UNIVERSIDADE FEDERAL DA GRANDE DOURADOS
FACULDADE DE CIÊNCIAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

Investigação química, botânica, toxicológica e cardiorrenal das sementes de *Citrullus lanatus* (Thunb.) Matsum. & Nakai

ALINE APARECIDA MACEDO MARQUES

Dourados - MS
2024

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Área do CNPq: Etnofarmacologia

Tese apresentada ao Programa de Pós-Graduação em Ciências da Saúde da Faculdade de Ciências da Saúde da Universidade Federal da Grande Dourados (UFGD), para obtenção do título de Doutor em Ciências da Saúde

Área de concentração: Farmacologia

Orientador: Prof. Dr. Arquimedes Gasparotto Junior

Dourados - MS
2024

Dados Internacionais de Catalogação na Publicação (CIP).

M357i Marques, Aline Aparecida Macedo
Investigação química, botânica, toxicológica e cardiorrenal das sementes de Citrullus lanatus (Thunb.) Matsum. & Nakai [recurso eletrônico] / Aline Aparecida Macedo Marques. -- 2024.
Arquivo em formato pdf.

Orientador: Arquimedes Gasparotto Junior.
Tese (Doutorado em Ciências da Saúde)-Universidade Federal da Grande Dourados, 2024.
Disponível no Repositório Institucional da UFGD em:
https://portal.ufgd.edu.br/setor/biblioteca/repositorio

1. Doenças cardiovasculares. 2. Hipertensão. 3. Periodontite. 4. Citrullus lanatus. I. Gasparotto Junior, Arquimedes . II. Título.

Ficha catalográfica elaborada automaticamente de acordo com os dados fornecidos pelo(a) autor(a).

Ministério da Educação
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DEDICATÓRIA

Dedico este trabalho a todos aqueles que contribuíram para sua realização.

AGRADECIMENTOS

“Quem caminha sozinho pode até chegar mais rápido, mas aquele que vai acompanhado, com certeza vai mais longe” (Clarice Lispector).

Agradeço primeiramente a Deus, pois Dele provém toda a bênção. Sou grata por nunca ter soltado a minha mão e por me guiar em todos os momentos da minha jornada.

À Nossa Senhora Aparecida, por me acalentar.

Aos meus avós José e Judite, o amor que sinto por vocês é imensurável, um grande exemplo de força e serenidade. Vocês são inspiração, equilíbrio e porto seguro nos momentos de tribulação. Obrigada por ensinar à nossa família os valores mais preciosos de um ser humano: a humildade, o amor e o respeito ao próximo. Sem vocês, nada teria graça.

Agradeço aos meus pais, Antonio e Maria, por terem me apoiado nesta caminhada, meu porto seguro nos momentos difíceis da minha vida. Agradeço por acreditarem em mim e não medirem esforços para a concretização dos meus sonhos, mesmo à distância, sempre participaram e ajudaram nesta trajetória. Sem vocês, nada seria possível. Amo vocês!

Ao Murilo, meu eterno companheiro, pela paciência, pela compreensão, por estar comigo durante todo o processo, sempre me apoiando e incentivando, sendo meu maior apoio nas horas mais difíceis. Ele, que em todos os momentos, mesmo longe, sempre foi meu maior conforto, minha fonte de alegria, meu pedaço do presente e do futuro.

Agradeço ao meu irmão Lucas e à minha cunhada Camilli, por terem me amparado nos meus momentos de aflição e ansiedade, por se fazerem presentes mesmo distantes, obrigada por acreditarem no meu sonho e sempre me motivarem a seguir em frente. É muito bom saber que posso contar com você**s** em todos os momentos.

Aos meus amigos Ge, Wagner, Juliana e Maria Alcione, vocês são anjos que Deus colocou em meu caminho. Mesmo à distância, sempre se fizeram presentes na minha vida e estarão sempre em meu coração. Obrigada pelo companheirismo, apoio e amizade incondicional.

Expresso minha sincera gratidão à Viviana Cezar, que chegou à minha vida de maneira despretensiosa. Agradeço por sua amizade genuína, pelos valiosos conselhos e pelos puxões de orelha. Sua positividade é verdadeiramente contagiante e sou imensamente grata por tê-la presente em minha vida.

Aos meus amigos que a pesquisa me trouxe: Gabriela, você é a melhor IC que o LaFaC já teve. Só você sabe terceirizar as funções. Karyne, Luana, Luciane, Maria Luiza e, claro, a outra Aline, nossa amizade foi selada em cada diurese, finalização, comes e bebes, mas principalmente em cada garrafa de vinho, em cada risada e caneca de café**.** Vocês foram essenciais para que eu pudesse realizar esse árduo trabalho. Cada perrengue e imprevisto que aconteceu ao longo da jornada, ficaram mais leves, pois eu tinha o apoio de vocês. Muitos experimentos que realizamos eram uma sessão de terapia, além do mais, muitas vezes vocês foram a minha família em Dourados. Nos tornamos as meninas superpoderosas da FCS.

Agradeço a toda a equipe do LaFaC, mestrandos, doutorandos e alunos de iniciação científica. Vocês com certeza foram essenciais para a realização deste trabalho.

Ao professor Arquimedes, por ter me dado a chance de entrar no laboratório, não mediu esforços para aprimorar nosso trabalho. Obrigada pela confiança e por atender com paciência todas as minhas solicitações. Agradeço por todos os ensinamentos compartilhados de forma admirável e por me guiar nos primeiros passos, desde a iniciação científica até a pós-graduação. Sempre foi muito solícito e nunca mediu esforços para que pudéssemos realizar esse projeto, na busca de recursos e parcerias que foram imprescindíveis na execução.

Agradeço aos membros da banca presentes na defesa, por terem aceitado o convite e por contribuírem de certa forma para minha formação e meu crescimento.

Aos voluntários da pesquisa, muito obrigada pela compreensão e dedicação ao estudo. Vocês foram fundamentais!

Aos órgãos de fomento CAPES, FUNDECT e CNPq por tornarem este doutorado possível.

A todos que, de alguma forma, contribuíram para a realização deste trabalho, o meu muito obrigada!

EPÍGRAFE

A mente que se abre a uma nova ideia jamais
volta ao seu tamanho original.
(Albert Einstein)

LISTA DE ABREVIATURAS E SÍMBOLOS

DCV- Doenças Cardiovasculares

OMS- Organização Mundial da Saúde

OPAS/OMS- Organização Pan-Americana da Saúde / Organização Mundial da Saúde

HAS- Hipertensão Arterial Sistêmica

PA- Pressão Arterial

ECA- Enzima Conversora de Angiotensina

SUS- Sistema Único de Saúde

RENAFITO- Relação Nacional de Plantas Medicinais e Fitoterápicos

FAO- Organização das Nações Unidas para a Agricultura e a Alimentação (Food and Agriculture Organization)

TG- Triglicerídeos

SFAs- Ácidos Graxos Saturados (Saturated Fatty Acids)

PUFAs- Ácidos Graxos Poli-insaturados (Polyunsaturated Fatty Acids)

ω-6- Ácidos Graxos Ômega-6

OCDE- Organização para a Cooperação e Desenvolvimento Econômico (Organisation for Economic Co-operation and Development)

SHR- Ratos Espontaneamente Hipertensos (Spontaneously Hypertensive Rats)

HCTZ- Hidroclorotiazida

Phe- Fenilefrina

ACh- Acetilcolina

NPS- Nitroprussiato de Sódio

Investigação química, botânica, toxicológica e cardiorrenal das sementes de *Citrullus lanatus* (Thunb.) Matsum. & Nakai

RESUMO

As doenças cardiovasculares (DCV) abrangem distúrbios que afetam o coração e os vasos sanguíneos, sendo a hipertensão arterial um fator de risco central. A hipertensão, caracterizada por níveis persistentemente elevados de pressão arterial, sobrecarrega o coração e danifica os vasos sanguíneos ao longo do tempo, levando a complicações graves. Nos últimos anos, o uso de plantas medicinais tem sido cada vez mais estudado como uma abordagem complementar no tratamento dessas doenças, devido à presença de compostos bioativos que podem exercer efeitos benéficos sobre o sistema cardiovascular. Neste contexto, destaca-se *Citrullus lanatus* (Cucurbitaceae), comumente conhecida como melancia, uma fruta de grande importância nutricional e etnobotânica, amplamente consumida no Brasil e utilizada na medicina popular como suplemento nutricional no tratamento de DCV. Este estudo teve como objetivo realizar uma análise morfológica e química das sementes de *Citrullus lanatus*, além de investigar a toxicidade e os efeitos farmacológicos em ratos. As sementes foram analisadas por microscopia óptica e eletrônica de varredura, além de serem submetidas a análises físico-químicas. Para isso, sementes de *C. lanatus* foram colhidas, secas, pulverizadas. Extratos das sementes foram obtidos através de maceração, turbólise e infusão, sendo analisados por cromatografia líquida (LC-DAD-MS). Foram realizados testes de toxicidade aguda e subaguda em ratos Wistar, além de estudos de efeitos diuréticos, hipotensores e vasodilatadores. A análise revelou sementes ricas em compostos fenólicos e pectina, com alto teor de lipídios e minerais. Os testes de toxicidade indicaram que os extratos são seguros, não causando mortalidade ou efeitos adversos. O extrato aquoso demonstrou reduzir a resistência vascular periférica, com efeitos cardioprotetores significativos em ratos hipertensos. Finalmente, e não menos importante, mostramos que o uso popular de *C. lanatus* pode ser eficaz como uma terapia complementar no tratamento de doenças cardiovasculares, e abre perspectivas para o desenvolvimento de um medicamento fitoterápico contra a hipertensão.

Palavras-chave: Doenças cardiovasculares. Hipertensão. *Citrullus lanatus.*

Chemical, botanical, toxicological, and cardiorenal investigation of *Citrullus lanatus* (Thunb.) Matsum & Nakai seeds.

ABSTRACT

Cardiovascular diseases (CVD) encompass disorders that affect the heart and blood vessels, with high blood pressure being a central risk factor. Hypertension, characterized by persistently high blood pressure levels, overloads the heart and damages blood vessels over time, leading to serious complications. In recent years, the use of medicinal plants has been increasingly studied as a complementary approach in the treatment of these diseases, due to the presence of bioactive compounds that can exert beneficial effects on the cardiovascular system. In this context, *Citrullus lanatus* (Cucurbitaceae), commonly known as watermelon, stands out. It is a fruit of great nutritional and ethnobotanical importance, widely consumed in Brazil and used in folk medicine as a nutritional supplement in the treatment of CVD. This study aimed to perform a morphological and chemical analysis of *Citrullus lanatus* seeds, in addition to investigating their toxicity and pharmacological effects in rats. The seeds were analyzed by optical and scanning electron microscopy, in addition to being subjected to physicochemical analyses. For this purpose, *C. lanatus* seeds were collected, dried and pulverized. Seed extracts were obtained through maceration, turbolysis and infusion, and analyzed by liquid chromatography (LC-DAD-MS). Acute and subacute toxicity tests were performed in Wistar rats, in addition to studies of diuretic, hypotensive and vasodilatory effects. The analysis revealed seeds rich in phenolic compounds and pectin, with high lipid and mineral content. Toxicity tests indicated that the extracts are safe, causing no mortality or adverse effects. The aqueous extract was shown to reduce peripheral vascular resistance, with significant cardioprotective effects in hypertensive rats. Finally, and no less important, we showed that the popular use of *C. lanatus* can be effective as an additional therapy in the treatment of cardiovascular diseases and opens perspectives for the development of an herbal medicine against hypertension.

Keywords: Cardiovascular diseases. Hypertension. *Citrullus lanatus*.

SUMÁRIO

1 INTRODUÇÃO

As doenças cardiovasculares (DCVs) atingem tanto os países desenvolvidos quanto os países emergentes, além de incidirem em todas as faixas etárias. Contudo, sua prevalência aumenta significativamente com o avanço da idade (LIBBY; PINKOSKY; NISSEN, 2023; RODGERS et al., 2019). Sua prevalência e impacto significativo enfatizam a importância de compreender os fatores de risco e desenvolver estratégias de prevenção eficazes (ROTH et al., 2020).

Segundo a Organização Mundial de Saúde (OMS), as DCVs são as principais causas de morte no mundo: mais pessoas morrem anualmente por essas enfermidades do que por qualquer outro motivo, representando 31% de todas as mortes no mundo (OPAS/OMS, 2016).

As DCVs são caracterizadas por um grupo de doenças que afetam o coração e os vasos sanguíneos, resultando em complicações como infarto do miocárdio, acidente vascular cerebral e insuficiência cardíaca. Suas causas são multifatoriais: hipertensão, diabetes, fatores hereditários e genéticos, além dos fatores ambientais como tabagismo, obesidade, dieta inadequada e sedentarismo estão associados ao risco de desenvolvimento dessas doenças (OPAS/OMS, 2016).

A hipertensão arterial sistêmica (HAS), além de figurar entre os maiores fatores de risco para DCV, é uma das principais causas de morte no Brasil e no mundo. Trata-se de uma doença multifatorial e assintomática, caracterizada por níveis elevados e persistentes da pressão arterial (PA), acarretada por uma série de fatores, como predisposição genética, estilo de vida sedentário, dieta rica em sódio e alta ingestão de álcool. Dentre os vários fatores que podem agravar a HAS, destaca-se a periodontite (SILVA et al., 2022; SOUZA et al., 2020).

A periodontite é uma doença inflamatória crônica multifatorial, iniciada e perpetuada por bactérias anaeróbicas gram-negativas que colonizam a área subgengival. Essa doença é caracterizada pela destruição do tecido periodontal de inserção, acarretando a reabsorção óssea, infiltração de leucócitos e formação de bolsa periodontal (PAPAPANOU et al., 2018).

Evidências crescentes sugerem uma conexão significativa entre a periodontite e a HAS, sugerindo que a inflamação crônica presente na periodontite pode contribuir para o aumento da pressão arterial. Ademais, indivíduos com periodontite muitas vezes apresentam níveis mais elevados de proteína C- reativa, um marcador de inflamação associado à DCV (SOUZA et al., 2020).

O tratamento para HAS é bem estabelecido, com inúmeras alternativas e classes farmacológicas de medicamentos anti-hipertensivos disponíveis no mercado. Entre os mais utilizados estão os bloqueadores de canais de cálcio, diuréticos, β-bloqueadores e inibidores do sistema renina-angiotensina (CAMPBELL et al., 2022). A escolha do medicamento ou da associação de medicamentos depende da condição clínica específica de cada paciente, incluindo idade, outras condições de saúde e possíveis efeitos colaterais.

Outro aspecto fundamental é o investimento em pesquisa e inovação, visando aprimorar o diagnóstico, tratamento e reabilitação de pacientes com DCV. Avanços na tecnologia médica, terapias inovadoras e abordagens personalizadas estão contribuindo significativamente para a melhoria dos desfechos clínicos e a qualidade de vida dos pacientes (STANO; FRAGA; ANDRADE, 2022).

Dessa forma**,** a utilização de espécies vegetais é uma prática importante na área da saúde e pode ser uma abordagem eficaz e acessível para o tratamento de diversas doenças e condições de saúde. A disponibilização de plantas medicinais e de fitoterápicos pelo Sistema Único de Saúde (SUS) tem impulsionado a utilização da fitoterapia baseada em evidências científicas, extraída do conjunto de plantas utilizadas por gerações sucessivas de uma população que tinha como única opção para o tratamento de seus males, o uso empírico das plantas medicinais de fácil acesso em cada região do país (RIBEIRO, 2019).

O uso de espécies vegetais deve ser feito de forma consciente e responsável, levando em consideração as orientações e recomendações dos profissionais de saúde. Adicionalmente, é necessário realizar estudos científicos para comprovar a eficácia e segurança dessas espécies, a fim de garantir o uso correto e evitar possíveis riscos à saúde (CHOUDHURY et al., 2023).

Neste âmbito, encontra-se *Citrullus lanatus*, também conhecida como melancia, uma das frutas mais comercializadas no Brasil. Este fruto não é valorizado apenas pelo seu sabor aprazível, mas também pelos seus benefícios nutricionais e para a saúde, pois contém uma gama diversificada de compostos bioativos, incluindo carotenoides, compostos fenólicos, vitaminas, aminoácidos e alcaloides, com distribuição e concentração variadas na polpa, casca, folhas e sementes (JIBRIL et al., 2019).

Além do mais, contém diversos compostos que tem potencial para regular a pressão arterial ao converter os aminoácidos L-citrulina e arginina em óxido nítrico, o que promove a dilatação vascular e facilita o controle da PA. Os polissacarídeos isolados de *Citrullus lanatus* têm notável impacto na enzima conversora de angiotensina (ECA), que está envolvida na regulação da pressão arterial. Ademais, a presença de metabólitos secundários pode contribuir para a redução da PA através de mecanismos como vasodilatação e atividade antioxidante.

Essas propriedades fazem do *Citrullus lanatus* um candidato promissor para o controle da hipertensão arterial sistêmica (ISMAEL; MUSTAFA; AL-QAZAZ, 2022; ROTH et al., 2020).

Embora existam estudos sobre o uso de plantas medicinais na regulação da pressão arterial, muitos carecem de dados robustos sobre a eficácia e segurança de fitocomplexos específicos, justificando a necessidade de investigações adicionais focadas em compostos como os encontrados na *Citrullus lanatus*. Dessa forma, surge a necessidade de estudos científicos aprofundados acerca de suas propriedades medicinais. Assim, este estudo se propõe a testar a hipótese de que diferentes extrações do extrato da semente de *Citrullus lanatus* têm efeitos benéficos sobre os parâmetros cardiovasculares e renais em modelos experimentais de HAS.

2 REVISÃO DE LITERATURA

2.1. Doenças cardiovasculares

As DCV referem-se a uma série de condições que afetam o coração e os vasos sanguíneos, incluindo doença cardíaca coronária, doença cardíaca congênita, doença cardíaca valvular, distúrbios do ritmo cardíaco e doença vascular periférica. Essas condições podem resultar em complicações graves, como ataques cardíacos, acidentes vasculares cerebrais e insuficiência cardíaca (ROTH et al., 2020).

Os fatores de risco para as DCV incluem pressão arterial elevada, tabagismo, obesidade, falta de atividade física, dieta pouco saudável, diabetes e histórico familiar dessas condições (ROTH et al., 2020).

O impacto das DCV cria enormes desafios para os sistemas de saúde em todo o mundo. Os altos custos associados ao tratamento e à reabilitação dessas patologias são uma parte significativa desse desafio (ESTEL; CONTI, 2016).

Os custos de tratamento das DCV incluem não apenas as despesas diretas com medicamentos, procedimentos médicos e hospitalização, mas também os custos indiretos decorrentes da perda de produtividade devido a incapacidade, aposentadoria precoce e impacto nas famílias dos afetados (PEREIRA; PEREIRA, 2020; SANTOS et al., 2020).

A reabilitação após eventos cardiovasculares, como ataques cardíacos ou cirurgias cardíacas, também implica custos substanciais. Os programas de reabilitação cardíaca, embora fundamentais para a recuperação e a redução do risco futuro, podem demandar recursos consideráveis em termos de profissionais de saúde, instalações especializadas e acompanhamento contínuo dos pacientes (GHEORGHE et al., 2018).

Outrossim, as DCV frequentemente requerem tratamento contínuo e gerenciamento a longo prazo, o que contribui para a carga financeira nos sistemas de saúde (GHEORGHE et al., 2018).

Diante desses desafios, os sistemas de saúde precisam investir em estratégias eficazes de prevenção, diagnóstico precoce, educação para a saúde, promoção de estilos de vida saudáveis e acesso a tratamentos acessíveis e de qualidade. Isso não apenas reduzirá os custos de tratamento, mas também aliviará a pressão sobre os recursos médicos, melhorando a qualidade de vida dos pacientes e reduzindo a carga financeira global para o sistema de saúde. (TARRIDE et al., 2009).

2.1.1 Hipertensão arterial sistêmica

A HAS é uma doença crônica não transmissível, também conhecida como pressão alta, de alta prevalência global e que por ser oriunda de diversos fatores, torna-se difícil o seu controle (NILSON et al., 2020). Trata-se de uma condição médica na qual a pressão exercida pelo sangue nas paredes das artérias está consistentemente elevada, resultando do desequilíbrio entre a quantidade de sangue bombeada pelo coração e a resistência vascular periférica. Normalmente, a pressão arterial aumenta e diminui durante o dia em resposta às atividades e situações, mas quando permanece constantemente elevada, pode causar danos ao sistema circulatório (MENSAH et al., 2023).

Em consequência disso, é possível notar que a HAS é considerada um dos fatores de risco cardiovascular que pode ter uma variedade de causas. Em muitos casos, a hipertensão ocorre sem uma causa específica identificável e é denominada hipertensão primária ou essencial. A HAS envolve uma série de processos complexos que resultam no aumento da pressão arterial. Embora não haja uma causa única conhecida para a hipertensão primária, acredita-se que uma combinação de fatores genéticos, ambientais e de estilo de vida contribua para o seu desenvolvimento (BARROSO et al., 2021).

Ainda convém lembrar que a fisiopatologia HAS envolve uma série de processos complexos que resultam no aumento da PA. Vários fatores contribuem para o desenvolvimento da hipertensão, destacando-se as alterações no sistema renina-angiotensina-aldosterona, disfunção endotelial, atividade do sistema nervoso simpático, fatores genéticos e ambientais processos inflamatórios e de estresse oxidativo (VAROUNIS et al., 2017).

2.1.2 Doença periodontal e alterações cardiovasculares

A periodontite é uma doença inflamatória crônica que afeta os tecidos de suporte dos dentes, incluindo a gengiva e o osso alveolar. Essa condição pode resultar em perda progressiva do osso alveolar e, em casos graves, na perda dos dentes adjacentes. Essa inflamação pode desencadear uma resposta inflamatória sistêmica, afetando outros órgãos e sistemas, incluindo o cardiovascular (SANZ et al., 2020).

Uma vez que a periodontite é uma doença inflamatória crônica de origem bacteriana, o tratamento e a prevenção dessa condição são de extrema importância para a saúde geral. A quantidade de sítios afetados, a severidade da doença, a taxa de progressão e seus efeitos na saúde sistêmica devem ser levados em conta ao planejar um tratamento eficaz (CARDOSO et al., 2021).

A necessidade de ações efetivas no tratamento da periodontite é evidente, não apenas para preservar a saúde bucal do paciente, mas também para prevenir complicações sistêmicas. O impacto da inflamação causada pela periodontite crônica não se limita apenas à cavidade oral, mas também pode agravar condições como a HAS, devido à sua capacidade de contribuir para o aumento da pressão arterial (PAIZAN; VILELA-MARTIN, 2014).

A periodontite está ligada com as DCV, doenças pulmonares, diabetes mellitus e doenças autoimunes (ESPÍNDOLA-CASTRO et al., 2020). Pessoas com periodontite têm uma predisposição maior a desenvolver essas doenças. É importante ressaltar que, além dos impactos na saúde bucal, a periodontite também pode desencadear complicações graves em outras partes do corpo. As bactérias gram-negativas presentes na periodontite crônica causam uma inflamação local que, por sua vez, desencadeia uma resposta inflamatória sistêmica (CZESNIKIEWICZ-GUZIK, 2019).

Uma das principais complicações da periodontite é o agravamento do quadro da HAS que ocorrem de várias maneiras. Acredita-se que a inflamação crônica da periodontite possa contribuir para a elevação da pressão arterial, aumentando a produção de substâncias inflamatórias que podem afetar o sistema cardiovascular. Outro fator é o estresse oxidativo, que ocorre quando há um desequilíbrio entre os radicais livres e os antioxidantes no corpo. O estresse oxidativo pode contribuir para danos nas células vasculares e piorar a saúde cardiovascular, incluindo o controle da pressão arterial. Ademais, pode-se citar o aumento da resposta inflamatória sistêmica, que pode afetar não apenas a saúde bucal, mas também outros sistemas do corpo. Por conseguinte, a infecção periodontal pode ativar o sistema

imunológico, desencadeando uma série de eventos que aumentam sua atividade (KWON; LAMSTER; LEVIN, 2021; PAPAPANOU et al., 2018).

2.2. Plantas medicinais como terapia complementar

A utilização de plantas medicinais é uma prática comumente usada nos países em desenvolvimento. De acordo com a Organização Mundial de Saúde, 80% da população utiliza práticas tradicionais na atenção primária de saúde e, destes, 85% fazem uso de produtos derivados das plantas medicinais (BRASIL, 2006).

Adotando essa circunstância, o Ministério da Saúde brasileiro estabeleceu alguns critérios para a introdução de várias espécies medicinais no Sistema Único de Saúde (SUS). Uma de suas iniciativas foi a introdução da lista RENAFITO (Relação Nacional de Plantas Medicinais e Fitoterápicos) contendo as principais espécies a serem utilizadas como alternativa de tratamento no SUS (BRASIL, 2009).

Além do mais, elaborou-se a Política Nacional de Plantas Medicinais e Fitoterápicos, com o objetivo de promover o uso sustentável da biodiversidade brasileira na produção de medicamentos fitoterápicos e facilitar o acesso da população a esses recursos terapêuticos (BRASIL 2006).

Essa política visa regulamentar, incentivar e garantir a utilização de plantas medicinais e fitoterápicos de forma segura e eficaz, além de fomentar a pesquisa científica nesse campo, fortalecendo a cadeia produtiva e garantindo a qualidade, segurança e eficácia desses produtos. A política prevê a integração entre práticas tradicionais e conhecimentos científicos, estabelecendo parcerias entre instituições governamentais, universidades, comunidades tradicionais e a indústria farmacêutica. O objetivo é desenvolver políticas públicas e ações que ofereçam à população alternativas terapêuticas acessíveis e seguras (BRASIL, 2006).

O uso de tratamentos fitoterápicos está se tornando mais difundido como abordagem alternativa ou complementar no controle da hipertensão (MENEZES; PORTES; SILVA, 2020). O crescente interesse em tratamentos fitoterápicos é impulsionado pela perspectiva de minimizar os efeitos colaterais quando comparados aos medicamentos anti-hipertensivos convencionais (TABASSUM; AHMAD, 2011).

Portanto, é de suma importância valorizar e incentivar a pesquisa sobre plantas medicinais, a fim de desenvolver produtos e terapias alternativas para o tratamento de doenças.

2.2.1 *Citrullus lanatus*

A melancia (*Citrullus lanatus*) é uma fruta pertencente à família Cucurbitaceae, nativa da África do Sul, sendo uma fruta muito apreciada e comumente consumida em diversos países (ZAMUZ et al., 2021). O cultivo da melancia é mundialmente difundido e contribui significativamente para a produção vegetal, ocupando cerca de 7% da área total dedicada à produção vegetal (ANEES et al., 2021).

Dados da Organização das Nações Unidas para a Agricultura e a Alimentação (FAO), mostram que China, Turquia, Índia, Argélia e Brasil são os maiores produtores mundiais de melancia. Em todo o mundo, são produzidas cerca de 118 milhões de toneladas da fruta (FAO, 2022).

A melancia é uma fruta muito versátil e seu consumo tem se expandido rapidamente em todas as regiões brasileiras. O plantio da melancia é de grande importância econômica e social para o Brasil, contribuindo para geração de renda e empregos, principalmente em áreas rurais (NETO et al., 2016),

A melancia é uma fruta produzida em várias regiões do Brasil, sendo cultivada principalmente no Centro-Oeste e no Nordeste do país. O Brasil é um grande produtor da fruta e possui grande destaque na produção de melancia em todo o país, sobretudo no estado do Goiás (IBGE, 2022).

A produção da melancia é um setor promissor, visto que a demanda por essa fruta vem aumentando significativamente nos últimos anos. Isso se deve não apenas à riqueza de seus nutrientes, mas principalmente às propriedades fitoterápicas atribuídas ao fruto. Conhecida por seus benefícios nutracêuticos, a melancia se destaca por sua propriedade antioxidante, a qual está diretamente relacionada com a forma de consumo da fruta, podendo ser apreciada tanto in natura quanto em sua forma processada (MORIMOTO; ISEGAWA, 2023).

A melancia tem sido estudada por suas potenciais propriedades funcionais e benefícios para a saúde. Por exemplo, a entrecasca de melancia tem sido utilizada na produção de farinha, que tem demonstrado ser uma alternativa interessante na formulação de produtos alimentícios sem glúten e ricos em fibras (GUIMARÃES; FREITAS; SILVA, 2010). Tal inovação representa um avanço na utilização integral da fruta, promovendo sustentabilidade e ampliando as opções de consumo dessa deliciosa e nutritiva fruta.

Além das propriedades da fruta e da casca, as sementes de *Citrullus lanatus* também vêm atraindo a atenção de pesquisadores. As sementes são pequenas e ovais, normalmente pretas ou marrons, encontradas no interior da fruta. Elas são comestíveis e, na verdade, têm sido

consumidas em algumas culturas há séculos devido aos seus potenciais benefícios à saúde. As sementes também podem ser consideradas uma fonte de nutrientes, incluindo proteínas, ácidos graxos, vitaminas e minerais, como magnésio e ferro. Adicionalmente, contêm compostos como licopeno, antioxidantes e fitoesteróis, que podem ter propriedades benéficas para a saúde (MANIVANNAN et al., 2020).

O óleo das sementes de *Citrullus lanatus* é composto principalmente de ácidos graxos poli-insaturados, como oleico, linoleico e vestígios de ácidos linolênicos, juntamente com ácidos graxos saturados, como ácidos palmítico e esteárico, além de glicerídeos de armazenamento. A partir do peso seco, as sementes de *Citrullus lanatus* contêm principalmente ácido linoléico (50-60%), ácido oleico (15%) e quantidades significativamente menores de ácidos palmítico e esteárico. Composto principalmente de ácidos graxos insaturados ω-6, sobretudo o ácido linoleico, que auxilia na diminuição dos níveis de colesterol e hipertensão. O ácido linoleico e o ácido β-linolênico em concentrações moderadas promovem uma boa saúde. A abundância de triglicerídeos (TG), ácidos graxos saturados (SFAs) e ácidos graxos poli-insaturados u-6 (PUFAs) atendem às necessidades de óleos culinários, cosméticos e terapêuticos, sendo uma fonte medicinal e clínica popular de ω-PUFAs usados no tratamento de DCV, atuando nos níveis epitelial e de membrana (IBRAHIM et al., 2018; JARRET; LEVY, 2012; LOGARAJ, 2011; ZAMUZ et al., 2021).

2.2.2 Segurança farmacológica

Estudos de segurança são importantes para o desenvolvimento de novos medicamentos (TORNATORE et al., 2019). Este tipo de estudo tem como propósito investigar os potenciais efeitos indesejáveis de novas drogas, utilizando doses presentes na janela terapêutica e acima da mesma (BASS; KINTER; WILLIAMS, 2004). Um dos motivos que levam aos estudos de segurança farmacológica são os efeitos adversos que podem ser observados após a administração, principalmente nos sistemas nervoso central, respiratório e cardiovascular (BRIGGS et al., 2015; HAMDAM et al., 2013).

Ao padronizar os protocolos de estudos de segurança farmacológica, foram estabelecidas disposições importantes para a realização de estudos com esse fim. Afinal, todos os testes realizados são baseados em documentos publicados que regulamentam os detalhes da metodologia científica relacionada ao processo. Vale ressaltar que, embora existam protocolos bem definidos para avaliação de segurança e toxicidade farmacológica, essas publicações ainda não resolveram todos os desafios na investigação de novos medicamentos, especialmente no

que diz respeito à identificação de riscos raros e fatais ou efeitos adversos. Uma das dificuldades mais importantes é como conduzir experimentos que detectem efeitos adversos precocemente e com precisão. Isto é especialmente um problema para efeitos colaterais raros, mas potencialmente letais (PUGSLEY; AUTHIER; CURTIS, 2008).

A regulamentação dos medicamentos fitoterápicos no Brasil é debatida desde 1995 para normatizar e padronizar o uso desses medicamentos no país, buscando garantir sua segurança, eficácia e qualidade. Em 2006, a Agência Nacional de Vigilância Sanitária (Anvisa) publicou a RDC (Resolução da Diretoria Colegiada) 14, que estabelece critérios para registro de medicamentos fitoterápicos no Brasil. Esta resolução define requisitos específicos para a produção, controle de qualidade, rotulagem e registro desses medicamentos (BRASIL, 2014).

A RDC 14/2006 estabeleceu critérios para a comprovação de segurança e eficácia dos fitoterápicos por meio de estudos clínicos, farmacológicos e toxicológicos, assim como critérios de boas práticas de fabricação. Essa resolução foi um passo importante para regulamentar o setor e criar padrões para a produção e comercialização de fitoterápicos no país. Finalmente, em 2013, a ANVISA publicou um guia para realização de testes pré-clínicos para desenvolvimento de fitoterápicos (BRASIL, 2013).

Em consequência disso, é possível notar que o tema tem sido continuamente revisado e atualizado pela Anvisa e outros órgãos competentes, buscando aprimorar as diretrizes e regulamentações para os medicamentos fitoterápicos no Brasil, garantindo a segurança e eficácia desses produtos (BRASIL, 2006).

Atualmente, todos os protocolos de análises toxicológicas seguem as diretrizes da Organisation for Economic Co-operation and Development (OCDE, 2008), que são implementadas no Brasil em conjunto com o Guia para a Condução de Estudos Não Clínicos de Toxicologia e Segurança Farmacológica Necessários ao Desenvolvimento de Medicamentos (BRASIL, 2013), detalhando os protocolos mais utilizados na literatura, que são estudos de toxicidade aguda, subaguda e genotoxicidade.

Os protocolos de ensaio que investigam a segurança e a toxicidade de possíveis medicamentos são definidos como estudos que analisam possíveis efeitos farmacodinâmicos indesejados de uma substância nas funções fisiológicas em relação à exposição. Portanto, estes estudos são parte integrante da avaliação pré-clínica de novos agentes. Antes de novos medicamentos serem administrados pela primeira vez em pacientes humanos, os testes realizados devem incluir uma variedade de testes toxicológicos e estudos adicionais que os justifiquem (CLAUDE; CLAUDE, 2004).

3 OBJETIVOS

OBJETIVO GERAL

- Investigar os efeitos cardiovasculares e renais da *Citrullus lanatus* em modelo experimental de periodontite induzida em ratas SHR.

OBJJETIVOS ESPECÍFICOS

- Coletar, processar e realizar a caracterização morfoanatômica da espécie;
- Obter extratos por três diferentes métodos extrativos (infusão, turbólise e maceração);
- Realizar a caracterização fitoquímica dos extratos;
- Realizar análise centesimal da semente;
- Investigar a toxicidade aguda em ratas Wistar fêmeas com diferentes extratos;
- Investigar a toxicidade prolongada em ratos Wistar com diferentes extratos;
- Realizar o tratamento oral das ratas Wistar por 7 dias com diferentes extratos

(30, 100 e 300 mg/kg), hidroclorotiazida (HCTZ; 25 mg/kg) e água filtrada (controle negativo; 0,2 ml/100 g);

-Avaliar diariamente a função renal dos animais em estudo;

- Determinar o perfil eletrocardiográfico dos diferentes grupos experimentais;
- Avaliar de forma direta (invasiva) a pressão arterial sistólica, diastólica, média e frequência cardíaca após o tratamento agudo com os diferentes extratos;
- Induzir a periodontite em fêmeas SHR;
- Realizar o tratamento oral das ratas Wistar por 28 dias com extrato aquoso (30, 100 e 300 mg/kg), hidroclorotiazida (HCTZ; 25 mg/kg) e água filtrada (controle negativo; 0,2 ml/100 g);
- Avaliar de forma direta (invasiva) a pressão arterial sistólica, diastólica, média e frequência cardíaca após o tratamento prolongado;
- Avaliar a reatividade vascular em leito mesentérico isolado e perfundido sob a administração de diferentes substâncias vasoativas (Phe, ACh e NPS);
- Mensurar diferentes parâmetros bioquímicos séricos (ureia, creatinina, sódio, potássio, cloreto, alanina aminotransferase e aspartato aminotransferase);
- Realizar histopatologia da lesão periodontal;
- Determinar o peso relativo dos órgãos-alvo, realizar análises histopatológicas e a morfometria do coração;
- Investigar a natureza do estresse oxidativo tecidual (aorta, coração e rins).

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5 APÊNDICES

Artigo 1: Revista Planta Medica
Qualis A1
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Morphological and qualitative-quantitative analysis of the seeds of *Citrullus lanatus* and its extracts

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Abreviations

Thunb.: Thunberg
Matsum.: Matsumura
Nakai: Nakai
LC-DAD-MSLiquid Chromatography-Diode Array Detection-Mass Spectrometry
Embrapa: Empresa Brasileira de Pesquisa Agropecuária
pH: Potential of Hydrogen
µm: Micrometer
DRI: Dietary Reference Intakes
cm: Centimeter
g: Gram
mg: Milligram
HCl: Hydrochloric Acid
°C: Degrees Celsius
SEM: Scanning Electron Microscopy
AOAC INTERNATIONAL: Association of Official Analytical Collaboration International
RDC: Resolução da Diretoria
Anvisa: Agência Nacional de Vigilância Sanitária
EECL: Ethanolic Extract of *Citrullus lanatus*
TECL: Turbo-extract of *Citrullus lanatus*
SECL: Ethanol-Soluble Fraction of *Citrullus lanatus*
v/v: Volume/Volume

Abstract

Citrullus lanatus (Cucurbitaceae) is an important fruit widely utilized in Brazil as a nutritional supplement. The aim of this study was to evaluate morphologically and chemically the seeds of *Citrullus lanatus* and its extracts. Before cultivation, soil analyses were conducted to ensure quality control of the production process. Seeds from *Citrullus lanatus* fruit were collected and analyzed using light and scanning electron microscopy. Moreover, the seeds were subjected to centesimal composition and physical-chemistry analysis. Finally, seed extracts were obtained through maceration, turbolysis, and infusion. These extracts were later analyzed using liquid chromatography coupled with a diode array detector and mass spectrometer (LC-DAD-MS). The botanical analysis showed oval-shaped, flattened seeds that are notable for their smaller size compared to other species in the same family. Cross-sectional analysis revealed a palisade epidermis rich in phenolic compounds and pectin. The inner layers consisted of brachysclereids and parenchymatic cells, while the endosperm surrounded the inner epidermis, confirmed by the presence of oil bodies as a source of polyunsaturated fatty acids. The centesimal and physical-chemistry analysis highlighted high levels of lipids, calcium, potassium, phosphorus, and magnesium. The LC-DAD-MS analysis revealed... The present study revealed important anatomical, nutritional, and phytochemical

data to provide reliability in obtaining raw materials and for quality control of *Citrullus lanatus* seeds.

Keywords: Curcubitaceae; chemical analysis; morphoanatomy; quality control; seeds

Introduction

The origin of watermelon comes from South Africa and this fruit has a significant presence in the global diet. It is annually cultivated in tropical areas with seasonal periods of drought [1]. Currently, China is the leading producer, with a production of 60.3 million tons, followed by Turkey with 3.46 million tons, and Brazil in fourth place with 1.91 million tons [2], cultivated in an area of approximately 91.922 acres. The state of Rio Grande do Norte leads the production, reaching up to 340.805 tons [3].

In Brazil, watermelon is one of the most popular fruits, especially during the summer season. Its cultivation requires an ideal average temperature between 20 and 30 °C and an adequate amount of water to ensure its development. Usually, it is grown in areas close to water sources such as rivers, lakes, or irrigated regions. For healthy growth, correcting soil acidity and ensuring high levels of nutrients are essential [4].

Scientifically known as *Citrullus lanatus* (Thunb.) Matsum. & Nakai, watermelon is a member of the Cucurbitaceae family (1). Its fruits have green tones with darker stripes and have a spherical or oval shape (**Fig. 1**). The fruit has a thick shell, called the exocarp, and a fleshy, water-rich center, known as the mesocarp and endocarp. Inside, there are seeds with an oval or elliptical outline, which can be smooth or slightly textured, usually in a dark color. (**Fig. 2**). The flowers on this plant are light green and appear in the axil of the leaves [5,6].

The *Citrullus lanatus* has remarkable antioxidant activity, providing a wide range of bioactive compounds such as carotenoids, phenolic compounds, vitamins, amino acids, and alkaloids. These elements are distributed and concentrated differently in the pulp, peel, leaves, and seeds. Carotenoids, particularly carotenes and xanthophylls, are notable as high-capacity antioxidant compounds and are responsible for the variety of coloring found in watermelon pulp [6].

The seeds of *Citrullus lanatus* are an abundant source of nutritional compounds, including essential fatty acids such as linoleic acid (54-68%) and oleic acid (13-16%). These seeds also contain vitamin E and a variety of carotenoids, such as beta-carotene, lycopene, lutein, and phytosteroids [7]. Despite the importance of this species, data regarding the morphoanatomical, nutritional, and chemical analysis of its seeds remains not fully investigated. Therefore, the objective of this study was to evaluate the morphological and qualitative-quantitative characteristics of the seeds of *Citrullus lanatus* and its extracts obtained through different extraction processes.

Results and discussion

Conducting a soil analysis is a crucial procedure before planting watermelon, as it helps accurately identify nutritional deficiencies. This analysis provides detailed information on the physical, chemical, and biological properties of the soil, allowing for an assessment of its condition and the detection of potential nutritional deficiencies [8].

The soil plays a vital role in watermelon cultivation. For successful tillage, the ideal soil must have a specific combination of physical and chemical characteristics. The soil needs to contain essential nutrients like nitrogen, phosphorus, potassium, and various micronutrients to support the healthy growth of the plants. Proper fertilization, whether using organic or inorganic fertilizers, is necessary to ensure that the plants have access to the nutrients they require [9,10].

According to Embrapa (Brazilian Agricultural Research Corporation) [11], watermelon cultivation is suitable for a variety of soils, including those with medium sandy textures. However, it is not recommended to grow watermelons in clayey or highly clayey soils. Watermelon is known to be a crop that requires a lot of nutrients. Throughout its

growing cycle, the crop needs a substantial amount of nutrients to support its development and fruit production [12].

Fig. 3 shows the soil evaluation before planting of *Citrullus lanatus*. The average pH detected was 4,7 indicating the need for adjustments with limestone to meet the specific needs of the species. The soil's pH level also plays a crucial role. For watermelon, the optimal pH is typically between 6 and 6.8, creating a setting where nutrients can be readily absorbed by plants. The ideal soil for growing watermelon should have a balanced combination of good drainage and the proper pH levels to guarantee strong growth and high-quality fruits [13].

In addition to pH, the soil needs to provide essential elements such as nitrogen, phosphorus, potassium, and a range of micronutrients to support the healthy growth of watermelon plants. This can be achieved through proper fertilization, whether through organic or inorganic fertilizers, ensuring that the plants have access to all the nutrients they need [14]. In our study, the values of zinc and vanadium were below the desired levels, requiring adjustments before planting the species. Zinc and vanadium deficiency can negatively impact the growth and development of watermelon plants.

Zinc deficiency is associated with several physiological disorders, including stunted growth due to its role in auxin synthesis, leaf chlorosis, especially in young leaves, and fruit malformation. In addition, zinc deficiency can reduce fruit set, compromising the quantity and quality of fruit produced [15].

On the other hand, although vanadium is not considered an essential micronutrient for most plants, its absence can affect microbiological processes in the soil, which are critical for the availability and absorption of other nutrients, such as phosphorus and iron. This indirect deficiency can consequently impair plant development, interfering with their overall nutrition and the efficiency of absorption of essential nutrients [16].

Morphoanatomical studies play a fundamental role in several areas of biology and natural sciences, focusing on the analysis of the shape (morphology) and internal structure (anatomy) of organisms. Morphoanatomical investigation is extremely important to understand the relationship between biological structure and function, as well as to elucidate the mechanisms by which different organisms adapt to their environments over time. These studies are indispensable for species identification and classification, and the analysis of intraspecific morphoanatomical variations provides valuable information about evolution, adaptation and potential subspecies formation [17].

The seeds of *Citrullus lanatus,* which are the focus of this study, have a yellow to dull brown or black color and an oval and flattened shape, as noted by Alka et al., 2018 [5]. The seeds measure 0.50 × 0.83 cm, smaller in size compared to the seeds of *Cucurbita moschata* Duchesne (1.1 × 0.7 cm), *Cucurbita maxima* Duchesne (2.0 × 1.2 cm), and *Cucurbita pepo* L. (1.8 × 0.9 cm), which belong to the Cucurbitaceae family [18].

In cross section, the seeds of *Citrullus lanatus* (**Fig. 4a-m**) have an oblong shape (**Fig. 4a**). The epidermis is made up of a palisade parenchyma consisting of long cells (**Fig. 4a-e**). Some palisade cells contain phenolic compounds, which were identified using ferric chloride (**Fig. 4f**) and potassium dichromate (**Fig. 4g**). The epidermis is covered by a thick layer that contains pectin and is approximately 50 µm thick. Pectin stains from pink to red with ruthenium red (**Fig. 4d**) and from pink to purple with toluidine blue (**Fig. 4e**).

Below the outer protective layer, there are approximately five layers of short sclerenchyma cells that reacted with phloroglucinol/HCl (**Fig. 4h**). The innermost layer consists of these short sclerenchyma cells arranged side by side (**Fig. 4h**), which encircle a columnar layer (**Fig. 4i**). In the innermost part, there is a layer of parenchyma cells (**Fig. 4i**). Oil droplets were found scattered within the columnar layer and were identified using Sudam

III in the microchemical tests (**Fig. 4j**). Additionally, there exists a layer of cells containing lipophilic compounds located between the columnar and parenchyma cells (**Fig. 4j**).

The endosperm surrounds the inner epidermis (**Fig. 1i**). The nutritional reserve of the seed consists of oil bodies that can be identified with Sudan black (**Fig. 1l**) and Nile blue (**Fig.1m**), which are present in high amounts in the cotyledons. This data supports previous studies that have mentioned the presence of oil compounds in the seeds of *Citrullus lanatus*, recognizing them as a significant source of polyunsaturated fatty acids [19,20].

According to Lima et al. [21], food production leads to a significant amount of waste and by-products from fruit processing. A portion of this waste includes seeds, which are often thrown away. Watermelon seeds are a source of protein, fiber, vitamins, and minerals. The fat content in them is relatively low, mainly containing unsaturated fatty acids such as oleic acid and linoleic acid. However, watermelon seeds are typically consumed in small quantities and are primarily recognized for their protein and fiber content [22]. Therefore, due to the significant presence of proteins, fats, fibers, and minerals, including iron, zinc, and magnesium, seeds can play an important role in consumer nutrition [23,24].

According to the centesimal analysis, the amounts of macro and micronutrients in 100g of seeds are shown in **Table 1**.

According to the requirements established by the Dietary Reference Intakes (DRI) [25], the recommended amount of macronutrients for men and women aged between 19 and 70 years is as follows: carbohydrates (100 g for both sexes), proteins (56 g/day for men and 46 g/day for women), and lipids (20-35 g for both sexes). Additionally, the recommended amount of minerals is as follows: calcium (1000-1200 mg for both sexes), sodium (1200-500 mg for both sexes), potassium (4700 mg for both sexes), phosphorus (700 mg for both sexes), and magnesium (400-420 mg for men and 310-320 mg for women). Therefore, our results showed that 100 grams of watermelon seeds meet 72.8% of the daily recommended lipid

intake, 74.5% of calcium, 113.6% of potassium, 570% of phosphorus, and 74.5% of magnesium, demonstrating their potential as a dietary supplement.

Materials and methods

Soil analysis

Soil analysis was conducted following the methods outlined by Teixeira et al., 2017[26]. To ensure an accurate representation of the area, a grid was set up, with collection points spaced every 2 to 4 hectares. Before sampling, surface debris like leaves and stones were cleared to expose the soil. Holes were drilled using an auger to a depth of about 40 cm, and the collected soil samples were carefully packaged. The analysis included determining soil texture through granulometric analysis, as well as evaluating physical properties such as density and porosity. Chemical analyses were also performed, including pH and both macro and micronutrients, including phosphorus, potassium, calcium, magnesium, aluminum, vanadium, sulfur, iron, manganese, zinc, copper, boron. The levels of organic matter in the soil were also assessed.

Plant material

Seeds of *Citrullus lanatus* were collected in Ouro Verde, São Paulo, Brazil (-21.510240817687322, -51.65258069269025). A voucher specimen (1345) was authenticated by Dr. Zefa Valdivina Pereira and deposited in the herbarium of Universidade Federal da Grande Dourados (UFGD). The fruit seeds were manually removed and dried by forced air circulation for 5 days. The seeds were stored in plastic bags at 2-8°C until analysis.

Morphoanatomical study

Initially, the plant material was fixed in a 70% solution of formalin, acetic acid, and ethanol [27]. After 5 days, the seeds were stored in a solution of 70% ethanol and then transverse free-hand sections were conducted [28]. Histochemical tests were conducted using the following reagents: phloroglucinol/HCl [29], Sudan III [30], 2% ferric chloride[27], 10% potassium dichromate[31], 1% iodine solution [27], 1% methylene blue[32], Schiff reagent, 0.002%, ruthenium red solution, 0.5%, hydrochloric vanillin solution, Dragendorff reagent, Wagner reagent, Sudan black, Nile blue sulfate, Xylidine Ponceau, Coomassie bright blue, and NADI reagent [33]. Photomicrographs were taken using an Olympus CX31 microscope with an Olympus C-7070 digital camera at the Laboratory of Pharmacognosy at the University of Ponta Grossa (UEPG), Brazil. Scanning electron microscopy (SEM) [34] was conducted at the Multi-user Laboratory at UEPG.

Centesimal composition and physical-chemistry analysis

The levels of total calcium, phosphorus, potassium, and sodium were determined using the methodology described in the Official Methods of Analysis of AOAC INTERNATIONAL[35]. The quantification of carbohydrates was carried out through the methods described in the RDC no. 360 of December 23, 2003, by the Brazilian Health Regulatory Agency (Anvisa) [36]. The levels of lipids and proteins were quantified using techniques previously described by the Adolfo Lutz Institute (Brazil) [37].

Phytochemical analysis

The phytochemical analysis was performed using three different extraction methods, including maceration in ethanol, turbolysis, and infusion.

Ethanol extract

Initially, dried *Citrullus lanatus* seeds were pulverized in a hammer mill. The ethanol extract was obtained by adding an ethanol-water solution (7:3, v/v) to the pulverized plant material (100 g/L). The extractive solution was constantly homogenized for 48 hours for the first extraction, and then every 24 hours for the subsequent solvent exchanges (a total of five exchanges). During each exchange, a new ethanol-water solution was added to the same plant material to ensure maximum extraction of phytochemical components. The solvent from the extract was concentrated using a rotary evaporator and freeze-dried. The yield of the ethanolic extract of *Citrullus lanatus* (EECL) was 18%.

Turbo-extraction

The turbo-extraction was performed using a high shear stirrer (Ika Ltda, Sao Paulo, Brazil). Powdered *Citrullus lanatus* seeds were added to filtered water (100 g/L at room temperature; 24 degrees Celsius) and subjected to high shear stirring for 5 minutes. The extract (TECL) was then filtered, lyophilized, and stored at -18◦C. The yield obtained was 22%.

Ethanol-soluble fraction from aqueous extract

Initially, an infusion was prepared by adding 1 liter of boiling water to 100 grams of dried and powdered fruit seeds. After 4 hours (to reach room temperature), the infusion was treated with 3 volumes of 95% ethanol, resulting in a precipitate and an ethanol-soluble fraction (SECL). The SECL was then filtered, and after removing the ethanol (using a rotary evaporator at 55 ºC), it was lyophilized (yield of 13%).

Chemical characterization

Acknowledgments

The authors would like to express their gratitude to the C-Labmu Center at the State University of Ponta Grossa (UEPG), Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT, Brazil; grant numbers 71/700.135/2018 and 83/013.186/2023), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil; grant number 407685/2018-9), and Coordenadoria de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil).

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Table 1. Centesimal analysis the *Citrullus lanatus* seeds

Parameter	**Results***
Carbohydrates (g/100 g)	12,4
Protein (g/100 g)	21,1
Fat (g/100 g)	25,5
Calcium (mg/100 g)	89,4
Sodium (mg/100 g)	9,7
Potassium (mg/100 g)	534,0
Phosphorus (mg/100 g)	399,0
Magnesium (mg/100 g)	184,5

***All measurements were carried out in triplicate.**

Legend to figures

Figure 1. *Citrullus lanatus* fruit. External view.

Figure 2. Representative image of the pulp and seeds of *Citrullus lanatus* fruit.

Figure 3. Chemical analysis data of soil from the cultivation area of *Citrullus lanatus* P: phosphorus, K: potassium, Ca: calcium, Mg: magnesium, Al: aluminum, V: vanadium, S: sulfur, Fe: iron, Mn: manganese, Zn: zinc, Cu: copper, B: boron

Figure 4. Anatomy and histochemistry of *Citrullus lanatus* (Thunb.) Matsum. & Nakai seed. a, b, d-m: light microscopy; c, n: electron scanning microscopy. a, b, i, and k: basic Fuchsin and Astra blue; d: ruthenium red; e: toluidine blue; f: ferric chloride; g: potassium dichromate; h: floroglucinol/HCl; j: Sudam III; l: Sudam black; m: Nile blue. [br: brachysclereids; cl: columnar layer; co: cotyledon; en: endosperm; ib: inner brachysclereid layer; ie: inner epidermis; li: inner layer; ob: oil bodies; pa: parenchymatous cells; pc: phenolic compounds; pe: pectin layer; pl: palisade layer; pp: palisade parenchyma]. Scale bars: a = 500 µm; b, d = 300 µm; i = 100 µm; c, e-h, k = 50 µm; m = 25 µm; n = 20 µm.

Figure 1

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Figure 2

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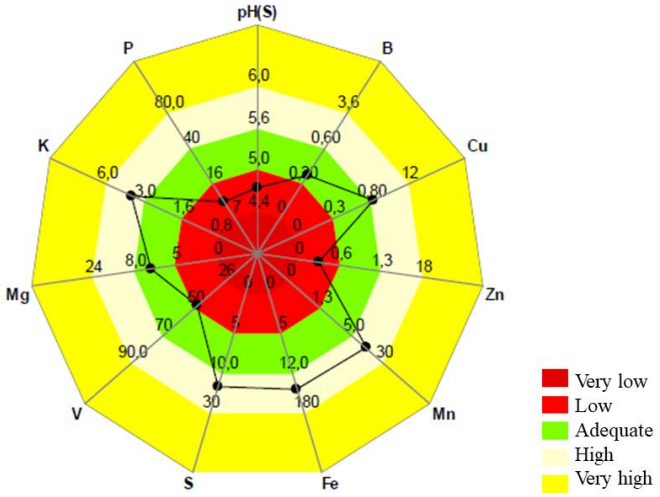

Figure 3

Marques et al

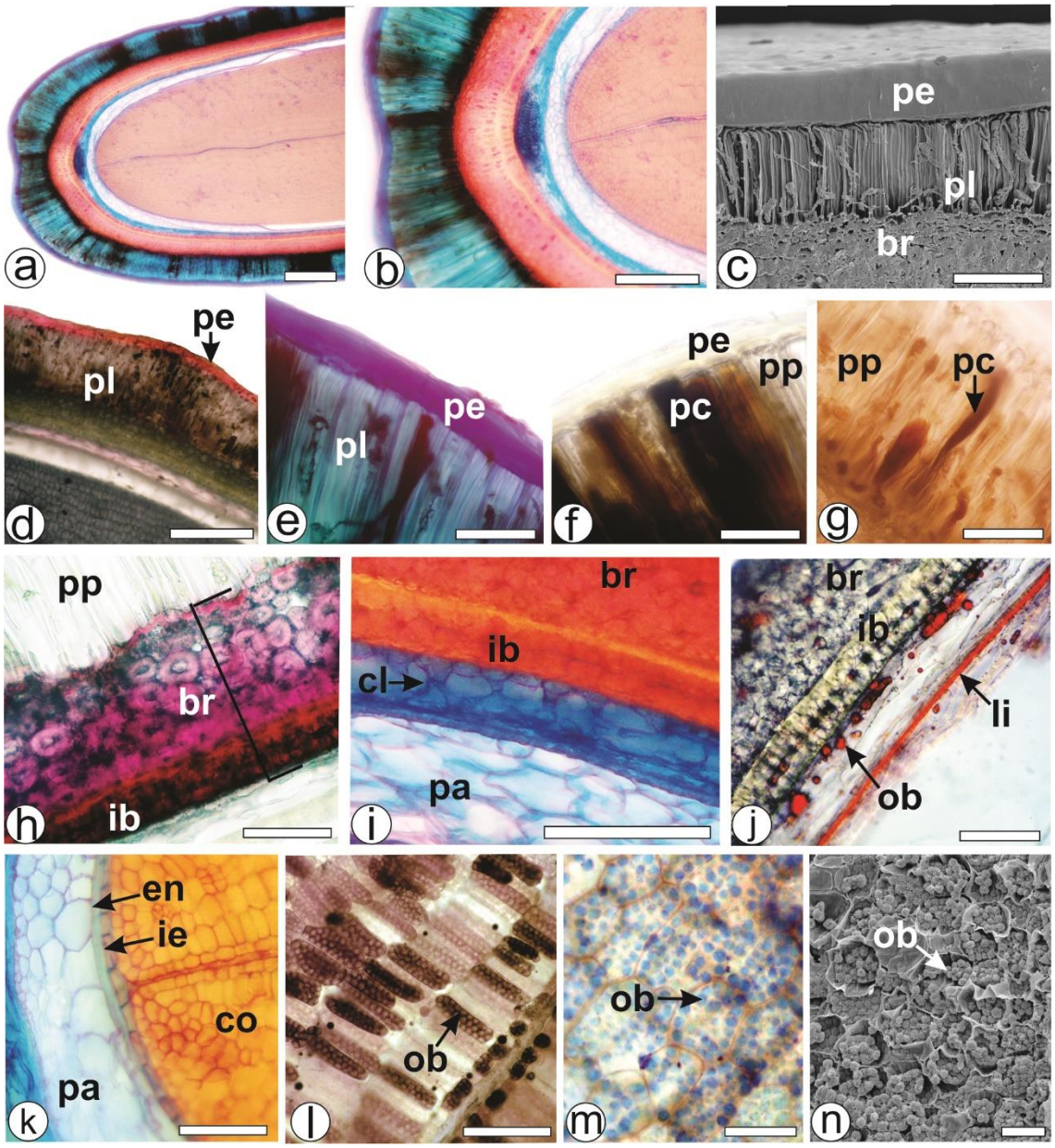

Figure 4

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Artigo 2: Journal of Medicinal Food
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Assessment of acute and subacute toxicity of different preparations obtained from *Citrullus lanatus* seeds

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ABSTRACT

Citrullus lanatus (Thunb.) Matsum. & Nakai, commonly known as watermelon, is native to South Africa and is a highly appreciated and commonly consumed fruit in several countries, including Brazil. The purpose of this study is to assess the acute and subacute toxicity of different extracts derived from *C. lanatus* seeds in Wistar rats. First, three different *C. lanatus* extracts obtained from dried and pulverized seeds were prepared. These included an ethanolic extract (EECL), an aqueous extract (TECL), and a pre-purified infusion (SECL). Then, acute toxicity was evaluated in female rats after a single oral administration containing 2,000 mg/kg of the different preparations. Additionally, for the assessment of subchronic toxicity, the EECL, TECL, and SECL were orally administered for 28 days at doses of 30, 100, and 300 mg/kg in male and female rats. The acute treatment did not induce mortality and did not affect the evaluated clinical and behavioral parameters. No clinical, hematological, biochemical, or histopathological alterations were observed after the administration of the different formulations for 28 days. Our findings suggest that different preparations obtained from *C. lanatus* seeds, traditionally used in Brazil, are safe and offer some protection to body organs. Furthermore, the extracts can be further evaluated in preclinical and clinical studies for their safety and therapeutic effects.

KEYWORDS: • *Cucurbitaceae • fruit • herbal medicine • watermelon • safety*

INTRODUCTION

Several studies have established that fruits have the ability to promote nutrients and bioactive components, making them beneficial for health. Fruits are rich in a variety of vitamins, minerals, dietary fiber, and antioxidants, all of which are essential for the proper functioning of the human body (1).

Citrullus lanatus (Thunb.) Matsum. & Nakai, commonly known as watermelon and belonging to the Cucurbitaceae family, is native to South Africa. It is a highly appreciated and commonly consumed fruit in several countries. Watermelon cultivation is widespread worldwide (2).

Watermelon has been researched for its potential health benefits and functional properties. Additionally, researchers have also taken an interest in the properties of the seeds of *C. lanatus* along with those of the fruit and peel. The seeds are small and oval, typically black or brown, and are found inside the fruit. They are edible and have been consumed in certain cultures for centuries due to their potential health benefits. Seeds are also a good source of nutrients, including proteins, fatty acids, vitamins, and minerals like magnesium and iron. Additionally, they contain compounds such as lycopene, antioxidants, and phytosterols, which may have beneficial effects (3).

Despite the high consumption, studies focusing on the toxicity of *C. lanatus* seeds are scarce. Toxicity assessment is crucial and essential to understand the possible adverse impacts that a substance being tested might have on various organic systems, as well as being mandatory for the development of new medicines (4,5). Additionally, one of the reasons for conducting pharmacological safety studies is the potential adverse effects that can be observed after administration, particularly on the central nervous, respiratory, and cardiovascular systems (6,7).

Therefore, the goal of this study is to evaluate the acute and subacute toxicity of different preparations obtained from *C. lanatus* seeds in Wistar rats.

MATERIALS AND METHODS

Drugs

Isoflurano was purchased from Syntec (São Paulo, SP, Brazil). Heparin was purchased from Hipolabor (Belo Horizonte, MG, Brazil). All other reagents were obtained in analytical grade.

Animals

Healthy male and female Wistar rats, aged 3 months, were obtained from the Central Vivarium of the Federal University of Grande Dourados (UFGD, Brazil). The rats were housed in the vivarium at a constant temperature of 22 °C ± 2 °C under a 12-hour light/12-hour dark cycle. They had access to food and water ad libitum. Prior to the start of the experiments, all animals were allowed to acclimatize to the laboratory conditions for seven days. All procedures involving the animals were approved by the Ethics Committee in Animal Experimentation at UFGD (protocol no. 07/2020) and were in compliance with the Brazilian Legal Framework on the Use of Scientific Animals.

Plant material

Seeds of *C. lanatus* were collected in Ouro Verde, São Paulo, Brazil (-21.510240817687322, -51.65258069269025). A voucher specimen (1345) was authenticated by Dr. Zefa Valdivina Pereira and deposited in the herbarium of Universidade Federal da Grande Dourados (UFGD). The fruit seeds were manually removed and dried by forced air circulation for 5 days. The seeds were stored in plastic bags at 2-8°C until analysis.

Extractive processes

We utilized three different methods for obtaining the extracts, including maceration in ethanol, turbolysis, and infusion. The phytochemical analysis was performed previously (in press).

Ethanol extract

Initially, dried *C. lanatus* seeds were pulverized in a hammer mill. The ethanol extract was obtained by adding an ethanol-water solution (7:3, v/v) to the pulverized plant material (100 g/L). The extractive solution was constantly homogenized for 48 hours for the first extraction, and then every 24 hours for the subsequent solvent exchanges (a total of five exchanges). During each exchange, a new ethanol-water solution was added to the same plant material to ensure maximum extraction of phytochemical components. The solvent from the extract was concentrated using a rotary evaporator and freeze-dried. The yield of the ethanolic extract of *C. lanatus* (EECL) was 18 %.

Turbo-extraction

The turbo-extraction was performed using a high shear stirrer (Ika Ltda, Sao Paulo, Brazil). Powdered *C. lanatus* seeds were added to filtered water (100 g/L at room temperature; 24 degrees Celsius) and subjected to high shear stirring for 5 minutes. The extract (TECL) was then filtered, lyophilized, and stored at -18◦C. The yield obtained was 22%.

Ethanol-soluble fraction from aqueous extract

Initially, an infusion was prepared by adding 1 liter of boiling water to 100 grams of dried and powdered fruit seeds. After 4 hours (to reach room temperature), the infusion was treated with 3 volumes of 95% ethanol, resulting in a precipitate and an ethanol-soluble fraction

(SECL). The SECL was then filtered, and after removing the ethanol (using a rotary evaporator at 55 ºC), it was lyophilized (yield of 13).

Toxicological studies

Acute toxicity

The acute toxicity test was conducted according to guideline number 425 from the Organization for Economic Co-operation and Development (OECD), which was published in 2022 (8). Twenty female rats were fasted for 6 hours before treatment. Then, the rats were weighed, randomized into groups of 5 individuals, and treated with SECL, EECL, and TECL at a dose of 2000 mg/kg orally. The animals in the control group (n=5) were treated with the vehicle (filtered water; 0.2 mL/100 g). Mortality and the clinical and behavioral signs of toxicity were evaluated for 8 hours after treatments and every 24 hours for 14 days. On the 15th day, the animals were euthanized using isoflurane anesthesia (inhalation) followed by exsanguination. During necropsy, vital organs (heart, lung, liver, spleen, and kidneys) and reproductive organs (ovaries and uterus) were extracted, weighed, and the relative organ weight was determined. Samples showing significant changes were subjected to histopathological analysis.

Subacute toxicity

To assess the subacute toxicity of the different formulations, we followed the recommendations presented in OECD guideline no. 407 (Repeated Dose 28-day Oral Toxicity Study in Rodents) (9). Sixty female and 60 male Wistar rats were used in this study. The animals were randomly divided into ten groups (n = 6/group/sex), as follows: 1) negative control (filtered water; 0.2 mL/100 g), SECL (30, 100, and 300 mg/kg); EECL (30, 100, and 300 mg/kg); and TECL (30, 100, and 300 mg/kg). The treatments were administered orally,

once a day, for 28 days. Throughout the study, body weight, and food and water consumption were monitored.

On the morning of the twenty-ninth day, all animals were anesthetized by inhalation with isoflurane (2-3%). Subsequently, blood samples were collected from the retro-orbital plexus and stored in BD Hemogard^{TM} (K2EDTA) tubes. Hemoglobin (Hb) (g/dL), hematocrit (Hct) (%), mean corpuscular volume (MCV) (fl), mean corpuscular hemoglobin (MCH) (pg), mean corpuscular hemoglobin concentration (MCHC) (g/dL), white blood cells, lymphocytes (10^3/μL), neutrophils (10^3/μL), platelets (10^3/μL), red cell distribution width (RDW), mean platelet volume (MPV) (fl) were measured using a Sysmex XN-3100 automated hematology system.

After blood collection via retro-orbital plexus, the animals were euthanized by decapitation and the blood was collected into SST Clot Activator and Polymer Gel Hemogard™ tubes. The serum was obtained by centrifugation (1,500 g for 10 min). Amylase (U/L), urea (mg/dL), creatinine (mg/dL), uric acid (mg/dL), potassium (mEq/L), sodium (mEq/L), total proteins (g/dL), albumin (g/dL), globulin (g/dL), alkaline phosphatase (U/L), triglycerides (mg/dL), total bilirubin (mg/dL), aspartate aminotransferase (AST) (U/L), alanine transaminase (ALT), and total cholesterol (mg/dL) were measured using a Cobas 8000 modular analyzer (Roche Diagnostics).

After euthanasia, a median xiphoid laparotomy was performed to remove the organs. The liver, heart, kidneys, lungs, spleen, testicles, epididymis, prostate, ovaries, uterus, and cervix were removed, cleaned, and weighed to determine absolute and relative organ weights (absolute organ weight × 100/body weight). Tissue samples from the lungs, heart, liver, kidneys, spleen, testicles, epididymis, ovaries, and vaginal canal were dehydrated in alcohol, cleared in xylene, and embedded in paraffin. Histological sections of 5 μm thickness were obtained, stained with hematoxylin and eosin, and examined under an optical microscope

(Olympus Optical Co., Tokyo, Japan). The samples were analyzed for general structural changes, degenerative alterations, evidence of necrosis, and signs of inflammation. The necropsy and histopathological evaluation were performed by a veterinary pathologist from the Faculty of Health Sciences at UFGD.

Statistical analysis

The results are expressed as the mean ± standard error of each experimental group. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's test. A p value less than 0.05 was considered statistically significant. The statistical analysis was performed using the GraphPad Prism software version 9.4.1 for macOS (GraphPad Software, Inc., La Jolla, CA, USA).

RESULTS

Acute toxicity

No significant changes in appearance or general behavior pattern were observed until the end of the 14-day observation period. The median lethal dose (LD50) of SECL, EECL, and TECL was not determined as no deaths were observed in animals treated with doses up to 2,000 mg/kg orally. Final body weight and body weight gains throughout the entire experimental period were similar among all experimental groups. Additionally, except for the first day after treatment, daily food and water consumption in animals treated with the different extracts followed the same pattern as observed in animals treated with the vehicle. There were no significant differences in absolute (g) or relative (%) weight of all isolated organs of rats after 14 days of treatment. Macroscopic examination of vital organs did not reveal any abnormalities among all experimental groups. No signs of inflammation, hemorrhage, fluid accumulation, or other suggestive changes of lesions were observed in all tissues studied.

Subacute toxicity

During the 28 days of the study, all animals displayed normal activity and behavior for their species and gender. There were no noticeable changes in appearance or general behavior until the end of the experimental period. No deaths or significant gastrointestinal issues, such as diarrhea or loss of appetite, were observed in animals treated with either the vehicle or the various formulations tested.

Table 1 displays the data concerning the initial and final body weight of male and female rats that were orally treated with various doses of SECL, EECL, and TECL or the vehicle. There were no significant changes observed in the final body weight or weight gain in animals treated with SECL and TECL compared to the group treated with the vehicle only. However, male rats treated with EECL showed a statistically higher final body weight than those treated with the vehicle alone. This change in body weight was not observed in female rats. Daily food and water consumption was not altered by any of the treatments, maintaining a regular pattern for the species and gender.

None of the treatments had a significant effect on the absolute or relative weight of isolated organs in male or female rats that were treated with various doses of SECL, EECL, and TECL or the vehicle after 28 days of experimentation, as shown in Tables 2 and 3.

Tables 4 and 5 display the hematological parameters of male and female rats treated for 28 days with SECL, EECL, and TECL or the vehicle. There were no statistically significant differences between the groups treated with the extracts (SECL, EECL, and TECL) and the animals that only received the vehicle.

Tables 6 and 7 display the results of serum biochemical analyses conducted on male and female rats that were treated with either SECL, EECL, TECL, or the vehicle, over a period of 28 days. None of the parameters measured showed any statistically significant changes when

comparing animals of both sexes treated with the extracts to those that received only the vehicle.

Tissues collected from male and female rats treated for 28 days with SECL, EECL, and TECL or the control did not show any morphological changes. The examined lung, heart, liver, kidney, and spleen samples did not display any signs of abnormal cell growth, inflammation, apoptosis, or necrosis (Figures 1 to 5). The heart tissues appeared normal under the microscope, showing no signs of scarring, enlargement, or areas of reduced blood supply (Figures 2). In the liver tissue, the structural organization was normal with hepatocytes arranged as expected, divided into zones 1 and 2 (Figures 3). The connective tissue capsule in the spleen and red pulp appeared normal, with no morphological changes observed. The white pulp also showed no changes in the germinal center (Figures 5). Kidney tissues did not display any abnormalities in the renal corpuscles, tubules, or renal pelvis (Figures 4). Lung tissues maintained their usual structure without any evidence of inflammation or damage to the epithelial cells in the bronchi, bronchioles, and alveoli (Figures 1).

DISCUSSION

In this study, safety data are presented for the use of three different preparations obtained from the seeds of *C. lanatus*, including a preparation that is traditionally used in Brazil for juice production, i.e. TECL. In addition, the use of the other two preparations, namely SECL and EECL, was employed with the aim of extracting and concentrating a greater quantity of secondary metabolites, in order to evaluate if the toxicity would be proportional to the concentration of different phytochemicals (10).

The acute toxicity of SECL, EECL, and TECL was assessed following the guidelines of OECD guideline 425 (8). Typically, acute toxicity studies investigate the effects of a test substance

when given in one or more doses over a 24-hour period and then followed by a 14-day observation (11) The method also enables the estimation of an LD_{50} with a confidence interval. Moreover, the results allow for the ranking and classification of a substance according to the Globally Harmonized System for the Classification of Chemicals that cause acute toxicity (12). In our study, female rats that were given a single dose of SECL, EECL, and TECL did not show any changes in behavior, food and water intake, body weight gain, relative organ weight, or in pathological analysis. Therefore, it can be concluded that all tested extracts are safe and have an LD_{50} above 2,000 mg/kg. With data similar to that obtained in our study, Belemkar and Shendge (13) showed that the ethanolic extract of *C. lanatus* seed administered orally at a dose of 2,000 mg/kg in Wistar rats did not induce any acute signs of toxicity, including behavioral or clinical changes.

For the evaluation of subacute toxicity, we chose to follow the recommendations presented in OECD guideline no. 407 (Repeated Dose 28-day Oral Toxicity Study in Rodents). This method provides a variety of information on the health hazards that are likely to arise from exposure to the test substance after repeated doses via oral administration. This guideline is primarily intended for use in rats, preferably with a minimum of 5 males and 5 females for each dose tested. The report of this study will include results from clinical and functional observations, body weight and food/water consumption measurements, hematology and clinical biochemistry, as well as gross necropsy and histopathology.

Our data enhances previous information and presents SECL, EECL, and TECL as preparations with low potential for inducing cumulative toxic effects. After 28 days of study, the animals showed normal behavior for the species and gender, as well as a hematological and biochemical profile similar to animals treated only with filtered water (vehicle). Furthermore, no histopathological alteration was evidenced in any of the experimental groups.

One data that caught our attention was the significant increase in weight in the male animals treated with all doses of the ethanolic extract (EECL). Speculatively, we imagine that, due to the absence of toxicity, this effect may be due to the high concentration of phytochemicals with nutritional properties present in this type of formulation. However, further studies should be conducted in order to explore this alteration in detail.

A study conducted previously by Belemkar and Shendge (13) also evaluated the effects of the ethanolic extract of *C. lanatus*. Administering doses of 250, 500, and 1000 mg/kg for 28 days in rats did not reveal any harmful effects on behavior, body weight, biochemical, hematological, or histopathological parameters. Similarly, other studies have already demonstrated the protective effects of *C. lanatus* in various experimental models, including the hepato- and neuro-protective effects on acute ethanol-induced oxidative stress in rats (14), protective effects against aluminum chloride-induced testosterone, testicular, and hematological changes in an experimental male rat model (15), as well as protection against deleterious effects of nicotine on some reproductive indices in male Wistar rats (16).

This study presented the results of various toxicity tests using three preparations obtained from the seeds of *C. lanatus* traditionally used in Brazil. Although the results may suggest potential safety in using SECL, EECL, and TECL, additional toxicological studies, particularly regarding the central nervous, respiratory, gastrointestinal, hematopoietic, reproductive, and cardiovascular systems, are essential before conducting clinical trials.

CONCLUSION

Our findings suggest that different preparations obtained from *C. lanatus* seeds, traditionally used in Brazil, are safe and offer some protection to body organs. Furthermore, the extracts can be further evaluated in preclinical and clinical studies for their safety and therapeutic effects.

ACKNOWLEDGMENTS

We are also grateful to the Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT, Brazil) for their grant's numbers 71/700.135/2018 and 83/013.186/2023. We also extend our gratitude to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) for their grant number 407685/2018-9 and 150258/2023-2, and to the Coordenadoria de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil) for their financial support.

AUTHOR CONTRIBUTIONS

All authors participated in the design, interpretation of the studies,
analysis of the data and review of the manuscript; AAMM, GPS, KGTM, LABL, LBP, KSL and MLFS conducted the experiments FFBJ responsible for hematologiacal analizes; RICS and ACS were responsible for histopathological analizes. AAMM and AGJ were responsible for data discussion and manuscript correction. AGJ was the senior researcher responsible for this work. All authors have read and agreed to the published version of the manuscript

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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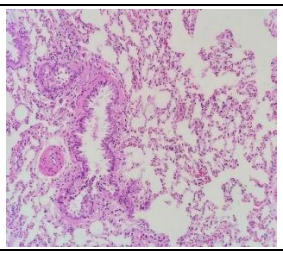
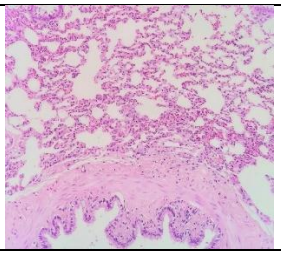
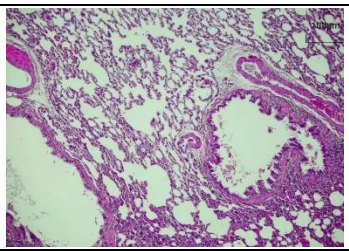
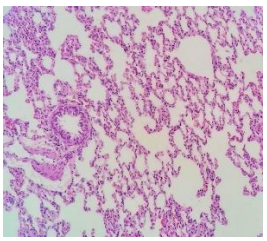
LEGEND TO FIGURES

Figure 1. Representative photomicrographs of histological slides obtained from the lungs of rats treated with the highest dose (300 mg/kg) of SECL, EECL, and TECL. H&E (hematoxylin and eosin) staining. 40X magnification.

MALE RATS			
			
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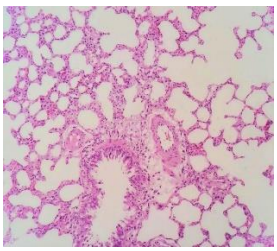
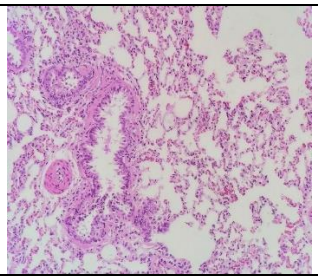
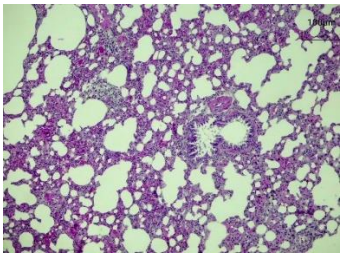
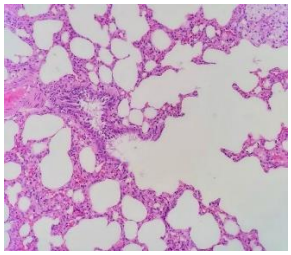
			
Control; Lung	SECL 300 mg/kg; Lung	EECL 300 mg/kg; Lung	TECL 300 mg/kg; Lung

Figure 1
Marques et al.

Figure 2. Representative photomicrographs of histological slides obtained from the heart of rats treated with the highest dose (300 mg/kg) of SECL, EECL, and TECL. H&E (hematoxylin and eosin) staining. 40X magnification.

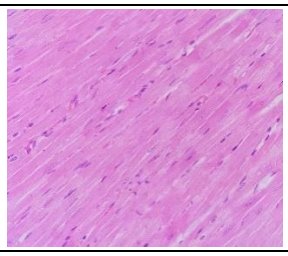
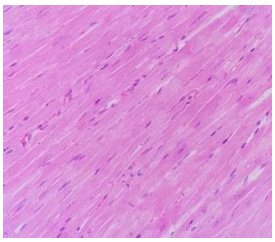
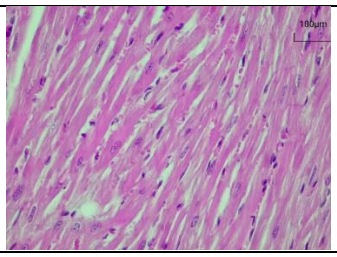
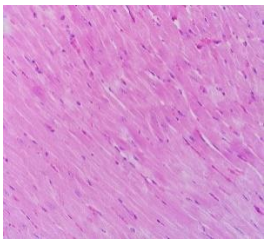
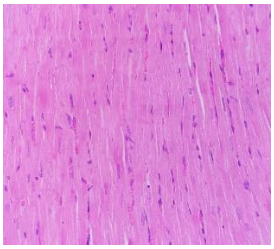
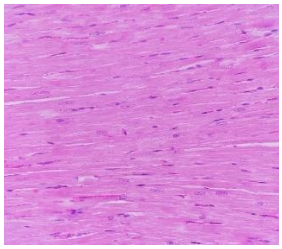
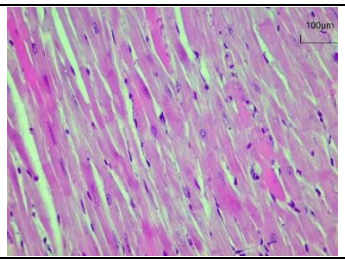
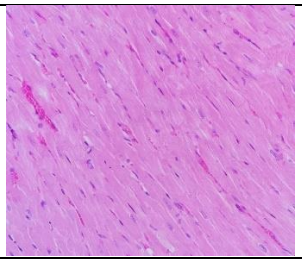
MALE RATS			
			
Control; Heart	SECL 300 mg/kg; Heart	EECL 300 mg/kg; Heart	TECL 300 mg/kg; Heart
FEMALE RATS			
			
Control; Heart	SECL 300 mg/kg; Heart	EECL 300 mg/kg; Heart	TECL 300 mg/kg; Heart

Figure 2
Marques et al.

Figure 3. Representative photomicrographs of histological slides obtained from the liver of rats treated with the highest dose (300 mg/kg) of SECL, EECL, and TECL. H&E (hematoxylin and eosin) staining. 40X magnification.

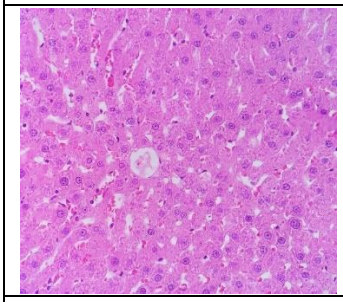
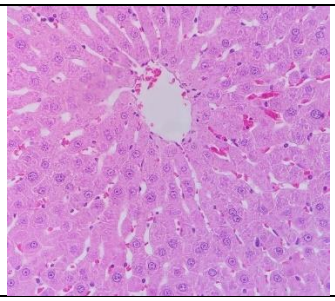
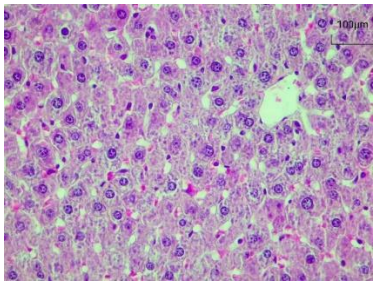
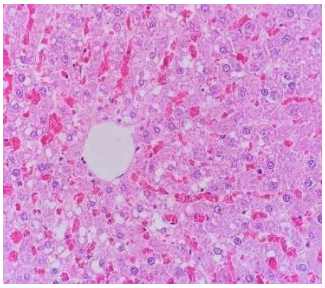
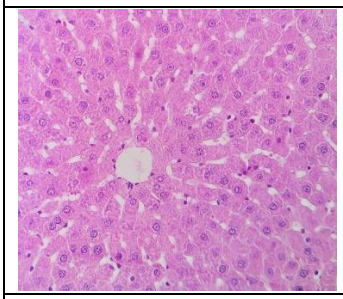
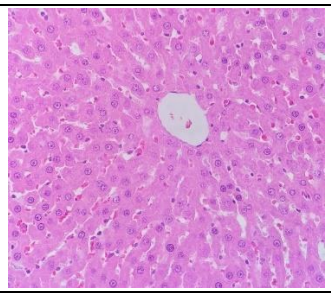
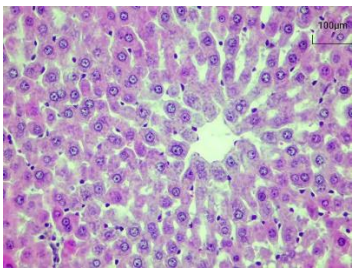
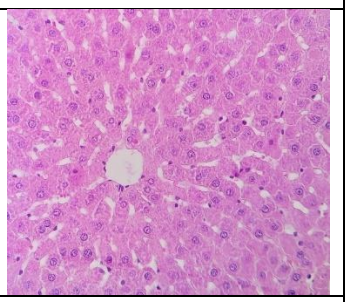
MALE RATS			
			
Control; Liver	SECL 300 mg/kg; Liver	EECL 300 mg/kg; Liver	TECL 300 mg/kg; Liver
FEMALE RATS			
			
Control; Liver	SECL 300 mg/kg; Liver	EECL 300 mg/kg; Liver	TECL 300 mg/kg; Liver

Figure 3
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Figure 4. Representative photomicrographs of histological slides obtained from the kidney of rats treated with the highest dose (300 mg/kg) of SECL, EECL, and TECL. H&E (hematoxylin and eosin) staining. 40X magnification.

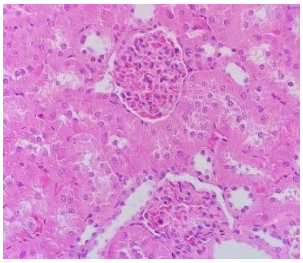
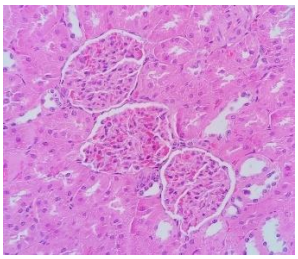
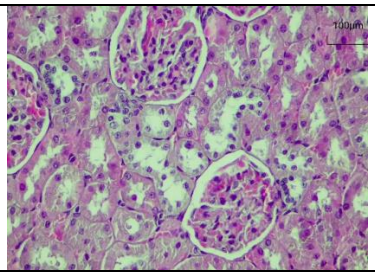
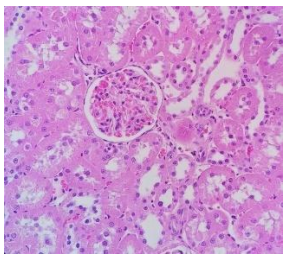
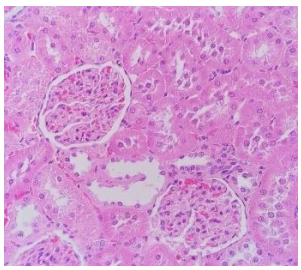
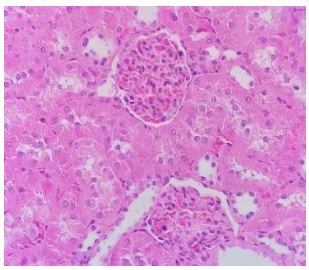
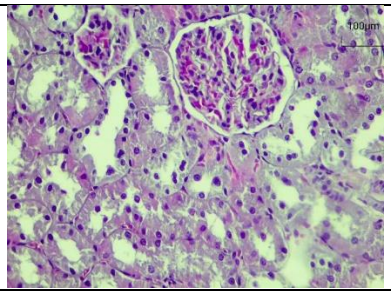
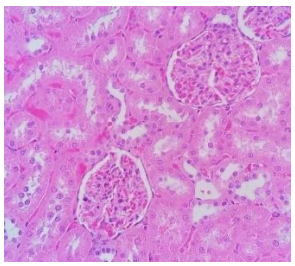
MALE RATS			
			
Control; Kidney	SECL 300 mg/kg; Kidney	EECL 300 mg/kg; Kidney	TECL 300 mg/kg; Kidney
FEMALE RATS			
			
Control; Kidney	SECL 300 mg/kg; Kidney	EECL 300 mg/kg; Kidney	TECL 300 mg/kg; Kidney

Figure 4
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Figure 5. Representative photomicrographs of histological slides obtained from the spleen of rats treated with the highest dose (300 mg/kg) of SECL, EECL, and TECL. H&E (hematoxylin and eosin) staining. 40X magnification.

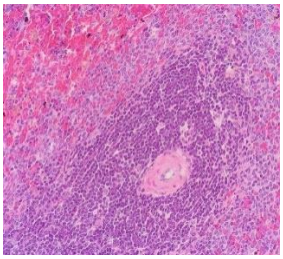
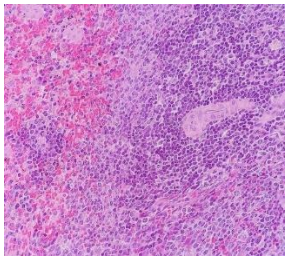
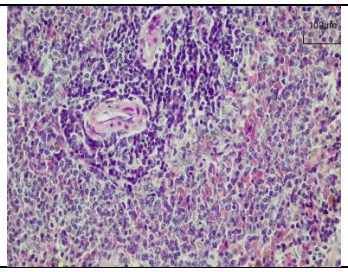
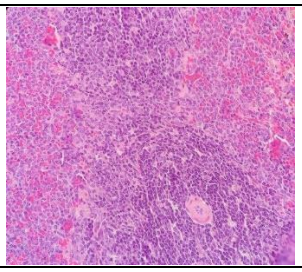
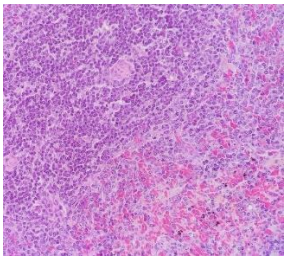
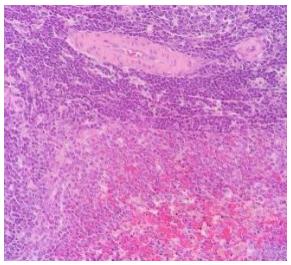
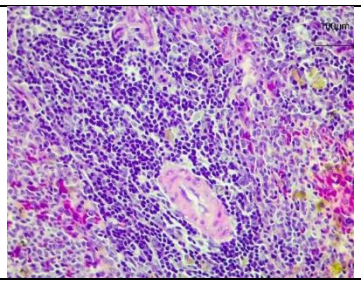
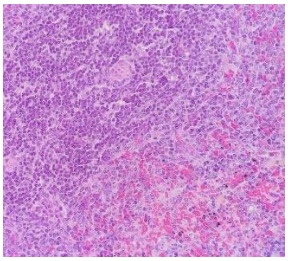
MALE RATS			
			
Control; Spleen	SECL 300 mg/kg; Spleen	EECL 300 mg/kg; Spleen	TECL 300 mg/kg; Spleen
FEMALE RATS			
			
Control; Spleen	SECL 300 mg/kg; Spleen	EECL 300 mg/kg; Spleen	TECL 300 mg/kg; Spleen

Figure 5
Marques et al.

Figure 6. Representative photomicrographs of histological slides obtained from the epididymis and uterus of rats treated with the highest dose (300 mg/kg) of SECL, EECL, and TECL. H&E (hematoxylin and eosin) staining. 40X magnification.

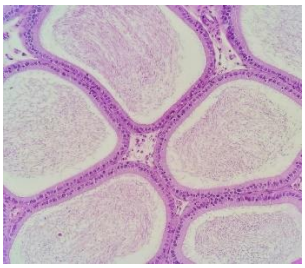
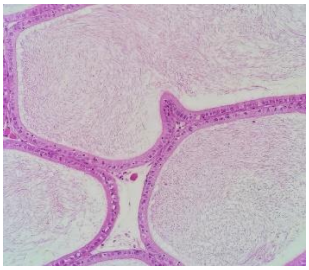
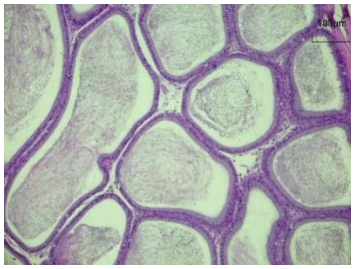
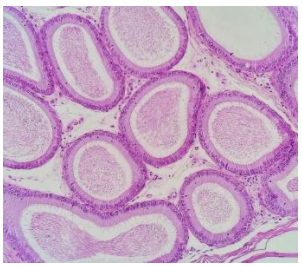
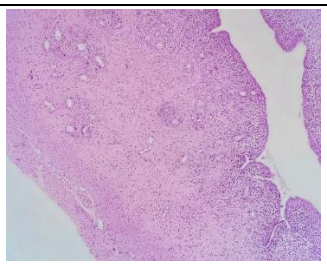
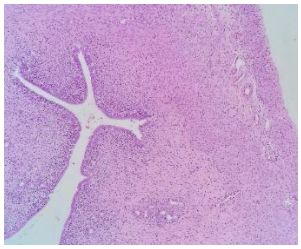
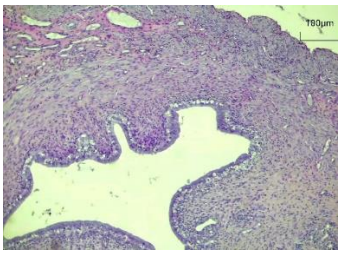
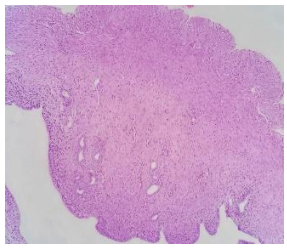
MALE RATS			
			
Control; Epididymis	SECL 300 mg/kg; Epididymis	EECL 300 mg/kg; Epididymis	TECL 300 mg/kg; Epididymis
FEMALE RATS			
			
Control; Uterus	SECL 300 mg/kg; Uterus	EECL 300 mg/kg; Uterus	TECL 300 mg/kg; Uterus

Figure 6
Marques et al.

Figure 7. Representative photomicrographs of histological slides obtained from the testicles and ovary of rats treated with the highest dose (300 mg/kg) of SECL, EECL, and TECL. H&E (hematoxylin and eosin) staining. 40X magnification.

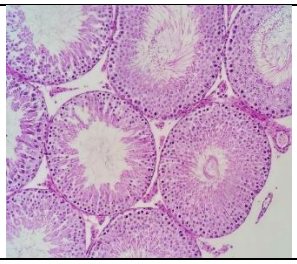
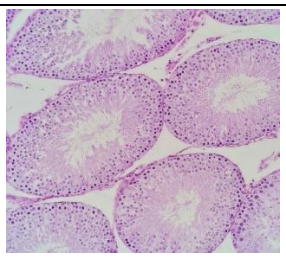
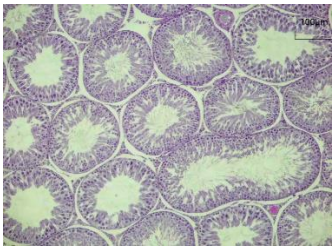
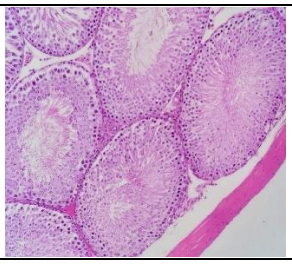
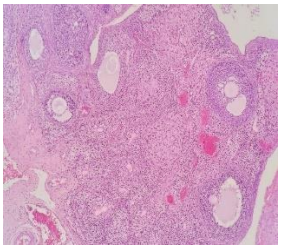
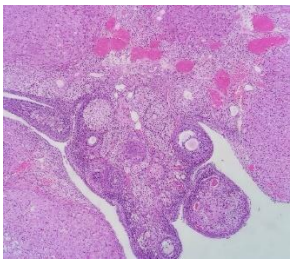
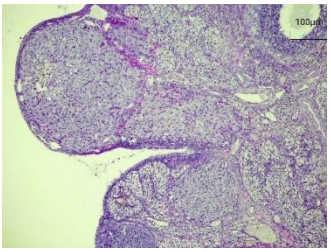
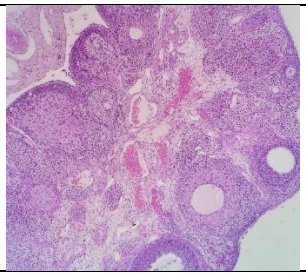
MALE RATS			
			
Control; Testicles	SECL 300 mg/kg; Testicles	EECL 300 mg/kg; Testicles	TECL 300 mg/kg; Testicles
FEMALE RATS			
			
Control; Ovary	SECL 300 mg/kg; Ovary	EECL 300 mg/kg; Ovary	TECL 300 mg/kg; Ovary

Figure 7
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Table 1. Effects of prolonged (28-day) treatment with different extracts from *C. lanatus* on the body weight of male and female Wistar rats.

Sex	Parameter	Control	SECl (30 mg)	SECL (100 mg)	SECL (300 mg)	EECL (30 mg)	EECL (100 mg)	EECL (300 mg)	TECL (30 mg)	TECL (100 mg)	TECL (300 mg)
Female	**Initial weight**	261.0±28.2	263.7±42.6	283.5±17.6	288.3±17.7	252.0±27.5	255.7±15.2	256.7±5.3	281.7±12.5	287.2±11.9	279.8±4.4
	Final weight	243.0±9.5	240.0±24.6	239.8±19.8	247.8±14.9	266.3±30.7	273.0±11.8	267.0±6.9	245.0±11.8	241.7±8.2	241.2±4.5
Male	**Initial weight**	357.6±6.6	318.3±160.2	371.3±28.5	368.2±29.3	410.7±26.9	410. ±36.9	454.1±30.4	373.0±17.1	374.5±20.6	359.6±8.8
	Final weight	355.5±4.6	379.8±50.1	379.7±15.8	373.3±32.3	438.3±30.8[a]	421.9±43.9[a]	458.9±28.5[a]	364.0±21.0	382.0±15.7	365.0±14.6

Statistical analyses were performed using one-way ANOVA followed by Dunnett's test. Values are expressed as mean ± SEM (standard error of the mean) of 6 rats per group. [a] $p \leq 0.05$ when compared to the control group. Control: animals treated with water; SECL: animals that received different doses of aqueous extract of *Citrullus lanatus*; EECL: animals that received different doses of ethanolic extract *Citrullus lanatus* TECL: animals that received different doses of Turbolysis of *Citrullus lanatus.*

Table 2. Effects of prolonged (28-day) treatment with different extracts from *C. lanatus* on the relative organ weight of male Wistar rats.

Parameter	Control	SECL (30 mg)	SECL (100 mg)	SECL (300 mg)	EECL (30 mg)	EECL (100 mg)	EECL (300 mg)	TECL (30 mg)	TECL (100 mg)	TECL (300 mg)
Heart (%)	0,28±0,009	0,27±0,01	0,27±0,01	0,26±0,007	0,22±0,008	0,22±0,006	0,22±0,005	0,29±0,02	0,26±0,01	0,23±0,05
Lung (%)	0,36±0,002	0,39±0,02	0,39±0,02	0,37±0,01	0,34±0,004	0,37±0,02	0,35±0,008	0,50±0,06	0,36±0,01	0,29±0,07
Spleen (%)	0,22±0,009	0,22±0,009	0,19±0,04	0,26±0,02	0,22±0,009	0,19±0,007	0,17±0,007	0,22±0,01	0,19±0,01	0,18±0,04
Kidney R (%)	0,32±0,007	0,33±0,009	0,32±0,02	0,31±0,01	0,28±0,01	0,29±0,004	0,29±0,01	0,33±0,01	0,37±0,01	0,27±0,05
Kidney L (%)	0,31±0,009	0,32±0,009	0,33±0,02	0,30±0,01	0,28±0,01	0,28±0,002	0,29±0,01	0,41±0,08	0,34±0,02	0,26±0,05
Liver (%)	3,04±0,15	3,24±0,14	0,08±0,08	2,90±0,08	2,99±0,08	2,39±0,13	2,71±0,06	4,01±0,38	3,66±0,17	2,60±0,53
Epididymis	0,33±0,01	0,31±0,02	0,33±0,01	0,32±0,02	0,27±0,1	0,26±0,01	0,31±0,04	0,34±0,04	0,29±0,01	0,40±0,10
Testicles	0,85±0,04	0,87±0,06	0,94±0,04	0,88±0,02	0,68±0,02	0,70±0,02	0,77±0,02	0,89±0,03	0,83±0,04	0,80±0,12

Statistical analyses were performed using one-way ANOVA followed by Dunnett's test. Values are expressed as mean ± SEM (standard error of the mean) of 6 rats per group. [a] $p \leq 0.05$ when compared to the control group. Relative organ weight (absolute organ weight × 100/body weight). Control: animals treated with water; SECL: animals that received different doses of aqueous extract of *Citrullus lanatus*; EECL: animals that received different doses of ethanolic extract *Citrullus lanatus* TECL: animals that received different doses of Turbolysis of *Citrullus lanatus.*

Table 3. Effects of prolonged (28-day) treatment with different extracts from *C. lanatus* on the relative organ weight of female Wistar rats.

Parameter	Control	SECL (30 mg)	SECL (100 mg)	SECL (300 mg)	EECL (30 mg)	EECL (100 mg)	EECL (300 mg)	TECL (30 mg)	TECL (100 mg)	TECL (300 mg)
Heart (%)	0,26±0,05	0,33±0,02	0,32±0,01	0,30±0,01	0,26±0,009	0,26±0,006	0,26±0,007	0,32±0,01	0,32±0,01	0,31±0,01
Lung (%)	0,38±0,01	0,50±0,02	0,48±0,02	0,41±0,01	0,42±0,01	0,47±0,01	0,44±0,009	0,47±0,01	0,50±0,02	0,44±0,01
Spleen (%)	0,21±0,05	0,24±0,006	0,24±0,01	0,23±0,01	0,23±0,01	0,21±0,004	0,21±0,009	0,23±0,01	0,24±0,01	0,24±0,02
Kidney R (%)	0,26±0,05	0,36±0,03	0,35±0,009	0,29±0,01	0,28±0,01	0,28±0,01	0,30±0,003	0,35±0,02	0,33±0,01	0,31±0,02
Kidney L (%)	0,24±0,05	0,33±0,02	0,31±0,01	0,29±0,01	0,27±0,01	0,27±0,006	0,28±0,006	0,33±0,01	0,31±0,01	0,30±0,01
Liver (%)	3,32±0,68	4,64±0,3	4,17±0,2	3,76±0,2	3,42±0,19	3,07±0,07	2,78±0,1	4,5±0,22	4,26±0,2	3,89±0,17
Uterus (%)	0,17±0,04	0,22±0,01	0,23±0,02	0,29±0,04	0,24±0,02	0,26±0,02	0,27±0,03	0,22±0,02	0,28±0,03	0,25±0,02
Ovary R (%)	0,03±0,01	0,11±0,06	0,04±0,002	0,03±0,001	0,02±0,004	0,03±0,0007	0,03±0,0006	0,09±0,04	0,04±0,003	0,03±0,002
Ovary (%)	0,07±0,03	0,10±0,06	0,03±0,002	0,03±0,003	0,25±0,001	0,26±0,006	0,27±0,002	0,04±0,004	0,03±0,003	0,04±0,004

Statistical analyses were performed using one-way ANOVA followed by Dunnett's test. Values are expressed as mean ± SEM (standard error of the mean) of 6 rats per group. [a] $p \leq 0.05$ when compared to the control group. Relative organ weight (absolute organ weight × 100/body weight). Control: animals treated with water; SECL: animals that received different doses of aqueous extract of *Citrullus lanatus*; EECl: animals that received different doses of ethanolic extract *Citrullus lanatus* TECL: animals that received different doses of Turbolysis of *Citrullus lanatus.*

Table 4. Effects of prolonged (28-day) treatment with different extracts from *C. lanatus* on the hematological parameters of male Wistar rats.

Parameter	Control	SECL (30 mg)	SECL (100 mg)	SECL (300 mg)	EECL (30 mg)	EECL (100 mg)	EECL (300 mg)	TECL (30 mg)	TECL (100 mg)	TECL (300 mg)
RBC (106/µl)	8.72 ± 0.26	8.01 ± 0.24	7.53 ± 0.11	7.54 ± 0.19	7.53 ± 0.19	8.72 ± 1.08	10.67 ± 0.10	11.61 ± 0.06	12.07 ± 0.19	11.97 ± 0.19
Hemoglobin (g/dL)	14.34 ± 0.40	15.07 ± 0.29	14.53 ± 0.20	14.73 ± 0.22	14.57 ± 0.41	15.83 ± 0.98	17.90 ± 0.11	16.27 ± 0.23	15.63 ± 0.18	15.77 ± 0.09
Hematocrit (%)	44.24 ± 1.52	44.10 ± 1.30	42.20 ± 0.35	42.53 ± 0.75	42.37 ± 1.58	48.33 ± 4.09	56.00 ± 0.36	55.33 ± 0.38	54.70 ± 0.76	54.57 ± 0.85
MCV (fL)	50.66 ± 0.37	55.07 ± 0.07	56.07 ± 0.38	56.40 ± 0.95	56.20 ± 1.04	55.93 ± 2.05	52.43 ± 0.30	53.30 ± 0.73	52.63 ± 0.39	53.43 ± 0.17
MCH (pg)	16.47 ± 0.16	18.83 ± 0.22	19.33 ± 0.07	19.57 ± 0.47	19.33 ± 0.07	18.43 ± 1.04	16.53 ± 0.12	17.37 ± 0.12	17.30 ± 0.06	17.53 ± 0.22
MCHC (g/dL)	32.50 ± 0.31	34.17 ± 0.39	33.53 ± 1.09	34.67 ± 0.26	34.40 ± 0.52	32.87 ± 0.70	31.60 ± 0.30	33.00 ± 0.47	32.67 ± 0.03	32.87 ± 0.03
WBC (103/µl)	8.80 ± 1.06	9.07 ± 1.05	8.57 ± 0.35	10.53 ± 0.74	9.17 ± 0.41	11.03 ± 1.65	13.23 ± 0.03	14.10 ± 0.06	14.00 ± 0.21	14.83 ± 0.18
Lymphocyte (103/µl)	8.26 ± 1.16	12.00 ± 2.08	9.33 ± 1.20	10.33 ± 0.67	13.33 ± 1.33	13.00 ± 1.73	11.67 ± 1.20	10.00 ± 0.58	10.67 ± 0.33	10.67 ± 0.33
Neutrophil (103/µl)	0.87 ± 0.15	84.67 ± 2.90	88.33 ± 0.88	87.00 ± 0.58	83.67 ± 1.85	84.00 ± 2.31	84.67 ± 0.88	87.00 ± 0.58	85.33 ± 0.33	86.33 ± 0.67
Platelets (103/µl)	769.60 ± 26.92	957.00 ± 83.03	842.00 ± 72.52	849.00 ± 30.66	980.33 ± 116.37	955.67 ± 33.53	917.67 ± 10.17	825.33 ± 3.28	937.67 ± 1.86	924.00 ± 3.61
RDW (%)	16.68 ± 0.47	26.60 ± 0.11	27.37 ± 0.20	27.63 ± 0.18	27.23 ± 0.38	28.00 ± 0.36	29.33 ± 0.12	29.07 ± 0.13	29.33 ± 0.17	28.83 ± 0.12
MPV (fL)	7.56 ± 0.11	7.10 ± 0.11	7.20 ± 0.06	7.20 ± 0.10	7.07 ± 0.07	7.27 ± 0.03	7.43 ± 0.03	7.43 ± 0.07	7.27 ± 0.03	7.27 ± 0.03

Statistical analyses were performed using one-way ANOVA followed by Dunnett post hoc test. The results are expressed as mean ± standard error of the mean (S.E.M.) and p-value of less than 0.05 was considered statistically significant. WBC: white blood cells; RBC: red blood cells; MCV: mean corpuscular volume; MHC: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red blood cell distribution width; MPV: mean platelet volume. Control: animals treated with water; SECL: animals that received different doses of aqueous extract of *Citrullus lanatus*; EECl: animals that received different doses of ethanolic extract *Citrullus lanatus* TECL: animals that received different doses of Turbolysis of *Citrullus lanatus.*

Table 5. Effects of prolonged (28-day) treatment with different extracts from *C. lanatus* on the hematological parameters of female Wistar rats.

Parameter	Control	SECL (30 mg)	SECL (100 mg)	SECL (300 mg)	EECL (30 mg)	EECL (100 mg)	EECL (300 mg)	TECL (30 mg)	TECL (100 mg)	TECL (300 mg)
RBC (106/µl)	8.24 ± 0.16	8.50 ± 0.24	8,78 ± 0.28	8.51 ± 0.59	8.95 ± 0.23	8.97 ± 0.13	8.84 ± 0.15	8.90 ± 0.006	8.78 ± 0.19	8.90 ± 0.36
Hemoglobin (g/dL)	14.23 ± 0.25	14.70 ± 0.66	15.70 ± 0.21	15.97 ± 0.59	15.70 ± 0.35	15.70 ± 0.15	15.57 ± 0.22	15.73 ± 0.08	15.40 ± 0.35	15.73 ± 0.33
Hematocrit (%)	44.25 ± 0.90	34.80 ± 9.68	46.97 ± 0.92	46.17 ± 2.49	47.80 ± 0.12	47.63 ± 0.64	46.63 ± 0.86	46.90 ± 0.11	45.67 ± 1.12	47.37 ± 1.52
MCV (fL)	53.70 ± 0.54	52.70 ± 0.06	53.53 ± 0.93	55.70 ± 1.32	53.40 ± 0.15	53.13 ± 0.32	52.77 ± 0.07	52.97 ± 0.12	53.07 ± 0.12	53.07 ± 0.13
MCH (pg)	53.70 ± 0.16	17.27 ± 0.28	17.90 ± 0.46	18.14 ± 0.69	17.53 ± 0.09	17.47 ± 0.12	17.60 ± 0.06	17.63 ± 0.08	17.43 ± 0.22	17.80 ± 0.15
MCHC (g/dL)	32.16 ± 0.16	32.77 ± 0.53	33.43 ± 0.23	32.80 ± 0.75	32.83 ± 0.09	32.87 ± 0.23	33.37 ± 0.18	33.63 ± 0.17	33.33 ± 0.03	33.60 ± 0.40
WBC (103/µl)	11.12 ± 1.57	5.57 ± 0.55	7.30 ± 1.28	6.53 ± 0.47	6.67 ± 0.86	9.57 ± 0.35	7.77 ± 0.62	8.13 ± 0.13	6.47 ± 0.13	7.60 ± 0.66
Lymphocyte (103/µl)	10.03 ± 1.43	7.33 ± 1.33	8.33 ± 1.33	9.67 ± 0.88	8.33 ± 2.03	8.00 ± 0.58	10.33 ± 0.33	9.00 ± 2.08	9.67 ± 0.33	10.00 ± 2.31
Neutrophil (103/µl)	0.50 ± 0.08	89.33 ± 1.20	88.67 ± 0.33	89.00 ± 1.00	88.33 ± 1.20	89.00 ± 0.58	87.67 ± 0.88	87.33 ± 1.45	87.67 ± 0.33	87.00 ± 2.52
Platelets (103/µl)	882.60 ± 27.87	867.33 ± 24.83	869.00 ± 38.18	840.33 ± 15.32	925.33 ± 42.60	865.33 ± 29.79	976.67 ± 19.61	954.67 ± 10.09	810.00 ± 20.82	901.00 ± 16.80
RDW (%)	13.84 ± 0.19	29.07 ± 0.09	28.70 ± 0.76	28.00 ± 0.46	29.10 ± 0.21	29.47 ± 0.08	29.03 ± 0.12	28.87 ± 0.03	29.63 ± 0.08	28.93 ± 0.18
MPV (fL)	7.39 ± 0.09	7.43 ± 0.12	7.57 ± 0.18	7.50 ± 0.10	7.87 ± 0.44	7.70 ± 0.15	7.40 ± 0.10	7.33 ± 0.08	7.17 ± 0.03	7.30 ± 0.10

Statistical analyses were performed using one-way ANOVA followed by Dunnett post hoc test. The results are expressed as mean ± standard error of the mean (S.E.M.) and p-value of less than 0.05 was considered statistically significant. WBC: white blood cells; RBC: red blood cells; MCV: mean corpuscular volume; MHC: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red blood cell distribution width; MPV: mean platelet volume. Control: animals treated with water; SECL: animals that received different doses of aqueous extract of *Citrullus lanatus*; EECl: animals that received different doses of ethanolic extract *Citrullus lanatus* TECL: animals that received different doses of Turbolysis of *Citrullus lanatus.*

Table 6. Effects of prolonged (28-day) treatment with different extracts from *C. lanatus* on the biochemical parameters of male Wistar rats.

Parameter	Control	SECL (30 mg)	SECL (100 mg)	SECL (300 mg)	EECL (30 mg)	EECL (100 mg)	EECL (300 mg)	TECL (30 mg)	TECL (100 mg)	TECL (300 mg)
Amylase (U/L)	525±35	527±32	529±33	530±33	535±35	531±38	527±33	531±32	533±32	528±30
Urea (mg/dL)	43±8.1	45±6.8	48±7.5	47±8.9	42±9.3	45±8.1	52±9.2	46±8.2	46±8.4	48±8.1
Creatinine (mg/dL)	0.52±0.06	0.54±0.07	0.53±0.07	0.56±0.07	0.54±0.09	0.53±0.07	0.50±0.10	0.56±0.09	0.57±0.09	0.55±0.04
Uric acid (mg/dL)	3.15±0.54	3.14±0.66	3.13±0.51	3.11±0.52	3.12±0.51	3.14±0.51	3.12±0.48	3.19±0.50	3.21±0.66	3.16±0.54
Potassium (mEq/L)	5.44±0.41	5.39±0.55	5.36±0.42	5.34±0.44	5.40±0.51	5.30±0.63	5.38±0.51	5.42±0.63	5.39±0.44	5.32±0.40
Sodium (mEq/L)	142±6.27	138±7.21	137±8.22	139±7.33	133±9.21	134±7.12	140±8.11	135±7.77	139±6.66	135±7.44
TP (g/dL)	5.77±0.84	5.69±0.88	5.75±0.91	5.52±0.88	5.66±0.99	5.44±0.88	5.59±0.77	5.60±0.83	5.62±0.90	5.44±0.92
Albumin (g/dL)	3.16±0.55	3.20±0.47	3.19±0.55	3.42±0.47	3.32±0.57	3.20±0.63	3.30±0.55	3.27±0.41	3.29±0.39	3.24±0.53
Globulin (g/dL)	2.27±0.39	2.30±0.33	2.40±0.42	2.38±0.42	2.40±0.52	2.43±0.40	2.33±0.44	2.31±0.54	2.32±0.37	2.36±0.44
AP (U/L)	125±19	123±17	129±14	126±16	122±17	125±18	127±18	128±14	125±16	123±19
TG (mg/dL)	52±7.7	49±5.5	44±6.6	45±5.9	43±6.1	49±5.6	50±6.2	42±6.1	44±5.1	43±5.6
TC (mg/dL)	65± 8.3	62± 5.8	57± 6.8	58± 6.6	60± 6.6	55± 6.4	61± 5.9	56± 7.3	57± 5.0	56± 5.2
TB (mg/dL)	0.13±0.02	0.12±0.03	0.11±0.03	0.12±0.03	0.12±0.03	0.13±0.02	0.11±0.03	0.13±0.02	0.12±0.03	0.12±0.03
AST (U/L)	39±4.2	38±3.7	34±6.3	35±5.8	33±4.6	35±3.6	34±4.0	36±3.3	34±3.5	37±3.9
ALT (U/L)	35±3.8	36±4.4	35±4.6	37±4.8	34±5.3	36±4.9	34±4.9	35±4.9	36±4.4	38±4.6

Statistical analyses were performed using one-way ANOVA followed by Dunnett's test. Values are expressed as mean ± SEM (standard error of the mean) of 6 rats per group. ALT: alanine transaminase; AP: alkaline phosphatase; AST: aspartate aminotransferase; TB: total bilirubin; TC: total cholesterol; TG: triglycerides; TP: total protein. Control: animals treated with water; SECL: animals that received different doses of aqueous extract of *Citrullus lanatus*; EECl: animals that received different doses of ethanolic extract *Citrullus lanatus* TECL: animals that received different doses of Turbolysis of *Citrullus lanatus.*

Table 6. Effects of prolonged (28-day) treatment with different extracts from *C. lanatus* on the biochemical parameters of female Wistar rats.

Parameter	Control	SECL (30 mg)	SECL (100 mg)	SECL (300 mg)	EECL (30 mg)	EECL (100 mg)	EECL (300 mg)	TECL (30 mg)	TECL (100 mg)	TECL (300 mg)
Amylase (U/L)	504±54	510±35	514±33	508±43	511±39	513±31	507±42	512±41	510±31	503±52
Urea (mg/dL)	30±5.6	35±6.3	37±7.7	31±5.2	33±6.1	36±7.4	32±5.4	36±6.1	33±7.1	31±5.3
Creatinine (mg/dL)	0.34±0.06	0.36±0.07	0.36±0.05	0.37±0.05	0.39±0.09	0.35±0.06	0.33±0.09	0.34±0.08	0.32±0.07	0.35±0.09
Uric acid (mg/dL)	2.57±0.84	2.61±0.50	2.41±0.64	2.42±0.73	2.51±0.42	2.44±0.71	2.50±0.91	2.55±0.53	2.55±0.72	2.54±0.82
Potassium (mEq/L)	5.15±0.63	5.20±0.49	5.19±0.36	5.18±0.53	5.22±0.51	5.21±0.34	5.17±0.65	5.24±0.55	5.21±0.40	5.19±0.77
Sodium (mEq/L)	149±11.2	142±9.2	146±7.9	151±12.4	155±9.0	149±7.1	146±9.7	148±9.5	149±7.1	145±10.1
TP (g/dL)	5.13±0.88	5.31±0.90	5.25±0.74	5.23±0.64	5.40±0.99	5.22±0.71	5.17±0.81	5.23±0.98	5.30±0.88	5.11±0.74
Albumin (g/dL)	3.12±0.52	3.20±0.48	3.15±0.46	3.16±0.56	3.22±0.62	3.20±0.55	3.15±0.55	3.21±0.53	3.14±0.54	3.11±0.73
Globulin (g/dL)	2.13±0.44	2.14±0.35	2.20±0.35	2.18±0.41	2.17±0.42	2.22±0.31	2.19±0.41	2.21±0.40	2.18±0.40	2.10±0.42
AP (U/L)	110±11	115±12	120±17	114±12	116±13	122±21	119±14	117±15	120±12	113±14
TG (mg/dL)	54±6.6	49±6.1	50±5.9	51±6.1	45±9.2	53±6.6	51±6.9	46±6.6	53±5.1	53±6.1
TC (mg/dL)	69± 7.4	72± 6.7	65± 5.8	63± 7.7	71± 6.9	68± 6.1	61± 7.2	69± 6.2	64± 6.6	67± 7.3
TB (mg/dL)	0.11±0.03	0.12±0.02	0.10±0.03	0.12±0.03	0.11±0.03	0.11±0.02	0.12±0.03	0.12±0.03	0.10±0.03	0.12±0.03
AST (U/L)	31±4.2	34±4.6	33±3.9	32±4.7	38±7.1	31±3.2	35±5.1	32±4.2	32±4.1	33±4.1
ALT (U/L)	29±3.5	30±4.5	33±4.6	31±3.2	28±4.9	32±4.1	31±4.2	33±4.0	19±5.5	34±4.4

Statistical analyses were performed using one-way ANOVA followed by Dunnett's test. Values are expressed as mean ± SEM (standard error of the mean) of 6 rats per group. ALT: alanine transaminase; AP: alkaline phosphatase; AST: aspartate aminotransferase; TB: total bilirubin; TC: total cholesterol; TG: triglycerides; TP: total protein. Control: animals treated with water; SECL: animals that received different doses of aqueous extract of *Citrullus lanatus*; EECl: animals that received different doses of ethanolic extract *Citrullus lanatus* TECL: animals that received different doses of Turbolysis of *Citrullus lanatus.*

Artigo 3: Cardiovascular Therapeutics
Qualis A3/ FI 3.1

https://www.hindawi.com/journals/cdtp/guidelines/

Investigation of the cardiovascular and renal effects of different preparations obtained from *Citrullus lanatus* (Thunb.) Matsum. & Nakai seeds

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Abstract

Citrullus lanatus is a species widely cultivated in tropical regions of the world. It is often used for fresh consumption or to produce juices and cocktails. Therefore, this present study was conducted to determine if different preparations obtained from *C. lanatus* seeds can influence the renal or hemodynamic function of rats. *C. lanatus* seeds were collected, dried, pulverized, and used to prepare different preparations, including a purified aqueous extract (ESCL), an ethanolic extract (EECL), and an aqueous extract obtained by turbolysis (TECL). The diuretic and hypotensive effects of the ESCL, EECL, and TECL were evaluated in normotensive female Wistar rats. Furthermore, the vasodilatory effects in the mesenteric vascular beds were also investigated. Of all the extracts tested, only ESCL resulted in a response in the cardiorenal system of rats, inducing a reduction in peripheral vascular resistance. This study demonstrates that a purified aqueous extract obtained from the seeds of *C. lanatus* induces vasodilatory effects on resistance vessels of rats. It appears that the vasodilatory effects are not reliant on the release of endothelial mediators. This research advances our comprehension of the medicinal properties of *C. lanatus* seeds and indicates that this species has the potential for the development of a new herbal medicine.

Introduction

Cardiovascular diseases (CVDs) are a significant global health issue. They are among the main causes of morbidity and mortality worldwide, impacting millions of people and placing a considerable burden on health systems (1,2).

Among cardiovascular diseases, hypertension is the most prevalent, complex, and multifactorial chronic condition characterized by a persistent increase in blood pressure. This condition is known to be a significant risk factor for damage to blood vessels and vital organs such as the heart, brain, and kidneys (3).

The treatment for hypertension is well established, with multiple options and classes of antihypertensive drugs available, including diuretics, angiotensin-converting enzyme inhibitors, calcium channel blockers, beta-blockers, angiotensin II receptor blockers, among others. In Brazil, these treatments are provided free of charge by the Brazilian Unified Health System (SUS), which in turn leads to high expenses (4).

Despite the treatment, the control of hypertension is irregular (5), and there is often an association between synthetic drugs and herbal medicines. In this context, the use of herbal products has emerged as an alternative to reduce costs, minimize undesirable side effects, and avoid the irrational consumption of synthetic drugs (6). The intake should be done consciously and responsibly, considering the guidelines and recommendations of health professionals (7,8).

To demonstrate the effectiveness and safety of using plant species for treatment purposes, scientific studies are essential (9). *Citrullus lanatus* (Thunb.) Matsum. & Nakai (Cucurbitaceae), commonly known as watermelon, is a highly popular fruit in Brazil. It is prized not only for its delicious flavour but also for its nutritional and health benefits. Watermelon contains a variety of bioactive compounds, including carotenoids, phenolic compounds, vitamins, amino acids, and alkaloids, which are found in different concentrations in the pulp, peel, leaves, and seeds (10).

The purpose of this study was to assess the pharmacological effects of *C. lanatus* seed extracts on the cardiovascular and renal systems in female Wistar rats.

Materials and Methods

Drugs

Isoflurane was sourced from Cristália (São Paulo, SP, Brazil). Xylazine and ketamine were purchased from Syntec (São Paulo, SP, Brazil). Heparin was acquired from Hipolabor (Belo Horizonte, MG, Brazil). Hydrochlorothiazide, acetylcholine chloride, phenylephrine, indomethacin, Nω-Nitro-L-arginine methyl ester, tetraethylammonium, 4-aminopyridine, glibenclamide, NaCl, KCl, NaHCO3, MgSO4, CaCl2, KH2PO4, dextrose and ethylenediaminetetraacetic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were obtained in analytical grade.

Plant material

Seeds of *C. lanatus* were collected in Ouro Verde, São Paulo, Brazil (-21.510240817687322, -51.65258069269025). A voucher specimen (1345) was authenticated by Dr. Zefa Valdivina Pereira and deposited in the herbarium of Universidade Federal da Grande Dourados (UFGD). The fruit seeds were manually removed and dried by forced air circulation for 5 days. The seeds were stored in plastic bags at 2-8°C until analysis.

Extractive processes

We utilized three different methods for obtaining the extracts, including maceration in ethanol, turbolysis, and infusion. The phytochemical analysis was performed previously (in press).

Ethanol extract

Initially, dried *C. lanatus* seeds were pulverized in a hammer mill. The ethanol extract was obtained by adding an ethanol-water solution (7:3, v/v) to the pulverized plant material (100 g/L). The extractive solution was constantly homogenized for 48 hours for the first extraction, and then every 24 hours for the subsequent solvent exchanges (a total of five exchanges). During each exchange, a new ethanol-water solution was added to the same plant material to ensure maximum extraction of phytochemical components. The solvent from the extract was concentrated using a rotary evaporator and freeze-dried. The yield of the ethanolic extract of *C. lanatus* (EECL) was 18 %.

Turbo-extraction

The turbo-extraction was performed using a high shear stirrer (Ika Ltda, Sao Paulo, Brazil). Powdered *C. lanatus* seeds were added to filtered water (100 g/L at room temperature; 24 degrees Celsius) and subjected to high shear stirring for 5 minutes. The extract TECLwas then filtered, lyophilized, and stored at -18◦C. The yield obtained was 22%.

Ethanol-soluble fraction from aqueous extract

Initially, an infusion was prepared by adding 1 liter of boiling water to 100 grams of dried and powdered fruit seeds. After 4 hours (to reach room temperature), the infusion was treated with 3 volumes of 95% ethanol, resulting in a precipitate and an ethanol-soluble fraction (SECL). The SECL was then filtered, and after removing the ethanol (using a rotary evaporator at 55 ºC), it was lyophilized (yield of 13%).

Pharmacological study

Animals

Healthy female Wistar rats, aged 3 months, were obtained from the Central Vivarium of the Federal University of Grande Dourados (UFGD, Brazil). The rats were kept in the vivarium at a constant temperature of 22°C ± 2°C, with a 12-hour light/dark cycle. They had access to food and water freely. Prior to the start of the experiments, all rats were given seven days to acclimatize to the laboratory conditions. All procedures involving the rats were approved by the Ethics Committee in Animal Experimentation at UFGD (protocol no. 07/2020) and were

in compliance with the Brazilian Legal Framework on the Use of Animals in Scientific Research.

Diuretic activity

Diuretic activity was assessed following the methodology of Gasparotto et al. (2009) (11). Seventy-seven normotensive female rats were randomly assigned to twelve experimental groups (n = 7-8) as described below:

1) HCTZ (rats treated with hydrochlorothiazide; 25 mg/kg).

2) Negative control (NC; rats treated with filtered water; 0.2 mL/100 g).

3) SECL, EECL, and TECL 30 (rats treated with 30 mg/kg of each extract).

4) SECL, EECL, and TECL 100 (rats treated with 100 mg/kg of each extract).

5) SECL, EECL, and TECL 300 (rats treated with 300 mg/kg of each extract).

The animals were orally treated once a day for 7 days. On the first day of treatment, all animals received 5 mL/100 g of saline solution (NaCl 0.9%) to ensure body salt and water balance. Subsequently, each animal was placed in individual metabolic cages. Urine samples were collected over a 24-hour period on days 1 and 7, and their volumes were recorded. The pH and density data were measured using a digital pH meter (Q400MT; Quimis Instruments, Brazil) and handheld refractometer (NO107; Nova Instruments, Brazil), respectively. Urinary levels of sodium, potassium, and chloride were quantified using an automated biochemical analyzer (Cobas Integra 400 plus, Roche).

Effects on the heart's electrical system

After collecting urine on the seventh day of the experiment, the animals were anesthetized with isoflurane inhalation (2-3%). Four alligator clips were used to position electrodes on the animal's two forelimbs and two hindlimbs. A 5-minute acclimatization period was allowed, and ECG waves were recorded for 5 minutes. The following data were recorded: PR, QRS, and QT (ms); P, Q, R, and S wave amplitudes (mV). Electrocardiography (ECG) was recorded using a 12-lead ECG recorder (WinCardio, Micromed, Brasilia, Brazil).

Arterial pressure and heart rate evaluation

Immediately after recording the ECG, all animals were given a subcutaneous single bolus injection of heparin (30 IU). Following this, the left carotid artery was isolated, cannulated, and connected to a pressure transducer that was coupled to a PowerLab® recording system. The heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) were recorded by an application program (Chart, v 7.1; both from ADI Instruments, Castle Hill, Australia) for a duration of 20 minutes.

Biochemical analysis

Following the measurement of blood pressure (BP), blood samples (3 mL) were collected from the left carotid artery. Serum was obtained by centrifugation at 1,500 g for 10 minutes.

Potassium, sodium, creatinine, and urea levels were quantified using an automatic biochemical analyzer (Roche Cobas Integra 400 plus).

Effects on peripheral vascular resistance

Untreated normotensive female rats were given anesthesia with ketamine and xylazine (100 and 20 mg/kg) through the intraperitoneal route. The mesenteric vascular beds (MVBs) were then isolated and prepared using methods previously described by McGregor (12). The MVBs (n = 5) were placed in a water-jacketed organ bath and perfused with physiological saline solution (PSS) at a rate of 4 mL/min. The composition of PSS was as follows: NaCl 119 mM, KCl 4.7 mM, CaCl2 2.4 mM, MgSO4 1.2 mM, NaHCO3 25.0 mM, KH2PO4 1.2 mM, dextrose 11.1 mM, and EDTA 0.03 mM. The MVBs were maintained at a temperature of 37°C and gassed with a mixture of 95% O_2 and 5% CO_2. Changes in perfusion pressure (PP, mm Hg) were obtained through a pressure transducer connected to a PowerLab recording system and using an application program (LabChart 8.1.30 for Windows).

After stabilization of the preparations (45 minutes), their integrity was confirmed by a bolus injection of KCl (120 mmol). To evaluate the endothelial viability, different MVBs were continuously perfused with PSS plus Phe (3 μM) to promote a prolonged increase in PP. Then, a bolus injection of ACh (1 nmol) was administered and the PP reduction was measured. Some preparations were perfused with PSS containing sodium deoxycholate (1.8 mg/mL) for 30 seconds to chemically remove the endothelium. Then, MVBs containing or lacking functional endothelium were continuously perfused with PSS plus Phe (3 μM) and, after the stabilization of the contractile process, received bolus injections of ESCL, SECL, and TECL (at doses of 0.03, 0.1, 0.3, and 1mg), and the perfusion pressure was measured. A minimum interval of 3 minutes between doses was observed.

To evaluate the molecular mechanisms involved in vasodilatory activity, MVBs were perfused with PSS containing Phe (3 μM) plus indomethacin (1 μM; a nonselective cyclooxygenase inhibitor), and Nω-nitro-L-arginine methyl ester (L-NAME) (100 μM; a nonselective nitric oxide [NO] synthase inhibitor), used in combination or alone. After 15 minutes of continuous perfusion, the preparations were once again exposed to the doses of the tested extracts, and their ability to reduce perfusion pressure in the presence and absence of different inhibitors was evaluated.

Statistical analyses

The analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's test or Student's t-test when applicable. Results are expressed as mean ± standard error of the mean. A p-value of less than 0.05 was set as the level of significance. All graphs and analysis were performed using GraphPad Prism software version 9.4.1 for macOS (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Effects on renal function

The effects of treatments with ESCL, EECL, TECL, or with HCTZ on urinary volume and renal excretion of sodium, potassium, and bicarbonate are presented in Tables 1 and 2. The urinary volume excreted by different experimental groups did not show any statistically significant difference during the 7 days of the study. On the other hand, renal excretion of sodium, chloride, and potassium was significantly higher in animals treated with HCTZ compared to the animals in the negative control group. Treatments with ESCL, EECL, TECL did not result in any statistically significant effect on electrolyte excretion in urine compared to animals that received only the vehicle (NC). No significant changes were observed in urinary pH and density among all experimental groups.

Effects on the heart's electrical system

Table 3 displays quantitative electrocardiography data for female rats that were treated for 7 days with ESCL, EECL, TECL, or with HCTZ. We did not observe any significant changes in electrocardiographic characteristics of the PR, QRS, and QT segments, as well as the amplitude of the P, Q, R, and S waves in any of the experimental groups.

Effects on hemodynamic and serum biochemical parameters

The values obtained for BP and HR levels in normotensive rats treated for 7 days with vehicle, ESCL, EECL, TECL, or HCTZ are shown in Table 4. Only the animals that received HCTZ showed a significant reduction in SBP values when compared to all other experimental groups. The treatments with ESCL, EECL, and TECL were not able to alter the levels of SBP, DBP, MAP, or HR when compared to the animals that received only the vehicle (NC). None of the treatments performed altered the serum levels of urea, creatinine, sodium, and potassium (data not shown).

Effects on peripheral vascular resistance

Among the three tested extracts, only ESCS was able to induce vasodilatory response in the MVBs. At a dose of 0.1 mg, ESCS was able to induce a reduction in perfusion pressure of approximately 7% (**Figure 1**). The absence of endothelium or prior perfusion of indomethacin or L-NAME was not able to prevent the vasodilatory response of ESCS, suggesting the absence of involvement of endothelial mediators (**Figure 2**).

Discussion

C. lanatus is a species widely cultivated in tropical regions of the world. It is often used for fresh consumption or to produce juices and cocktails (13). However, due to its large mass, it generates waste in the form of peel and seeds. Its seeds are used for human consumption in various countries, including India, Arab countries, and African countries. Several studies have been conducted on the chemical composition and dietary importance of watermelon seeds. They can be used as an additive in various foods, as well as for filling cakes and sweets (14,15). *C. lanatus* seeds are also reported to treat chronic or acute eczema and are known to have high levels of proteins and lipids (15,16). Despite the available data, there is currently no information on the effects of the seeds on the cardiorenal system. Therefore, this present study was conducted to determine if different preparations obtained from watermelon seeds can influence the renal or hemodynamic function of rats, as a prelude to their effects in humans.

In our study, we chose to use three different preparations obtained from *C. lanatus* seeds, including a purified aqueous extract (ESCL), an ethanolic extract (EECL), and an aqueous extract obtained by turbolysis (TECL). Of all the extracts tested, only ESCL resulted in a response in the cardiorenal system of rats, inducing a reduction in peripheral vascular resistance. In this case, we observed that all three tested doses of ESCL showed a significant vasodilatory effect. However, it is worth noting that the dose of 0.1 mg showed the most significant vasodilator response, with a reduction in activity with increasing dose. A limitation of our study was that we did not identify the reason why significant vasodilator activity was only obtained with the intermediate dose of ESCL. This effect is relatively common in crude plant extracts due to the wide range of secondary metabolites (17). Therefore, it is likely that increasing the dose also increases the concentration of molecules that can counteract vasodilatory effects. Furthermore, while we cannot identify a single molecule responsible for the biological activity, we believe that the high amount of phenolic compounds identified in ESCL, acting synergistically, could be likely candidates. Phytochemical analysis showed several polyphenol compounds in *C. lanatus* seeds, including several flavonoids (18,19).

Data indicates that various flavonoids, such as rutin, vitexin, quercetin, and isoquercitrin, can enhance the expression of eNOS, reduce the breakdown of NO, as well as increase the release of prostaglandins, which are endothelial vasodilator mediators closely linked to different natural products (20,21). To investigate this theory, a set of experiments were carried out to assess the vasodilator effects of ESCL along with different inhibitory molecules. These experiments involved the removal of endothelium and the use of inhibitors for prostaglandin and NO synthesis, which are crucial pathways for decreasing peripheral vascular resistance. The results showed that ESCL could maintain the vasodilator response in the absence of endothelium or in the presence of inhibitors of NO and prostaglandin synthesis. This indicates that the effects induced by ESCL do not rely on the release of endothelial mediators. Further studies are required to understand how ESCL can decrease peripheral vascular resistance, which may involve the direct release of vasodilating agents or directly affecting the vascular contractile process.

Conclusion

In this study, we show that a purified aqueous extract obtained from the seeds of *C. lanatus* induces vasodilatory effects on resistance vessels of rats. It seems that the vasodilatory effects are not dependent on the release of endothelial mediators. This research enhances our understanding of the medicinal properties of *C. lanatus* seeds and suggests that this species shows potential for the development of a new herbal medicine.

Author Contributions

Conceptualization, funding acquisition, and project administration: AGJ. Methodology, investigation, and data curation: AAMM, KGTM, LBP, LABP KSL, RICS, ACS, GPS, BVS and MLFS Writing - original draft: AAMM. Supervision and writing - review and editing: AGJ.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding Statement

We are also grateful to the Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT, Brazil) for their grant's numbers 71/700.135/2018 and 83/013.186/2023. We also extend our gratitude to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) for their grant number 407685/2018-9 and 150258/2023-2, and to the Coordenadoria de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil) for their financial support.

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Legend to figures

Figure 1. ESCM promotes reduction in the perfusion pressure in the mesenteric vascular bed of rats. Effects of ESCL (A), EECL (B), and TECL (C) on the perfusion pressure of the mesenteric vascular bed perfused with physiological saline solution containing 3 μM phenylephrine (Phe). Values are expressed as mean ± S.E.M. of 6 experiments. + indicates $p < 0.05$ compared with the previous dose. All experiments were performed in endothelium-intact preparations.

Figure 2. Absence of endothelial mediator participation in ESCS-induced vasodilatory response. Effects of ESCL on endothelium-intact (End+) and endothelium-denuded (End-) preparations (A), or endothelium-intact mesenteric vascular beds continuously perfused with L-NAME (B) or indomethacin (C) are presented. The results show the mean ± S.E.M. of 6 preparations per group. # Indicate $p < 0.05$ compared with the effects of ESCS on the endothelium-intact (A) or the vehicle group (B and C). + indicates $p < 0.05$ compared with the respective previous dose.

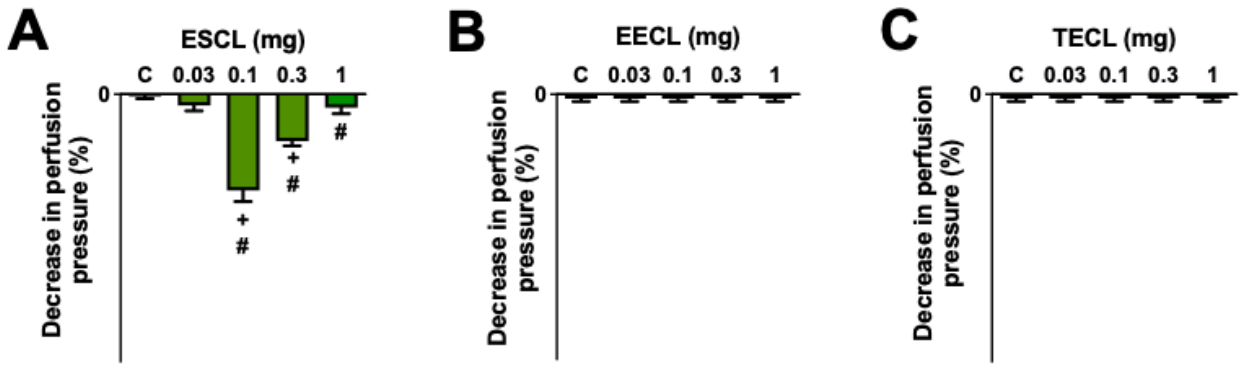

Figure 1

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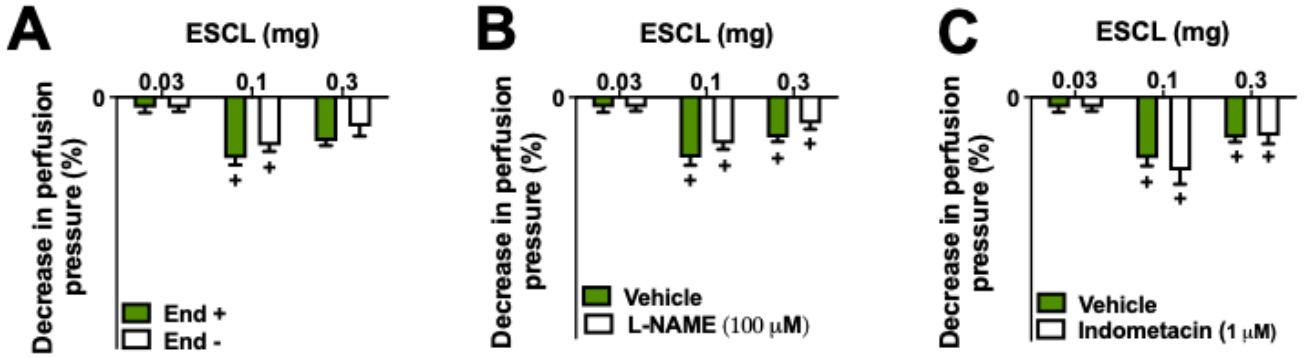

Figure 2

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Table 1. Effects of oral treatment with different extracts from *C. lanatus* at doses of 30, 100, and 300 mg/kg and hydrochlorothiazide (HCTZ) on urinary parameters on the first day of treatment.

Parameter	CN	HCTZ	ESCL 30	ESCL 100	ESCL 300	EECL 30	EECL 100	EECL 300	TECL 30	TECL 100	TECL 300
24-hour urine volume (mL/100g)	8.24± 5.05	8.51±2.65	7.21±1.52	5.90±2.24	8.54±2.30	7.32±3.22	5.88±1.92	8.42±3.35	6.63±1.75	8.77±5.55	9.53±4.03
Chloride (µmol/100g/24h)	999.2±4.26	1067±13.81[a]	998.0±6.00[b]	997.0±6.36[b]	996.8±6.85[b]	994.2±8.08[b]	997.8±9.56[b]	994.0±7.29[b]	997.0±6.48[b]	994.3±8.62[b]	995.0±8.81[b]
Potassium (µmol/100g/24h)	784.8±13.41	862.0±29.36[a]	784.0±9.88[b]	778.2±9.73[b]	780.8±8.04[b]	780.8±8.59[b]	783.7±6.09[b]	780.7±7.94[b]	780.2±8.18[b]	782.5±7.31[b]	781.3±10.54[b]
Sodium (µmol/100g/24h)	1315±11.39	1421±32.37[a]	1316±12.02[b]	1321±5.22[b]	1321±10.67[b]	1322±5.43[b]	1323±5.72[b]	1325±3.97[b]	1324±5.84[b]	1325±4.86[b]	1324±5.08[b]

Statistical analyses were performed using one-way ANOVA followed by Dunnett's test. Values are expressed as mean ± SEM (standard error of the mean). There were 7-8 rats per group. a $p \leq 0.05$ when compared to the negative control (NC); b $p \leq 0.05$ when compared to HCTZ.

Table 2. Effects of oral treatment with different extracts from *C. lanatus* at doses of 30, 100, and 300 mg/kg and hydrochlorothiazide (HCTZ) on urinary parameters on the seventh day of treatment.

Parameter	CN	HCTZ	ESCL 30	ESCL 100	ESCL 300	EECL 30	EECL 100	EECL 300	TECL 30	TECL 100	TECL 300
24-hour urine volume (mL/100g)	10.15±3.48	8.80±4.03	7.77±2.09	7.96±4.23	9.43±2.35	8.26±2.33	11.34±3.21	9.52±2.66	6.72±1.88	9.72±4.87	9.12±2.60
Chloride (µmol/100g/24h)	987.7±7.82	1069±23.49[a]	980.7±7.31[b]	917.2±7.51[ab]	984.3±7.89[b]	987.5±5.05[b]	985.2±8.91[b]	984.5±7.94[b]	985.3±7.55[b]	986.2±8.70[b]	983.2±6.79[b]
Potassium (µmol/100g/24h)	779±9.52	879.2±18.95[a]	780.5±6.35[b]	782.2±4.02[b]	784.3±7.00[b]	781.5±7.84[b]	783.5±7.20[b]	785.3±5.65[b]	782.7±6.62[b]	801.2±44.32[b]	786.2±5.35[b]
Sodium (µmol/100g/24h)	1315±10.30	1415±29.19[a]	1319±9.25[b]	1315±7.60[b]	1403±17.35[b]	1316±5.04[b]	1319±3.37[b]	1316±6.71[b]	1321±5.46[b]	1318±4.38[b]	1319±8.87[b]

Statistical analyses were performed using one-way ANOVA followed by Dunnett's test. Values are expressed as mean ± SEM (standard error of the mean). There were 7-8 rats per group. a $p \leq 0.05$ when compared to the negative control (NC); b $p \leq 0.05$ when compared to HCTZ.

Table 3. Effects of oral treatment with different extracts from *C. lanatus* at doses of 30, 100, and 300 mg/kg and hydrochlorothiazide (HCTZ) on electrocardiographic parameters on the seventh day of treatment.

Parameter	**CN**	**HCTZ**	**ESCL 30**	**ESCL 100**	**ESCL 300**	**EECL 30**	**EECL 100**	**EECL 300**	**TECL 30**	**TECL 100**	**TECL 300**
Segment (ms)											
PR	34,33±7,53	37,60±4,51	41,14±2,55	38,00±4,60	42,14±4,06	36,29±3,73	39,17±7,47	40,80±4,02	38,71±4,35	39,00±4,76	35,57±4,72
QRS	36,33±3,78	36,80±3,90	35,43±2,37	34,67±2,06	36,43±1,81	36,29±3,15	38,67±3,14	39,80±2,86	38,00±4,44	37,75±2,99	34,57±3,51
QT	70,33±10,09	75,00±11,25	75,00±16,96	84,33±16,45	77,86±9,39	76,14±12,17	85,17±14,82	88,00±14,82	77,00±16,31	81,00±5,89	87,14±7,08
Wave (mV)											
P	0,09±0,01	0,08±0,03	0,07±0,02	0,09±0,02	0,08±0,03	0,08±0,02	0,08±0,02	0,08±0,02	0,06±0,02	0,08±0,02	0,08±0,03
Q	-0,01±0,01	-0,01±0,01	-0,01±0,01	-0,01±0,01	-1,00±0,01	-0,02±0,01	-0,01±0,01	-0,01±0,01	-0,01±0,01	-0,02±0,01	-0,01±0,01
R	0,25±0,11	0,25±0,07	0,24±0,07	0,24±0,08	0,23±0,03	0,24±0,04	0,23±0,04	0,29±0,06	0,22±0,07	0,27±0,06	0,24±0,04
S	-0,02±0,01	-0,02±0,01	-0,02±0,01	-0,02±0,01	-0,02±0,01	-0,02±0,01	-0,02±0,01	-0,02±0,01	-0,02±0,01	-0,02±0,01	-0,02±0,01

Statistical analyses were performed using one-way ANOVA followed by Dunnett's test. Values are expressed as mean ± SEM (standard error of the mean). There were 7-8 rats per group.

Table 4. Effects of oral treatment with different extracts from *C. lanatus* at doses of 30, 100, and 300 mg/kg and hydrochlorothiazide (HCTZ) on blood pressure and heart rate on the seventh day of treatment.

Parameter	CN	HCTZ	ESCL 30	ESCL 100	ESCL 300	EECL 30	EECL 100	EECL 300	TECL 30	TECL 100	TECL 300
SBP (mm Hg)	120.8±9.46	100.8±6.68[a]	137.9±8.62[b]	126.8±9.62[b]	122.0±9.72[b]	120.4±11.69[b]	127.8±9.06[b]	120.5±0.54[b]	120.6±8.68[b]	121.8±9.26[b]	117.0±9.05[b]
DBP (mm Hg)	70.76±8.89	71.62±7.06	71.11±7.11	61.90±11.04	58.62±6.06	58.94±11.11	63.30±8.77	59.34±10.14	76.24±11.54	56.88±9.34	50.35±9.46
MAP (mm Hg)	106.19±12.89	102.4±12.26	103.6±11.04	91.87±11.41	87.11±7.98	92.41±11.47	97.23±10.70	82.19±11.18	104.0±11.31	93.10±7.99	96.46±11.46
HR (bpm)	395.1±34.09	420.0±35.57	415.1±31.72	391.5±24.57	396.9±30.12	408.5±35.92	394.2±45.72	407.7±33.74	368.2±38.2	391.3±24.86	375.6±44.44

Statistical analyses were performed using one-way ANOVA followed by Dunnett's test. Values are expressed as mean ± SEM (standard error of the mean). There were 7-8 rats per group. DBP: diastolic blood pressure; HR: heart rate; MAP: mean arterial pressure; SBP: systolic blood pressure.

5.4 Artigo 4: Pharmaceuticals
Qualis A1/ FI 4.6

https://www.mdpi.com/journal/pharmaceuticals/instructions#preparation

The cardioprotective effects of *Citrullus lanatus* (Thunb.) Matsum. & Nakai seeds on periodontitis-induced in hypertensive rats.

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Abstract: *Citrullus lanatus* (Cucurbitaceae) is a fruit species known as watermelon. Chemically, the species contains carotenoids, phenolic compounds, vitamins, amino acids, and alkaloids. Particularly, carotenes and phenolic compounds are notable for their high-capacity antioxidant properties. Although these compounds are presumed to have cardioprotective activities, few studies have been conducted to investigate the effects of watermelon on pre-clinical hypertension models. Therefore, this study aims to evaluate the cardiovascular and renal effects of the ethanol-soluble fraction from *C. lanatus* seeds (ESCL) in hypertensive rats with periodontitis. *C. lanatus* seeds were collected, dried, pulverized, and the ESCL was obtained. Periodontal disease was induced in spontaneously hypertensive rats by inserting a ligature at the left first molar. After the establishment of periodontitis (30 days), the ESCL was administered orally once daily for 28 days. The renal function was evaluated on days 1 and 28. On day 29, the animals underwent electrocardiographic evaluation and measurement of blood pressure and heart rate. The serum renal function and oxidative stress markers were investigated. The mesenteric vascular bed reactivity and the heart histopathological analysis were also performed. The treatment with ESCL at a dose of 300 mg/kg was able to reverse the elevation of markers of renal function and serum oxidative stress. In addition, it reversed the endothelial dysfunction induced by hypertension, as well as significantly reduced blood pressure levels, resulting in the reversal of left ventricular hypertrophy. We have demonstrated that the *C. lanatus* seed extract can have cardioprotective effects on periodontitis-induced on hypertensive rats. This discovery opens up possibilities for the development of an herbal medicine for treating hypertension.

Keywords: cardiovascular diseases; Cucurbitaceae; hypertension; watermelon

Citation: To be added by editorial staff during production.

Academic Editor: Firstname Lastname

Received: date
Revised: date
Accepted: date
Published: date

1.Introduction

Hypertension is a significant risk factor for the development of cardiovascular and renal diseases. The relationship between periodontitis and hypertension is not yet fully understood, but studies suggest that the inflammation caused by periodontitis can trigger systemic inflammatory responses that may contribute to the development of hypertension [1]. This connection can be explained by the fact that periodontitis is a chronic disease caused by the presence of bacteria in the mouth, which can lead to a chronic inflammatory response in the body. As a result, it is observed that the chronic inflammatory response is associated with changes in the immune system and the production of inflammatory mediators such as C-reactive protein and interleukin 1b and 6. These inflammatory mediators can affect blood vessel function, including reduced vasodilation and decreased nitric oxide production, which can lead to increased blood pressure [2].

The drug treatment for controlling hypertension is well-established and supported by vast scientific data [3]. However, natural products and herbal medicines have been explored as complementary options for controlling hypertension [4]. *Citrullus lanatus* (Thunb.) Matsum. & Nakai (Cucurbitaceae) is a fruit species known as watermelon. Chemically, the species contains carotenoids, phenolic compounds, vitamins, amino acids, and alkaloids [5]. Particularly, carotenes and phenolic compounds are notable for their high-capacity antioxidant properties and are responsible for the variety of coloring found in watermelon pulp [6]. Although these compounds are presumed to have cardioprotective activities [7], few studies have been conducted to investigate the effects of watermelon on pre-clinical hypertension models.

Therefore, this study aims to evaluate the cardiovascular and renal effects of the ethanol-soluble fraction from *C. lanatus* seeds in hypertensive rats with periodontitis.

2. Results

2.1 Effects on renal function

The urinary volume and renal elimination of electrolytes recorded on the first day of the experiment are presented in **Table 1**. The animals in the NC group showed a significant reduction in renal sodium elimination when compared to the naïve group. On the other hand, animals treated with ESCL at a dose of 300 mg/kg showed a significant increase in urinary volume and renal sodium excretion, with values statistically higher than those observed in the NC or naïve group. As expected, animals treated with HCTZ showed a significant increase in urinary volume and excretion of sodium, potassium, and chloride, with values higher than those observed in both control groups. The pH and urinary density were not significantly altered in any of the experimental groups.

Table 1. Effects of oral treatment with ESAP (30, 100, and 300 mg/kg) and hydrochlorothiazide (HCTZ) on urinary parameters on the 1st day of treatment.

Parameter	Naïve	NC	HCTZ (25 mg/kg)	ESCL (30 mg/kg)	ESCL (100 mg/kg)	ESCL (300 mg/kg)
Urine volume mL/100g/24h	4.1±1.38	5.9±1.59	13.4±1.87[ab]	3.83±0.65	3.33±1.27	11.71±19.18[ab]
Sodium (µmol/100g/24h)	1315±100.3	977.2±75.2[a]	1676±112.8[ab]	1345±98.4	1294±112.5	1588±85.2[ab]
Potassium (µmol/100g/24h)	779±69.5	704±55.1	982±72.2[ab]	823±72.3	748±55.8	719±77.2
Chloride (µmol/100g/24h)	934±56.3	873±75.2	1311±83.2[ab]	911±69.3	928±88.1	914±77.1

Statistical analyses were performed using one–way ANOVA followed by Dunnett's test. Values are expressed as mean ± SEM (standard error of the mean). n = 7-8 rats per group. [a] p < 0.05 when compared to naïve; [b] p < 0.05 when compared to NC. NC: negative control.

The urinary volume and renal elimination of electrolytes recorded on the twenty-eighth day of the experiment are presented in **Table 2**. The urinary volume produced in 24 hours was not significantly altered in any of the experimental groups. On the other hand, animals in the NC group, or those treated with the lower doses of ESCL (30 and 100 mg/kg), exhibited a significant decrease in renal sodium excretion compared to the naïve animals. The animals treated with ESCL at a dose of 300 mg/kg showed a significant increase in renal sodium excretion, with values statistically higher than those observed in the NC group. The animals treated with HCTZ showed a significant increase in the excretion of urinary sodium and potassium, with values higher than those observed in both control groups. The pH and urinary density were not significantly altered in any of the experimental groups.

Table 2. Effects of oral treatment with ESAP (30, 100, and 300 mg/kg) and hydrochlorothiazide (HCTZ) on urinary parameters on the 28th day of treatment.

Parameter	Naïve	NC	HCTZ (25 mg/kg)	ESCL (30 mg/kg)	ESCL (100 mg/kg)	ESCL (300 mg/kg)
Urine volume mL/100g/24h	4.13±1.38	4.43±1.00	3.69±1.05	3.47±1.49	3.83±0.61	3.31±0.46
Sodium (µmol/100g/24h)	912±92.2	556.6±56.1[a]	1333±101.3[ab]	586±77.1[a]	654±77.2[a]	1066±81.1[b]
Potassium (µmol/100g/24h)	701±77.1	723±66.2	912±66.1[ab]	716±71.0	721±73.2	723±71.0
Chloride (µmol/100g/24h)	855±66.2	869±71.9	899±81.6	819±80.3	827±83.4	842±88.4

Statistical analyses were performed using one–way ANOVA followed by Dunnett's test. Values are expressed as mean ± SEM (standard error of the mean). n = 7-8 rats per group. [a] p < 0.05 when compared to naïve; [b] p < 0.05 when compared to NC. NC: negative control.

2.2 Effects on cardiac electrical activity

The electrocardiographic data recorded on the twenty-ninth day of the experiment are presented in **Table 3**. The animals in the NC group, as well as the rats treated with ESCL at a dose of 30 mg/kg, showed a significant reduction in the QT interval when compared to the animals in the naïve group. Treatment with ESCL at doses of 100 and 300 mg/kg was able to reverse these changes, maintaining QT segment values similar to those found in naïve animals. All other electrocardiographic parameters were not altered by any of the treatments performed.

Table 3. Effects of oral treatment with ESAP (30, 100, and 300 mg/kg) and hydrochlorothiazide (HCTZ) on electrocardiographic parameters

Parameter	Naïve	NC	HCTZ (25 mg/kg)	ESCL (30 mg/kg)	ESCL (100 mg/kg)	ESCL (300 mg/kg)
Segment (ms)						
P	35.6±3.0	35.6±4.2	32.8±1.3	32.0±3.2	31.6±1.7	37.6±2.0
PR	45.1±3.2	40.7±3.7	36.0±1.7	40.2±2.3	40.0±1.9	42.6±2.1
QRS	43.7±2.2	42.0±1.4	38.3±1.5	40.0±0.93	41.2±1.3	41.3±1.8
QT	102.1±9.3	94.83±9.5	86.7±8.4	97,83±7,4	101.7±9.0	102.5±10.7
Wave (mV)						
P	0.06±0.01	0.07±0.01	0.07±0.01	0.06±0.01	0.07±0.01	0.08±0.01
Q	-0.03±0.003	-0.03±0.01	-0.03±0.008	-0.02±0.006	-0.02±0.004	-0.02±0.005
R	0.31±0.03	0.27±0.03	0.31±0.02	0.34±0.05	0.29±0.03	0.30±0.03
S	-0.01±0.004	-0.01±0.002	-0.01±0.002	-0.01±0.001	-0.01±0.001	-0.01±0.002

Statistical analyses were performed using one–way ANOVA followed by Dunnett's test. Values are expressed as mean ± SEM (standard error of the mean). n = 7-8 rats per group. [a] $p < 0.05$ when compared to naïve; [b] $p < 0.05$ when compared to NC. NC: negative control.

2.3 Effects on blood pressure and heart rate

The blood pressure (BP) and heart rate (HR) data recorded on the twenty-ninth day of the experiment are presented in **Table 4**. The animals in the NC groups or those treated with ESCL at a dose of 30 mg/kg had significantly elevated levels of systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) compared to the rats in the naïve group. Animals treated with ESCL at a dose of 100 mg/kg showed a significant reduction in SBP and MAP levels compared to animals in the NC group. On the other hand, the groups treated with ESCL at the highest dose (300 mg/kg) or with HCTZ showed a significant reduction in BP levels for all evaluated parameters (SBP, DBP, and MAP), while maintaining DBP and MAP levels similar to those observed in naïve animals. All hypertensive animals had statistically lower HR values compared to those observed in the naïve group.

Table 4. Effects of oral treatment with ESAP (30, 100, and 300 mg/kg) and hydrochlorothiazide (HCTZ) on hemodynamic parameters

Parameter	Naïve	NC	HCTZ (25 mg/kg)	ESCL (30 mg/kg)	ESCL (100 mg/kg)	ESCL (300 mg/kg)
SBP (mm Hg)	128,3±7,28	180,9±10,36[a]	163,3±7,50[ab]	169,6±7,27[a]	159,3±7,24[ab]	156,9±7,78[ab]
DBP (mm Hg)	71,32±7,10	92,53±9,91[a]	85,13±26,57	96,57±9,07[a]	90,17±9,50[a]	86,37±9,19
MAP (mm Hg)	101,8±10,33	136,9±10,11[a]	124,7±19,66	133,1±10,73[a]	124,8±16,02	121,3±13,17
HR (bpm)	399,5±23,44	341,5±21,50[a]	332,6±35,61[a]	332,2±37,25[a]	314,6±24,86[a]	335,0±22,80[a]

Statistical analyses were performed using one–way ANOVA followed by Dunnett's test. Values are expressed as mean ± SEM (standard error of the mean). n = 7-8 rats per group. [a] p < 0.05 when compared to naïve; [b] p < 0.05 when compared to NC. DBP: diastolic blood pressure; HR: heart rate; MAP: mean arterial pressure; NC: negative control; SBP: systolic blood pressure.

2.4 Effects on serum biochemical parameters

The serum biochemical parameters recorded on the twenty-ninth day of the experiment are presented in **Table 5**. The animals from the NC group and the rats treated with ESCL at doses of 30 and 100 mg/kg showed a significant increase in serum creatinine levels when compared to the rats from the naïve group. On the other hand, treatment with ESCL at a dose of 300 mg/kg and with HCTZ was able to significantly reduce this parameter when compared to the animals from the NC group.

Levels of oxidized low-density lipoprotein (oxLDL), nitrotyrosine (NT), and malondialdehyde (MDA) were significantly elevated in animals from the NC, HCTZ, and ESCL (30 mg/kg) groups when compared to the naïve group. On the other hand, treatments with ESCL at doses of 100 and 300 mg/kg were able to significantly reduce these parameters, presenting values close to those obtained in animals from the naïve group. Serum levels of sodium, potassium, and urea were not altered by any of the treatments.

Table 5. Effects of oral treatment with ESAP (30, 100, and 300 mg/kg) and hydrochlorothiazide (HCTZ) on serum biochemical parameters

Parameter	Naïve	NC	HCTZ (25 mg/kg)	ESCL (30 mg/kg)	ESCL (100 mg/kg)	ESCL (300 mg/kg)
Sodium (mmol/L)	121,1±10.4	123,4±12.2	125,5±13.3	129,7±12.1	124,2±13.2	125,5±11.9
Potassium (mmol/L)	5,12±0.51	5,23±0.66	5,11±0.54	5,09±0.66	5,13±0.55	5,14±0.73
Urea (mg/dL)	33.5±3.5	35.6±4.1	37.1±3.7	35.1±3.9	32.8±3.0	35.1±4.1
Creatinine (mg/dL)	0.47±0.07	1.23±0.10[a]	0.66±0.09[b]	1.12±0.10[a]	1.07±0.12[a]	0.83±0.08[ab]
oxLDL (ng/mL)	0.20± .05	0.47±0.04[a]	0.39±0.07[a]	0.37±0.07[a]	0.30±0.06[b]	0.27±0.05[b]
NT (μmol/L)	0.012±0.002	0.030±0.005[a]	0.027±0.006[a]	0.025±0.005[a]	0.016±0.004[b]	0.015±0.004[b]
MDA (mmol/L)	1.21±0.08	2.13±0.13[a]	2.03±0.11[a]	1.98±0.10[a]	1.37±0.10[b]	1.33±0.11[b]

Statistical analyses were performed using one–way ANOVA followed by Dunnett's test. Values are expressed as mean ± SEM (standard error of the mean). n = 7-8 rats per group. [a] p < 0.05 when compared to naïve; [b] p < 0.05 when compared to NC. MDA: malondialdehyde; NC: negative control; NT: nitrotyrosine; oxLDL: oxidized low-density lipoprotein.

2.5 Effects on vascular reactivity

The vascular reactivity assessed in the mesenteric vascular beds (MVBs) on the twenty-ninth day of the experiment is presented in **Table 6**. All animals in the NC group exhibited a significant increase in the vasoconstrictor response induced by Phe (1 to 100 nmol) when compared to the naïve group. Only the dose of 300 mg/kg of ESCL was able to prevent this alteration, maintaining the reactivity to Phe similar to that obtained in the animals of the naïve group. On the other hand, animals treated with HCTZ showed normalization of vascular reactivity to Phe only at doses of 10 and 30 nmol. The vascular reactivity to Ach and SNP remained without significant changes among all experimental groups.

Table 6. Effects of oral treatment with ESAP (30, 100, and 300 mg/kg) and hydrochlorothiazide (HCTZ) on mesenteric vascular reactivity

Parameter	Naïve	NC	HCTZ (25 mg/kg)	ESCL (30 mg/kg)	ESCL (100 mg/kg)	ESCL (300 mg/kg)
Phe						
1 nmol	2.5±2.14	18.9±6.12[a]	19.8±6.10[a]	18.1±1.63[a]	19.7±5.49[a]	7.3±6.87
3 nmol	3.2±2.27	34.1 ± 11.14[a]	40.3 ± 7.3[a]	32.2 ± 15.1[a]	23.6 ± 9.43[a]	5.7±8.65[b]
10 nmol	24.6 ± 13.78	174.6 ± 13.0[a]	81.2 ± 13.5[ab]	161.0 ± 40.9[a]	190.6 ± 62.30[a]	50.6±20.96[b]
30 nmol	129.7± 37.4	268.6 ±37.8[a]	167.4 ±32.7[b]	220.8±72.2[a]	253.9±51.8[a]	99.8±33.40[b]
100 nmol	231.1±55.9	336.1±49.3[a]	300.7±59.4	322.2±52.3[a]	307.1 ±86.1	235.7±46.4[b]
ACh						
10 pmol	-19.1 ±8.5	-23.5±7.1	-14.2±7.7	-15.1±6.3	-12.1±8.3	-13.6±6.6
30 pmol	-25.2±5.8	-27.4±7.2	-27.9±8.9	-25.1±8.5	-22.88±8.8	-19.8±9.1
100 pmol	-38.3±10.2	-38.1±8.0	-31.0±9.2	-50.9±10.8	-32.21 ±11.6	-41.8±17.2
300 pmol	-56.2±19.0	-63.0±13.5	-54.6±10.0	-72.8±14.5	-50.55±16.3	-64.1±12.7
1 nmol	-60.8±10.3	-77.7±12.4	-67.7±16.2	-75.6±12.2	-71.34±15.5	-78.6±8.5
SNP						
1 nmol	-8.6±5.7	-9.6±4.5	-7.2±4.0	-7.1±3.0	-6.6±3.7	-9.9±4.4
3 nmol	-73.7±17.5	-73.3±22.7	-63.6±23.3	-55.2±21.3	-55.9±12.8	-53.8±10.9
10 nmol	-75.6±33.06	-77.9±17.9	-68.9±19.6	-59.6±12.5	-74.0±7.83	-70.8±11.6
30 nmol	-144.4±28.5	-145.8±26.9	-145.3±28.33	-135.2±28.2	-145.4±29.9	-154.4±19.1

Statistical analyses were performed using one–way ANOVA followed by Dunnett's test. Values are expressed as mean ± SEM (standard error of the mean). n = 7-8 rats per group. [a] $p < 0.05$ when compared to naïve; [b] $p < 0.05$ when compared to NC. ACh: acetylcholine; Phe: phenylephrine; SNP: sodium nitroprusside.

2.6 Effects on relative organ weight

The **Table 7** shows the relative organ weight in different experimental groups. The relative weight of the kidney, liver, and heart did not show any significant changes among all experimental groups.

Table 7. Effects of oral treatment with ESAP (30, 100, and 300 mg/kg) and hydrochlorothiazide (HCTZ) on relative organ weight

Parameter	Naïve	NC	HCTZ (25 mg/kg)	ESCL (30 mg/kg)	ESCL (100 mg/kg)	ESCL (300 mg/kg)
Right kidney (%)	0.34±0.02	0.37±0.03	0.36±0.04	0.38±0.05	0.36±0.02	0.34±0.02
Left kidney (%)	0.34±0.02	0.37±0.03	0.34±0.03	0.37±0.04	0.36±0.03	0.34±0.02
Heart (%)	0.43±0.05	0.41±0.02	0.41±0.04	0.45±0.05	0.43±0.04	0.44±0.05
Liver (%)	3.80±0.33	3.87±0.26	3.80±0.42	3.79±0.31	3.88±0.16	3.80±0.33

Statistical analyses were performed using one–way ANOVA followed by Dunnett's test. Values are expressed as mean ± SEM (standard error of the mean). n = 7-8 rats per group. [a] p < 0.05 when compared to naïve; [b] p < 0.05 when compared to NC.

2.7 Effects on cardiac morphometry

The cardiac measurements recorded on the twenty-ninth day of the experiment are presented in **Table 8.** The animals in the NC group showed a significant increase in the thickness of the left ventricle wall and heart area compared to the rats in the naive group. Treatment for 28 days with ESCL at a dose of 300 mg/kg or with HCTZ was able to prevent these changes, maintaining heart size and left ventricle wall thickness similar to that observed in naive animals.

Table 8. Effects of oral treatment with ESAP (30, 100, and 300 mg/kg) and hydrochlorothiazide (HCTZ) on cardiac morphometry

Parameter	Naïve	NC	HCTZ (25 mg/kg)	ESCL (30 mg/kg)	ESCL (100 mg/kg)	ESCL (300 mg/kg)
Heart area (mm)	25. 9±0.9	35.6±2.1[a]	25.7±1.6[b]	40.1±4.1[a]	32.5±2.0[a]	26.9 ± 0.2[b]
LV wall (mm)	1.1±0.1	1.3±0.1[a]	1.2±0.1	1.5±0.2[a]	1.3±0.1[a]	1.0±0.1[b]
RV wall (mm)	1.1±0.2	0.9±0.2	0.8±0.2	1.0±0.1	1.0±0.1	0.8±0.2
IS (mm)	1.0±0.1	0.8±0.1	0.8±0.1	1.0±0.1	1.0±0.1	0.9±0.1

Statistical analyses were performed using one–way ANOVA followed by Dunnett's test. Values are expressed as mean ± SEM (standard error of the mean). n = 7-8 rats per group. [a] p < 0.05 when compared to naïve; [b] p < 0.05 when compared to NC. IS: interventricular ventricle; LV: left ventricle; RV: right ventricle.

2.8 Histopathological alterations

Representative cross-sections of the heart from the groups of naïve, NC, HCTZ and ESCL (30, 100, 300 mg/kg) are shown in **Figure 1**. There was

no evidence of fibrillar derangement, fibrosis, inflammation, apoptosis, or cellular necrosis found in any of the experimental groups.

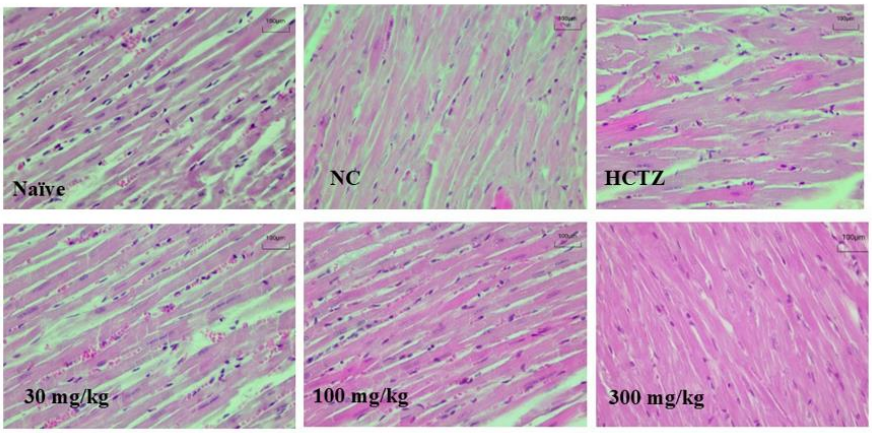

Figure 1. Representative histological sections of heart H&E (40_).

3. Discussion

In this study, we explored the potential cardioprotective effects of ESCL in spontaneously hypertensive rats (SHRs). The choice of this strain is due to its similarity to human essential hypertension, including the undefined cause, increased total peripheral **[8]** resistance, and similar responses to pharmacological treatments **[8]**. In addition to hypertension, we chose to associate a common comorbidity that can catalyze underlying cardiovascular changes, i.e., periodontitis. Patients with periodontitis are more likely to develop hypertension, and the presence of the clinical condition negatively affects the course of the disease, favoring early morphological changes, including left ventricular hypertrophy **[9]** In fact, all periodontitis-induced hypertensive rats that did not receive pharmacological treatment presented hypertension, reduced renal function, alterations in vascular reactivity, as well as electrocardiographic and morphometric changes characteristic of ventricular hypertrophy.

Hypertension and renal function are closely linked. Hypertension can lead to a decrease in renal function, while kidney disease can aggravate hypertension. The pathophysiology of hypertension-induced kidney damage is complex and involves an increase in activity of the sympathetic nervous system and the renin-angiotensin-aldosterone system, as well as vascular dysfunction. **[10]**. The 28-day treatment with ESAP, especially at its highest dose (300 mg/kg), showed significant renal and vasculoprotective effects. It normalized serum creatinine levels and vascular reactivity in resistance arteries. One characteristic of hypertension-induced vascular dysfunction is the increased vasoconstrictor response to catecholamines, which helps maintain high blood pressure levels **[11]**. In vascular reactivity to Phe (alpha adrenergic agonist), treatment with 300 mg/kg of ESCS showed a statistically superior response compared to animals treated with lower doses of ESCS (30 and 100 mg/kg), indicating a dose-dependent effect.

In biological systems, there is an equilibrium between the production and neutralization of reactive oxygen species. Disruptions in the redox state can generate free radicals and peroxides that can affect cell components such as proteins and lipids, primarily contributing to endothelial dysfunction and hypertension. Furthermore, oxidative modifications in important protein targets for redox signaling and inflammasome activation, as well as endoplasmic reticulum stress, seem to be directly related to the development and maintenance of hypertension **[12]** In this regard, the ESCS appears to play a significant protective role, as we observed that at higher doses (100 and 300 mg/kg), the extract was able to decrease LDL oxidation and lipid peroxidation. Additionally, we observed a reduction in serum levels of nitrotyrosine, a marker of tyrosine nitration mediated by reactive nitrogen species. Nitrotyrosine can be considered as an indicator or marker of cellular damage and inflammation during hypertension or other diseases, such as periodontitis **[13]**.

It is widely known that the increase in oxidative stress associated with the cardiac workload present in hypertension contributes to ventricular hypertrophy **[14]**. Therefore, the synergistic effects on oxidative stress and blood pressure levels induced by ESCL are among the main factors behind the reduction of left ventricular wall thickness and heart area in periodontitis-induced hypertensive rats. Although ESAP (300 mg/kg) is relatively effective in preventing the mentioned changes, some structural alterations were not reversed at doses of 30 and 100 mg/kg. This may be due to the increase in the concentration of cardioprotective molecules in ESCL only when larger amounts are administered.

Three limitations have been identified in this study. First, we do not know the pharmacological mechanisms by which ESCL is able to induce cardioprotective effects. Additionally, we were not able to definitively identify the secondary metabolites responsible for the cardioprotective activity. While the concept of a single pharmacologically active molecule is intriguing, we believe that the effect comes from the combined actions of various secondary metabolites. Lastly, we did not evaluate the potential synergistic or additive effects of ESCL and HCTZ. Future studies should investigate whether the cardioprotective effects of ESCL could be enhanced by co-administering a traditional cardioprotective drug.

4. Materials and Methods

4.1. Drugs

Isoflurane was sourced from Cristália (São Paulo, SP, Brazil). Xylazine and ketamine were purchased from Syntec (São Paulo, SP, Brazil). Heparin was acquired from Hipolabor (Belo Horizonte, MG, Brazil). Hydrochlorothiazide, acetylcholine chloride, phenylephrine, Nitroprusside sodium NaCl, KCl, $NaHCO_3$, $MgSO_4$, $CaCl_2$, KH_2PO_4, dextrose and ethylenediaminetetraacetic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were obtained in analytical grade.

4.2 Plant material

Seeds of *C. lanatus* were collected in Ouro Verde, São Paulo, Brazil (-21.510240817687322, -51.65258069269025). A voucher specimen (1345) was authenticated by Dr. Zefa Valdivina Pereira and deposited in the herbarium of Universidade Federal da Grande Dourados (UFGD). The fruit seeds were manually removed and dried by forced air circulation for 5 days. The seeds were stored in plastic bags at 2-8°C.

4.3 Obtaining the ethanol-soluble fraction

Initially, an infusion was prepared by adding 1 liter of boiling water to 100 grams of dried and powdered fruit seeds. After 4 hours (to reach room temperature), the infusion was treated with 3 volumes of 95% ethanol, resulting in a precipitate and an ethanol-soluble fraction (SECL). The SECL was then filtered, and after removing the ethanol (using a rotary evaporator at 55 ºC), it was lyophilized (yield of 13%). The phytochemical characterization of ESCL was submitted for publication along with another manuscript.

4.4 Animals

Thirty-five female SHR and seven female WKY rats, 6 months old, were obtained from the Central Vivarium of the Federal University of Grande Dourados (UFGD, Brazil). The animals were housed in the vivarium at a constant temperature of 22 °C ± 2 °C under a 12-hour light/dark cycle. They had access to food and water ad libitum. Before the start of the experiments, all animals were given seven days to acclimatize to the laboratory conditions. All procedures involving animals were approved by the Ethics Committee in Animal Experimentation from UFGD (protocol no. 07/2020) and followed the Brazilian Legal Framework on the Use of Scientific Animals.

4.5 Induction of experimental periodontitis

Initially, the animals were given anesthesia through intramuscular administration of ketamine (100 mg/kg) and xylazine (20 mg/kg). Their jaws were kept open using a modified Doku apparatus (**Figure 2A**), and their teeth were gently spaced using a modified hypodermic needle (BD® 30 x 7) (**Figure 2C**). This tool was inserted between the first and second lower left molars to create the necessary space for inserting the cotton bandages. The ligatures were placed below the gumline around the first lower left molar of a randomly chosen animal (**Figure 2B**). Three simple knots were tied to secure them in place, with the knot positioned on the mesial surface of the lower first molar (**Figure 2D**).

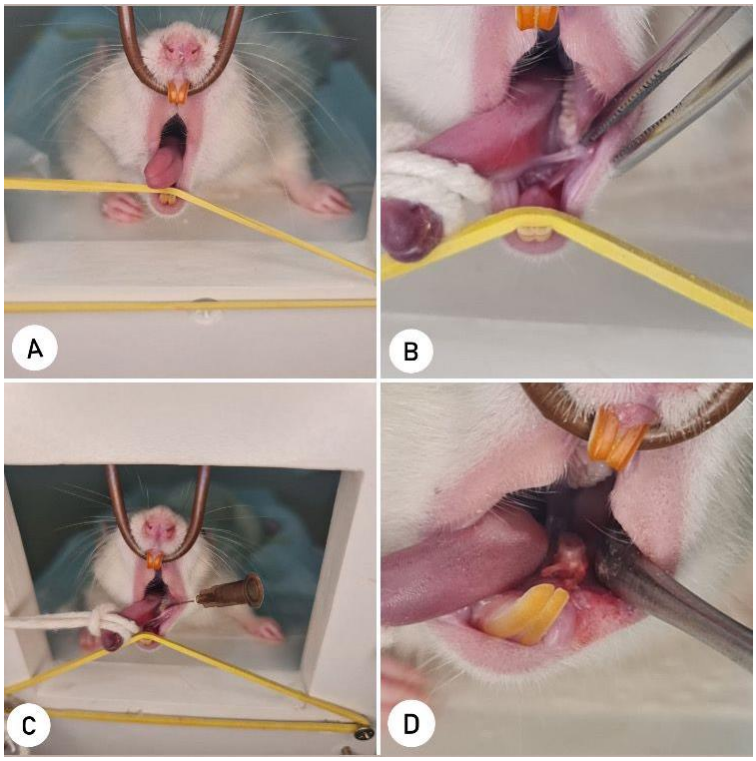

Figure 2. Induction of periodontitis

4.6 Experimental design

Thirty days after the induction of periodontitis, the animals were randomly divided into six experimental groups (n = 7-8), as follows:

1) HCTZ (SHR treated with hydrochlorothiazide; 25 mg/kg);
2) Negative control (NC; SHR treated with filtered water; 0.2 mL/100 g);
3) ESCL 30 (SHR treated with ESCL 30 mg/kg);
4) ESCL100 (SHR treated with ESCL 100 mg/kg);
5) ESCL 300 (SHR treated with ESCL 300 mg/kg); and
6) Naïve (WKY rats treated with filtered water; 0.2 mL/100 g).

The animals were treated orally, once a day, for 28 days.

4.6.1 Renal function

Renal function was evaluated in accordance with Gasparotto et al. [15]. On days 1 and 28, all animals received orally (via gavage) 5 mL/100g of saline solution (NaCl 0.9%) to ensure uniform body salt and water levels. Subsequently, each animal was placed in individual metabolic cages. Urine samples were collected over a 24-hour period and their volumes were recorded (all results were expressed as mL/100g of body weight). The pH and density were determined using a digital pH meter (Q400MT; Quimis Instruments, Brazil) and handheld refractometer (NO107; Nova Instruments, Brazil), respectively. Urinary sodium, potassium, chloride,

urea, and creatinine levels were measured using an automated biochemical analyzer (Cobas Integra 400 plus, Roche).

4.6.2 Electrocardiography

On the morning of the 29th day of treatment, all rats underwent ECG. Initially, the animals were anesthetized with isoflurane inhalation (2-3%). The electrodes were then placed on the rat's two front legs and two back legs using four alligator clips. After a 5-minute acclimatization period, ECG waves were recorded for another 5 minutes. The following data were recorded: PR, QRS, QT, and QTc segments (ms); P, Q, R, and S wave amplitudes (mV). The electrocardiography was carried out using a 12-lead ECG recorder (WinCardio, Micromed, Brasilia, Brazil).

4.6.3 Blood pressure and heart rate

After conducting the ECG, all animals were given a subcutaneous injection of heparin (30 IU). Subsequently, the left carotid artery was isolated, cannulated, and connected to a pressure transducer linked to a PowerLab® recording system. The HR, SBP, DBP, and MAP were monitored using an application program (LabChart 8; ADInstruments Pty Ltd, New South Wales, Australia) for a duration of 20 minutes.

4.6.4 Biochemical analysis

After recording the blood pressure levels, blood samples were collected from the left carotid artery. Serum was obtained by centrifugation at 1,500 ×g for 10 minutes. Sodium, potassium, urea, and creatinine levels were measured using an automated Roche cobas ® 6000 biochemical analyzer. Serum NT and oxLDL levels were measured using enzyme-linked immunosorbent assays (Merck KGaA, Darmstadt, Germany). The MDA assay kit determined MDA levels (Cayman Chemical, Ann Arbor, MI, USA).

4.6.5 Vascular reactivity

After the blood collection, the MVBs were isolated, cannulated and prepared according to methods described by McGregor (1965) [16]. The MVBs were then coupled in an perfusion system and continuously perfused with PSS (composition in mM: NaCl 119; KCl 4.7; CaCl2 2.4; MgSO4 1.2; NaHCO3 25.0; KH2PO4 1.2; dextrose 11.1; and EDTA 0.03), and gassed with 95% O_2/5% CO_2 at 37°C. Changes in perfusion pressure (PP, mm Hg) were detected by a pressure transducer connected to a PowerLab® recording system and its application software (LabChart 8; ADInstruments Pty Ltd, New South Wales, Australia). After a 30-min stabilization period, tissue integrity was assessed with a bolus injection of KCl (120 mmol). Different doses of Phe were then administered in the MVBs (1, 3, 10, 30, and 100 nmol; 10-30 μL). After another 30-min stabilization period, tissues were continuously perfused with PSS plus 3 μM Phe to induce a prolonged increase in PP. After stabilization of the contractile process, vascular reactivity to acetylcholine (Ach: 10, 30, 100, 300, and 1 pmol; 10-30 μL) and sodium nitroprusside (SNP: 1, 3, 10, and 30 nmol; 10-30 μL) were evaluated. At the end of the experiments, animals were euthanized using isoflurane deep anesthesia (at concentrations above 30%).

4.6.6 Relative organ weight, histopathology, and morphometry

After euthanasia, the heart, kidneys, and liver were removed, cleaned, weighed, and longitudinally sectioned. The relative organ weight (RW%) was determined as follows: RW% = absolute organ weight × 100 / body weight. Then, organ fragments were fixed in 10% buffered formalin. After fixation, samples were cleaved, dehydrated with increasing absolute ethanol concentrations, diaphanized in xylol and embedded in paraffin. Sections were then cut at a thickness of 4 μm and stained with hematoxylin and eosin for evaluation under light microscopy (40 X). The parameters analyzed were based on the presence or absence of reversible and/or irreversible cell lesions. The total area of the heart and the area of the lumen of each ventricle were determined. Moreover, right and left ventricles and interventricular septum were measured. The area of the microscope was adjusted using an ocular micrometer with a 100 X objective to determine the size of the field per μm2. All images were obtained and evaluated using Motic Images Plus 2.0 software.

4.7 Statistical analysis

The data was analyzed to check for normal distribution and homogeneity of variance. Statistical analyses were conducted using one-way ANOVA, followed by Dunnett's test. The significance level was set at 95% ($p < 0.05$). The statistical analyses were carried out using GraphPad Prism version 10 for macOS.

4. Conclusion

We have demonstrated that the *C. lanatus* seed extract can have cardioprotective effects on periodontitis-induced on hypertensive rats. This discovery opens up possibilities for the development of an herbal medicine for treating hypertension.

Author Contributions: Conceptualization, funding acquisition, and project administration: AGJ. Methodology, investigation, and data curation: AAMM, KGTM, GPS, BRL, CSF, PRTL, ACS, RICS, and LIS. Writing - original draft: KGTM. Supervision and writing - review and editing: AGJ.

Funding: We are also grateful to the Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT, Brazil) for their grant's numbers 71/700.135/2018 and 83/013.186/2023. We also extend our gratitude to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) for their grant number 407685/2018-9 and 150258/2023-2, and to the Coordenadoria de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil) for their financial support.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author

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

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6 CONCLUSÃO

Neste estudo, apresentamos importantes dados anatômicos que apoiam a identificação e o controle de qualidade de *Citrullus lanatus*. Ademais, demonstramos que diferentes extrações da semente de melancia é seguro após administração aguda e prolongada em ratos Wistar. Além disso, demonstramos que a semente de *Citrullus lanatus* tem importantes efeitos após administração prolongada em SHRs. Finalmente, e não menos importante, mostramos que o uso popular *Citrullus lanatus* pode ser eficaz como uma terapia adicional no tratamento de doenças cardiovasculares, e abre perspectivas para o desenvolvimento de um medicamento fitoterápico contra a hipertensão.

7 ANEXOS

7.1 Parecer De Aprovação Do Comitê De Ética

MINISTÉRIO DA EDUCAÇÃO
FUNDAÇÃO UNIVERSIDADE FEDERAL DA GRANDE DOURADOS
PRÓ-REITORIA DE ENSINO DE PÓS-GRADUAÇÃO E PESQUISA

COMISSÃO DE ÉTICA NO USO DE ANIMAIS – CEUA

Dourados-MS, 8 de outubro de 2021.

CERTIFICADO

Certificamos que a proposta intitulada ***"PROSPECÇÃO ETNOFARMACOLÓGICA DE ESPÉCIES NATIVAS DOS CAMPOS GERAIS DO PARANÁ APLICADA ÀS DOENÇAS CARDIOVASCULARES"***, registrada sob o protocolo de nº 07/2020, sob a responsabilidade de *Arquimedes Gasparotto Junior* – que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo *Chordata, subfilo Vertebrata* (exceto o homem), para fins de pesquisa científica (ou ensino), encontra-se de acordo com os preceitos da Lei nº 11.794, de 08 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovada pela Comissão de Ética no Uso de Animais (CEUA/UFGD) da Universidade Federal Da Grande Dourados, em reunião de 19/06/2020.

Finalidade	*( ) Ensino (X) Pesquisa Científica*
Vigência da autorização	*01/01/2021 a 31/12/2024*
Espécie/linhagem/raça	*Rattus norvegicus, Wistar e da linhagem Spontaneously Hypertensive Rats (SHR)*
Nº de animais	*195 – 135 Wistar e 60 SHR*
Peso/idade	*90 dias*
Sexo	*Machos – 45 Wistar e 60 SHR / Fêmeas 90 Wistar*
Origem	*Biotério Central UFGD*

Melissa Negrão Sepulvida

Melissa Negrão Sepulvida
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