UNIVERSIDADE FEDERAL DO PARANÁ

ARIANNE JUNG KLUCK

EFEITOS HEPATO E NEFROPROTETORES DA *Petiveria alliacea* L. EM UM MODELO ANIMAL MULTI-HIT DE DOENÇA HEPÁTICA GORDUROSA ASSOCIADA AO METABOLISMO

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Dissertação apresentada ao curso de Pós-Graduação em Farmacologia, Setor de Ciências Biológicas, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Mestre em Farmacologia.

Orientadora: Profa. Dra. Francislaine Aparecida dos Reis Lívero

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NOTA EXPLICATIVA

Esta dissertação é apresentada em formato alternativo – artigo para publicação – de acordo com as normas do Programa de Pós-Graduação em Farmacologia da Universidade Federal do Paraná, constando de uma revisão de literatura, objetivos do trabalho e um artigo científico abordando os experimentos realizados, com resultados e discussão, além da conclusão. O artigo foi formatado conforme as normas propostas pelo periódico Naunyn-Schmiedeberg's Archives of Pharmacology, ao qual o artigo foi submetido. Durante a elaboração deste trabalho foi utilizada ferramenta de inteligência artificial para revisão gramatical e tradução.

RESUMO

A doença hepática gordurosa associada ao metabolismo (DHGAM) caracteriza-se pela presença de esteatose hepática, podendo evoluir para cirrose e carcinoma hepatocelular. Afeta até 30% da população adulta e resulta da interação de fatores genéticos e ambientais, incluindo alterações epigenéticas, dietas ricas em lipídios e/ou frutose, obesidade, resistência à insulina, consumo de álcool, tabagismo e disbiose intestinal. Apesar da relevância clínica, não há tratamento específico, e o manejo é focado na etiologia subjacente. A doença constitui importante problema de saúde pública, pela morbimortalidade e os custos econômicos associados, reforçando a necessidade de estratégias terapêuticas eficazes. Estudos etnofarmacológicos surgem como alternativa promissora ao manejo da DHGAM. Destaca-se Petiveria *alliacea* L. (guiné, tipi ou erva-de-alho), tradicionalmente utilizada como analgésico, hipoglicemiante, diurético e antitérmico. Este estudo avaliou a segurança toxicológica e a eficácia terapêutica da fração etanólica solúvel de *P. alliacea* em modelo pré-clínico inovador de DHGAM induzida por diabetes e dislipidemia associadas ao álcool. Inicialmente, o extrato foi testado quanto à citotoxicidade *in vitro* em células HepG2 e à toxicidade oral aguda em ratos Wistar machos, não apresentando efeitos adversos, evidenciando segurança. Posteriormente, durante cinco semanas, estabeleceu-se um modelo multi-hit de DHGAM em ratos Wistar machos (n = 6-7), combinando diabetes (induzido pela administração de estreptozotocina 60 mg/kg, i.p.), dislipidemia (promovida pelo consumo de ração enriquecida com 0,5% de colesterol) e consumo de álcool (5%, na água de beber), sendo este último um fator agravante da doença. Nas duas últimas semanas, os animais receberam diariamente por gavagem veículo (água filtrada, grupo controle negativo, C-), *P. alliacea* (30, 100 ou 300 mg/kg) ou a combinação de sinvastatina e insulina [2,5 mg/kg e 6 UI (s.c.), respectivamente, grupo controle positivo, C+], enquanto ratos não expostos aos fatores de risco constituíram o grupo basal. Foram avaliados parâmetros bioquímicos séricos, cortes histológicos hepáticos e renais, e marcadores de estresse oxidativo hepático. Os animais do grupo C- apresentaram disfunção metabólica e hepática, com aumento da glicemia, de lipídios plasmáticos e hepáticos, aspartato e alanina

aminotransferase, bem como alterações histopatológicas hepáticas e renais. O tratamento com o extrato de *P. alliacea* reduziu parcialmente a hiperglicemia e, de forma dose-dependente, as concentrações plasmáticas e hepáticas de colesterol e triglicerídeos, além de normalizar as enzimas hepáticas. A administração da dose de 300 mg/kg restaurou integralmente as defesas antioxidantes hepáticas e reduziu a peroxidação lipídica. A disfunção renal observada no grupo C- (aumento do peso relativo dos rins, elevação de ureia e creatinina e lesões histopatológicas) foi revertida nos animais tratados com 300 mg/kg do extrato, enquanto o tratamento com doses menores promoveu melhorias intermediárias. Por sua vez, a combinação de sinvastatina e insulina normalizou o perfil lipídico e reduziu a glicemia, mas não restaurou completamente as enzimas hepáticas nem os marcadores de estresse oxidativo e dano renal. Histologicamente, os animais do grupo C+ exibiram alterações hepáticas mínimas, sem evidência de esteatose e alterações renais intermediárias. Esses achados indicam que *P. alliacea* possui um perfil de segurança favorável e ação multialvo, evidenciando potencial como agente hepato e nefroprotetor no manejo da DHGAM.

Palavras-chave: Álcool; Antioxidante; Diabetes Mellitus; Esteatose Hepática; Guiné; Fígado.

ABSTRACT

Metabolism-associated fatty liver disease (MASLD) is characterized by hepatic steatosis and may progress to cirrhosis and hepatocellular carcinoma. It affects up to 30% of the adult population and results from a complex interaction between genetic and environmental factors, including epigenetic modifications, diets rich in lipids and/or fructose, obesity, insulin resistance, alcohol consumption, smoking, and intestinal dysbiosis. Despite its clinical relevance, there is no specific treatment, and current management focuses on addressing the underlying causes. MASLD represents a major public health concern due to its high morbidity, mortality, and economic burden, underscoring the urgent need for effective therapeutic strategies. Ethnopharmacological studies have emerged as promising alternatives for MASLD management. *Petiveria alliacea* L. (commonly known as guiné, tipi, or garlic weed) stands out for its traditional use as an analgesic, hypoglycemic, diuretic, and antipyretic agent. This study evaluated the toxicological safety and therapeutic efficacy of the ethanol-soluble fraction of *P. alliacea* in an innovative preclinical model of MASLD induced by diabetes and dyslipidemia associated with alcohol intake. Initially, the extract was tested for in vitro cytotoxicity in HepG2 cells and for acute oral toxicity in male Wistar rats. No adverse effects were observed, demonstrating its safety. Subsequently, a multi-hit MASLD model was established over five weeks in male Wistar rats (n = 6–7), combining diabetes (induced by streptozotocin, 60 mg/kg, i.p.), dyslipidemia (via a 0.5% cholesterol-enriched diet), and alcohol consumption (5% in drinking water), with alcohol acting as an aggravating factor. During the final two weeks, animals received daily oral administration (gavage) of either vehicle (filtered water; negative control, C–), *P. alliacea* extract (30, 100, or 300 mg/kg), or a combination of simvastatin and insulin [2.5 mg/kg and 6 IU (s.c.), respectively; positive control, C+]. Rats not exposed to risk factors comprised the basal group. Serum biochemical parameters, hepatic and renal histology, and hepatic oxidative stress markers were evaluated. Rats in the C– group exhibited metabolic and hepatic dysfunction, evidenced by elevated blood glucose, plasma and hepatic lipids, aspartate and alanine aminotransferases, and histopathological alterations in the liver and kidneys. Treatment with *P. alliacea* extract partially reduced hyperglycemia and dose-dependently decreased plasma and hepatic cholesterol and triglycerides,

while normalizing liver enzyme levels. The 300 mg/kg dose fully restored hepatic antioxidant defenses and reduced lipid peroxidation. Renal dysfunction observed in the C– group—including increased relative kidney weight, elevated urea and creatinine levels, and histopathological damage—was reversed in animals treated with 300 mg/kg of the extract, while lower doses produced intermediate improvements. In contrast, treatment with simvastatin and insulin normalized the lipid profile and reduced glycemia but failed to fully restore hepatic enzyme activity or oxidative stress and renal damage markers. Histologically, C+ animals showed minimal hepatic alterations without steatosis and moderate renal changes. These findings demonstrate that *P. alliacea* exhibits a favorable safety profile and multi-target action, highlighting its potential as a hepato and nephroprotective agent in the management of MASLD.

Keywords: Alcohol; Antioxidant; Diabetes Mellitus; Guiné; Hepatic Steatosis; Liver.

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LISTA DE ABREVIATURAS OU SIGLAS

ALT – *Alanine aminotransferase*

ANOVA - *Analysis of variance*

AST- *Aspartate aminotransferase*

DHGAM - Doença hepática gordurosa associada ao metabolismo

DM2 - Diabetes mellitus tipo 2

DRC - Doença renal crônica

GSH - *Reduced glutathione*

H&E - H*ematoxylin 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*

SUMÁRIO

1. INTRODUÇÃO

1.1 Caracterização e epidemiologia da doença hepática gordurosa associada ao metabolismo

A doença hepática gordurosa associada ao metabolismo (DHGAM), anteriormente denominada doença hepática gordurosa não alcoólica, consiste na presença de esteatose hepática, a qual se caracteriza pelo excesso de triglicerídeos no fígado (ESLAN et al., 2020; POLYZOS et al., 2019). Na esteatose, a gordura acumulada no citoplasma dos hepatócitos é igual ou superior a 5% do peso hepático total (CHALASANI et al., 2018).

A DHGAM atinge entre 25% e 30% da população adulta mundial, sendo uma das principais causas de morte por doenças hepáticas crônicas (ESLAN et al., 2020; POLYZOS et al., 2019; YOUNOSSI et al., 2016; YOUNOSSI et al., 2023). A enfermidade vem crescendo em paralelo à epidemia global de obesidade e diabetes mellitus (BENCE et al., 2020; CHALASANI et al., 2018). De fato, Estes et al. (2018) estimam um aumento global de 30% nos casos de DHGAM até 2030, caso a obesidade e o diabetes mellitus continuem a crescer.

Allen et al. (2018) realizaram um estudo populacional na comunidade no Minnesota (EUA), acompanhando 3.869 pacientes com DHGAM e 15.209 indivíduos controle, durante 20 anos. Neste estudo, o índice de mortalidade foi significativamente maior entre os pacientes com DHGAM (10,2%) em comparação à população controle (7,6%), sendo o risco de morte associado principalmente à presença de comorbidades metabólicas, como diabetes mellitus, e à progressão para cirrose.

1.2 Fatores de risco para a DHGAM

A DHGAM desenvolve-se devido a múltiplos fatores genéticos e ambientais. Entre eles estão polimorfismos genéticos, modificações epigenéticas, dieta com excesso de gordura e/ou frutose, falta de exercício físico, obesidade e resistência à insulina, estresse oxidativo, alcoolismo, tabagismo e disbiose da microbiota intestinal. Por meio da combinação desses fatores, lipídeos se acumulam nos hepatócitos, causando esteatose hepática, que, se não controlada, gera uma infiltração de células imunes no fígado, causando um processo inflamatório, o qual

pode gerar fibrose hepática e evoluir para carcinoma hepatocelular (ESLAN et al., 2018; MAHADY et al., 2016; MOTA et al., 2016; POLYZOS et al., 2019; RAO et al., 2023).

Considerando a complexidade da DHGAM, fatores sociodemográficos influenciam na prevalência da doença. Em 2021, observou-se que homens apresentaram uma prevalência global de DHGAM, ajustada por idade, superior à das mulheres. Entre os homens, estima-se que cerca de 15.731 casos por 100 mil habitantes, enquanto entre as mulheres a taxa foi de aproximadamente 14.311 casos por 100 mil (FENG et al., 2025). Cho et al. (2025) realizaram um estudo populacional na Coréia do Sul com 70.276 indivíduos com idade ≥19 anos, dos quais 29.169 eram homens (41,51%). Observou-se que a prevalência de DHGAM foi maior entre mulheres de baixa renda e apresentou tendência a ser mais elevada entre homens de alta renda. Além disso, sexo masculino, meia-idade, níveis elevados de estresse e tabagismo foram identificados como fatores de risco significativos para DHGAM.

A prevalência global de DHGAM em pacientes com diabetes mellitus tipo 2 (DM2) é de 55,5% (YOUNOSSI et al., 2019). A DHGAM e o DM2 apresentam-se de forma bidirecional, sendo que o DM2, por meio da glicotoxicidade agravada pela hiperglicemia, produz resistência periférica e hepática à insulina. Como resposta, há maior secreção pancreática e menor *clearance* hepático de insulina, visando aumentar a concentração sérica do hormônio e prevenir o desenvolvimento de hiperglicemia. Dessa forma, há supressão da lipólise e acúmulo de triglicerídeos nos hepatócitos, gerando esteatose. Na outra direção, a DHGAM aumenta o risco de desenvolvimento de DM2 por meio da lipotoxicidade criada pelo acúmulo de gordura ectópica. A lipotoxicidade contribui para um estado inflamatório, o qual provoca resistência à insulina. Além disso, há desregulação da produção hepática de glicose, resultando em hiperglicemia (GASTALDELLI et al., 2019; MALDONADO-ROJAS et al., 2024; POWELL et al., 2021; TOMAH et al., 2020).

A dislipidemia pode ser causada por fatores genéticos ou pelo estilo de vida e constitui um importante fator de risco para a DHGAM, principalmente devido à lipotoxicidade decorrente do acúmulo excessivo de gordura nos hepatócitos. Esse acúmulo resulta de um desequilíbrio entre a síntese, a oxidação e o transporte de lipídios, de modo que, quando a captação de ácidos graxos livres e a síntese de triglicerídeos superam a capacidade do fígado de oxidá-los ou exportá-los na forma

de lipoproteínas, ocorre acúmulo intracelular de lipídios. Esse excesso de triglicerídeos induz estresse oxidativo, disfunção mitocondrial e ativação de vias inflamatórias, fatores que favorecem o desenvolvimento da esteatose hepática e a progressão para lesões hepáticas mais graves (KATSIKI et al., 2016; RAO et al., 2023; RESS et al., 2016). Zang et al. (2021) analisaram dados representativos da população adulta dos Estados Unidos, provenientes do *National Health and Nutrition Examination Survey* (NHANES) entre 1999 e 2016, totalizando 19.617 participantes. O estudo estimou que a prevalência de DHGAM foi de 35,8%, sendo que 57,8% desses indivíduos apresentaram dislipidemia, evidenciando a elevada carga metabólica associada à doença.

O consumo de álcool constitui um fator de risco, isolado ou como cofator, para a progressão da DHGAM e influencia o risco de mortalidade (CRABB et al., 2020; YOUNOSSI et al., 2019). A DHGAM associada ao consumo de álcool inclui indivíduos que ingerem quantidades moderadas da substância, atualmente definidas como 140–350 g por semana para mulheres e 210–420 g por semana para homens, equivalentes a uma ingestão média diária de 20–50 g e 30–60 g, respectivamente (RINELLA et al., 2023b).

Aproximadamente 90% do álcool absorvido pelo organismo é transportado para o fígado (CEDERBAUM, 2012). O álcool é inicialmente oxidado a acetaldeído pela álcool desidrogenase no citosol dos hepatócitos e, de forma parcial, metabolizado pelo sistema do Citocromo P-450, processo que gera espécies reativas de oxigênio. O acetaldeído é posteriormente convertido em acetato pela acetaldeído desidrogenase, principalmente nos macrófagos hepáticos, conhecidos como células de Kupffer. A ativação dessas células promove a liberação de citocinas pró-inflamatórias e a geração adicional de espécies reativas de oxigênio, contribuindo para o processo inflamatório hepático (LIU, 2014; SLEVIN et al., 2020). O consumo de álcool provoca alterações significativas no metabolismo lipídico hepático. A oxidação do álcool aumenta a razão entre de nicotinamida adenina dinucleotídeo e de sua forma reduzida, comprometendo a oxidação β-mitocondrial de ácidos graxos, ao mesmo tempo em que estimula a lipogênese. Esse desequilíbrio metabólico favorece o acúmulo de triglicerídeos nos hepatócitos, contribuindo para o desenvolvimento da esteatose alcoólica (JEON et al., 2020; SUN; WANG, 2021). Além disso, o álcool pode modificar a composição da

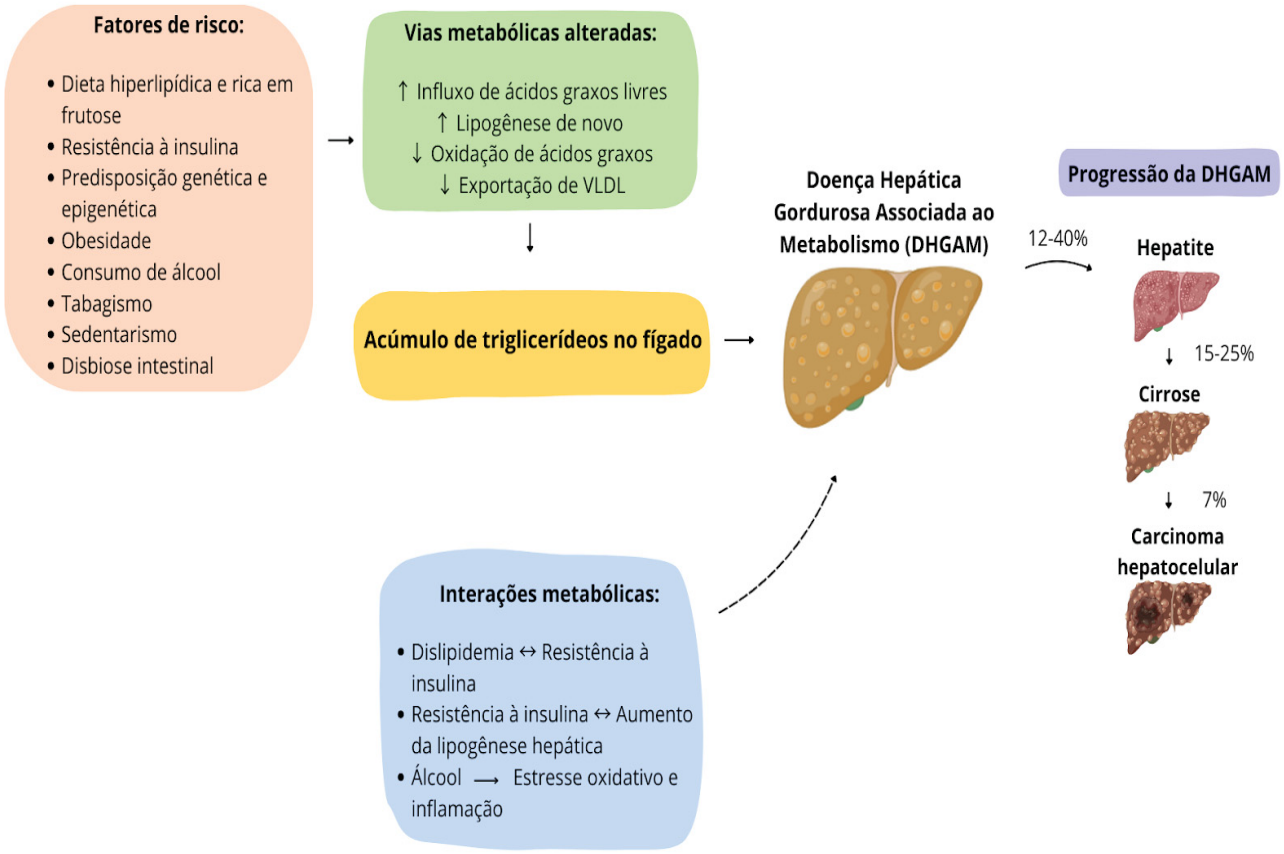
microbiota intestinal e aumentar a permeabilidade da barreira intestinal. Como resultado, endotoxinas bacterianas, especialmente lipopolissacarídeos, alcançam a circulação sistêmica e o fígado, ativando as células de Kupffer e amplificando a inflamação hepática (SZABO, 2015).

Indivíduos que consomem álcool geralmente apresentam uma ingestão elevada de carboidratos, o que pode estar relacionado ao uso substituto do álcool pelo açúcar em períodos emocionais negativos ou de abstinência de bebidas alcoólicas (BRAUN et al., 2021). A ingestão de álcool e açúcar apresenta mecanismos glicolíticos similares, ao gerar um aumento de glicose no organismo, o que, por sua vez, eleva a liberação de dopamina no núcleo *accumbens*, atuando no sistema de recompensa cerebral (ABRANTES et al., 2022; AVENA et al., 2006; VOLKOW et al., 2007).

Policarpo et al. (2022) analisaram 161 pacientes com DHGAM, os quais foram classificados de acordo com o consumo de álcool (alto – acima de 20 g/dia em mulheres e 30 g/dia em homens; moderado – abaixo de 20 g/dia em mulheres e 30 g/dia em homens; e não consumo). Juntamente, foi aplicado um questionário validado de consumo de alimentos, utilizado para avaliar a dieta dos pacientes. Como resultado, os indivíduos com DHGAM que consomem álcool apresentaram maior ingestão de calorias e tendência a consumir mais carboidratos e açúcares em comparação aos indivíduos que não consomem álcool.

A Figura 1 apresenta, de forma esquemática, os principais fatores de risco e mecanismos fisiopatológicos envolvidos no desenvolvimento e progressão da DHGAM, destacando o papel da dislipidemia, da resistência à insulina e do consumo de álcool.

Figura 1. Principais fatores de risco e mecanismos envolvidos na Doença Hepática Gordurosa Associada ao Metabolismo.



Fonte: Elaborado pela autora, 2025.

1.3 DHGAM e doença renal crônica

A doença renal crônica (DRC) é caracterizada pela presença de lesão renal ou por uma baixa taxa de filtração glomerular estimada, persistindo por 3 meses ou mais. A DRC envolve uma perda progressiva da função renal, frequentemente levando à necessidade de terapia de substituição renal, como diálise ou transplante. As implicações da DRC são amplas, que pode surgir a partir de diversos processos patológicos e afeta a saúde cardiovascular, a função cognitiva, o metabolismo ósseo, a pressão arterial e muitos outros indicadores de saúde (VAIDYA; AEDDULA, 2024).

A DHGAM é vista como uma condição multissistêmica e está correlacionada com o desenvolvimento de patologias extra-hepáticas, como a DRC. Estudos recentes vêm mostrando que indivíduos com DHGAM apresentam maior risco de desenvolver DRC (JUNG et al., 2022; QUEK et al., 2022; SUN et al., 2021; ZHANG et al., 2020). Gao et al. (2024) realizaram uma análise com 79.540 pacientes com DHGAM por 12,9 anos. Após esse período, exames clínicos e laboratoriais foram realizados, e pôde-se avaliar que, desses pacientes, 20.465 (25,72%) indivíduos desenvolveram DRC.

A DHGAM e a DRC compartilham mecanismos fisiopatológicos semelhantes, como resistência à insulina (ALKERWI et al., 2017; DUAN et al., 2019; KRISHNAN et al., 2024; THONGNAK et al., 2020; YOUNOSSI et al., 2019), dislipidemia (CHEN et al., 2023; HSU et al., 2021; LIANG et al., 2020), disfunção endotelial (CHEN et al., 2015; JIANG et al., 2020; PASARIN et al., 2012; RECIO-MAYORAL et al., 2011; STINGHEN et al., 2009; WANG et al., 2019; YILMAZ et al., 2011), estresse oxidativo (KIM; VAZIRI, 2010; LI; LIU, 2024), inflamação crônica (AMDUR et al., 2016; MONSEU et al., 2016; TONELLI et al., 2005) e disfunção da microbiota intestinal (JÄCKEL et al., 2017; NANTO-HARA et al., 2020; TANG et al., 2015).

1.4 Diagnóstico da DHGAM

A DHGAM é a manifestação hepática de uma desordem multissistêmica, que pode se apresentar de formas variadas em relação à maneira como é causada e se desenvolve. Por ter uma fisiopatologia complexa, não possui um diagnóstico único. A detecção da DHGAM deve ser baseada em ultrassonografia, histologia e biomarcadores sanguíneos que indiquem a presença de lesões hepáticas, em adição a pelo menos um dos fatores a seguir: sobrepeso, obesidade, presença de DM2 ou evidência de desregulação metabólica. A doença deve ser descrita pelo grau de atividade e pelo estágio da fibrose hepática (ESLAN et al., 2020; RINELLA et al., 2023a).

1.5 Tratamento da DHGAM

Atualmente, o tratamento para a DHGAM e disfunções relacionadas é limitado e frequentemente associado a efeitos adversos. Usualmente, o manejo da condição é realizado com base na causa subjacente da doença, como DM2, obesidade, dislipidemia ou tabagismo (RINELLA et al., 2023a). Recomenda-se aos pacientes a mudança no estilo de vida, dieta e exercício físico, associados ou não a intervenções farmacológicas (ESLAN et al., 2020).

De acordo com a Diretriz da Sociedade Brasileira de Diabetes, para o tratamento da DHGAM na ausência de fibrose, é recomendada a mudança no estilo de vida e uma reavaliação hepática do paciente em três anos. Nos casos em que há presença de fibrose, deve ser realizada a mudança no estilo de vida, a redução do peso corporal em casos de sobrepeso e o uso de farmacoterapia com agonistas do receptor ativado por proliferadores de peroxissoma gama (pioglitazona) e inibidores do cotransportador de sódio-glicose 2 (empagliflozina, dapagliflozina ou canagliflozina). Agonistas do peptídeo semelhante ao glucagon 1 (liraglutida ou semaglutida) e agonistas do peptídeo semelhante ao glucagon-1 e do polipeptídeo insulinotrópico dependente de glicose (tirzepatida) são utilizados em casos de DM2 e/ou obesidade. Entre os efeitos adversos desses medicamentos estão o ganho de peso, aumento do risco de insuficiência cardíaca e fraturas, infecções do trato urinário, poliúria, hipotensão e sintomas gastrointestinais (LIPSCOMBE et al., 2018; LYRA et al., 2025).

Em relação ao manejo terapêutico da dislipidemia, condição frequentemente relacionada à DHGAM, recomenda-se mudança no estilo de vida e, se necessário, o uso de estatinas, as quais, como efeitos adversos, podem causar dores musculares, hiperglicemia (que pode predispor ao desenvolvimento de DM2), hepatotoxicidade, além de distúrbios neurológicos e gastrointestinais (GODOY-MATOS et al., 2023; KHATIB et al., 2022).

Entre 2024 e 2025, o *Food and Drug Administration* (FDA) aprovou dois medicamentos para o tratamento da esteato-hepatite: resmetirom (Rezdriffra®, Madrigal Pharmaceuticals), um agonista do receptor β-tireoidiano, e a semaglutida (Wegovy®, Novo Nordisk). Entre os efeitos adversos desses medicamentos estão cefaleia, náuseas, vômitos, diarreia e alterações hepáticas e na vesícula biliar

(HARRISON et al., 2024; SANYAL et al., 2025). Ambos os medicamentos apresentam custos elevados e são indicados para casos em que o paciente apresenta fibrose moderada a avançada. Sendo assim, para os casos de esteatose hepática, os tratamentos farmacológicos atuais continuam limitados, seja por suas restrições em abranger todos os mecanismos da DHGAM, seja pelos custos elevados e/ou efeitos adversos.

1.6 Modelos experimentais de DHGAM

Diversos modelos de roedores têm sido utilizados para o estudo da dislipidemia e das doenças hepáticas. Ratos, devido ao metabolismo acelerado e à capacidade de reverter rapidamente alterações metabólicas, são particularmente adequados para modelar essas condições, que se assemelham de forma próxima à progressão da doença em humanos (JOHNSTON; FRANCIS; KISS-TOTH, 2018).

O modelo animal ideal para o estudo da DHGAM deve abranger todos os aspectos de sua fisiopatologia complexa, incluindo a evolução temporal e as lesões histopatológicas nos diferentes estágios da doença. Até o momento, os modelos disponíveis contemplam apenas características patogênicas e histológicas principais, com enfoque em abordagens genéticas e dietéticas. Modelos de ratos geneticamente modificados incluem Zucker obesos (BRAY, 1977; GUZZARDI et al., 2021), Zucker obesos com dieta rica em gordura (KUNDU et al., 2020) e Otsuka Long-Evans Tokushima obesos (KAWANO et al., 1992). Os modelos dietéticos de DHGAM têm sido descritos em ratos com dietas enriquecidas em gordura (CUI et al., 2020; LIOU et al., 2019; VELÁZQUEZ et al., 2019; ZAMANI-GARMSIRI et al., 2021) ou sacarose (LIMA et al., 2016; VALE et al., 2020), e deficiências de colina e metionina (KARATZAS et al., 2018).

Além disso, a maioria dos estudos em animais tradicionalmente avaliou fatores de risco isoladamente, embora haja crescente interesse em modelos que integrem múltiplos fatores, permitindo investigar a fisiopatologia complexa da DHGAM e testar potenciais terapias. Exemplos incluem ratos com combinações de diabetes, dislipidemia, hipertensão e exposição ao tabaco (AUTH et al., 2022; BARBOSA et al., 2020; DWIYEDI et al., 2020; LO et al., 2011; MENDES et al., 2021; RODRIGUES ALBUQUERQUE et al., 2023).

A DHGAM destaca-se como uma preocupação de saúde pública devido à sua alta morbimortalidade e aos custos econômicos substanciais. Dessa forma, é essencial promover estudos aprofundados que investiguem a DHGAM e possíveis estratégias terapêuticas (ESLAN et al., 2020; SAYIENER et al., 2017). A proposição e o refinamento de modelos animais que reproduzam de forma mais fidedigna as características metabólicas e histopatológicas observadas em humanos são fundamentais para o avanço do conhecimento sobre a DHGAM e para a avaliação de novas abordagens terapêuticas. Paralelamente, os estudos etnofarmacológicos e suas aplicações despontam como estratégias complementares e promissoras para a compreensão e o tratamento da doença (RODRIGUES et al., 2020; SÜNTAR, 2020).

1.7 *Petiveria alliacea* L.: dos usos populares ao estudo farmacológico

Popularmente conhecida no Brasil como guiné, tipi, erva-de-alho, mucuracaá, amansa-senhor ou ouoembo, *Petiveria alliacea* L. (Figura 2) é um arbusto pertencente à família *Phytolaccaceae*, da ordem de plantas *Caryophyllales*, amplamente distribuído pelo continente americano, com ocorrência principalmente na América do Sul. A planta ocorre em todos os estados do Brasil e desenvolve-se, principalmente, em localidades úmidas e sombreadas, podendo atingir até 1,5 m de altura. Apresenta folhas simples, de formato elíptico, com ápice e base agudos, raiz fusiforme de coloração parda, flores pequenas de coloração branca, branco-rosa ou esverdeada e odor aliáceo (CRONQUIST, 1988; DUARTE et al., 2005; MARCHIORETTO et al., 2014; NEVES et al., 2006).

Figura 2. Espécie adulta de *Petiveria alliacea***.**

Fonte:https://plantidtools.fieldmuseum.org/pt/nlp/search-results/PETIVERIA+ALLIACEA+L.

A espécie é utilizada pela população para tratar diversas condições. As folhas e raízes são usadas em infusões para fazer chás e topicamente em casos de traumas, contusões e dores localizadas. Também se atribui à utilização de *P. alliacea* propriedades anti-inflamatórias, hipoglicemiantes, diuréticas, antitérmicas, anti-helmínticas e para congestão hepática. Além desses usos populares, a planta também é empregada em rituais para induzir visões ou alucinações no México, Nicarágua, Guatemala e Brasil (BELTRESCHI et al., 2019; BREMM et al., 2020; CARBOLIM et al., 2025; CASAGRANDE et al., 2023; GUIMARÃES et al., 2022; MAGALHÃES et al., 2022; MONTENEGRO PAZ, 2024; PAZ PERAFAN; SANTIAGO et al., 2025; TAYLOR, 2002).

Estudos fitoquímicos identificaram a presença de taninos, glicosídeos, terpenoides, flavonoides e saponinas (ABDUL RAHEEM et al., 2018; KANMODI et al., 2022). Na caracterização fitoquímica de *P. alliacea*, também foi possível determinar a presença de óleo essencial (KERDUDO et al., 2015), cumarina, enxofre, isoarborinol e ácido benzóico (KIM et al., 2005), além de esteroides e alcaloides (AROGBODO et al., 2021). A composição química — e, consequentemente, a atividade biológica — dos extratos de *P. alliacea* estão relacionadas às variações de clima, solo, coleta, preparo da planta e obtenção dos extratos. De fato, macerados frescos das plantas apresentam-se mais vantajosos

em relação a preparações com plantas secas ou aquecidas. As extrações das raízes apresentam maior atividade antimicrobiana em comparação às extrações das folhas (KIM et al., 2005; LUZ et al., 2016).

Estudos pré-clínicos realizados com extrato etanólico 80% das folhas e raízes de *P. alliacea* mostraram potencial antimicrobiano frente às bactérias *Staphylococcus aureus*, *Pseudomonas aeruginosa* e *Klebsiella pneumoniae* (LOZANO et al., 2021). Khadka et al. (2023), em uma análise *in vitro*, demonstraram que o tratamento com o extrato acetato de etila das folhas, caule e raízes de *P. alliacea* apresentou atividade antimicrobiana e antifúngica contra *S. aureus*, *Escherichia coli*, *Bacillus subtilis*, *Candida albicans* e *Aspergillus fumigatus*. Pesquisas desenvolvidas com as folhas de *P. alliacea* apresentaram efeitos antifúngicos: o tratamento com extrato etanólico das folhas secas da planta inibiu 100% do crescimento de *Aspergillus flavus* (MACHADO; RAMOS, 2020). Análises também descrevem que o óleo essencial de *P. alliacea* apresenta atividade acaricida em *Rhipicephalus microplus* (ARCEO-MEDINA et al., 2017), nematicida em *Meloidogyne incognita* e inseticida em *Bemisia tabaci* (BEZERRA, 2006).

Em um estudo conduzido por Caicedo-Pinto et al. (2019), avaliou-se as propriedades ansiolíticas e antidepressivas de *P. alliacea*. Utilizou-se a fração aquosa das folhas da planta para o tratamento em ratos submetidos a testes comportamentais e cognitivos. O tratamento com *P. alliacea* promoveu efeitos ansiolíticos e antidepressivos nos animais tratados. Zavala-Ocampo et al. (2024) indicaram que o tratamento com o extrato etanólico das folhas reduziu o dano oxidativo e melhorou a potência de memória em camundongos com comprometimento de aprendizagem e memória. Além disso, o tratamento com o extrato hidroetanólico das folhas promoveu melhora cognitiva, reforço da aprendizagem, memória de curta e longa duração e memória espacial em ratos Wistar (SILVA et al., 2015).

Lopez-Martins et al. (2002) descreveram a atividade anti-inflamatória do extrato das raízes administrado oralmente em ratos Wistar. Houve redução de neutrófilos, monócitos e eosinófilos migrantes, bem como efeito analgésico prolongado, quando comparado ao ácido acetilsalicílico. Por meio de análises *in silico*, identificou-se o potencial anti-inflamatório de *P. alliacea* em inibir citocinas pró-inflamatórias, fator de necrose tumoral alfa e ciclooxigenase-2 (OLAJUBUTU et

al., 2022), além de interleucina 1 e fator de necrose tumoral alfa (FATIMAH et al., 2024).

O tratamento com extrato etanólico de folhas e caules de *P. alliacea* mostrou atividade antitumoral em linhagens de células de melanoma (URUEÑA et al., 2008) e de adenocarcinoma mamário (CARLOSAMA et al., 2024; HERNÁNDEZ et al., 2014). Murillo et al. (2023) avaliaram o efeito do extrato etanólico das folhas e caule da planta em um modelo murino de leucemia mieloide aguda. Os animais tratados com o extrato apresentaram redução dos níveis de blastos circulantes e no baço, aumento da hemoglobina, hematócrito, plaquetas e citocinas anti-inflamatórias, quando comparados ao grupo controle.

Em relação aos efeitos anti inflamatórios, o estudo conduzido por Mustika et al. (2021) mostrou que o tratamento com uma nanoformulação do extrato das folhas de *P. alliacea* reduziu a resistência à insulina, interleucina 6 e fator de necrose tumoral alfa em um modelo pré-clínico de diabetes induzido por estreptozotocina em ratos Wistar. Entretanto, ainda não há investigações científicas em modelos animais mais complexos de doenças metabólicas.

1.8 Justificativa do estudo

A DHGAM constitui uma questão de saúde pública, associando-se a elevados gastos econômicos. A ocorrência de cirrose e de doenças cardiovasculares está diretamente relacionada ao aumento da utilização de recursos financeiros. Dessa forma, em razão da crescente mortalidade e das implicações econômicas decorrentes da DHGAM, torna-se fundamental o desenvolvimento de estudos voltados para ao melhor entendimento de sua fisiopatologia e, consequentemente, de seu tratamento (ESLAN et al., 2020; SAYIENER et al., 2017). A DHGAM é uma condição multifatorial cuja abordagem terapêutica ainda é limitada, em virtude da escassez de opções eficazes, muitas vezes associadas a efeitos adversos relevantes e a elevados custos, tanto para os pacientes quanto para os sistemas de saúde.

Neste contexto, estudos etnofarmacológicos são uma via alternativa promissora para a compreensão e o tratamento da DHGAM. Ao longo dos séculos, produtos naturais têm sido a base terapêutica de diversas enfermidades e, atualmente, a etnobotânica tem se consolidado como uma importante ferramenta na

descoberta de novas drogas e sistemas terapêuticos. A etnofarmacologia, enquanto área multidisciplinar de pesquisa, vem acumulando um vasto corpo de conhecimento sobre o uso de plantas medicinais na medicina local e tradicional a nível global, configurando-se como uma estratégia relevante para o desenvolvimento de novos fármacos (LEONTI et al., 2017; YEUNG et al., 2020). De fato, grande parte dos compostos farmacológicos atualmente empregados derivam do uso tradicional de plantas medicinais (RODRIGUES et al., 2020; SÜNTAR, 2020).

O emprego de fitoterápicos em sistemas de saúde auxilia na redução de custos relacionados à fabricação de fármacos e ao tratamento de doenças, por utilizar recursos vegetais disponíveis localmente (AZEVEDO, 2008). Além disso, destaca-se que a etnofarmacologia está relacionada aos estudos de biodiversidade, contribuindo para a descoberta de diversos compostos bioativos e para a aplicabilidade do uso de fitoterápicos no progresso socioeconômico, a partir do manejo sustentável de fontes naturais e renováveis. Os estudos etnofarmacológicos permitem a avaliação das propriedades farmacológicas e toxicológicas de plantas medicinais previamente utilizados de forma empírica pela população, colaborando para o uso eficaz e seguro desses compostos. Dessa forma, promove-se o conhecimento tecno-científico na área, aliado ao conhecimento tradicional (BRASIL, 2006; BRASIL, 2016; SALES et al., 2015).

Apesar de seu uso tradicional para problemas hepáticos, a *P. alliacea* ainda não possui validação farmacológica em modelos experimentais robustos de doenças metabólicas. Além disso, a disponibilidade de modelos animais multi-hit para o estudo da DHGAM permanece limitada, o que reforça a necessidade de investigações que integrem agentes botânicos promissores a abordagens experimentais mais complexas.

Assim, o presente projeto propõe-se a avaliar a segurança toxicológica e os efeitos terapêuticos da *P. alliacea* em um modelo de doença hepática gordurosa associada a múltiplos fatores de risco.

2. OBJETIVOS

2.1. Objetivo geral

Investigar os efeitos terapêuticos da fração solúvel em etanol das forlhas de *P. alliacea* em um modelo de doença hepática associada à dislipidemia, diabetes e ao consumo de álcool, além de avaliar sua segurança toxicológica aguda por meio de testes *in vivo* e *in vitro*.

2.2. Objetivos específicos

2.2.1. Estudos de segurança *in vitro* e em ratos tratados oralmente com o extrato de *P. alliacea*:

- Analisar a viabilidade de células HepG2;
- Determinar o valor da dose letal 50 (DL50)*;*
- Avaliar alterações clínicas em ratos após a administração de diferentes doses do extrato*;*
- Examinar alterações no peso corporal, fígado, baço, rins e coração dos animais;
- Determinar as concentrações séricas de glicose, aspartato e alanina aminotransferase, ureia e creatinina dos ratos;
- Averiguar os parâmetros hematológicos de ratos na presença de *P. alliacea;*
- Investigar alterações sistêmicas por meio de análises histopatológicas do fígado, rins, baço e coração dos animais.

2.2.2. Estudos de eficácia do extrato de *P. alliacea* em ratos submetidos ao modelo de estudo:

- Avaliar os níveis séricos de glicose, aspartato e alanina aminotransferase, ureia e creatinina;
- Realizar análises morfométricas e histopatológicas do fígado e rins, visando avaliar alterações sistêmicas provocadas pelo modelo, bem como efeitos terapêuticos decorrentes do tratamento com o extrato*;*

- Qualificar e quantificar os lipídios presentes no plasma e nos hepatócitos por meio da dosagem de triglicerídeos e colesterol;
- Estudar a natureza do estresse oxidativo hepático por meio de medidas de lipoperoxidação, glutationa reduzida e superóxido dismutase.

3. ARTIGO CIENTÍFICO

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

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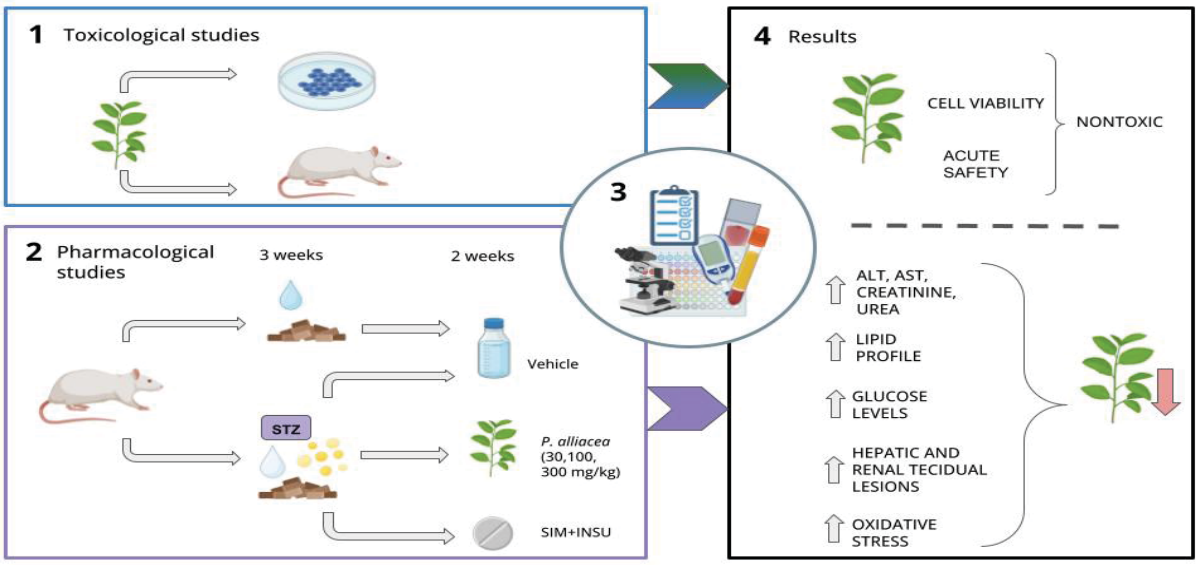
Abstract

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 native to the tropical Americas and 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) analyze *in vitro* cytotoxicity; (ii) assess *in vivo* acute toxicological safety; (iii) evaluate hepatoprotective and nephroprotective effects; and (iiii) 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), butylated hydroxytoluene (Synth®, Diadema, Brazil), chloroform (Biotec®, Curitiba, Brazil), dipotassium phosphate (Êxodo Científica®, Sumaré, Brazil), Doxorubicin (Sigma-Aldrich®, Missouri, USA) EDTA (Neon Reagentes®, Suzano, Brazil), Ellman's reagent - DTNB (Sigma-Aldrich®, Saint Louis, USA), ethanol (Êxodo Científica®, Sumaré, Brazil), eosin (Êxodo Científica®, Sumaré, Brazil), formalin (Êxodo Científica®, Sumaré, Brazil), glutathione-reduced (USB®, Cleveland, USA), Hayem's solution (Êxodo Científica®, Sumaré, Brazil), Harris hematoxylin (Êxodo Científica®, Sumaré, Brazil), hexane (Dinâmica®, Indaiatuba, Brazil), hydrochloric acid (Êxodo Científica®, Sumaré, Brazil), isoflurane (Isoforine®, Cristália, Itapira, Brazil), isopropanol (Biotec®, Curitiba, Brazil), methanol (Neon Reagentes®, Suzano, Brazil), monopotassium phospate (Êxodo Científica®, Sumaré, Brazil), MTT (Sigma-Aldrich®, Missouri, USA), paraffin (Allkimia®, Campinas, Brazil), pyrogallol (Êxodo Científica®, Sumaré, Brazil), saline solution (Sigma-Aldrich®, Saint Louis, USA), simvastatin (Novartis®, Basel, Switzerland), sodium citrate dihydrate (Êxodo Científica®, Sumaré, Brazil), streptozotocin (Sigma-Aldrich®, Missouri, USA), sulfuric acid (Êxodo Científica®, Sumaré, Brazil), tris (Invitrogen™, Carlsbad, USA), Turk's solution (NewProv®, Pinhais, Brazil), trichloroacetic acid (Proquímios®, Bangu, Brazil), xylene (Allkimia®, Campinas, Brazil), xylenol orang'e (Sigma-Aldrich®, Saint Louis, USA).

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 DDMS Herbarium of the Federal University of Grande Dourados (UFGD). The botanical identification was confirmed

using the 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-2-yl)-2,5-diphenyltetrazolium 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 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 4h. 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.

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**.

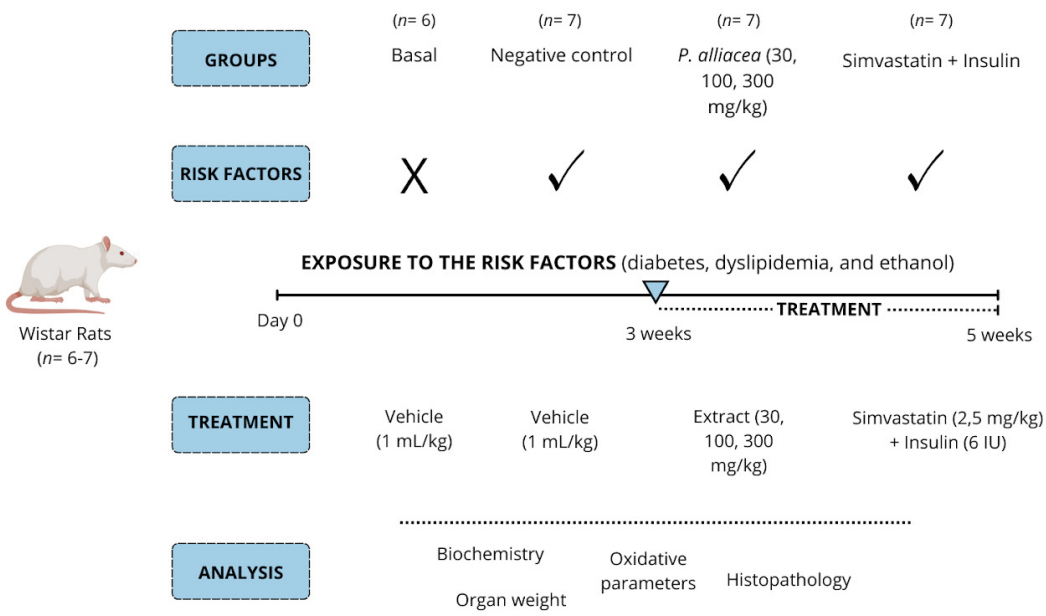


Fig 1. Experimental design. The model of metabolic dysfunction-associated steatotic liver disease was induced with diabetes, dyslipidemia, and ethanol in male Wistar rats during 5 weeks. In the last 2 weeks of the experiment the baseline group and the negative control group were treated with vehicle (filtered water, 1 mL/kg). Meanwhile the other 3 groups were treated with the *P. alliacea* extract (30, 100 and 300 mg/kg) and 1 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 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 100 μg/mL, with significant decreases observed at 300 and 900 μg/mL. The 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.

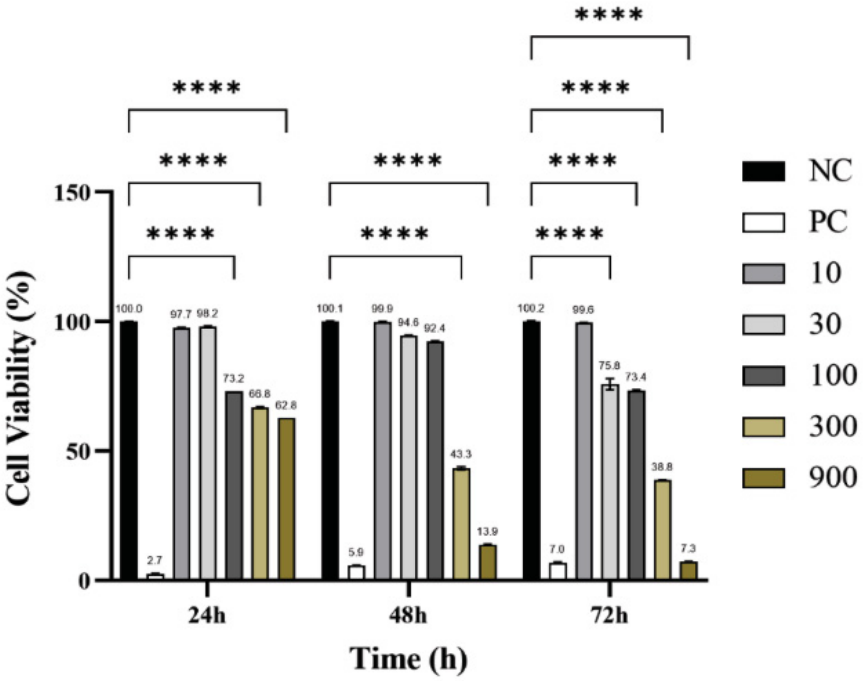


Fig 2. Effect of *P. alliacea* on cell viability. HepG2 cells after 24, 48, and 72 h of treatment with *Petiveria alliacea* extract at different concentrations (10, 30, 100, 300, and 900 µg/mL). Saline solution was used as the negative control (NC), and doxorubicin (200 µg/mL; 2%) as the positive control (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 concentrations 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 contraction	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 contraction	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 contraction	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 slight but 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**).

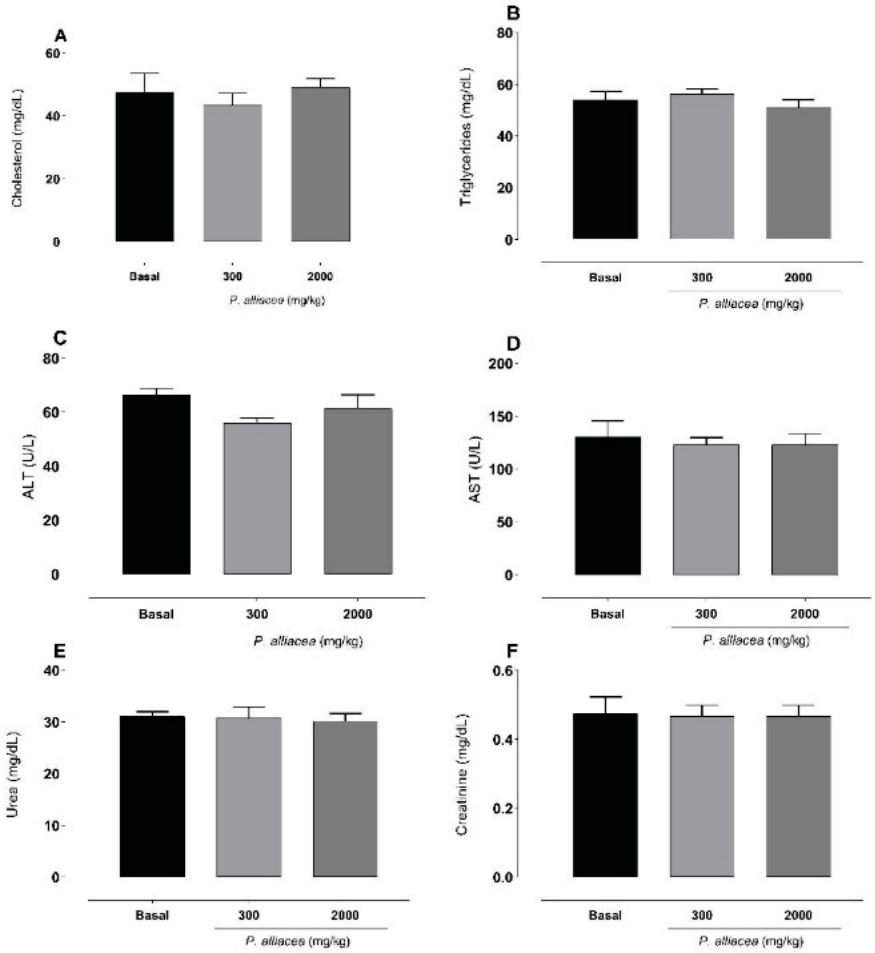


Fig 3. Serum biochemical parameters of Wistar rats treated orally with a single dose of *Petiveria alliacea* extract (300 or 2000 mg/kg) or vehicle (filtered water). Measured parameters include **(a)** total cholesterol, **(b)** triglycerides, **(c)** alanine aminotransferase (ALT), **(d)** aspartate 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**).

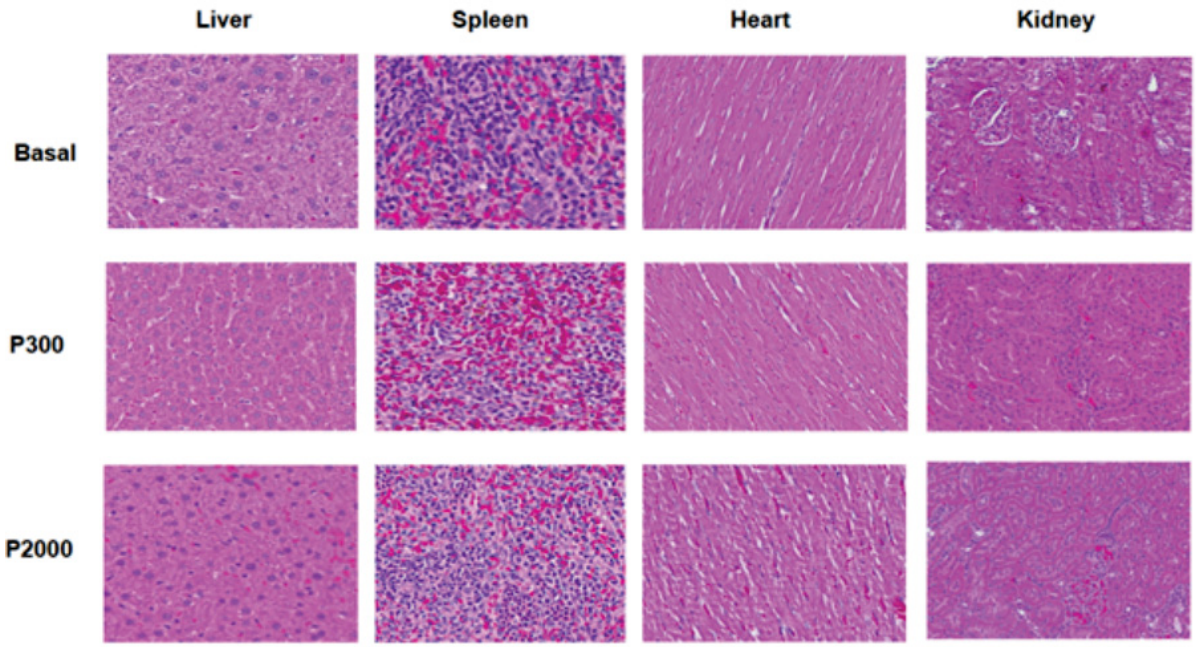


Fig 4. Histopathological analysis of the liver, spleen, heart, and kidney from Wistar rats treated with *Petiveria alliacea* (P) extract (300 mg/kg and 2000 mg/kg), compared to the basal (vehicle) group. Representative H&E-stained tissue sections show no significant morphological alterations in the liver,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

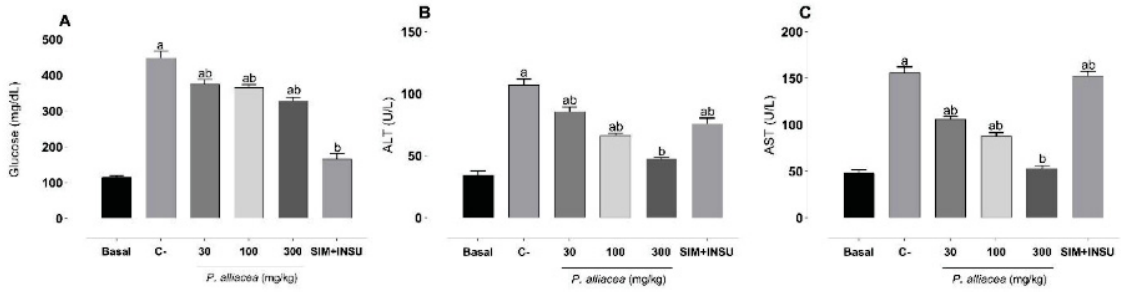


Fig 5. Serum levels of **(a)** glucose, **(b)** alanine aminotransferase (ALT), and **(c)** aspartate aminotransferase (AST). Basal: healthy rats, not subjected to the disease model, treated with vehicle. C–: diabetic, dyslipidemic rats exposed to ethanol, treated with vehicle. *P. alliacea*: diabetic, 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).

3.2.2. 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 behaviour 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**).

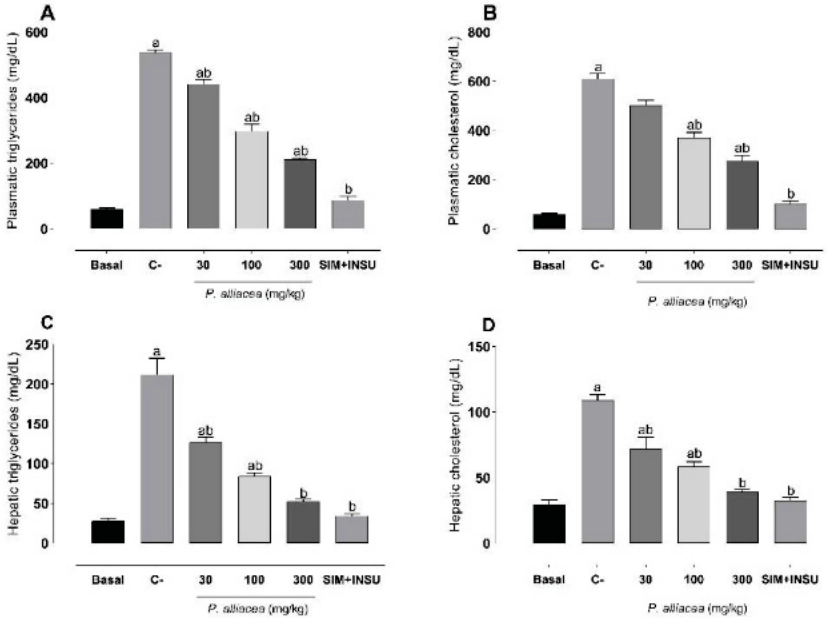


Fig 6. Plasmatic levels of **(a)** triglycerides and **(b)** total cholesterol, and hepatic levels of **(c)** triglycerides and **(d)** cholesterol. Basal: healthy rats, not subjected to the disease model, treated with vehicle. C–: diabetic, dyslipidemic rats exposed to ethanol, treated with vehicle. *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**).

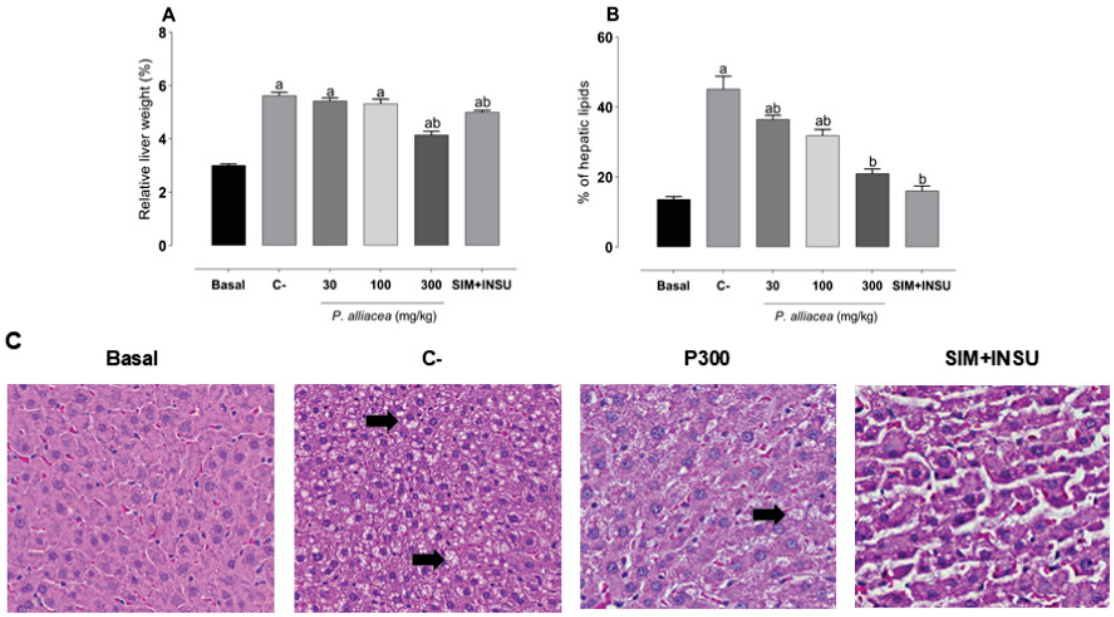


Fig 7. Relative liver weight **(a)**, percentage of hepatic lipid content **(b)**, and representative histological sections of the liver stained with hematoxylin and eosin (H&E) **(c)**. Basal: healthy rats, not subjected to the disease model, treated with vehicle. C–: diabetic, dyslipidemic rats exposed 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 U SOD/g of tissue) compared to the basal group (39.12 ± 0.97 U SOD/g of tissue). Treatment with *P. alliacea* at 30 mg/kg (32.36 ± 1.42 U SOD/g of tissue) and 100 mg/kg (34.77 ± 1.12 U SOD/g of tissue), as well as SIM+INSU (31.56 ± 0.65 U SOD/g of tissue), partially restored SOD activity, whereas the 300 mg/kg dose normalized enzyme activity (41.40 ± 0.54 U SOD/g of tissue), with values not statistically different from the basal group. Similarly, GSH levels were markedly depleted in the C– group (10.48 ± 2.25 µg GSH/g of tissue) relative to basal animals (58.46 ± 2.14 µg GSH/g of tissue). Partial recovery of GSH was observed with *P. alliacea* at 30 mg/kg (26.39 ± 2.93 µg GSH/g of tissue), 100 mg/kg (36.48 ± 4.02 µg GSH/g of tissue), and with

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

Table 4. Hepatic oxidative stress markers in Wistar rats subjected to the MASLD model and treated with *Petiveria alliacea* extract or simvastatin + insulin (SIM+INSU)

			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., *n*= 7-8. 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).

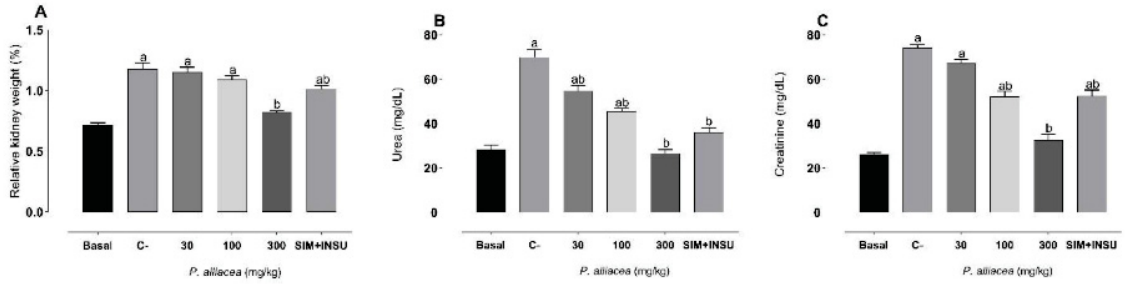


Fig 8. Relative kidney weight **(a)**; plasma urea levels **(b)**; and plasma creatinine levels **(c)**. Basal: healthy rats, not subjected to the disease model, treated with vehicle. C–: diabetic, dyslipidemic rats exposed to ethanol, treated with vehicle. *P. alliacea*: diabetic, dyslipidemic rats 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**).

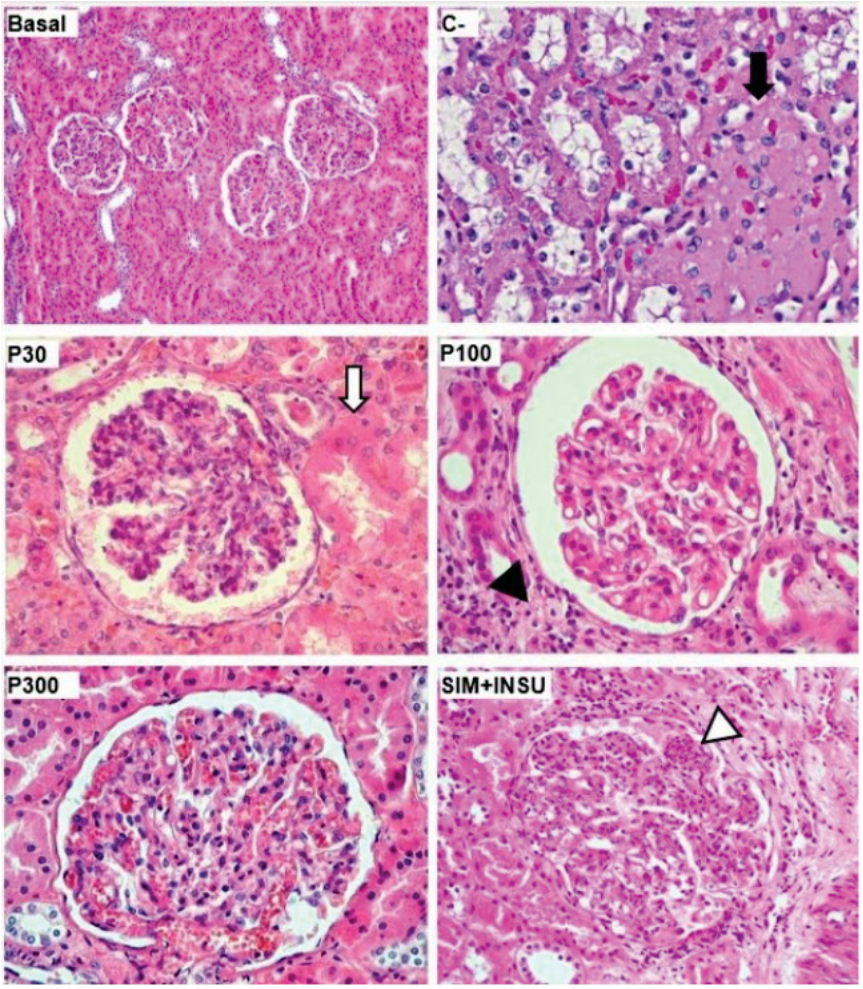


Fig 9. Representative histological sections of the kidneys stained with hematoxylin and eosin (H&E). Basal: healthy rats, not subjected to the disease model, treated with vehicle. C–: diabetic, dyslipidemic rats exposed to ethanol, treated with vehicle. *P. alliacea*: diabetic, dyslipidemic rats exposed to ethanol, treated with *P. alliacea* extract (30, 100, or 300 mg/kg). 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 damage acts 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 up to 100 µg/mL, 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 hepatotoxicity studies, 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, at least in part, 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. 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 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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4. CONCLUSÃO

O tratamento com o extrato de *P. alliacea* apresentou baixo potencial de toxicidade, evidenciando biocompatibilidade em células HepG2 e segurança em testes de toxicidade aguda em roedores. A ausência de efeitos adversos, tanto *in vivo* quanto *in vitro*, corrobora o uso tradicional da planta. Além disso, este estudo possibilitou estabelecer um modelo *in vivo*, inovador e complexo de DHGAM, por meio da associação de múltiplos fatores de risco. Em relação à planta analisada, o tratamento com o extrato de *P. alliacea* promoveu efeitos protetores hepáticos e renais, restaurando alterações bioquímicas e histopatológicas induzidas pelo modelo de DHGAM. Os resultados obtidos neste estudo apontam *Petiveria alliacea* como uma candidata botânica segura e promissora, com efeitos protetores multi-orgânicos, particularmente no contexto de complicações hepáticas e renais associadas à DHGAM. Entretanto, investigações adicionais sobre os mecanismos de ação do extrato, seus efeitos em longo prazo e sua aplicabilidade clínica. Estudos futuros também devem avaliar ações sinérgicas de *P. alliacea* em associação a terapias convencionais, assim como sua incorporação em formulações fitofarmacêuticas.

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