskia grandifolia* on fibroblast cell viability: percentage of viability at 24, 48, and 72 hours, and IC_{50}% determination.

Treatments	24 h	48 h	72 h
NC	100 ± 0.323	99 ± 0.03	100 ± 0.47
PC	5.94 ± 0.037*	4.92 ± 0.097*	2.80 ± 0.40*
100 μg/mL	91.94 ± 1.59*	100 ± 0.758	100 ± 0.157
200 μg/mL	85.91 ± 0.075*	73.65 ± 0.517*	93.01 ± 0.157*
400 μg/mL	74.33 ± 1.02*	68.68 ± 0.714*	85.37 ± 0.414*
800 μg/mL	38.61 ± 3.72*	67.83 ± 0.621*	20.24 ± 0.031*
1600 μg/mL	9.31 ± 0.06*	12.31 ± 0.68*	5.43 ± 0.36*
IC_{50}%	598.6 μg/Ml	567 μg/mL	540.4 μg/mL

IC_{50}%, half maximal inhibitory concentration; NC, negative control; PC, positive control. The data are expressed as mean ± SEM. * $p < 0.05$, *vs.* NC (one-way ANOVA followed by Dunnett's *post hoc* test).

Table 2: Effect of *Pereskia grandifolia* on carcinoma HUH7 cells viability: percentage of viability at 24, 48, and 72 hours, and IC_{50}% determination.

Treatments	24 h	48 h	72 h
NC	100 ± 0.17	100 ± 0.22	100 ± 0.22
PC	3.77 ± 0.09*	3.15 ± 0.15*	3.13 ± 0.221*
100 μg/mL	51.58 ± 0.44*	41.07 ± 0.08*	17.99 ± 0.061*
200 μg/mL	41.79 ± 0.09*	37.61 ± 0.11*	17.74 ± 0.169*
400 μg/mL	35.99 ± 0.08*	35.35 ± 0.22*	15.62 ± 0.23*
800 μg/mL	6.01 ± 0.55*	13.20 ± 0.27*	14.28 ± 0.400*
1600 μg/mL	5.83 ± 0.25*	10.26 ± 0.17*	14.28 ± 0.0.370*
IC_{50}%	446 μg/Ml	523.4 μg/mL	183.3 μg/mL

IC_{50}%, half maximal inhibitory concentration; NC, negative control; PC, positive control. The data are expressed as mean ± SEM. * $p < 0.05$, *vs.* NC (one-way ANOVA followed by Dunnett's *post hoc* test).

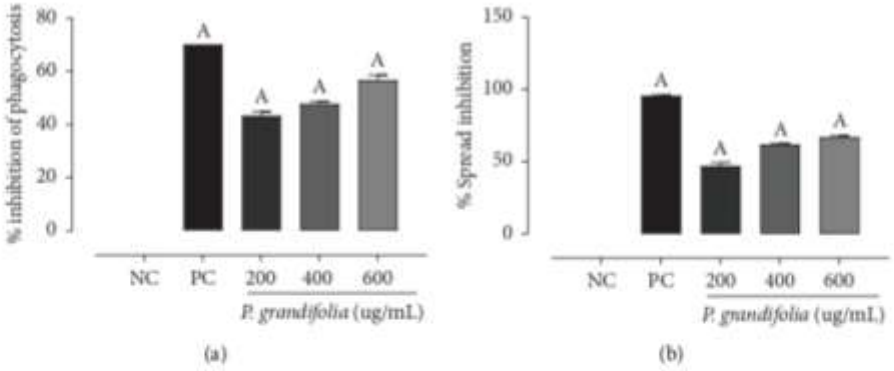

Figure 2: Percentage inhibition of phagocytosis (a) and inhibition of spreading (b) for negative control (NC; physiologic solution 0.9%), positive control (PC; 40 μg/mL, dexamethasone), and *P. grandifolia* (200 μg/mL, 400 μg/mL, and 600 μg/mL). The data are expressed as mean ± SEM. $^{A}p < 0.05$, *vs.* NC group (one-way ANOVA followed by Dunnett's post hoc test).

(93.3 ± 3.1 mg/dL). Administration of SIM + INS resulted in a decrease in blood glucose levels (141.0 ± 29.1 mg/dL), whereas treatment with *P. grandifolia* partially reverse these alterations (Figure 3(a)). Diabetes, dyslipidaemia, and smoking resulted in a significant rise of approximately 185.1% in ALT and 174.7% in AST levels compared to the basal group (46.3 ± 2.0 U/L and 105.7 ± 4.3 U/L, respectively). Nonetheless, administering 100 mg/kg of *P. grandifolia* extract entirely normalized the heightened levels of ALT and AST. Treatment with 30 and 300 mg/kg *P. grandifolia* extract partially restored these changes, while the combination of SIM + INS showed no significant effect (Figures 3(b) and 3(c)).

3.6. Pereskia grandifolia Extract Exerted Lipid-Lowering Effects. Figure 4 illustrates the effects of *P. grandifolia* treatment on triglyceride and cholesterol levels in the plasma, liver, and feces. The presence of diabetes, dyslipidaemia, and smoking significantly increased plasmatic levels of triglycerides (304.9 ± 21.6 mg/dL) and cholesterol (117.0 ± 6.4 mg/dL) than in the basal group (30.4 ± 1.9 mg/dL and 44.1 ± 2.3 mg/dL, respectively). These risk factors also induced a significant increase in hepatic triglyceride (833.9%) and cholesterol (394.9%) levels compared to that of the basal group (30.3 ± 1.4 mg/dL and 41.8 ± 2.6 mg/dL, respectively). However, administering 100 mg/kg of *P. grandifolia* extract and SIM + INS completely

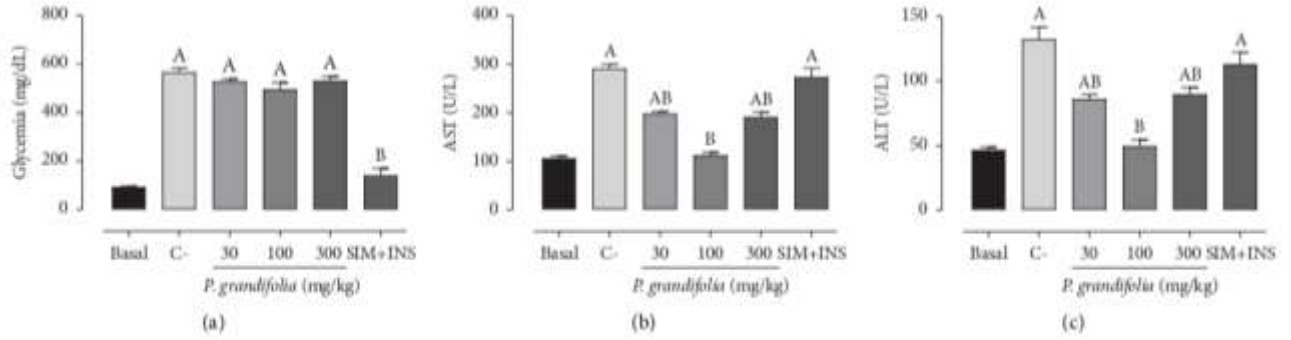

FIGURE 3: Levels of glucose (mg/dL), hepatic damage biomarkers aspartate aminotransferase (AST; U/L), and alanine aminotransferase (ALT; U/L) in plasma ((a–c), respectively) were measured in normoglycemic, nondyslipidemic, and nonsmoker Wistar rats (basal group) as well as diabetic, dyslipidemic, and smoker Wistar rats treated with vehicle (negative control [C−]), *Pereskia grandifolia* (30, 100, and 300 mg/kg), or simvastatin + insulin (SIM + INS). The presented data represent mean ± SEM. $^{A}p < 0.05$, *vs.* basal; $^{B}p < 0.05$, *vs.* C− (one-way ANOVA followed by Newman–Keuls post hoc test). Glucose and hepatic damage biomarkers levels. Plasma values of (a) glucose (mg/dL), (b) aspartate aminotransferase (AST; U/L), and (c) alanine aminotransferase (ALT; U/L).

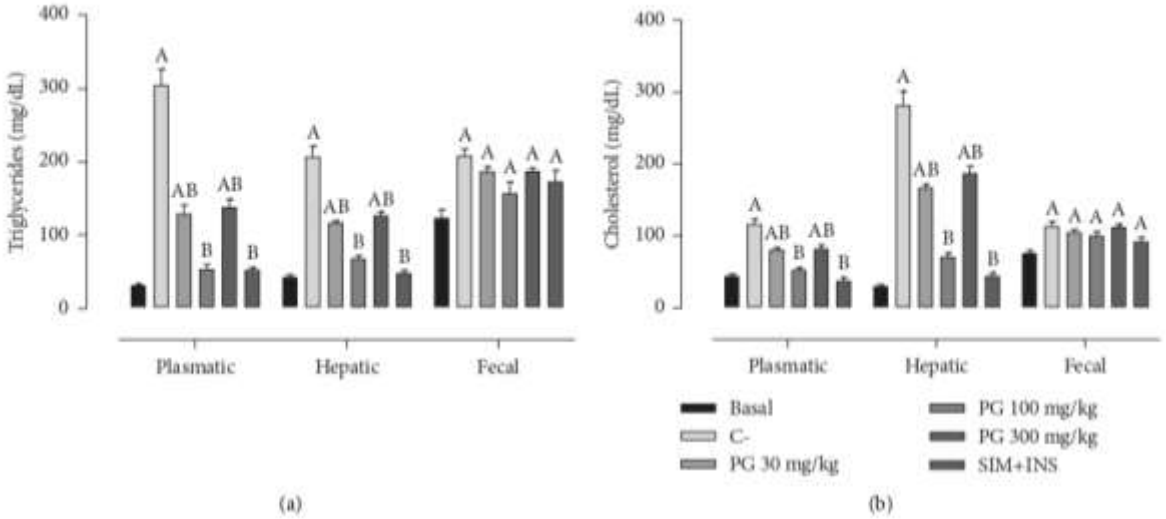

FIGURE 4: Levels of triglycerides and cholesterol in plasma, liver, and feces ((a and b) respectively) were assessed in normoglycemic, nondyslipidemic, and nonsmoker Wistar rats (basal group) and diabetic, dyslipidemic, and smoker Wistar rats subjected to treatment with vehicle (negative control [C−]), *Pereskia grandifolia* (30, 100, and 300 mg/kg), and simvastatin + insulin (SIM + INS). The study involved $n = 8$ rats per group. The data are expressed as mean ± SEM. $^{A}p < 0.05$, *vs.* basal; $^{B}p < 0.05$, *vs.* C− (one-way ANOVA followed by Newman–Keuls post hoc test). Plasma, hepatic, and fecal levels of (a) triglycerides and (b) cholesterol.

normalized these alterations in both the plasma and liver. Treatment with 30 and 300 mg/kg of *P. grandifolia* extract partially reinstated triglyceride and cholesterol levels in both the plasma and liver. Regarding fecal lipids, the negative control group exhibited a statistically significant increase in fecal cholesterol (75.93 ± 3.3 mg/dL) and triglyceride (113.7 ± 5.8 mg/dL) levels than in the basal group (75.9 ± 3.3 mg/dL and 123.3 ± 10.6 mg/dL, respectively). Interestingly, none of the treatments utilized in this study exhibited the capability to counteract the elevated levels of fecal cholesterol observed in the negative control group.

3.7. Pereskia grandifolia Reversed Hepatocellular Alterations. The results depicted in Figure 4 reveal several important findings regarding liver weight, lipid percentage, and histopathological analyses. First, the relative liver weight in the C− group exhibited a notable increase (3.9% ± 0.1%) than that in the basal group (2.7% ± 0.1%). However, the administration of 100 mg/kg *P. grandifolia* extract and SIM + INS effectively normalized this parameter (Figure 5(a)). In addition, the risk factors notably increased the percentage of hepatic lipids by 75.96% in the C− group than that in the basal group (18.9% ± 0.6%). On the contrary, treatment with 100 mg/kg of

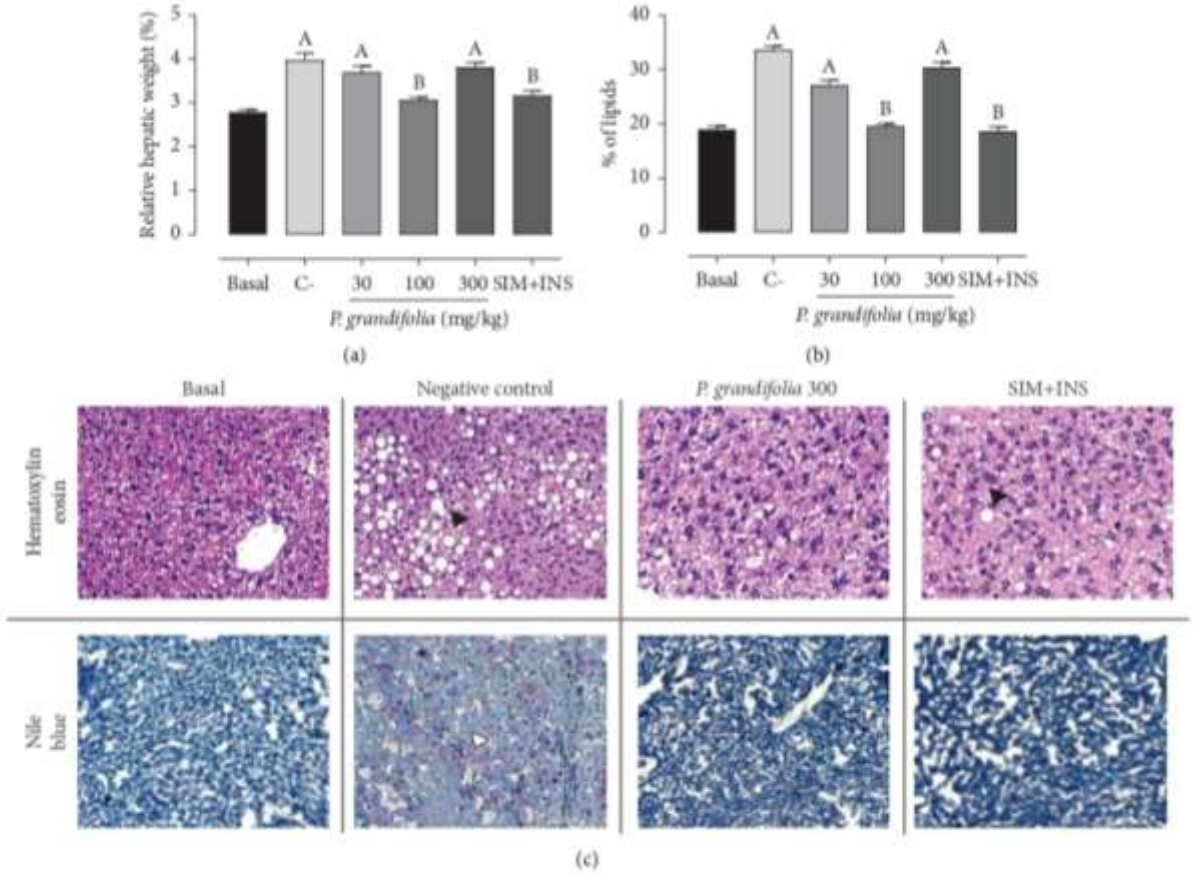

FIGURE 5: Relative (%) hepatic weight (a), % of hepatic lipids (b), and hepatic histopathological analysis with hematoxylin/eosin and Nile Blue (c) are presented in the figure. The study encompasses two groups: normoglycemic, nondyslipidemic, and nonsmoker Wistar rats (referred to as the basal group) and diabetic, dyslipidemic, and smoker Wistar rats. The latter group was subjected to treatments with vehicle (considered the negative control [C–]), *Pereskia grandifolia* (administered at doses of 30, 100, and 300 mg/kg), and simvastatin + insulin (referred to as SIM + INS). The data are expressed as mean ± SEM. $^{A}p < 0.05$, *vs.* basal; $^{B}p < 0.05$, *vs.* C– (one-way ANOVA followed by Newman–Keuls post hoc test). In the histopathological analysis, hepatic fatty degeneration (steatosis) is indicated by black closed arrows, while triglycerides are indicated by white closed arrows. The magnification used for the images is 20x.

P. grandifolia extract and SIM + INS successfully reversed these changes (Figure 5(b)). Hematoxylin/eosin staining of liver samples confirmed the presence of steatosis, revealing macroscopic alterations and indicating successful induction of hepatic injury (Figure 5(c)). Moreover, Nile Blue staining (Figure 5(c)), which highlights triglycerides in pink, verified the accumulation of lipids. All three risk factors caused cellular alterations, characterized by steatosis. Conversely, no microscopic changes were observed in the liver of the basal group. The combination of the three risk factors resulted in pronounced hepatic steatosis, yielding a lesion score of 3 in vehicle-treated animals. However, treatment with SIM + INS and 100 mg/kg of *P. grandifolia* extract significantly reduced lipid droplets, resulting in a lesion score of 0.5. Furthermore, treatment with 100 mg/kg and 300 mg/kg of *P. grandifolia* extract partially reversed hepatic steatosis, yielding lesion scores of 1 and 2, respectively (data were not shown).

3.8. Effects of Pereskia grandifolia on Hepatic Redox State. In the rat study, the concurrent presence of diabetes, dyslipidemia, and smoking led to hepatic oxidative stress. These conditions resulted in a decline in the levels of glutathione (GSH), a crucial antioxidant, while simultaneously causing an increase in lipoperoxidation levels when compared to the control group. However, when different doses of *P. grandifolia* were administered, these negative changes were reversed. Notably, treatment with *P. grandifolia* at doses of 30 and 100 mg/kg effectively reduced the heightened activity of SOD observed in the

C− group, bringing it closer to the levels seen in the control group. In contrast, treatment with SIM + INS partially restored SOD activity, GSH levels, and lipoperoxidation levels (Table 3).

4. Discussion

This study explored the hepatoprotective potential of *Pereskia grandifolia* utilizing an experimental model that integrated three significant risk factors for MAFLD: diabetes, dyslipidaemia, and exposure to tobacco smoke. Rats were exposed to these risk factors for a span of 4 weeks, leading to the onset of dyslipidemia, oxidative stress, and evident hepatic anomalies. Nevertheless, daily administration of an ethanol-soluble fraction obtained from *P. grandifolia* leaves effectively reversed these adverse alterations, signifying its promise as a hepatoprotective agent (Figure 6).

Animal models that incorporate multiple risk factors are invaluable for screening new hepatoprotective substances and enable researchers to comprehensively evaluate the efficacy and safety of potential treatments under conditions that closely mirror the complexity of human diseases [35]. By subjecting these models to a combination of risk factors associated with MAFLD, such as diabetes, dyslipidaemia, and tobacco use, it becomes possible to assess the effects of candidate substances on multiple disease mechanisms simultaneously. This multifactorial approach provides a more realistic representation of the disease and enhances our understanding of how potential treatments may interact with various aspects of its pathogenesis.

Moreover, the utilization of animal models that incorporate multiple risk factors enables the identification of synergistic effects of pharmacological treatments [36]. For instance, evaluating the efficacy of a substance in a model encompassing MAFLD, insulin resistance, and other pertinent risk factors allows for the assessment of the combined benefits derived from targeting multiple facets of the disease simultaneously. This approach facilitates an examination of how a drug effectively ameliorates glucose levels and reduces liver fat accumulation within such a model, potentially leading to more pronounced therapeutic benefits compared to substances exclusively focused on a singular aspect of the disease. By comprehensively considering the intricate interplay between diverse disease mechanisms, it is possible to glean valuable insights into potential treatment strategies that optimize therapeutic outcomes for multifactorial diseases such as MAFLD. The results of this study substantiate the promising therapeutic effects of *P. grandifolia* in a model of MAFLD with multiple risk factors. The observed antioxidative, lipid-lowering, and hepatoprotective effects imply that the plant operates through multiple synergistic pathways within the studied model, thus presenting a multifaceted approach to the treatment of MAFLD.

The antioxidant effects of *P. grandifolia* are of great importance in the context of MAFLD, as oxidative stress plays a crucial role in disease progression. Increased levels of reactive oxygen species and lipid peroxide are associated with inflammation and oxidative injury in the liver [37]. The reduction of these markers of oxidative stress following administration of the plant extract suggests its ability to neutralize free radicals and protect liver cells against oxidative damage. Preclinical studies involving multifactorial risk-induced liver disease previously conducted support the results of this research and have highlighted the importance of the antioxidant effect of medicinal plants as a fundamental component in the construction of therapeutic effect [12–14].

The observed hypolipidemic activity is another important characteristic of the therapeutic effects of *P. grandifolia* in MAFLD. The excessive accumulation of lipids, such as triglycerides and cholesterol, in the liver contributes to the development and progression of the disease [8, 38]. The decrease in serum lipid levels, especially when associated with the reduction of hepatic fat accumulation observed in histological analysis, suggests that the plant may modulate lipid metabolism and promote efficient breakdown and transportation of lipids, thereby reducing the lipid burden in the liver. The effect of the plant on reducing hepatic lipids and protecting the liver was previously described by Almeida et al. [22]. Following a 10-week hypercaloric diet, 21 animals were categorized into three groups and provided distinct diets for 4 weeks: control, hypercaloric diet with 5% *P. grandifolia* flour, and hypercaloric diet with 10% *P. grandifolia* flour. Rats subjected to the flour diet exhibited diminished food intake and body weight, as well as reduced body mass and Lee indices in comparison to the control group. In the second week, the 10% *P. grandifolia* flour group demonstrated significantly lower weight compared to the control group. By the fourth week, the 10% *P. grandifolia* flour group experienced the most notable body weight reduction than the other two groups, along with lower hepatic lipid levels and reduced liver weight. However, there was no significant difference in total lipid and moisture levels between the groups [22]. As observed in the referenced study, the rats were exposed to a hypercaloric diet and were provided with feed enriched with 10% *P. grandifolia* for a period of 2 weeks, emphasizing variations in the study design and treatment approach when compared to the current study.

Furthermore, the observed hepatoprotective effects of *P. grandifolia* are particularly encouraging. The decrease in ALT and AST enzyme activities indicates an enhancement in the liver's functional integrity and metabolic functioning, implying a potential safeguard against liver injury [39]. Moreover, *P. grandifolia* demonstrates notable anti-inflammatory activity by inhibiting phagocytosis and macrophage spreading, critical processes in inflammation. Histological analysis further supports these findings, revealing a reduction in liver tissue fat accumulation following treatment with *P. grandifolia* extract. These results suggest that the plant may regulate key metabolic pathways implicated in MAFLD pathogenesis, such as lipogenesis, inflammation, and oxidative stress, thereby providing protection to the liver against damage.

In the study conducted by Barbosa et al. [12], Wistar rats with diabetes were subjected to cigarette smoke exposure and the induction of dyslipidemia for a period of 4 weeks. During the last two weeks of the experiment, the animals received oral treatment with different doses of the ethanol-soluble fraction of *Baccharis trimera* (30, 100, and 300 mg/kg), vehicle (group C−), or a combination of insulin and simvastatin (group C+).

Table 3: Hepatic markers of oxidative stress in diabetic, dyslipidemic, and smoker Wistar rats that were treated with vehicle, *Pereskia grandifolia*, and simvastatin + insulin.

	Basal	C−	*Pereskia grandifolia* (mg/kg)			SIM + INS
			30	100	300	
GSH	184.50 ± 3.52	29.50 ± 2.11[a]	146.20 ± 7.18[b]	186.20 ± 6.01[b]	151.30 ± 4.11[a,b]	117.60 ± 10.97[a,b]
LPO	63.91 ± 2.74	194.30 ± 3.82[a]	69.13 ± 4.70[b]	57.41 ± 3.22[b]	74.10 ± 2.15[b]	121.40 ± 9.66[a,b]
SOD	1210.0 ± 34.87	1624.2 ± 64.30[a]	1293.0 ± 19.42[b]	1199.0 ± 30.95[b]	1447.5 ± 23.19[a]	1501.8 ± 74.29[a]

C−, negative control; SIM + INS, simvastatin + insulin; GSH, reduced glutathione (μg GSH/g tissue); LPO, lipoperoxidation (nmol LPO/min/mg tissue); SOD, superoxide dismutase (U SOD/mg of tissue). $n = 8$/group. The data are expressed as mean ± SEM. [a] $p < 0.05$, *vs.* basal; [b] $p < 0.05$, *vs.* C− (one-way ANOVA followed by Newman-Keuls *post hoc* test).

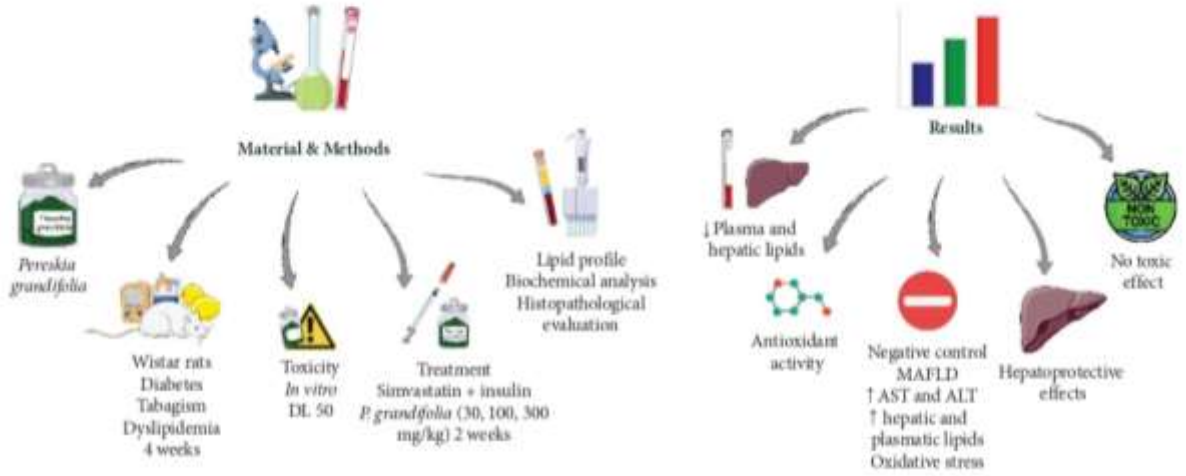

Figure 6: Preclinical ethnopharmacological study examining the impact of *Pereskia grandifolia* on metabolic-associated fatty liver disease (MAFLD) in diabetic rats exposed to a diet high in cholesterol and cigarette smoke. The rats were subjected to various treatments, including vehicle, *P. grandifolia* extract at different doses (30, 100, and 300 mg/kg), or a combination of insulin and simvastatin. The figure showcases the MAFLD model that induced liver abnormalities, such as increased AST and ALT levels, disrupted lipid profile, oxidative stress, and significant liver damage. The results of the study demonstrate the potential of *P. grandifolia* as a promising hepatoprotective agent against MAFLD. The treatment with *P. grandifolia* extract led to improvements in metabolic parameters, lipid levels, and liver histology, without causing any signs of toxicity.

This experimental model was designed to induce hepatic steatosis in the animals. Remarkably, the *B. trimera* extract at doses of 30 and 100 mg/kg showed a significant reduction in hepatic lipid levels, including cholesterol and triglycerides, than the animals in the C+ group. Similar results were observed in the group treated with insulin and simvastatin. As for markers of liver injury, diabetic animals with dyslipidemia and cigarette smoke exposure treated with the vehicle showed an increase in AST and ALT levels than that in the C− group. However, all three doses of *B. trimera* demonstrated efficacy in reversing this liver damage. The results suggest that this species holds promise for future studies aimed at the development of a hepatoprotective herbal remedy.

Mendes and colleagues [13] proposed an investigative model associating systemic arterial hypertension, dyslipidemia, and smoking as cardiovascular risk factors. Wistar rats were subjected to a 2K1C Goldblatt hypertension model, received a diet rich in 0.5% cholesterol and were exposed to 9 commercial cigarettes per day for 8 weeks. During the last 4 weeks, the animals received oral treatment with the ethanol-soluble fraction of *Baccharis trimera* (30, 100, and 300 mg/kg) or with a combination of simvastatin (2.5 mg/kg) and enalapril (15 mg/kg). The results revealed that treatment with *B. trimera* was able to reverse all observed alterations, displaying similar or superior effects to the standard treatment, indicating its potential as a promising therapy for cardiovascular and hepatic disorders, especially those associated with multiple risk factors.

In the study conducted by Auth et al. [14], spontaneously hypertensive rats were exposed to a high-cholesterol diet and cigarette smoke for a period of 10 weeks. In the final 5 weeks of this period, the rats were subjected to various treatments including different doses of *Croton urucurana* extract, simvastatin + enalapril, or a vehicle (serving as the negative control). This model induced liver damage, leading to an increase in AST, ALT, cholesterol, and triglyceride levels. Treatment with *C. urucurana* (300 mg/kg) and simvastatin + enalapril effectively mitigated these parameters, successfully reversing the hepatic alterations. These findings underscore the therapeutic potential of *C. urucurana* extract in managing cardiovascular and hepatic conditions associated with multiple risk factors.

Together, the results of this research contribute to the growing evidence supporting the therapeutic potential of

medicinal plants in the management of liver diseases, providing valuable insights for the development of treatments and the advancement of hepatic investigations [40–42]. It is important to highlight that the observed therapeutic effects can be attributed to the presence of bioactive compounds in *P. grandifolia*, such as phenolic compounds, flavonoids and saponins, for example [43–45]. However, further research is necessary to identify and characterize the specific compounds present in the extract utilized in this study. In addition, delving into the molecular mechanisms that drive the observed effects is crucial for a more in-depth understanding.

The results of toxicity tests conducted on *P. grandifolia* indicate a high level of tolerance and safety for this plant. Both *in vitro* cell cultures and rat studies have demonstrated its excellent performance. In the cytotoxicity assay, it was observed that the plant had no significant impact on cell viability and proliferation, indicating the absence of direct toxicity on the tested cells. This finding suggests that *P. grandifolia* does not compromise the integrity and functionality of the studied cells, which is a crucial factor to consider in terms of its safety profile. The IC50% values indicate that *P. grandifolia* demonstrates varying inhibitory potencies on cell growth or viability in the two studied cell lines. *P. grandifolia* exhibits a relatively higher potency against HUH7 cells than NIH/3T3 cells. The discovery of *P. grandifolia* selective cytotoxic effect on cancer cells holds great significance in the field of cancer research. Its ability to preserve cell viability in healthy cells shows promising therapeutic potential, as it minimizes the undesirable side effects commonly associated with many conventional therapies. Moreover, the plant cytotoxic effect specifically targeting cancer cells underscores its potential selectivity towards specific molecular targets present in tumor cells.

In the toxicity assay in rats, administration of *P. grandifolia* extract did not result in significant changes in behavior, body weight, or feeding patterns. No lesions or abnormalities were observed in organs and blood biochemical parameters, indicating the absence of systemic toxicity and relevant structural or functional damage in the evaluated tissues. These results suggest that the plant did not cause immediate adverse effects in the studied animals and support the potential therapeutic use of *P. grandifolia*. However, it is important to note that these investigations are an initial step in assessing the safety of the extract. For a comprehensive and more accurate evaluation, further studies are needed, including testing in more diverse animal models, long-term toxicity assessment, and eventually clinical studies in humans [46, 47].

In summary, the results of this study reveal the potential importance of *P. grandifolia* as a hepatoprotective agent in an experimental model of MAFLD. This may have significant implications for public health, medical research, and the appreciation of traditional medicine and culture. MAFLD is an increasingly prevalent liver disease and is associated with risk factors such as diabetes, dyslipidaemia, and smoking [2]. By demonstrating that *P. grandifolia* has hepatoprotective properties, this study may have an impact on public health by providing a possible natural therapeutic approach for the treatment or prevention of MAFLD.

Furthermore, the discovery of the hepatoprotective effects of *P. grandifolia* highlights the potential for further research on this plant and its therapeutic properties. This could lead to the development of drugs or treatments derived from the plant, offering alternatives for addressing liver diseases. In addition, depending on the region, *P. grandifolia* may play a significant role in the traditional medicine of certain communities [48]. This study can provide scientific evidence supporting the traditional use of the plant as a hepatoprotective agent, helping to preserve and promote cultural practices and traditional knowledge related to health.

Although the study provided evidence of the positive impact of *P. grandifolia* treatment on dyslipidaemia, oxidative stress, and hepatic abnormalities, the precise mechanisms responsible for these effects were not fully clarified. To gain a comprehensive understanding, further research is required to unravel the specific molecular targets involved in the observed hepatoprotective effects. Furthermore, while this study investigated the effects of *P. grandifolia*, its clinical relevance and safety in human subjects have yet to be established. To address these aspects, additional studies, including rigorous clinical trials, are necessary. These trials should focus on evaluating the efficacy of *P. grandifolia*, determining the optimal dosage, and assessing potential side effects when administered to humans. Only through such comprehensive investigations will it be possible to gain a clearer understanding of the potential benefits and risks associated with the use of *P. grandifolia* in a clinical setting.

5. Conclusion

The experimental model used, which incorporated major risk factors for MAFLD, provided valuable insights into the potential therapeutic benefits of *P. grandifolia* in combating liver damage. This suggests that *P. grandifolia* holds promise as a natural hepatoprotective agent.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Edilson Rodrigues Albuquerque, Gustavo Ratti da Silva, Fernanda de Abreu Braga, Ester Pelegrini Silva, and Karina Sposito Negrini conceptualized the study, wrote the original draft, performed formal analysis, and wrote and reviewed the manuscript. Julia Amanda Rodrigues Fracasso and Lucas Pires Guarnier performed formal analysis and wrote and reviewed the manuscript. Ezilda Jacomassi, João Tadeu Ribeiro-Paes, Roberto da Silva Gomes, and Arquimedes Gasparotto Junior conceptualized the study and wrote,

reviewed, and edited the manuscript. Francislaine Aparecida dos Reis Lívero conceptualized the study, wrote the original draft, and reviewed and edited the manuscript. Francislaine Aparecida dos Reis Lívero was the senior researcher who was responsible for the project.

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3. CONCLUSÃO

1 *Petiveria alliacea* é uma planta promissora na abordagem terapêutica de hepatoproteção, inclusive nas condições hepáticas e renais relacionadas à síndrome metabólica. Os resultados obtidos requerem investigações adicionas sobre a aplicabilidade clínica de *P. alliacea* e os efeitos diretos de seu mecanismo de ação a longo prazo, bem como suas possíveis interações quando associada aos fármacos de referência na terapia medicamentosa convencional.

2 Os resultados apontam que o modelo experimental utilizado, incorporando os principais fatores de risco para MAFLD, foi extremamente eficaz para os parâmetros adotados neste estudo, fornecendo informações significantes acerca dos potenciais terapêuticos de *P. grandifolia* e sua atuação no combate aos danos hepáticos. O estudo sugere ainda que *P. grandifolia* é uma fonte promissora de agentes hepatoprotetores naturais, que necessita de investigações adicionais que possam trazer segurança na compreensão mais aprofundada dos mecanismos envolvidos.

4 ANEXOS

ANEXO 1 - Normas do Periódico *Naunyn-Schmiedeberg's Archives of Pharmacology*

Naunyn-Schmiedeberg's Archives of Pharmacology

Writing and formatting

Manuscript Submission

Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.

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Please make sure your title page contains the following information.

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The title should be concise and informative.

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- The affiliation(s) of the author(s), i.e. institution, (department), city, (state), country
- A clear indication and an active e-mail address of the corresponding author
- If available, the 16-digit ORCID of the author(s)

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Abstract

Please provide a structured abstract of 150 to 250 words which should be divided into the following sections:

- Purpose (stating the main purposes and research question)
- Methods
- Results
- Conclusion

For life science journals only (when applicable)

- Trial registration number and date of registration for prospectively registered trials
- Trial registration number and date of registration followed by “retrospectively registered”, for retrospectively registered trials

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Please provide 4 to 6 keywords which can be used for indexing purposes.

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Authors must provide a short description of the contributions made by each listed author (please use initials). This will be published in a separate section in front of the Acknowledgments.

Example: AM and DB conceived and designed research. AM and BB conducted experiments. GR contributed new reagents or analytical tools. AM and GR analyzed data. AM wrote the manuscript. All authors read and approved the manuscript.

Note by the editors:

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ICMJE advice

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Manuscripts should be submitted in Word.

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- Do not use field functions.
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- Use the table function, not spreadsheets, to make tables.
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Abbreviations should be defined at first mention and used consistently thereafter.

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Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.

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Always use footnotes instead of endnotes.

Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section on the title page. The names of funding organizations should be written in full.

Specific remarks

- IntroductionThe Introduction should state the purpose of the investigation and give a short review of the pertinent research.

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- Nomenclature: Insofar as possible, authors should use systematic names similar to those used by IUPAC.

Manuscripts submitted to the journal are expected to adhere to internationally accepted nomenclature

- for receptors:

www.guidetopharmacology.org

- and enzymes:

International Union of Biochemistry and Molecular Biology

References

Citation

Cite references in the text by name and year in parentheses. Some examples:

- Negotiation research spans many disciplines (Thompson 1990).
- This result was later contradicted by Becker and Seligman (1996).
- This effect has been widely studied (Abbott 1991; Barakat *et al*. 1995a, b; Kelso and Smith 1998; Medvec *et al*. 1999, 2000).

Reference list

The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text.

Reference list entries should be alphabetized by the last names of the first author of each work. Please alphabetize according to the following rules: 1) For one author, by name of author, then chronologically; 2) For two authors, by name of author, then name of coauthor, then chronologically; 3) For more than two authors, by name of first author, then chronologically.

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- Journal article

Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. https://doi.org/10.1007/s00421-008-0955-8

Ideally, the names of all authors should be provided, but the usage of “*et al*” in long author lists will also be accepted:

Smith J, Jones M Jr, Houghton L *et al* (1999) Future of health insurance. N Engl J Med 965:325–329

- Article by DOI

Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med. https://doi.org/10.1007/s001090000086

- Book

South J, Blass B (2001) The future of modern genomics. Blackwell, London

- Book chapter

Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

- Online document

Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

- Dissertation

Trent JW (1975) Experimental acute renal failure. Dissertation, University of California

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"The authors declare that no funds, grants, or other support were received during the preparation of this manuscript."

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- Vector graphics containing fonts must have the fonts embedded in the files.
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- Supply all figures electronically.
- Indicate what graphics program was used to create the artwork.
- For vector graphics, the preferred format is EPS; for halftones, please use TIFF format. MSOffice files are also acceptable.
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A Graduate Student's Guide to Determining Authorship Credit and Authorship Order, APA Science Student Council 2006

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MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL DO PARANÁ
SETOR DE CIÊNCIAS BIOLÓGICAS
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

Nº 1536

CERTIFICADO

A Comissão de Ética no Uso de Animais do Setor de Ciências Biológicas da Universidade Federal do Paraná (CEUA/BIO – UFPR), instituída pela Resolução Nº 86/11 do Conselho de Ensino Pesquisa e Extensão (CEPE), de 22 de dezembro de 2011, **CERTIFICA** que os procedimentos utilizando animais no projeto de pesquisa abaixo especificado estão de acordo com a Diretriz Brasileira para o Cuidado e a Utilização de Animais para fins Científicos e Didáticos (DBCA) estabelecidas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA) e com as normas internacionais para a experimentação animal.

STATEMENT

The Ethics Committee for Animal Use from the Biological Sciences Section of the Federal University of Paraná (CEUA/BIO – UFPR), established by the Resolution Nº 86/11 of the Teaching Research and Extension Council (CEPE) on December 22nd 2011, **CERTIFIES** that the procedures using animals in the research project specified below are in agreement with the Brazilian Guidelines for Care and Use of Animals for Scientific and Teaching purposes established by the National Council for Control of Animal Experimentation (CONCEA) and with the international guidelines for animal experimentation.

PROCESSO/PROCESS: 23075.026096/2023-62

APROVADO/APPROVAL: 13/06/2023 – R.O. 05/2023.

TÍTULO: Padronização de um modelo de estudo de doença hepática para triagem de compostos bioativos com atividade hepatoprotetora.

TITLE: Standardization of a liver disease study model for screening bioactive compounds with hepatoprotective activity.

AUTORES/AUTHORS: Francislaine Aparecida dos Reis Livero.

DEPARTAMENTO/DEPARTAMENT: Farmacologia.

Coordenador(a) da CEUA

Documento assinado eletronicamente por **ANGELA CRISTINA DA FONSECA DE OLIVEIRA**, **INSTITUCIONAL**, em 03/07/2023, às 14:22, conforme art. 1º, III, "b", da Lei 11.419/2006.

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COMITÊ DE ÉTICA EM PESQUISA ENVOLVENDO EXPERIMENTAÇÃO ANIMAL (CEPEEA)

CERTIFICADO

Certificamos que o projeto intitulado **"PADRONIZAÇÃO DE UM MODELO DE ESTUDO DE DOENÇA HEPÁTICA PARA TRIAGEM DE COMPOSTOS BIOATIVOS COM ATIVIDADE HEPATOPROTETORA",** protocolo nº. **SPP2020031000167,** sob a responsabilidade de **FRANCISLAINE APARECIDA DOS REIS LIVERO** – que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) – encontra-se de acordo com os preceitos da lei nº. 11.794, de 08 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 aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA). DO(A)Universidade Paranaesnse – UNIPAR, em reunião de 12/10/2021.

Umuarama, PR 12/10/2021.

Prof Dr. Salviano Tramontin Belettini
Presidente CEPEEA/UNIPAR

Lucilene N. Castilho Monteiro
Secretária CEPEEA/UNIPAR

UNIVERSIDADE PARANAENSE – UNIPAR

Reconhecida pela Portaria – MEC N.º 1580, de 09/11/93 – D.O.U. 10/11/93

Mantenedora: Associação Paranaense de Ensino e Cultura – APEC

Coordenadoria de Pós-Graduação - COPG

COMITÊ DE ÉTICA EM PESQUISA ENVOLVENDO EXPERIMENTAÇÃO ANIMAL (CEPEEA)

Finalidade	( ) Ensino (X) Pesquisa científica
Vigência do Projeto	10/2021 a 12/2022
Espécie/Linhagem	Wistar
Nº de animais	48
Peso/Idade	200 a 300g / 90 dias
Sexo	48 machos
Origem	Biotério da Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP)