UNIVERSIDADE FEDERAL DE PERNAMBUCO

CENTRO DE BIOCIÊNCIAS

PROGRAMA DE PÓS-GRADUAÇÃO EM BIOQUÍMICA

LUCIANE VASCONCELOS SANTOS

Avaliação dos Efeitos Antinociceptivo, Anti-inflamatório e Gastroprotetor do Extrato Hidroalcóolico das Folhas do Jatobá (*Hymenaea cangaceira* Pinto, Mansano & Azevedo)

RECIFE

2022

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Dissertação apresentada à coordenação do Programa de Pós-graduação em Bioquímica da Universidade Federal de Pernambuco, apresentado como um dos requisitos para o cumprimento parcial das exigências para obtenção do título de Mestre em Bioquímica e Fisiologia pela Universidade Federal de Pernambuco.

Orientadora: Maria Tereza dos Santos Correia

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2022

Dados Internacionais de Catalogação na Publicação (CIP) de acordo com ISBD

Santos, Luciane Vasconcelos
Avaliação dos efeitos antinociceptivo, anti-inflamatório e gastroprotetor do extrato hidroalcóolico das folhas do Jatobá (*Hymenaea cangaceira* Pinto, Mansano & Azevedo / Luciane Vasconcelos Santos – 2022.

100 f. : il., fig., tab.

Orientadora: Maria Tereza dos Santos Correia
Coorientadora: Márcia Vanusa da Silva
Dissertação (mestrado) – Universidade Federal de Pernambuco. Centro de Biociências. Programa de Pós-Graduação em Bioquímica e Fisiologia, Recife, 2022.
Inclui referências.

1. Plantas medicinais 2. Úlcera péptica 3. Caatinga I. Correia, Maria Tereza dos Santos (orient.) II. Silva, Márcia Vanusa (coorient.) III. Título

615.321 CDD (22.ed.) UFPE/CB – 2023 -062

Elaborado por Elaine C Barroso - CRB-4/1728

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Aprovada em: 13/06/2022

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AGRADECIMENTOS

A Deus e a Nossa Senhora! Por possibilitar que eu chegasse até aqui, com dedicação e persistência.

RESUMO

Úlceras pépticas são lesões no trato digestivo, com a intensificação deste processo inflamatório, o paciente pode experimentar dor crônica e intensa devido ao forte estado inflamatório, além de grande desconforto causado por outros sintomas, como náuseas. Neste sentido, os medicamentos fitoterápicos podem ser alternativas eficazes para tratar estes distúrbios do trato gastrointestinal. Assim, o presente trabalho visou caracterizar a composição química, toxicidade aguda, antioxidante,antinocéptico, atividades anti-inflamatórias, bem como investigar o efeito protetor gastrointestinal e os mecanismos do extrato hidroalcoólico das folhas da Hymenaea cangaceira (EHHc). Para isto, o extrato foi sintetizado e diferentes doses foram aplicadas a ratos albinos suíços, com os controles apropriados para comparação. Primeiramente, foi obtido um rendimento de aproximadamente 4% de EHHc, além disso, o principal composto fenólico encontrado foi ácido gálico (39,82±2,82 mg/g). Diante da toxicidade, o EHHc não apresentou toxicidade oral aguda (2.000 mg/kg). O resultado da atividade de limpeza radical da DPPH+ mostrou um valor de IC50 de 466,28 ± 1,44 μg/mL. No ensaio de eliminação do radical ABTS+ houve um valor de IC50 de 2.163,95 ± 48,53 μg/mL. A ação antinociceptiva foi confirmada pela redução da contorção nos animais. Além disso, o efeito terapêutico do EHHc era comparável à droga padrão (indometacina) mesmo com uma dosagem relativamente baixa de 50 mg/kg; e a ação anti-inflamatória foi comprovada pelo efeito inibidor de até 82,4% no inchaço das patas traseiras. Foi demonstrado que grupos de indivíduos tratados com EHHc, em todas as concentrações possíveis, mostraram uma boa atividade gastroprotetora, como o uso de carbenoxolona. Verificou-se que as vias de óxido nítrico e prostaglandinas eram os principais mecanismos de ação gastroprotetora e antiulcerogênica da EHHc. Além de valorizar uma espécie endêmica da Caatinga brasileira, os resultados do presente estudo podem impulsionar o uso desta espécie pela comunidade local e pelas indústrias farmacêuticas, bem como favorecer futuras pesquisas que testem estes efeitos em humanos.

Palavras-chaves:Úlcera; Gastroproteção; Inflamação; Extrato Hidroalcóolico; Caatinga.

ABSTRACT

Peptic ulcers are lesions in the digestive tract, with the intensification of this inflammatory process, the patient may experience chronic and intense pain due to the strong state of inflammation, in addition to great discomfort caused by other symptoms such as nausea. In this sense, herbal medicines can be effective alternatives to treat these disorders of the gastrointestinal tract. Thus, the present work aimed to characterize the chemical composition, acute toxicity, antioxidant, antinociceptive, anti-inflammatory activities as well as investigate the gastrointestinal protective effect and mechanisms of the hydroalcoholic extract of the leaves of Hymenaeacangaceira (EHHc). For this, the extract was synthesized and different doses were applied to Swiss albino mice, with the appropriate controls for comparison. Primarily, a yield of approximately 4% of EHHc was obtained, in addition the main phenolic compound found was gallic acid (39.82±2.82 mg/g). Faced with toxicity, EHHc did not present acute oral toxicity (2,000 mg/kg). The result of the DPPH+ radical scavenging activity showed an IC50 value of 466.28 ± 1.44 μg/mL. In the assay of elimination of the ABTS+ radical there was an IC50 value of 2,163.95 ± 48.53 μg/mL. The antinociceptive action was confirmed by the reduction of writhing in the animals. In addition, therapeutic effect of EHHc was comparable to the standard drug (indomethacin) even with a relatively low dosage of 50 mg/kg; and the anti- inflammatory action was proven by the inhibitory effect of up to 82.4% in the swelling of the hind paws. It was shown that groups of individuals treated with EHHc, in all possible concentrations, showed a good gastroprotective activity, such as using carbenoxolone. It was seen that the nitric oxide and prostaglandin pathways were found to be the main mechanisms of gastroprotective and antiulcerogenic actions of EHHc. In addition to valuing an endemic species of the Brazilian Caatinga, the results of the present study can boost the use of this species by the local community and pharmaceutical industries, as well as favoring future research that tests these effects in humans.

Keywords: Ulcer; Gastroprotection; Inflammation; Hydroalcoholic Extract; Caatinga.

LISTA DE ABREVIATURAS E SIGLAS

AINE Anti-inflamatórios não esteróides
CaSR Receptor sensível ao cálcio
CAT Catalase
CCK Colecistocinina
CCK2R Receptor de colecistocinina tipo 2
Cd Cádmio
Cl^- íon cloreto
CO2 Dióxido de carbono
COX1 Ciclo-oxigenase 1
COX2 Ciclo-oxigenase 2
ECL Célula tipo-enterocromafim
GPx Glutationa peroxidase
GMPc Monofosfato cíclico de guanosina
GLP-1 peptídeo semelhante ao Glucagon
GRP peptídeo liberador de gastrina
H+ Íon de hidrogênio
HCL Ácido clorídrico
H2CO3 Ácido carbônico
HCO3- Bicarbonato
HCO^- Grupamento formila
K+ Íon potássio
Hg Mércurio
H2O2 Peróxido de hidrogênio
IL-1β Interleucina 1 beta
LPAR5 receptor de ácido lisofosfatídico 5 acoplado à proteína
G LOOH Hidroperóxido lipídico
MUC Mucina
NANC transmissor não noradrenérgico e não colinérgico
Na+ Íon de sódio
NO Óxido nítrico
-OH Grupamento hidroxila
PAF Fator ativador de plaquetas
PG Prostaglandina
Ph Potencial hidrogeniônico
PP polipeptido pancreático
RENAME Relação Nacional de Medicamentos Essenciais
ROS Espécies reativas de oxigênio
SNC Sistema nervoso central
SOD Superóxido dismutase
TGI Trato gastroinstestinal
TNF-α Fator de necrose tumoral alfa
GSH Glutationa reduzida
MDA Malondialdeído

SUMÁRIO

1. INTRODUÇÃO

Dor e inflamação, apesar da interligação, apresentam conceitos diferentes. A inflamação é definida como reação complexa de tecidos vascularizados à infecção, exposição a toxinas ou lesão celular, seguido do extravasamento de proteínas plasmáticas e leucócitos. Embora a inflamação desempenhe um papel protetor no controle de infecções, também pode causar dano tecidual (ABBAS; LICHTMAN; PILLAI,2008). A dor é definida pela Associação Internacional para o Estudo da Dor como uma experiência sensorial e emocional desagradável, associada a um dano real ou potencial dos tecidos, e é considerada uma experiência subjetiva de percepção multidimensional, variando tanto na qualidade quanto na intensidade (GOMES et al., 2006).

A úlcera gástrica é uma doença que apresenta como sintomas a dor e a inflamação, na maioriadas vezes na sua forma mais grave, está associada ao não tratamento da gastrite, onde as bactérias vão perfurando as camadas do estômago, formando feridas e podendo causar sangramentos graves, necessitando de tratamento cirúrgico de urgência. Em casos mais graves, a úlcera pode ser mais profunda e produzir sangramento significativo, além dos ácidos poder causar corrosão completa da parede do trato digestório (DE LIMA, Andressa Gomes et al., 2021).

A caatinga, também conhecida como floresta branca, é um bioma que só existe no Brasil e se localiza, principalmente, na região nordeste, chegando a ocupar 70% do território. A caatinga apresenta grande diversidade biológica, suas características, como clima com altas temperaturas, chuvas irregulares e diferentes tipos de solos, caracterizam a singularidade desse ecossistema. Suas plantas nativas são utilizadas pelas comunidades locais, principalmente pelas suas atividades terapêuticas, contudo, ainda são pouco validadas cientificamente (RICARDO et al., 2018).

As espécies da *Hymenaea* têm sido estudadas, em virtude da grande utilização das mesmas, nas práticas terapêuticas (DA SILVA OLIVEIRA et al., 2016). Essa planta é conhecida em todo o território brasileiro como “jatobá”, “jetaíba” “jetaí”, “jataí-uva” (DA SILVA OLIVEIRA et al., 2016), etêm sido comumente usadas na medicina tradicional no tratamento de infecções, inflamação, reumatismo, dor, efeitos antidiarreicos, gastroprotetores e cicatrizantes em úlceras gástricas (VEGGI et al., 2014). Atividades dos extratos das folhas e raízes desta espécie são concretizadas em presença de vários elementos químicos como

compostos fenólicos, especificamente os flavonóides (VEGGI et al., 2014; DA SILVA OLIVEIRA et al., 2016).

As comunidades tradicionais empregam as espécies da planta *Hymenaea*sem distinções, no entanto, há pouco tempo novas espécies desse gênero foram caracterizadas na região nordeste do Brasil (PINTO et al., 2017). No que diz respeito à morfologia, a espécie *H. cangaceira* tem sido relatada semelhante à espécie da *H. martiana* e *H. longifólia*. Distingue-se no arranjo de folíolos glabros, ovados a largos elípticos, ovários glabros e um fruto comparativamente reduzido e cilíndrico (OLIVEIRA DE VERAS et al. 2019).

A ampla ocorrência de úlcera gástrica vem aumentando no decorrer dos anos (OFUSORI; MOODLEY; JONNALAGADDA, 2020), tornando-se um problema mundial de saúde pública (ZHOU et al., 2020). Essa condição produz efeitos graves sobre a qualidade de vida dos pacientes, pois o processo inflamatório instaurado, quando contínuo e intenso, causa lesão grave na mucosa do TGI, gerando um microambiente repleto de padrões moleculares associados a dano (DAMPs) que recruta ainda mais células imunes e amplifica o processo. Com esse progresso da patologia, a sinalização das vias de nocicepção se intensifica e o paciente apresenta dores fortes e crônicas, além de absorção de nutrientes prejudicada, pré-disposição para surgimento de processos neoplásicos e possíveis hemorragias. Ademais, alguns fármacos utilizados no tratamento dessa patologia geram efeitos colaterais desagradáveis, levando assim a procura de formas alternativas de tratamento (OFUSORI; MOODLEY; JONNALAGADDA, 2020).

Diante do efeito antiulcerativo apresentado pelo Jatobá e da dificuldade mundial que abrange a úlcera gástrica e suas possível consequências como a dor e inflamação, é primordial investigar a aplicação do extrato da *H. cangaceira* sobre o processo ulcerativo gástrico. Pois, são raros os estudos da atividade biológica relacionada a essa espécie na literatura científica. Nesta circunstância, o presente projeto tem como objetivo investigar o efeito gastroprotetor, bem como avaliar in vivo os possíveis mecanismos envolvidos no processo antiulcerativo do extrato das folhas do Jatobá (*Hymenaea cangaceira*).

2.FUNDAMENTAÇÃO TEÓRICA

2.1. Caatinga

2.1.1 Características gerais

A caatinga faz parteda região semiárida do Brasil sendo composta predominantemente por plantas xerófitas, lenhosas, espinhosas e decíduas. O nome Caatinga vem do Tupi-Guarani e significa “floresta branca”, em alusão as características da vegetação, que, em períodos de seca que podem durar por mais de 11 meses, fica sem folhas e apenas os galhos embranquecidos das árvores e arbustos permanecem sobre a terra e montam a paisagem desse bioma (DE OLIVEIRA et al., 2012). A Caatinga ocupa mais de 750,000 km² no nordeste do Brasil com um rico patrimônio biológico, sendo o único bioma no mundo com presença restrita ao território brasileiro (SOARES, 2016).

Dentre os biomas brasileiros, a Caatinga ainda é bastante desconhecida pela ciência, principalmente por existir uma crença que sua floresta é resultado apenas da transformação de outra formação vegetal. Apesar de estar realmente alterada em algumas regiões, principalmente nas terras mais baixas, a Caatinga é um bioma de grande biodiversidade, com relevância biológica e beleza peculiar, possuindo cerca de 3.150 espécies distribuídas em 950 gêneros e 152 famílias de angiospermas, das quais 23% do total de espécies e 29 gêneros são endêmicos (FERNANDES; QUEIROZ, 2018).

O amplo espaço da Caatinga é bastante heterogêneo e inclui espécies representantes de diversos biomas globais, além da vegetação típica. Essa fragmentação da vegetação é determinada por microvariações locais de clima e solo e os fragmentos são estruturais, funcional e floristicamente distintos. Essa gama de combinações e variações microclimáticas apresenta uma proporção expressiva de táxons raros e endêmicos, muitos deles utilizados pela população por suas propriedades terapêuticas. As interações da população com a Caatinga formam um sistema socioecológico agropastoril altamente dependente dos recursos da vegetação (TABARELLI et al., 2018).

Figura 1. Área de ocorrência do Bioma Caatinga.

Fonte: IBGE- Instituto Brasileiro de Geografia e Estatística. 2004. Mapas de Biomas do Brasil.

2.1.2 Aspectos Socioecológicos

Dentre os biomas brasileiros, a Caatinga é, provavelmente, o menos conhecido e estudado pela Botânica, e até 2005, 41% da região nunca havia sido investigada e 80% permanecem subamostrada. As formações vegetais da caatinga são constituídas por plantas que podem produzir óleos, ceras, fibras, bioextratos, plantas frutíferas, ornamentais e madeireiras, e com algumas espécies podendo apresentar múltiplos usos. Além disso, esse bioma também

apresenta uma coleção de espécies com potencial para produção de agentes praguicidas, matérias primas para indústria química, alimentar, cosmética e farmacêutica, incluindo plantas produtoras de óleos essenciais e de uso medicinal (MAIA et al., 2017).

Apesar de pouco estudada, a flora da Caatinga é muito conhecida pelas comunidades que habitam nesse bioma. Diversos estudos realizaram entrevistas com os moradores e constataram um rico conhecimento das comunidades não só sobre quais espécies vegetais estão presentes nos arredores, mas também quais têm propriedades medicinais e suas indicações (SILVA; FREIRE, 2010). Porém, esse conhecimento popular necessita de confirmação científica, como aponta um estudo da Universidade Federal de Pernambuco (UFPE). Foram realizadas entrevistas com moradores de uma comunidade local e constatou-se que tais moradoresconhecem e utilizam efeitos terapêuticos de plantas como a *Myracrodruonurundeuva*, por exemplo. Porém, nas entrevistas foi revelado que essa utilização não é otimizada, pois os métodos de extração e as partes da planta escolhidas muitas vezes não são as mais eficientes para atingir o pico de seu potencial terapêutico (ALBUQUERQUE; MELO, 2018).

2.2. Plantas medicinais

2.2.1 Cenário Histórico

Na história, sempre foi necessário lidar com o surgimento de sintomas causados por inúmeros fatores no cotidiano e para isso utilizava-se o conhecimento sobre plantas medicinais obtido com observação de seu uso pelos animais e de experiências compartilhadas. Mas, o desenvolvimento da indústria farmacêutica causou uma redução no interesse no uso de produtos medicinais naturais. Atualmente, há um elenco de 12 plantas medicinais e fitoterápicos constituindo a Relação Nacional de Medicamentos Essenciais (RENAME) (FERREIRA et al., 2019). O conceito de planta medicinal é toda planta que quando administrada por qualquer via em seres humanos ou animais, são capazes de produzir algum efeito terapêutico, sendo utilizada como remédio caseiro ou matéria-prima para fabricação de fitoterápicos e outros medicamentos (FIRMO et al., 2011).

Nos anos recentes, a utilização de tratamentos alternativos para doenças baseado em plantas demonstrou um crescimento considerável e atualmente é uma prática mundialmente disseminada, representando um recurso terapêutico que pode ser de grande importância para

muitas comunidades através dos chamados compostos bioativos. Além da utilidade para comunidades locais, esses compostos vêm chamando atenção de pesquisadores, que procuram testar e determinar atividades benéficas desses extratos, que podem ser exploradas como tratamento farmacológico alternativo, entre outras funções. Algumas plantas do gênero *Croton*, por exemplo, possuem propriedades medicinais e inseticidas já comprovadas cientificamente (GALHAS, 2015).

2.2.2 Uso de plantas medicinais por comunidades da Caatinga

Como dito anteriormente, as populações que habitam em regiões de Caatinga possuem um conhecimento único sobre as plantas de suas áreas, que normalmente é repassado de maneira informal a cada geração e disseminado na comunidade. O uso dessas plantas é feito de diversas maneiras, dentre chás, xaropes e lambedores, dependendo da planta e do efeito desejado. Diversas pesquisas realizadas com os residentes a fim de confirmar a validez e segurança dessas informações e costumes já foram realizadas, comprovando que apesar de não ser completamente ideal, o conhecimento repassado dentre os habitantes se mostra válido e eficaz na maioria das circunstâncias (MAIA et al., 2017).

Um desses estudos foi realizado no município de Picuí (Paraíba), no qual se entrevistou representantes das residências que citaram 48 espécies de plantas medicinais utilizadas no bairro de Monte Santo, área mais urbanizada do município, e 42 espécies utilizadas no Sítio de Massapê (totalizando 64 espécies distintas). Porém as variedades de plantas mais citadas nesse município não são espécies nativas da Caatinga, mostrando que até mesmo algumas comunidades familiarizadas com a área e vegetação da Caatinga, desconhecem o potencial terapêutico único de certas espécies endêmicas e nativas de sua região (COSTA; MARINHO, 2016).

Uma comunidade no estado da Bahia destacou representantes da família *Fabaceae* em conjunto com a família *Astraceae*, no uso terapêutico pela população. Entre esses destaques está a espécie*Hymenaeacourbaril*(Jatobá), citada como tendo maior valor de usopara comunidades de Lagoa Grande (PE), com indicações populares para tratamento de sintomas localizados em diferentes sistemas corporais, principalmente os sistemas digestório, respiratório e cardiovascular (ALBERGARIA; SILVA; SILVA, 2019).

2.3.A família *Fabaceae*

2.3.1 Características Gerais

Essa família possui duas nomenclaturas científicas igualmente válidas, chamada de *Fabaceae*, proveniente do termo latino *faba*, que significa fava ou feijão, e também denominada de *Leguminosae* remetendo ao fruto típico dessas plantas comumente conhecidas como leguminosas. Sendo uma das maiores famílias botânicas, possui representantes em quase todas as regiões do mundo (distribuição cosmopolita) e os vegetais que a compõe são de hábito variado, podendo ser herbáceas, trepadeiras, arbustivas e arbóreas. Uma característica importante e muito presente nessa família é a capacidade de formar complexos simbióticos com bactérias diazotróficas, que são capazes de utilizar moléculas de nitrogênio atmosférico (N_2) para sintetizar compostos nitrogenados que participam de processos metabólicos das plantas (BULEGON et al., 2016).

2.3.2 Importância Socioeconômica

Essa família é uma das mais economicamente importantes e possui grande relevância medicinal, pois têm abundância desubstâncias bioativas como flavonoides e compostos biossinteticamente relacionados. No Brasil, *Fabaceae* é a mais rica das famílias presentes na flora e apesar de sua biodiversidade vir sendo progressivamente perdida, representa um importante grupo de plantas que ainda compõe a maior parte da diversidade florística nas regiões brasileiras, incluindo a da Caatinga (AMORIM et al., 2016). Os indivíduos pertencentes a essa família possuem efeitos terapêuticos conhecidos e estudados. Um exemplo é o potencial farmacológico do gênero *Myroxylon*, que é utilizado na medicina popular para sintomas variando desde dores de ouvido até úlceras (atividade antibiótica cientificamente comprovada contra Helicobacterpylori) (PEREIRA etal., 2019).

2.3.3 Características Fitoquímicas

Na família *Fabaceae* existe uma riqueza particular em flavonoides e compostos relacionados como rotenóides e isoflavonas. Os flavonoides são um dos metabólitos

secundários mais abundantes no reino *Plantae,* estando ausentes apenas em organismos marinhos, já as isoflavonas ocorrem principalmente na *Fabaceae*. Alcaloides, terpenóides são outros de compostos bioativos com presença significativa nessa família (IGNOATO, 2012).

2.3.3.1 Flavonóides

Os vegetais da família *Fabaceae* são ricos em compostos flavonoides, que são metabólitos secundários de baixo peso molecular. Encontrados em locais da planta como fruto e flores, esses compostos possuem diversos efeitos biológicos atribuídos. Os potenciais terapêuticos mais associados aos flavonóides são os efeitos anti-inflamatório, antioxidante, antiviral, e até regulação hormonal através de ligação às enzimas desidrogenases de esteroides. Além disso, também é investigada certa ação anticancerígena vinda desses compostos, relacionada ao seu efeito antioxidante (DOS SANTOS; RODRIGUES, 2017).

2.3.3.2 Alcalóides

Os alcaloides, também muito presentes nas plantas da família *Fabacea*, são compostos amina de cadeia fechada que têm como função original afastar insetos e animais das plantas, provavelmente por seu sabor desagradável. Porém, seu principal uso para a humanidade atualmente é na forma de medicamentos. Uma das importantes utilidades de um fármaco à base de alcalóide é o tratamento antineoplásico, possuindo classes de compostos inibidores da topoisomerase I, compostos que inibem a mitose e outros que impedem a desmontagem de microtúbulos, todos produzindo efeitos antitumorais (MARQUES; LOPES, 2015).

2.3.3.3 Terpenóides

Os terpenos são compostos vegetais que constituem parte dos óleos essenciais, são formados por condensação de outros compostos e quando oxidados, são chamados de terpenoides. Essas substâncias já são conhecidas na literatura por exercer ação sobre o sistema nervoso central, tendo sido relatadas atividades anticonvulsivante, ansiolítica, antinociceptiva e até efeitos psicoativos e alucinógenos (PASSOS et al., 2009). Estudos mais recentes também

relacionaram os óleos à base de terpenos à inibição de crescimento bacteriano e micológico, sendo capazes de inibir a respiraçã e induzir a perda de componentes celulares.

2.4.O gênero *Hymenaea*

O gênero *Hymenaea* agrupa diversas espécies dos chamados Jatobás, que possui representantes nativos de várias partes do globo, como *Hymenaea verrucosa* que é nativa da África do Sul e *Hymenaea cangaceira*, espécie endêmica da Caatinga no Nordeste do Brasil. Dentro do território brasileiro se encontram a maioria dos representantes desse gênero, abrigando mais espécies desse gênero que qualquer outro país. São árvores retas de troncos cilíndricos e com "cascas" lisas e geralmente de cor acinzentada. Têm folhas compostas com estípulas e suas flores e frutos começam a aparecer em uma média de 10 anos de idade da planta. Esse gênero de plantas possui importância socioeconômica que vai desde seu fruto com alto teor de fibra alimentar e rico em Magnésio e Cálcio, muito utilizado na alimentação de humanos e animais, até óleos e chás utilizados no âmbito medicinal (CIPRIANO et al., 2014).

As variedades de Jatobá já vêm sendo estudadas e apresentando inúmeros efeitos e potenciais terapêuticos como planta medicinal. O extrato das folhas de *Hymenaea rubriflora*, por exemplo, possui ação antioxidante e inibidora da acetilcolinesterase comprovadas, através de seus metabólitos secundários (MIRANDA et al., 2020). A subfamília *Detarioideae*, à qual esse gênero pertence, é conhecida por sua capacidade de produzir resina, que tem utilidade tanto na medicina como em joalheria e artesanato. A resina produzida por essas plantas também é de valor paleontológico por sua capacidade de fossilização de animais e outros vegetais (PINTO, 2017).

2.5 Espécie *Hymenaea cangaceira*

Por ser morfologicamente parecida com outras espécies de Jatobá (*H. martiana*e *H. longifólia*)a *Hymenaea cangaceira* teria passado despercebida por especialistas que estudaram a área. Sua descoberta revela como a biodiversidade e endemismo excepcionais da região da Caatinga permanecem pouco documentados, e grande parte se encontra em risco por mudanças climáticas além de explorações e extrativismo inadequados. Portanto, fica evidente a necessidade de aprofundar o conhecimento existente na literatura sobre as propriedades e

benefícios dos diversos representantes endêmicos da Caatinga, incluindo a espécie *Hymenaeacangaceira* (PINTO et al., 2017).

Vista a singularidade dessa espécie e a falta de dados a respeito de suas propriedades, *H. cangaceira*se mostra um alvo de interessepara estudos visando investigar seu potencial terapêutico. Um efeito particularmente pouco testado e relatado no espectro de ação dessa planta, porém já visto em vegetais do gênero *Hymenae spp.*, é a gastroproteção, que possui algumas vias de sinalização e moléculas efetoras em comum com outras atividades já citadas da espécie (ORSI etal., 2012). Essa variante do Jatobá já é utilizada na medicina popular para combater infecções de maneira geral, agravos respiratórios, reumatismo, neoplasias, dor e processos inflamatórios. Porém, nenhuma dessas atividades foi cientificamente validada de maneira consistente, embora já existam estudos relatando atividade antioxidante, protetora de danos ao DNA e antimicrobiana com espectro de ação relativamente abrangente. Além disso, também foi confirmada a ação antinociceptiva relatada na medicina popular, inibindo a nocicepção em 75% com farmacodinâmica através do sistema opióide (OLIVEIRA DE VERAS et al., 2020).

Estudos a respeito de *H. cangaceira* e outros representantes do gênero *Hymenaea spp*. geralmente utilizam a formulação e metodologia de óleo essencial para veicular os compostos farmacologicamente ativos. Uma das formas de extração desse óleo é feita através da técnica de hidrodestilação, em que água é destilada na presença das folhas e o óleo resultante é coletado e armazenado. Outro tipo de formulação para extração dos compostos bioativos é o extrato hidroalcóolico das folhas, o qual pode ser obtido por maceração das folhas utilizando etanol como solvente. A última formulação pode apresentar efeitos diferenciados do óleo essencial e potencialmente mais eficazes por ter todo o conteúdo das folhas macerado e dissolvido no etanol (FILHO et al., 2019).

2.6 Extratos bioativos

2.6.1 Definição

Em linhas gerais, compostos bioativos são produtos naturais essenciais e não essenciais presentes na cadeia alimentar e que podem exercer efeito sobre a saúde humana. Por conta de variações na composição e estrutura química, esses compostos se diferenciam quanto à ação

biológica. Entretanto, possuem características em comum como baixo peso molecular, origem vegetal e caráter orgânico, incapacidade de síntese no corpo humano (embora seja possível síntese artificial parcial ou total esses compostos) e apresentam efeito protetor da saúde humana. Alguns compostos bioativos são encontrados na dieta habitual do ser humano. E existem evidências que a depender das quantidades consumidas e biodisponibilidade, esses compostos naturalmente consumidos podem exercer papéis na redução do risco de doenças como o câncer e as doenças cardiovasculares, entre outras doenças crônicas não transmissíveis (SILVA, Maria Daniela Nunes; PINHEIRO, Elayne Bessa Ferreira., 2021).

Mais especificamente, esses extratos são soluções concentradas debioprodutos de matéria- prima vegetal com determinadas propriedades socioeconomicamente relevantes para a população. Com frequência e espectro de uso progressivamente maiores, esses extratos estão presentes na indústria cosmética, alimentícia, e desde a medicina popular até a clínica. Para viabilizar essa aplicabilidade, a pesquisa científica envolvendo plantas medicinais e extratos bioativos vê um grande avanço e ampliação. Testar propriedades terapêuticas de novas plantas e avaliar e aperfeiçoar metodologias dos procedimentos estão dentre os objetivos desses estudos. Além disso, também é preciso confirmar/retificar o conhecimento popular que é disseminado sobre inúmeras espécies de plantas medicinais, para a segurança da população (BRITO, Amanda Laura Vieira.2021).

2.6.2 Técnicas de Extração

A respeito das técnicas para extração dessas substâncias, existem algumas mais comumente utilizadas como a hidrodestilação, maceração com solvente e a extração de Soxhlet, que consiste em um aparelho laboratorial que trabalha com um processo intermitente de refluxo de solvente que resulta numa extração rica e sem perda dos materiais a serem analisados. Existem outros métodos menos convencionais para obtenção de compostos bioativos como a extração líquida pressurizada, extração supercrítica com CO_2, utilização de solventes não convencionais e extração por pressão negativa. Apesar de serem pouco empregadas em larga escala na esfera agroindustrial principalmente por seu custo elevado, as técnicas não convencionais apresentam algumas vantagens. Dentre os benefícios da extração por esses métodos está uma maior seletividade e teor elevado de compostos obtidos, além de

menor demanda energética e uso de solventes com menos impacto ecológico (CARVALHO; BERMAMASCO; GOMES, 2018).

A maceração com solvente é um dos métodos convencionais de extração de compostos bioativos, esse método pode seradaptado e modificado conforme a espécie vegetal a ser trabalhada. Fatores como variação entre solventes, que podem variar dentro de um mesmo grupo funcional (como os álcoois metanol, etanol etc.) e solventes com outro grupo funcional como as cetonas, que tem propriedades distintas e podem resultar em diferentes concentrações de compostos bioativos e efeito terapêutico variante. Alguns estudos apontaram a extração com acetona como mais eficiente em questão de concentração do composto bioativo e atividade biológica para seus extratos específicos, em contraste a alguns outros que relataram ter encontrado melhor desempenho em extrações com metanol. A escolha do solvente e das adaptações do processo depende do tipo de vegetal utilizado e do conteúdo do extrato específico (SANTOS et al., 2016).

Uma configuração que aparece como promissora na literatura é a maceração das folhas com o solvente etanol. Essa técnica constitui em macerar o material biológico e mantê-lo em contato com oetanol em diferentes concentrações (50%, 70%) por um período que pode variar de três horas até três semanas em temperatura ambiente, para que os compostos bioativos das folhas sejam dissolvidos nele. O uso do etanol é mais eficiente quando utilizado na extração de compostos como os terpenos, polifenóis, flavonoides, alcaloides e taninos (DALOSTO; COLTURATO; PASQUALETTO, 2016).

2.6.3 Extratos Bioativos com Atividade Gastroprotetora

Alguns desses metabólitos secundários estão presentes nas plantas do gênero *Hymenaea*, como os taninos, terpenos e flavonóides, que demonstram auxiliar na gastroproteção através de diversos mecanismos. Um estudo que analisou colite ulcerativa, uma doença na mucosa do intestino, discutiu sobre como os fármacos normalmente utilizados para essa doença têm baixa responsividade e diversos efeitos colaterais indesejáveis, frisando a necessidade da busca de novos compostos úteis para tratamento dessa complicação e outros acometimentos relacionados. Alguns autores relatam que os taninos são capazes de formar uma camada protetora sobre a pele e mucosas de mamíferos, além de promover contração de capilares (PEDRO, 2015).

Já se pode encontrar na literatura diversos artigos que investigam a capacidade

gastroprotetora de extratos bioativos. Um deles relata uma pesquisa com modelo de gastrite experimental induzida por etanolpublicada no ano de 2021 que descreve uma atividade de gastroproteção associada ao extrato das folhas de *Stemodiamaritima L.*, composto principalmentepor flavonoides e terpenos, através da quantificação de indicadores de estresse oxidativo e parâmetro inflamatório. O sucesso na utilização do extrato demonstrou que o pré-tratamento com esses metabólitos foi capaz de fornecer proteção à mucosa gástrica, diminuindo significativamente o índice de lesão ulcerativa (DE SOUSA et al., 2021).

2.7. Anatomia e fisiologia do estômago

O estômago é um órgão presente no tubo digestivo e pode ser dividido anatomicamente em cinco porções: a junção gastroesofágica, fundo, corpo, o antro e o piloro. Possui também face anterior e posterior, que são demarcadas uma da outra por curvaturas maiores e menores. O órgão também é limitado por dois sistemas de esfíncteres: o esfíncter esofagiano inferior, através do qual o estômago se comunica com o esôfago, e na parte distal do órgão fica o esfíncter pilórico, ligando-o com o duodeno (SOYBEL, 2005).

A junção gastroesofágica é facilmente distinguível quando vista endoscopicamente, partindo do epitélio estratificado e escamoso do esôfago e transitando para um epitélio glandular e colunar do lúmen do estômago. Esse epitélio glandular é responsável pela secreção de uma mucosa gástrica contendo glicoproteínas, mucinas e água. Esse muco tem um pH alcalino e serve como proteção do lúmen do estômago contra lesões mecânicas e do ácido gástrico. O interior do estômago é totalmente rugoso e, por tal razão, há um aumento na área de superfície e que também permite maior expansão quando ocorre a chegada de alimento. (PHILLIPSON et al., 2008).

A mucosa gástrica pode ser subdividida em três camadas: (a) a superfície epitelial simples colunar, o (b) tecido conjuntivo frouxo (chamado de lâmina própria), e a (c) mucosa muscular (de músculo liso). A camada de epitélio superficial adentra a lâmina própria dando forma as fossetas gástricas e terminando nas glândulas. Essas glândulas podem ser formadas por diferentes tipos celulares a depender da região do estômago que se encontra. A porção glandular cardíaca, localizada abaixo do esfíncter esofágico, contém majoritariamente células glandulares secretoras de muco. Na porção do corpo e fundo do estômago está presente

diversos tipos celulares, como as parietais secretoras (produtoras de ácido clorídrico), células principais (produtoras de pepsinogênio), células D antrais (somatostatina) e células enterocromafins que liberam histamina (JAINU; DEVI, 2006).

2.7.1 Fisiologia da secreção gástrica

As células parietais são células epiteliais que possuem a capacidade de secretar ácido gástrico (HCL) no lúmen do estômago, deixando o pH mais ácido e facilitando a ação de enzimas digestivas. Adicionalmente, as células parietais secretam uma glicoproteína chamada fator intrínseco, necessário para a absorção de Vitamina B12 no íleo terminal do intestino. O pepsinogênio, enzima liberada pelas células principais, é uma das enzimas digestivas dependente do pH ácido do estômago (entre 1,8 a 3,5) para realizar sua atividade catalítica. A sua forma ativa, chamada de pepsina, é necessária para digerir proteínas em unidades menores como os peptídeos.

A produção do ácido gástrico se dá pela secreção de prótons de hidrogênio H^+ através bomba de H^+-K^+-ATPase presente em canalículos intracelulares na membrana apical das células parietais. A bomba de prótons captura os K+ no líquido extracelular em troca da liberação de H+, o qual provém da dissociação do ácido carbônico (H_2CO_3). O dióxido de carbono (CO_2) é o precursor desse ácido através de uma reação de hidratação. Já o ânion do cloro ($Cl^{-)}$ é liberado no interstício por um antitransportador localizado na membrana basolateral das células parietais, que em troca da liberação desse ânion, resgata o bicarbonato (HCO^-) proveniente da dissociação do bicarbonato de sódio (ENGEVIK, et al 2020).

A secreção ácida está relacionada com a digestão de proteínas, como também absorção de ferro, cálcio, vitamina B12, além de agir como proteção imunológica contra o crescimento bacteriano indesejado na mucosa do estômago. A regulação da secreção gástrica envolve o sistema nervoso, através de peptídeos como a colecistocinina (CCK) e leptina, produzidos no intestino que atravessam a barreira hematoencefálica e estabelecem uma sinalização entre o cérebro e intestino. O nervo vagal contém fibras aferentes que convertem sinais mecânicos e químicos do estômago e levam essas informações ao cérebro. O núcleo motor dorsal do vago localizado na parte dorsal parece ser o principal envolvido na integração dessas informações e já é demonstrado em ratos que estimulação elétrica das células desse nervo provoca a secreção

gástrica (SCHUBERT, 2007).

2.7.1.1 Gastrina

As células G estão localizadas próximo ao piloro e produz um hormônio proteico neuroendócrino chamado gastrina. A liberação desse hormônio é influenciada pela ingestão de alimentos com alto teor proteico, pelo peptídeo liberador de gastrina (GRP), pela distensão gástrica e um pH elevado. É relatado a presença de vários receptores acoplados nas células G, como o CaSRe LPAR5, que detectam peptonas e aminoácidos, e agem como um mecanismo de indução da secreção gástrica (PROSAPIO et al. 2021).

O hormônio tem influência no aumento da produção de HCL pelas células parietais. O receptor de gastrina, denominado de CCK2R encontra-se geralmente acoplado à proteína G na membrana basolateral das células parietais. A gastrina é liberada na corrente sanguínea e segue para o fundo gástrico onde a maioria das células parietais estão localizadas (PROSAPIO et al. 2021). Entretanto, a ação direta da gastrina na produção de ácido gástrico é diminuta, como já foi demonstrado que fármacos como a atropina e famotidina inibem quase que completamente esse mecanismo.

Adicionalmente, os receptores CCK2 estão presentes no sistema nervoso central, células endoteliais como também em alguns leucócitos, apontando que a gastrina pode também participar de outros mecanismos fisiológicos. A via de sinalização gastrina-CCK2R é essencial para a diferenciação e maturação das células parietais. É demonstrado que, em ratos deficientes de CCK2R, ocorre uma atrofia e decréscimo nas células parietais e ECL. Essa via de sinalização também parece contribuir para inibição do apoptose de células do epitélio gástrico e facilitar a migração celular nesse tecido (PROSAPIO et al. 2021).

2.7.1.2 Histamina

Outro tipo celular localizado próximo as glândulas gástricas do fundo e corpo do estômago são as células tipo-enterocromafim ou células ECL, que ao estímulo da gastrina liberam histamina. Essas células são do tipo enteroendócrinas fechadas, que não possuem contato direto com o lúmen do estômago pois são próximas a membrana basal e não possuem

microvilosidades. A histamina produzida nas células ECL aumenta diretamente a produção de ácido gástrico pela via das histaminas, através dos receptores H2 presentes na superfície das células parietais. Essa liberação de histamina está diretamente ligada a via da gastrina/CCK2R e o peptídeo ativador da adenilato- ciclase pituitária. A ligação da histamina aos receptores H2 e consequente ativação leva a um aumento na atividade da adenilato ciclase, o que provoca um acúmulo de AMP cíclico intracelular. Isso assegura a molécula cAMP como um importante mensageiro dos receptores H2 dentro da célula parietal (LATORRE et al., 2016).

A função de regulação do ácido gástrico está limitada apenas ao receptor H2 da família de receptores histamínicos. De tal forma, antagonistas de receptores H1, como a mepiramina, não apresentam ação na secreção gástrica. O advento da criação de fármacos anti-histamínicos contribuiu bastante para o tratamento de úlceras duodenais e esofagite erosiva, e era padrão ourono tratamento desses acometimentos até a criação da classe de inibidores da bomba de prótons. Entre os fármacos da classe de antagonistas do H2 destaca-se no uso comercial a ranitidina,cimetidina, famotidina e nizatidina.

2.7.1.3 Somatostatina

Já as células D estão localizadas no piloro do estômago e secretam a molécula inibitória chamada somatostatina. Essa molécula age suprimindo a liberação de gastrina, reduzindo a produção geral de ácido gástrico. A somatostatina é secretada pelas células D do TGI, mas também por células presente no pâncreas, hipotálamo e sistema nervoso central (O'TOOLE et al. 2019).

A somatostatina foi primeiramente isolada como um peptídeo hipotalâmico contendo 14 aminoácidos, e identificado como um inibidor do GH, o hormônio de crescimento. Tempos depois, outra forma ativa da somatostatina, contendo 28 resíduos de aminoácidos e chamada de somatostatina-28 foi encontrada em intestino de suínos. A somatostatina-14 é a forma predominanteno SNC e na maioria dos órgãos periféricos, incluindo o TGI. Já a somatostatina-28 é majoritariamente produzida pelas células enteroendócrinas do intestino (MARTINEZ, 2013).

Em ratos, é no TGI que se encontra aproximadamente 65% da somatostatina total do corpo. Fatores nutricionais e pH ácido são os principais estimulantes da liberação de

somatostatina na região antral do estômago. (FERRAND; WANG, 2006). Adicionalmente, a chegada dos nutrientesna porção inicial do intestino provoca esse estímulo da somatostatina, efeito provavelmente advindo da ativação de reflexos neurais adjacentes as células D. A liberação da secreção gástrica também influencia a produção de somatostatina, a qual apresenta um feedback negativo em relação a secreção.

As moléculas mais conhecidas como estimulantes da liberação somatostatina são, gastrina e colecistoquinina (CCK). Alguns peptídeos da família secretina, como o peptídeo liberador de gastrina (GRP), a Oxintomodulina, o peptídeo semelhante ao Glucagon (GLP-1), e o peptídeorelacionado ao gene da calcitonina, os quais estão envolvidos diretamente com a inibição da secreção gástrica, podem também estimular a liberação da somatostatina. Por outro lado, insulina, glucagon, polipeptídeo pancreático (PP), e opioides são potentes inibidores da liberação da somatostatina (ADAMSKA et al., 2014).

2.8. Mecanismos de defesa da mucosa gástrica

O suco gástrico liberado no estômago possui pH bastante ácido, capaz de lesionar as células epiteliais que revestem o lúmen. Apesar do mecanismo de liberação desse ácido possuir uma regulação fisiológica, outros agentes externos podem ser causadores de lesão ao tecido epitelial. O álcool, por exemplo, pode causar lesão direta às células, ou o tabagismo, que prejudica o fluxo sanguíneo e cicatrização do estômago. Em resposta aos vários tipos de agentes lesivos, existe mecanismos de defesa (pré-epitelial, epitelial e subeptelial) que impedem a lesão tecidual esurgimento de úlceras gástricas(DONG; KAUNITZ, 2006).

2.8.1 Mecanismos epiteliais – Muco e células epiteliais

O epitélio gástrico possui uma camada de muco por toda sua superfície, que atua como a primeiro mecanismo de defesa aos agentes lesivos. O muco é secretado em todo o TGI, desde o estômago até o cólon, formando um gel aderente à superfície, agindo como uma barreira física às enzimas proteolíticas, como a pepsina. Esse muco é formado principalmente por água (95%) e glicoproteínas, denominadas mucinas (aproximadamente 5%), e também o epitélio adiciona

HCO-, formando assim um complexo muco/bicarbonato de sódio de caráter viscoso e com o pH neutro que dificulta agentes lesivos alcançarem o epitélio(LAINE; TAKEUCHI; TARNAWSKI, 2008).

As estruturas de várias mucinas como MUC1, MUC5AC, MUC5B e MUC6, têm sido elucidadas. A MUC5AC é normalmente expressa na superfície epitelial de células da cárdia, fundo e antro, e juntamente com a MUC1 compõem a primeira camada da membrana de muco. Já a MUC6 é expressa nas glândulas da região do antro e fundo do estômago, e deposita-se sobre a MUC5AC como uma camada mais densa de mucina, apresentando também um potencial bactericida (NIV; BANIĆ, 2014).

O tecido epitelial também tem sua importância para a manutenção da integridade da mucosa. Ocorre uma constante renovação celular e restituição do epitélio após injúrias, através de uma rápida migração de células epiteliais próximas às glândulas gástricas. Essa migração precede e ocorre independentemente da proliferação de células progenitoras, a qual ocorre apenas algumas horas após acontecer a injúria (NIV; BANIĆ, 2014). Sanders *et al*. (1985) demonstraram que a membrana apical de células principais possui uma forte resistência in vitro ao efeito lesivo de uma solução ácida (pH 2, por 4 h), embora a parte basolateral dessas células tenham sofrido com agentequímicos de pH levemente ácido (pH 5,5).

2.8.2 Microcirculação

Nas últimas décadas alguns estudos experimentais têm evidenciado a participação da microcirculação gástrica na proteção do epitélio contra agentes lesivos. A hipotensão visceral que alguns pacientes com enfermidades críticas apresentam pode ser um dos principais problemas para o desenvolvimento de úlceras pépticas associadas ao estresse. A consequência de uma diminuição da microcirculação gástrica pode ser a redução da motilidade gástrica, atrasando assim a remoção do suco gástrico e outros agentes lesivos e assim aumentando o risco de úlceras (BARDOU; QUENOT; BARKUN, 2015). Mersereau e Hinchey (1973) demonstraram que, em ratos submetidos a um choque hemorrágico por 10 minutos desenvolveram úlceras sob uma concentração de H+ de 25 mEq/l. Nenhuma ulceração ocorreu sob concentração de H+ a 10 mEq/l, ao menos que a microcirculação seja interrompida por 10 min. Já em estudo com estômagos de ratos submetidos a hipotensão hemorrágica e uma infusão intragástrica de 0.1 HCL (240–340 mmol/rat), as lesões gástricas

apareceram apenas quando a pressão arterial e consequente microcirculação gástrica (medida pela técnica de depuração de gás hidrogênio) foram reduzidas para um valor 40% menor que o basal (LEUNG et al., 1985).

O óxido nítrico (NO) apresenta um papel chave na microcirculação gastrointestinal, pois é um ativador da guanilato ciclase solúvel, que resulta na formação de GMP cíclico, um importante mensageiro das células musculares. A produção constitutiva de NO estabelece uma via de sinalização não adrenérgica não colinérgica (NANC) que medeia o relaxamento da musculatura do esfíncter esofagiano, estômago, duodeno, intestino salgado. A vasodilatação provocada pelo NO é importante para manter a barreira protetora da mucosa gastrintestinal e estabilizar a ação dos mastócitos. A atividade do NO é modulada pelo conteúdo de Ca^{2+} intracelular. O bloqueio dessa cadeia de sinalização provoca um aumento no estresse oxidativo, ativando mastócitos da mucosa gástrica, os quais aumentam a quantidade de histamina e Fator Ativador de Plaqueta (PAF). Esses efeitos em conjunto causam um aumento na permeabilidade epitelial e na liberação de HCL.

2.8.3 Prostaglandinas (PGs) e Cicloxigenases (COXs)

As prostaglandinas (PGs) são lipídeos biologicamente ativos produzidos pelos músculos do TGI. A síntese se dá pela liberação do ácido aracdônico da membrana celular pela fosfolipase A2 e subsequentemente convertido em prostaglandina H2 (PGH2) pela prostaglandina endoperoxidase sintase ou chamada também de cicloxigenase (COX). Duas isoformas de COX são importantes da periferia do estômago, a COX-1, que é constitutiva no tecido, e a COX-2, que é induzível. A COX-1 contribui para a homeostase do órgão, funcionando na manutenção do fluxo sanguíneo local, como também secreção de muco e bicarbonato. Já a COX-2 é induzida quando ocorre lesões ou ulcerações, agindo na cicatrização e angiogênese, como também a enzima é mais expressa quando há aumento de citocinas, fatores de crescimento e estimulantes de crescimento tumoral, sugerindo sua relação também com processos neoplásicos e inflamatórios (LOPES et al., 2020).

A ação das drogas anti-inflamatórias não esteroidais (AINEs) sobre a mucosa evidencia o papel das prostaglandinas na manutenção da integridade da mucosa gástrica. A atividade ulcerogênica de alguns AINES advém da inibição dos efeitos da COX-1. Por exemplo, a indometacina é um anti-inflamatório não esteroidal (AINE), e serve como um fármaco antinociceptico, anti-inflamatório e antipirético, sendo usado no tratamento de

doenças inflamatórias crônicas (MORAIS et al., 2020).

2.8.4 Enzimas e agentes antioxidantes

Para a proteção epitelial gástrica, algumas enzimas como, a superóxido dismutase (SOD), catalase (CAT), glutationa peroxidase (GPx) e glutationa S-transferase formam juntas um sistema antioxidante que atua no combate às espécies reativas do oxigênio. As ROS podem provocar um processo de peroxidação lipídica, que atinge lipídeos das membranas celulares, gerando desordens e alterando a permeabilidade das mesmas. A segunda linha de defesa antioxidante é realizada por alguns compostos de moléculas químicas pequenas, incluindo vitaminas, flavonóides da dieta, carotenóides, ácido úrico e a glutationa (GSH).

Alguns mecanismos de ação antioxidante podem ser exemplificados, como o zinco, que participa na proteção de grupos sulfidrilas e importante catalisador para a superóxido dismutase. Além disso, o zinco influencia para um aumento da expressão de metaloproteinas, importante classe de proteínas que tem como função biológica o transporte de íons metálicos e sequestro de metais tóxicos como Cadmio (Cd) e Mercurio (Hg)(AKINRINDE; FAPURO; SOETAN, 2021). Já o ascorbato, forma reduzida da vitamina C, é conhecido por prevenir o estresse oxidativo através da estabilização de radicais livres como hidroxilas (-OH), peróxido de hidrogênio (H2O2). Também previne a peroxidação lipídica via redução de um único elétron de hidroperóxidos lipídicos (LOOH) (PADAYATTY; LEVINE, 2016).

2.9 Lesão gástrica

Antes do contexto de lesão gástrica é necessário abordar sobre dor e inflamação. A definição de dor, pela Associação Internacional do Estudo da Dor (IASP),é uma experiência sensitiva e/ou emocional desagradável, ligada a um dano tecidual, potencial ou já pré-existente, podendo ser caracterizada, de acordo com a intensidade e duração, em dor aguda ou crônica. A dor atua como um mecanismo de proteção pelo qual o corpo reage contra uma agressão que, por sua vez, responde, afastando-se da fonte de estímulo doloroso. Quando persistente por

tempos prologados, o seu significado biológico é perdido(Ferreira, SH.; Neto, AO. 2009; Merskey H; Bogduk N., 1994). Já a inflamação é geralmente definida como uma resposta à infecção ou lesão. Isso leva a uma definição natural de inflamação como uma resposta a desvios da homeostase que não podem ser revertidos apenas por mecanismos homeostáticos. Descrevemos como os sinais inflamatórios atuam nas mesmas funções celulares envolvidas na organização e homeostase do tecido normal, a fim de coordenar as respostas de emergência às perturbações e, finalmente, retornar o sistema a um estado homeostático (MEIZLISH, Matthew L. et al., 2021).

A lesão ao epitélio gástrico, se não tratada e cicatrizada, pode ocasionar um processo inflamatório agressivo, chamado de gastrite. Essa doença inflamatória atinge cerca de 5 a 10% da população mundial geral, e pode incluir a formação de úlceras pépticas gástricas e duodenais que causam muitas complicações para o paciente, incluindo dor crônica forte, chegando a ser potencialmente fatais pois também provocam sangramento e /ou perfuração, aumentando assim a taxa de morbidade e mortalidade da doença (CARLOTTO et al., 2019). As úlceras se desenvolvem sobre o desequilíbrio entre os fatores lesivos e os de proteção, e exemplos de agentes lesivos estão a infecção por *Helicobacterpylori*, ácidos biliares, uso contínuo de anti-inflamatórios não esteroidais (AINEs), processo de isquemia e hipóxia, assim como o hábito do tabagismo e alcoolismo (PEREIRABARBOSA et al., 2019).

O consumo de etanol em excesso tem sido associado com diversos processos inflamatórios do TGI, como esofagite, doença do refluxo gastroesofágico, gastrite, úlcera péptica e câncer (MEDEIROS et al., 2009). O efeito do etanol na camada de muco envolve a degranulação de mastócitos e liberação de mediadores do processo inflamatório, inibição da síntese de prostaglandinas endógenas, síntese e liberação de citocinas como Interleucina 1(IL-1β) e fator de necrose tumoral (TNF-α). O etanol também solubiliza os componentes que formam o muco do estômago, expondo a mucosa gastrointestinal a agressões por perder um de seus principais mecanismos de proteção (ANTONISAMY et al., 2015). Com isso, além de intensificar o processo inflamatório, o que contribui para o desenvolvimento do quadro patológico, a área fica sensibilizada com estímulo intenso da nocicepção, o que pode prejudicar o cotidiano do indivíduo por estar sentindo dor forte constantemente.

No estudo de lesões gástricas, os laboratórios tentam recriar as formas de gastrite e úlcera gástrica que acometem os seres humanos, sendo bastante utilizado os modelos de lesão por

etanol e lesão por indometacina em estômago de ratos. O mecanismo de ação lesiva provocada pelo etanol é multifatorial e permite estudar várias vias de proteção gástrica, como também testar potenciais fármacos para potencializar a proteção à mucosa. As lesões gástricas induzidas por etanol são facilmente produzidas, através da administração intragástrica de pequenas doses (0,5- 2,0 mL). Cerca de 10 a 40% das porções glandulares do estômago de ratos ficam com lesões hemorrágicas após horas da administração.

A indometacina é um fármaco anti-inflamatório não esteroidal bastante utilizado na clínica e terapêutica, principalmente para alivio da dor, febre, e com eficiência também para tratamentos a longo prazo de patologias imunológicas como artrite reumatoide e espondilite anquilosante (NAGAI; ITO, 2014). O surgimento de lesões gástricas como resultado do tratamento com AINES é reconhecidamente um dos mais sérios efeitos adversos desta classe de medicamentos. Esse fármaco tem dois mecanismos bem caracterizados de indução a lesão gástrica, como o bloqueio das enzimas COX, inibindo a produção de PGs, como também favorece um gradiente de íons H+ para dentro do meio intracelular e efluxo de íons Na+ e K+ para o lúmen gástrico (FIORUCCI; ANTONELLI; MORELLI, 2001). Os mecanismos pelos quais ocorre o bloqueio da COX e a mudança no gradiente de íons são objetos de estudos atualmente, levando a popularização no uso modelo de lesão por indometacina em avaliação de extratos de plantas com potencial farmacológico.

Visto que o quadro de gastroenterite estabelecida é caracterizado por um intenso estado de inflamação, e com a intensificação desse processo inflamatório há a possibilidade do surgimento de úlceras, os pacientes acometidos por essas patologias precisam de tratamento eficaz e consistente. Porém, os principais fármacos com o propósito de justamente reduzir inflamação e estímulos dolorosos causam efeitos adversos no sistema gastrointestinal capazes de induzir dano tecidual e desencadear processos contrários ao objetivo de melhora clínica do paciente. Portanto, se mostra necessária a pesquisa e consolidação de novas substâncias terapêuticas no mercado que sejam capazes de aliviar os sintomas da patologia gástrica enquanto contribuem para resolução e reversão do estado patológico, sem produção de efeitos tóxicos relacionados a seu uso consistente.

3.OBJETIVOS

3.1 OBJETIVO GERAL

Objetivo investigar o efeito gastroprotetor, bem como avaliar in vivo os possíveis mecanismos envolvidos no processo antiulcerativo do extrato das folhas do Jatobá (*Hymenaea cangaceira*).

3.2 OBJETIVO ESPECÍFICO

- Preparação e avaliação do perfil fenólico do extrato hidroalcóolico das folhas de *Hymenaeacangaceira (EHHc);*
- Avaliação da Toxicidade aguda e dose letal mediana do *EHHc;*
- Avaliação da atividade antioxidante de *EHHc* por Eliminação do radical ABTS e eliminação do radical DPPH;
- Avaliação da atividade antinociceptiva e anti-inflamatória de *EHHc*;
- Avaliação da Atividade gastroprotetora de*EHHc*por ulceração gástrica induzida por etanol e por etanol/HC;
- Investigação dos mecanismos envolvidos na atividade gastroprotetora de *EHHc* por: Canais de K+ dependentes de ATP (K+ - ATP), Óxido Nítrico, compostos sufidrila (-SH) e Síntese de PGE2;
- Avaliação do estresse oxidativo após uso do *EHHc*por ensaio de glutationa e malondialdeído;
- Quantificação de citocinas e óxido nítrico em tecidos após uso do *EHHc*.

4. ARTIGO

Evaluation of Antinociceptive, Anti-inflammatory and Gastroprotective Effects of the hydroalcoholic extract from Jatobá leaves (*Hymenaea cangaceira* Pinto, Mansano & Azevedo)

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Journal of Ethnopharmacology

Evaluation of Antinociceptive, Anti-inflammatory and Gastroprotective Effects of thehydroalcoholic extract from Jatobá leaves (Hymenaea cangaceira Pinto, Mansano & Azevedo)

Manuscript Number:	
Article Type:	Research Paper
Keywords:	Ulcer; Gastroprotection; Inflammation; Hydroalcoholic Extract; Caatinga.
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Abstract:	Peptic ulcers are lesions in the digestive tract, with the intensification of this inflammatory process, the patient may experience chronic and intense pain due to the strong state of inflammation, in addition to great discomfort caused by other symptoms such as nausea. In this sense, herbal medicines can be effective alternatives to treat these disorders of the gastrointestinal tract. Thus, the present work aimed to characterize the chemical composition, acute toxicity, antioxidant, antinociceptive, anti- inflammatory activities as well as investigate the gastrointestinal protective effect and mechanisms of the hydroalcoholic extract of the leaves of Hymenaea cangaceira (EHHc). For this, the extract was synthesized and different doses were applied to Swiss albino mice, with the appropriate controls for comparison. Primarily, a yield of approximately 4% of EHHc was obtained, in addition the main phenolic compound found was gallic acid (39.82±2.82 mg/g). Faced with toxicity, EHHc did not present acute oral toxicity (2,000 mg/kg). The result of the DPPH+ radical scavenging activity showed an IC50 value of 466.28 ± 1.44 µg/mL. In the assay of elimination of the ABTS+ radical there was an IC50 value of 2,163.95 ± 48.53 µg/mL. The antinociceptive action was confirmed by the reduction of writhing in the animals. In addition, therapeutic effect of EHHc was comparable to the standard drug (indomethacin) even with a relatively low dosage of 50 mg/kg; and the anti- inflammatory action was proven by the inhibitory effect of up to 82.4% in the swelling of the hind paws. It was shown that groups of individuals treated with EHHc, in all possible concentrations, showed a good gastroprotective activity, such as using carbenoxolone. It was seen that the nitric oxide and prostaglandin pathways were found to be the main mechanisms of gastroprotective and antiulcerogenic actions of EHHc. In addition to valuing an endemic species of the Brazilian Caatinga, the results of the present study can boost the use of this species by the local community and pharmaceutical industries, as well as favoring future research that tests these effects in humans.
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Cover Letter

UNIVERSIDADE FEDERAL DE PERNAMBUCO

70 ANOS TEMPOS TRANSVERSOS

Recife (Pernambuco State, Brazil),

23th May 2022.Dear Editor,

Please find attached the manuscript "**Evaluation of Antinociceptive, Anti-inflammatory and Gastroprotective Effects of the hydroalcoholic extract from Jatobá leaves (*Hymenaea cangaceira* Pinto, Mansano & Azevedo)**", to be considered for publication under the category Original Research Article in *Journal of Ethnopharmacology*.

We are particularly excited about this contribution for several reasons. We believe that our findings provide a significant contribution to the literature, as our study was the first to investigate the chemical composition and biological activities (antioxidant, antinociceptive, activity anti-inflammatory, gastroprotective activity and investigation of mechanisms involved in gastroprotection in addition to toxicological safety) of the hydroalcoholic extract this species. All the results obtained are in accordance with the ethnopharmacological knowledge linked to the species.

We believe that the results of the study will be relevant to the journal's readers. Our work discusses the validation of traditional knowledge and the potential applications of natural products for use against diseases that culminate in the establishment of the evaluated activities.The results obtained indicate that the hydroalcoholic extract this species has promising activities for the future development of industrial pharmaceutical products.

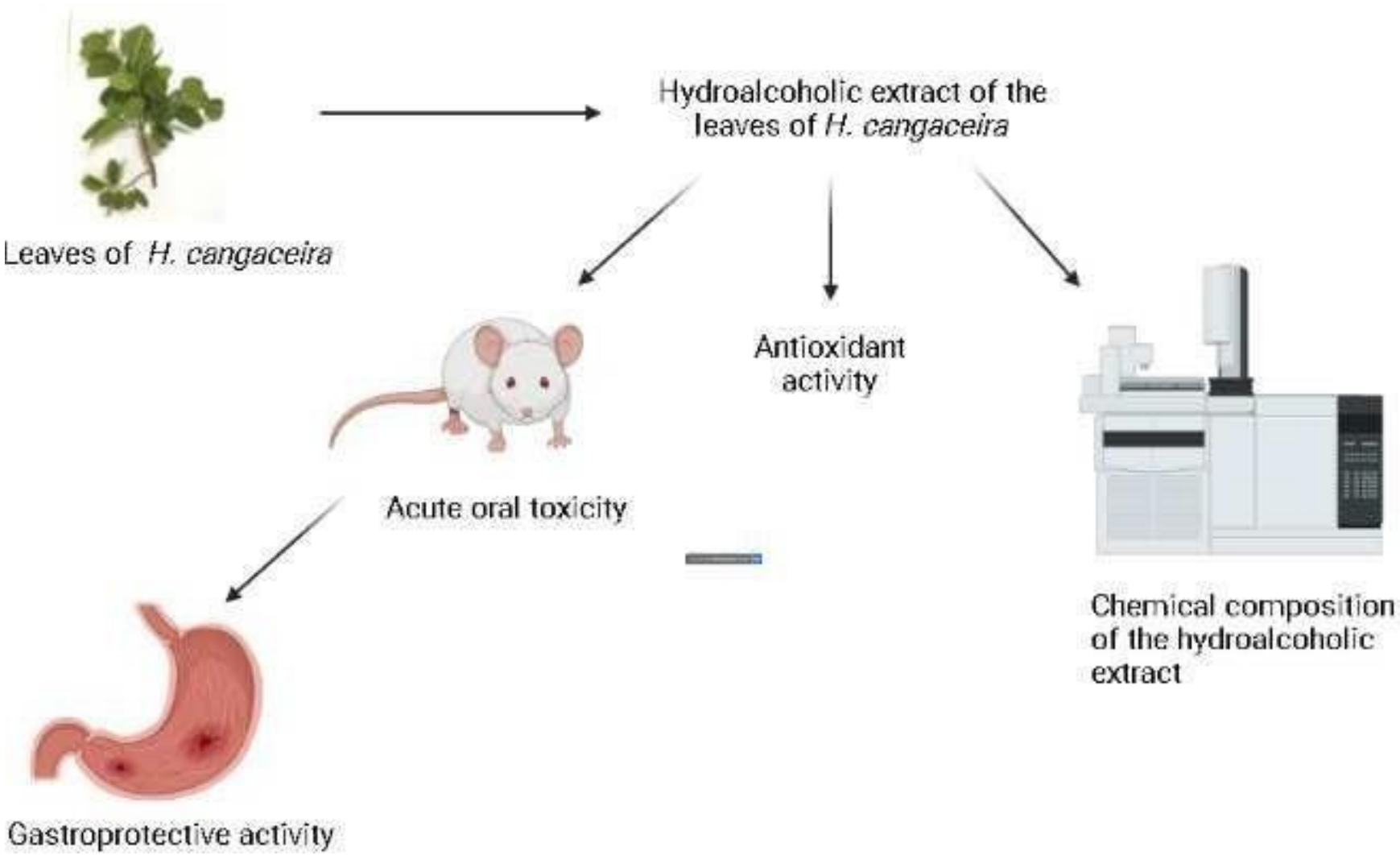
No material from the current manuscript is included in another manuscript, has been previously published, or is currently considered for publication elsewhere. In addition, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically, without the written consent of the copyright holder.

The ethical guidelines were followed by the researcher when carrying out

animal studies. We read and understand your journal's policies, and neither the manuscript nor the study violates any of them.
All authors participated sufficiently in the work to assume public responsibility for the content of the article, approved the final version of the manuscript and there is no conflict of interest to declare.

Thank you very much and I hope for your consideration. I look forward to hearing from you.Yours sincerely,
Maria Tereza dos Santos Correia and Weslley Felix de Oliveira Departamento de BioquímicaUniversidade Federal de Pernambuco, Recife, Brazil

Graphical Abstract Click here to access/download;Graphical Abstract;Untitled.png



ABSTRACT

Ethnopharmacological relevance

Peptic ulcers are lesions in the digestive tract, with the intensification of this inflammatory process, the patient may experience chronic and intense pain due to the strong state of inflammation, in addition to great discomfort caused by other symptoms such as nausea. In this sense, herbal medicines can be effective alternatives to treat these disorders of the gastrointestinal tract.

Aim of the study

Thus, the present work aimed to characterize the chemical composition, acute toxicity, antioxidant, antinociceptive, anti-inflammatory activities as well as investigate the gastrointestinal protective effect and mechanismsof the hydroalcoholic extract of the leaves of *Hymenaeacangaceira* (EHHc).

Materials and methods

For this, the extract was synthesized and different doses were applied to Swiss albino mice, with the appropriate controls for comparison.

Results

Primarily, a yield of approximately 4% of EHHc was obtained, in addition the main phenolic compound found was gallic acid (39.82±2.82 mg/g). Faced with toxicity, EHHc did not present acute oral toxicity (2,000 mg/kg). The result of theDPPH+ radical scavenging activity showed an IC50 value of 466.28 ± 1.44 µg/mL.In the assay of elimination of the ABTS+ radical there was an IC50 value of 2,163.95 ± 48.53 µg/mL. The antinociceptive action was confirmed by the reduction of writhing in the animals. In addition, therapeutic effect of EHHcwas comparable to the standard drug (indomethacin) even with a relatively low dosage of 50 mg/kg; and the anti-inflammatory action was proven by the inhibitory effect of up to 82.4% in the swelling of the hind paws. It was shown that groups of individuals treated with EHHc, in all possible concentrations, showed a good gastroprotective activity, such as using carbenoxolone. It was seen that the nitric oxide and prostaglandin pathways were found to be the mainmechanisms of gastroprotective and antiulcerogenic actions of EHHc.

Conclusions

In addition to valuing an endemic species of the Brazilian Caatinga, the results of the present study can boost the use of this species by the local community

and pharmaceutical industries, as well as favoring future research that tests these effects in humans.

Keywords: Ulcer, Gastroprotection, Inflammation, Hydroalcoholic Extract, Caatinga.

1. INTRODUCTION

Pain and inflammation, despite the interconnection, present different concepts. Inflammation is defined as a complex reaction of vascularized tissues to infection, exposure to toxins or cellular injury, followed by extravasation of plasma proteins and leukocytes. Although the inflammation plays a protective role in controlling infections, it can also cause tissue damage. The International Association for the Study of Pain defines pain as an unpleasant sensory and emotional experience, associated with actual or potential tissue damage, and is considered a subjective experience of multidimensional perception, varying in both quality and intensity (GOMES et al., 2006).

Gastric ulcer is a disease that has symptoms of pain and inflammation, most often in its most severe form, it is associated with the non-treatment of gastritis, where bacteria perforate the layers of the stomach, forming wounds and causing bleeding severe, requiring urgent surgical treatment. In more severe cases, the ulcer can be deeper and produce significant bleeding, and acids can cause complete corrosion of the digestive tract wall (DE LIMA, Andressa Gomes et al., 2021).

The Caatinga, also known as the white forest, is a biome that only exists in Brazil and is located mainly in the northeast region, occupying 70% of the territory. The Caatinga has great biological diversity, its characteristics, such as a climate with high temperatures, irregular rainfall and different types of soils, characterize the uniqueness of this ecosystem. Its native plants are used by local communities, mainly for their therapeutic activities, however, they are still poorly validated scientifically (RICARDO et al., 2018).

Hymenaea species have been studied, due to their wide use in therapeutic practices (DA SILVA OLIVEIRA et al., 2016). This plant is known throughout Brazil as “jatobá”, “jetaíba”, “jetaí”, “jataí-uva” (DA SILVA OLIVEIRA et al., 2016), and has been commonly used in traditional medicine to treat infections, inflammation, rheumatism, pain, antidiarrheal, gastroprotective and healing effects in gastric ulcers (VEGGI et al., 2014). Activities of extracts from the leaves and roots of this species are carried out in the presence of various

chemical elements such as phenolic compounds, steroids, alkaloids, amino acids, carbohydrates, coumarins, fatty acids and tannins (VEGGI et al., 2014; DA SILVA OLIVEIRA et al., 2016).

Traditional communities employ Hymenaea plant species without distinction, however, recently new species of this genus were characterized in the northeast region of Brazil (PINTO et al., 2017). With regard to morphology, the species H. cangaceira has been reported similar to the species of H. martiana and H. longifolia. It is distinguished by the arrangement of glabrous leaflets, ovate to wide elliptical, glabrous ovaries and a comparatively reduced and cylindrical fruit (OLIVEIRA DE VERAS et al. 2019).

It was observed in a mouse model that the antiulcer activity of the stem extract of one of the species of Hymenaea spp. presented similarity to lansoprazole, which is one of the most used drugs in the treatment of peptic ulcer (ORSI et al. 2012).

The wide occurrence of gastric ulcers has increased over the years (OFUSORI; MOODLEY; JONNALAGADDA, 2020), becoming a global public health problem (ZHOU et al., 2020). This condition has serious effects on the quality of life of patients, as the inflammatory process, when continuous and intense, causes serious injury to the GI mucosa, generating a microenvironment full of damage-associated molecular patterns (DAMPs) that recruit even more cells. immune system and amplifies the process. With this progress of the pathology, the signaling of the nociception pathways intensifies and the patient presents severe and chronic pain, in addition to impaired nutrient absorption, predisposition for the emergence of neoplastic processes and possible hemorrhages. Furthermore, given the anti-ulcerative effect presented by Jatobá and the worldwide difficulty that covers gastric ulcers, it is essential to investigate the application of *H. cangaceira* extract on the gastric ulcerative process. Therefore, studies of the biological activity related to this species are rare in the scientific literature. In this circumstance, the present work aims to investigate the gastroprotective effect as well as to evaluate *in vivo* the possible mechanisms involved in the anti-ulcerative process of the extract of the leaves of Jatobá.

2. Material and methods

2.1. Extract preparation

The aerial parts (leaves) of *H. cangaceira* (Pinto, Mansano&Azevedo) were collected in Catimbau National Park, Pernambuco, Brazil, Coordinates: 8°36′35"(S) and 37°14′40" (W). The sample was obtained in March 2018 by the team formed by researchers from the Federal University of Pernambuco (UFPE). A voucher sample used in this work was deposited in the herbarium of the InstitutoAgronômico de Pernambuco (IPA) under number 84888. After collection, the leaves of *Hymenaeacangaceira* were dried at room temperature for 15 days and crushed. The material (300g) was subjected to extraction by maceration where 70% ethanol was used as a liquid extractor in the proportion of 1:10. The procedure was performed twice in a row, for 12 hours each. At the end of each extraction process, the solvent used was separated from the extract using a rotary evaporator at reduced pressure at temperatures between 65-70°C. Approximately 12g of dry extract was obtained (4% yield).

2.2. Extract phenolic profile

The phenolic profile of the *H. cangaceira* extract was analyzed by High Performance Liquid Chromatography (HPLC) as described by Kim et al. (2013) with some modifications. The phenolic compounds in the extract were quantified using reversed-phase HPLC from Thermo Scientific (Waltham, USA) equipped with a quaternary pump, an autosampler and a Diode Array Detector (DAD). Reference phenolic standards and samples were filtered through an organic nylon membrane (0.22 µm) prior to injection. Data were processed using ChromQuest 5.0 (Thermo Scientific, Waltham, USA). Separation was performed using a Shim-pack CLC-ODS (M) C18 column (250 × 4.6 mm; Shimadzu, Kyoto, Japan) maintained at 30°C. The mobile phase consisted of 1% acetic acid (A) and acetonitrile (B). The column was eluted with a gradient of 0-30% (acetonitrile) for 0-10 min, 30-70% (acetonitrile) for 10-15 min, 70-100% (acetonitrile) for 15-20 min, and 100% (acetonitrile) for 20-25 min. The flow was 1.0mL/min and the injection volume was 10 µL. The detection wavelengths were optimized according to the wavelength of maximum absorption of reference compounds, such as: gallic acid (GA), syringic acid, catechin, vanillin, eugenol and vinyl acid. These compounds

were detected at 280 nm; where ellagic acid and quercetin were detected at 256 nm. Phenolic compounds were quantified based on retention time and absorbance spectrum of the extract measured with DAD by comparison with reference phenolic standards. An external calibration curve was constructed for each standard. Values were expressed in micrograms of phenolic compound per gram of sample.

2.3. Experimental model and ethical procedures

Males and females of albino Swiss mice (*Mus musculus*) aged 10-12 weeks and weighing 25-30g were obtained from the central vivarium of the Keizo Azami Immunopathology Laboratory (LIKA) at the Federal University of Pernambuco (UFPE).). The animals were housed in polypropylene cages with a solid bottom (size: 18cm x 34 cm x 41 cm) and sawdust floor at room temperature between 21°C and 25°C, following a 12-hour light/dark cycle, with access ad libitum to food and water. The animals were monitored and the experiments were carried out in accordance with the rules and procedures of the National Council for the Control of Animal Experimentation in Brazil (CONCEA) and theInternational Guidelines for Biomedical Research Involving Animals (CIOMS/ICLAS). All described procedures had prior approval from the Ethics Committee on the Use of Animals (CEUA) of the UFPE, under No. 71/2020.

2.4. Acute toxicity and median lethal dose (LD_{50})

Acute toxicity studies to assess safety were performed using the Organization for Economic Co-operation and Development (OECD, 2008). Initially, female Swiss albino were divided into groups (n=3), and treated orally (p.o.) by gavage with 100µL of the following doses of hydroalcoholic extract 1.75, 5.5, 17.5, 55, 175, 550, 1,750 and 5,000 mg/kg. The animals were

observed at 30, 60, 120, 180 and 240 minutes after oral treatment and daily for 14 days. Possible signs of alteration, such as tremors, convulsions, salivation, piloerection, hyperactivity, bleeding, among other signs of toxicity were observed. Mortality was also assessed for 14 days and the LD_{50} was calculated.

2.5. Antioxidant activity

2.5.1 ABTS radical elimination

Antioxidant activity of the hydroalcoholic extract by the2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was evaluated as described by OLIVEIRA DE VERASet al (2019). The radical cation ABTS+ was produced by the reaction between 7 mMof ABTS in H_2O and 140 ofmM potassium persulfate, stored in the dark at room temperature for 16 h. Before use, the ABTS+ solution was diluted in ethanol to gain an absorbance of 0.700 ± 0.05 at 734 nm. Dilutions ofhydroalcoholic extractwere mixed with the ethanolic solution of ABTS, being incubated at 25°C in the dark, with subsequent measurement of absorbance at 734 nm. Butylatedhydroxytoluene (BHT)and a Vitamin E analogue (Trolox®) were used as positive controls, and ethanol as the blank. The inhibition percentage (I%) was calculated using the following equation: I% =

{(A_{734}b – A_{734}s) / (A_{734}b)} x 100, where A_{734}b is the absorbance of the blank, and A_{734}s is the absorbance of the samples. All assays were performed in triplicate. The individual concentrations of the samples responsible for decreasing the initial activity of the free radical ABTS by 50% (IC_{50}) were calculated by linear regression.

2.5.2 Elimination of the DPPH radical

The evaluation of antioxidant capacity using themethodof the free radical 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was performed as described by OLIVEIRA DE VERAS et al. (2020). BHT and Trolox® were used as positive controls and methanol as a blank. The inhibition percentage (I%) was calculated using the following equation: I% = [(A517b – A517s) / (A517b)] x 100, where A517b represents the absorbance of the blank, and A517s the absorbance of the samples. All assays were performed in triplicate. The concentrations of samples that showed the ability to decrease the initial DPPH free radical activity by 50% (IC_{50}) were calculated by linear regression.

2.6. Antinociceptive activity

The evaluation was performed using the writhing test induced by acetic

acid according toRadulović et al., 2015. Sixgroups (n=6) of male mice were pre- treated orally (p.o.) by gavage with 100 µL of control (0.9% w/v saline), *H. cangaceira* hydroalcoholic extract (25, 50, and 100 mg/kg) , indomethacin (50mg/kg) or morphine (10mg/kg). After one hour, acetide acid (0.8% v/v) (10 mL/kg) was injected intraperitoneally (i.p.) to simulate nociception. Nociception intensity was quantified by the number of writhes during 15 min of observation after acetic acid administration, being expressed in relation to the number of abdominal writhes.

2.7. Anti-inflammatory activity

The evaluation was performed according to OU et al. (2019). Male mice were divided into five groups (n=6), being pre-treated orally with 100 µL of control (saline 0.9% w/v), hydroalcoholic extract of *H. cangaceira* (25, 50 and 100 mg/kg) or indomethacin (20 mg/kg). After one hour, the animals received carrageenan (1% w/v) (100 µL/paw) in the right hind paw and saline (0.9% w/v) (100 µL/paw) in the left paw. The volume of the right and left hind paws of each animal was recorded with the aid of a caliper at 1, 2, 3, 4 and 5 h after injection of carrageenan and saline (0.9% w/v). Edema inhibition was calculated by (right paw – left paw) and expressed in mm.

2.8. Gastroprotective activity

2.8.1. Ethanol-induced gastric ulceration

Acute gastric lesions were induced by absolute alcohol according to the method described by Robert et al. (1979) with modifications. Briefly, male Swiss albino mice that had fasted for 24 hours, with free access to water before the experiment, were divided into six groups (n=6). The animals were orally treated with negative control (0.9% saline solution), positive control (100 mg/kg carbenoxolone) or *H. cangaceira* hydroalcoholic extract (25, 50 and 100 mg/kg), except for the native group, which did not receive treatment. After one hour, 0.2 mL of absolute alcohol was administered orally. One hour after ethanol application, mice were sacrificed by cervical dislocation and their stomachs were removed. After removal, the stomachs were opened along the greater curvature and washed with cold saline, being subsequently compacted between two glass slides and the images were investigated to determine

the surface area of the gastric lesion in the ImageJ software (Bethesda, MD, USA). The extent of lesions was measured and expressed as Gastric Lesion Area (%) using ImageJ software (Version 1.45). Then, gastric tissue samples were frozen in liquid nitrogen and stored at -80°C for further biochemical evaluation.

2.8.2. Ethanol/HCl-induced gastric ulceration

Gastroprotective activity was assessed as described by Robert et al. (1979). Briefly, the mice after 24 h fasting with free access to water before the experiment were randomly assigned to seven groups (n = 6), as follows: negative control (0.9% saline solution), positive control (100 mg/kg carbenoxolone) or hydroalcoholic extract of *H. cangaceira* (25, 50 and 100 mg/kg), except for the native group, which did not receive treatment. After one hour, 0.2 mL of 3.0M ethanol/HCl 60% is administered orally. One hour after ethanol application, mice were sacrificed by cervical dislocation and their stomachs were removed. After removal, the stomachs were opened along the greater curvature and washed with cold saline, being subsequently compacted between two glass slides and the images were investigated to determine the surface area of the gastric lesion in the ImageJ software (Bethesda, MD, USA). The extent of lesions was measured and expressed as Gastric Lesion Area (%) using ImageJ software (Version 1.45).

2.8.3. Investigation of the mechanisms involved in gastroprotection

2.8.3.1. ATP-dependent K^+ channels (K^+ - ATP)

The evaluation was performed according to Rahgozar et al. (2003). Two groups of animals (n=6) were treated with 10 mg/kg, ip glibenclamide (selective potassium channel inhibitor) 30 minutes before administration of 100 mg/kg HEHc or 0.9% saline. One hour after administration of thehydroalcoholic extractor saline, the animals were treated orally with 0.2 mL of absolute ethanol. One hour after the ethanol application, the rats were sacrificed by cervical dislocation, having their stomachs removed and lesions evaluated.

2.8.3.2. Nitric oxide

To analyze whether the gastroprotective mechanism of HEHc is

associated with pathway, the protocol described by Matsuda and Yoshikawa et al. (1999). Briefly, the animals (n=6) were treated with 10 mg/kg ip, L-NG-nitro-arginine-methyl-ester (L-NAME) (non-specific inhibitor of the enzyme NO synthetase) 30 minutes before the administration of HEHc 100 mg/kg or 0.9% saline solution. One hour after administration ofhydroalcoholicextract or saline solution, the animals were treated with 0.2 mL of absolute ethanol orally. After one hour of ethanol application, the rats were sacrificed by cervical dislocation, having their stomachs removed and lesions evaluated.

2.8.3.3. Suphydryl compounds (-SH)

The participation of sufhydryl compounds associated with the gastroprotective effect of HEHc was determined according to Matsuda and Yoshikawa et al. (1999). The compound N-ethylmaleimide (NEM) 10 mg/kg, i.p. (blocker of sulfhydryl compounds) was administered to two groups of animals (n=6) 30 minutes before the administration of HEHc 100 mg/kg or saline 0.9%. One hour after administration of thehydroalcoholicextract or saline solution, the animals were treated orally with 0.2 mL of absolute ethanol to induce gastric injury. One hour after ethanol application, the rats were sacrificed by cervical dislocation and their stomachs were removed for lesion analysis.

2.8.3.4. PGE_2 synthesis

The evaluation was made according to Peskar, Ehlich and Peskar (2002). First, the animals (2 groups/n=6) were being treated with 10 mg/kg, i.p. of indomethacin (non-steroidal anti-inflammatory anti-inflammatory PGE2 synthesis inhibitor) 30 minutes before the administration of HEHc 100 mg/kg or 0.9% saline. One hour after administration of thehydroalcoholicextract or saline solution, the animals were treated orally with 0.2 mL of absolute ethanol to induce gastric injury. After one hour of ethanol application, the rats were sacrificed by cervical dislocation, having their stomachs removed for evaluation of the lesions.

2.9. Oxidative stress

2.9.1. Glutathione Assay

The reduced glutathione content in stomach tissues, such as non-protein

sulfhydryls, was estimated according to the method described by Sedlak and Lindsay, 1968. A glandular segment from each stomach was homogenized in 5 mL of ice-coldsolution 0.02 M of EDTA (1 mL/100 mg of tissue). Aliquots (400µL) of tissue homogenate were mixed with 320 µL of distilled water and 80 µL of 50% (w/v) trichloroacetic acid in glass tubes. The samples were then centrifuged at 3000 × g for 15 min, and the supernatants (400 µL) were mixed with 800 µL of Tris buffer (0.4 M, pH 8.9), followed by the addition of 20 µL of 5.5-dithio-bis (2-Nitrobenzoic acid) (DTNB; 0.01 M). After stirring the reaction mixture, the absorbance was measured at 412 nm within 5 min of the additionof DTNB against a blank without homogenate.

2.9.2. Malondialdehyde (MDA) test

The level of MDA in the homogenate of each group was measured using the method of Uchiyama and Mihara (1978), involving the determination of the MDA precursor in tissues by thiobarbituric acid tests. Briefly, 250 µL of 10% tissue homogenate was added to 1.5 mL of 1% H_3PO_4 and 0.5 mL of 0.6% tert-butyl alcohol (aqueous solution), and the mixture was then stirred and heated in a boiling water bath for 45 min. After cooling, 2 mL of n-butanol was added. The mixture was then stirred, and the butanol layer was separated by centrifugation. The optical density of the butanol layer was determined at 535 and 520 nm, and the optical density difference between the two determinations was calculated (as the value of tert-butyl alcohol). MDA concentration was expressed as nmol MDA/g tissue.

2.10. Quantification of cytokines and nitric oxide in tissues

Gastric tissue samples were homogenized in ice-cold PBS buffer (pH=7.4) containing protease inhibitors. Homogenized samples were centrifuged at 20,000 X g for twenty minutes in a 4°C refrigerated centrifuge. Supernatants were collected and frozen at -80°C until assay, according to the methodology adapted by Salaga et al. (2017). Cytokine levels Tumor Necrosis Factor Alpha (TNF-α), Interleukin 1, 6 and 10 (IL-1β, IL-6 and IL-10) were evaluated using the ELISA kit for mouse cytokines, high sensitivity in ELISA, Thermo Fisher (Waltham, USA) according to with the manufacturer's instructions. Results were expressed in pg/g tissue.

2.11. Statistical analysis

Data were analyzed using GraphPad Prism® software (version 8.4.3; San Diego, California, USA). Statistical analysis was performed using one-way ANOVA followed by Tukey-Kramer and Bonferroni tests. Unless otherwise specified, results are expressed as mean ± standard deviation. Values of $p<0.05$ were considered statistically significant.

3. RESULTS

3.1. Obtaining the extract and yield

The hydroalcoholic extract using by solvent maceration of the leaves of *Hymenaeacangaceira* obtained a yield of approximately 4% (300g of plant material yielding 12g of extract).

3.2. Chemical composition of the hydroalcoholic extract

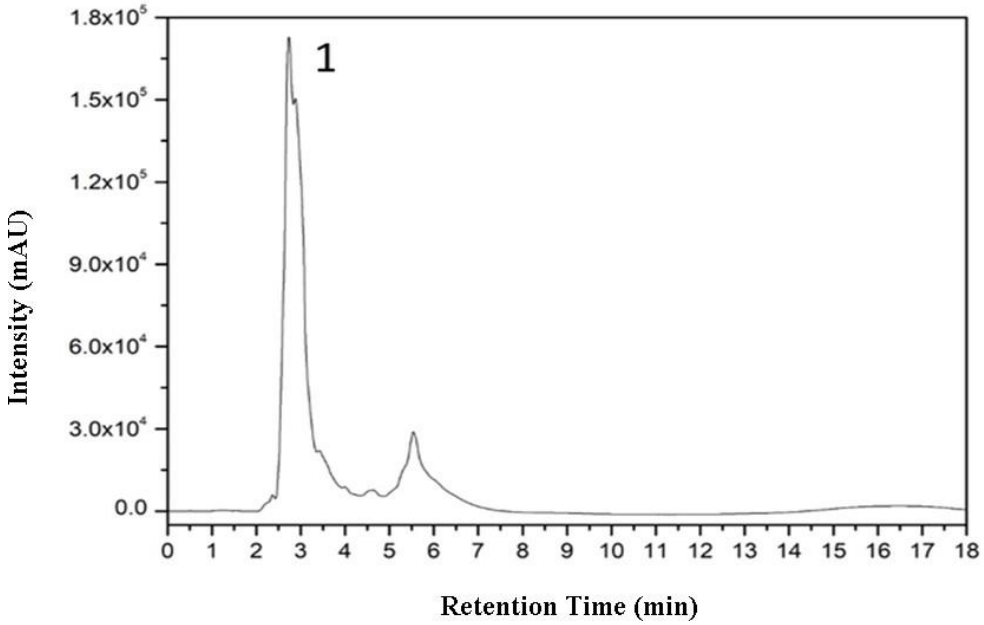
Through of the HPLC analysis it was possible to determine the phenolic profile of the hydroalcoholic extract of *H. cangaceira*, allowing a better understanding of which secondary metabolites of the plant are present in the extract and make it capable of producing their effects in the body. The main phenolic compound found in HEHc was gallicacid (or 3,4,5-trihydroxy acidbenzoic) (Table 1 and Figure 2).

Table 1. Phenolic profile of hydroalcoholic extract from leaves of *Hymenaea cangaceira* (HEHc).

Phenolic Compound	Concentration (mg/g dry extract)
Eugenol	0.00±0.00
Vanillin	0.00±0.00
Quercetin	0.00±0.00
Ellagic Acid	0.00±0.00
Catechin	0.00±0.00
Gallic Acid	39.82±2.82

Values expressed as mean ± standard deviation (n = 3).

Figure 2. High Performance Liquid Chromatography (HPLC). (1) Peak of the gallic acid metabolite in the chemical composition of HEHc.



3.3. Acute oral toxicity

The hydroalcoholic extract of *Hymenaeacangaceira* did not present acute oral toxicity (Table 2), being compatible with its biochemical composition, with gallic acid as its main constituent.

Table 2. Acute oral toxicity of the hydroalcoholic extract of leaves of *Hymenaeacangaceira* (HEHc) in mice.

Treatment	Dose (mg/kg; p.o.)	Gross behavior effect	Nº. of dead animals	Mortality (%)
HEHc	1.75	No change	0/3	0.00
	5.5	No change	0/3	0.00
	17.5	No change	0/3	0.00
	55	No change	0/3	0.00
	175	No change	0/3	0.00
	550	No change	0/3	0.00
	2,000	Piloerection	0/3	0.00

D/T: number of mice killed/number of mice treated. No symptoms of toxicity were observed during the observation period.

3.4. Antioxidant activity

For this, different conformations of the experiment were carried out to evaluate the antioxidant capacity of the HEHc, as an example of the ability to inhibit the formation of radicals of $DPPH^+$ and $ABTS^+$ molecules. The results ofinhibition of free radical scavenging in terms of IC_{50} values are shown in Table

3. The result of $DPPH^+$ radical scavenging activity showed an IC_{50} value of 466.28 ± 1.44 µg/mL. The elimination of the $ABTS^+$ radical by the extract was greater than that of the $DPPH^+$ radical, presenting an IC_{50} value of 2,163.95 ± 48.53 µg/mL.The data demonstrate the antioxidant effect of the extract of *H. cangaceira* in the two tests carried out, with values ofIC_{50}in1.44 µg/mL and 48.53 µg/mL for the reduction of $DPPH^+$ and $ABTS^+$ radicals, respectively.

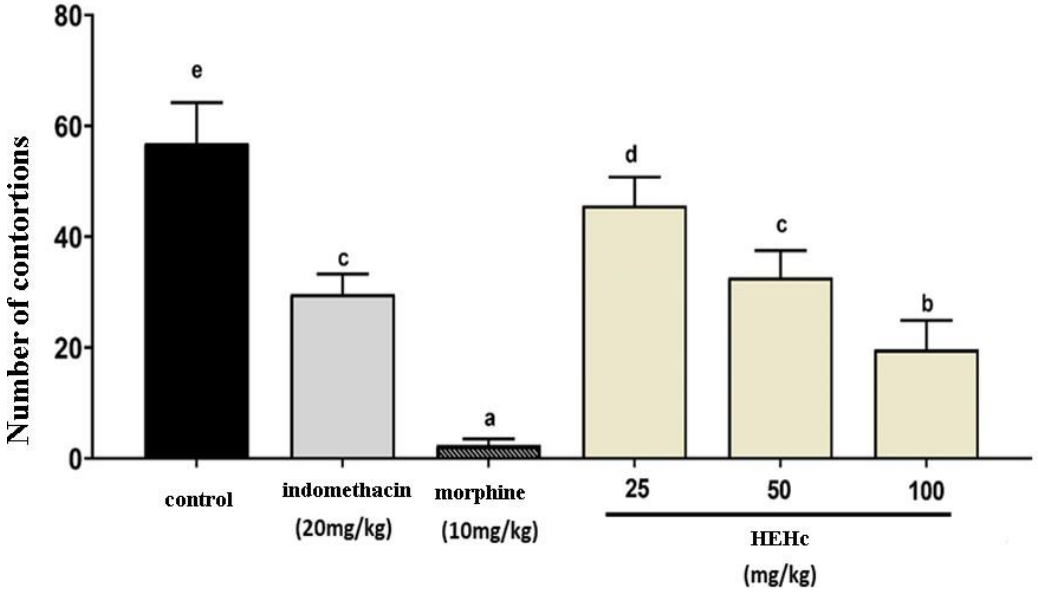
Table 3. Antioxidant activity of the hydroalcoholic extract of leaves of *Hymenaea cangaceira* (HEHc) expressed in IC50 (µg/mL).

samples	Methods	
	$DPPH^+$	$ABTS^+$
Trolox®	12.62±0.36 a	383.93±25.29 a
Ascorbic acid	24.12±0.61 b	769.12±12.68 b
HEHc	466.28±1.44c	2,163.95±48.53c

Values in means ± SD (n = 6). Means overwritten by different lowercase letters in the column are significantly different ($p<0.05$) by the Bonferroni test. ABTS Radical: 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid); DPPH Radical: 2,2-diphenyl-1-picrylhydrazyl; NT: Not Tested.

3.5. Antinociceptive activity by acetic acid (0.8%)

Figure2. Effect of the hydroalcoholic extract of the leaves ofHymenaeacangaceira (HEHc), Indomethacin and morphine in writhing induced by intraperitoneal acetic acid in mice.



All results were expressed as mean ± standard deviation (SD) (n=6). Means superscripted by different lowercase letters in the column are significantly different ($p<0.05$) by the Bonferroni test.

In this study, the test demonstrated a significant reduction ($p < 0.01$) in the number of writhes due to the hydroalcoholic extract of *Hymenaeacangaceira*, reducing the harmful stimulus at the concentrations of 25, 50 and 100 mg/kg tested (Figure 3). As a standard antinociceptive drug, Indomethacin (20mg/kg) showed a lower degree of reduction than the optimal concentration of HEHc (100mg/kg), with an effect close to that of HEHc at a concentration of 50mg/kg, and morphine (10 mg/kg) showed the highest inhibitory degree, reducing the number of acetic acid-induced abdominal twitching.

3.6. Anti-inflammatory activity

Extending the knowledge of the area, it was proved by the paw edema test induced by carrageenan that the hydroalcoholic extract of *Hymenaeacangaceira* has excellent anti-inflammatory activity. This fact was confirmed by observing the progress of edema formation in the paws of mice after the application of the immunogenic agent.

With this, it was possible to show that the therapeutic effect of HEHc is comparable to the standard drug on the market (Indomethacin) even with a relatively low dosage of 50 mg/kg. Moreover, also surpassing some

milestones in the literature and reaching, at the optimal dosage of 100mg/kg an inhibitory effect of up to 82.4% on the swelling of the hind legs of the tested animals, showing protection against the inflammatory process stimulated by the immunogenic agent (Table 4). Since demonstrated in the HEHc, the inhibition of the synthesis of prostaglandins and nitric oxide are part of the mechanism of action of this metabolite, consistent with its biochemical characterization that presentsgallic acid as the predominant compound.

Table 4. Anti-inflammatory effect of the hydroalcoholic extract of the leaves of *Hymenaeacangaceira* (HEHc) and Indomethacin on carrageenan-induced paw edema at different times (h).

Pre-treatment	Dose (mg/kg)	Edema formation (mm) in hours (h)					Mean edema formation (mm)	Average of inhibition (%)
		1h	2 h	3 h	4 h	5 h		
Control	-	0.855±0.145^{d}	1.155±0.292^{d}	0.995±0.219^{d}	0.877±0.271^{d}	0.892±0.191^{c}	0.955	-
Indomethacin	20	0.553±0.105^{b}	0.362±0.148^{b}	0.197±0.091^{b}	0.077±0.026^{a}	0.020±0.030^{a}	0.241	74.76b
	25	0.712±0.089^{c}	0.478±0.137^{c}	0.268±0.085^{c}	0.137±0.091^{c}	0.097±0.017^{b}	0.338	64.60c
HEHc	50	0.539±0.131^{b}	0.311±0.078^{b}	0.189±0.101^{b}	0.101±0.083^{b}	0.023±0.024^{a}	0.232	75.70b
	100	0.412±0.098^{a}	0.259±0.102^{a}	0.102±0.084^{a}	0.059±0.027^{a}	0.011±0.018^{a}	0.168	82.40a

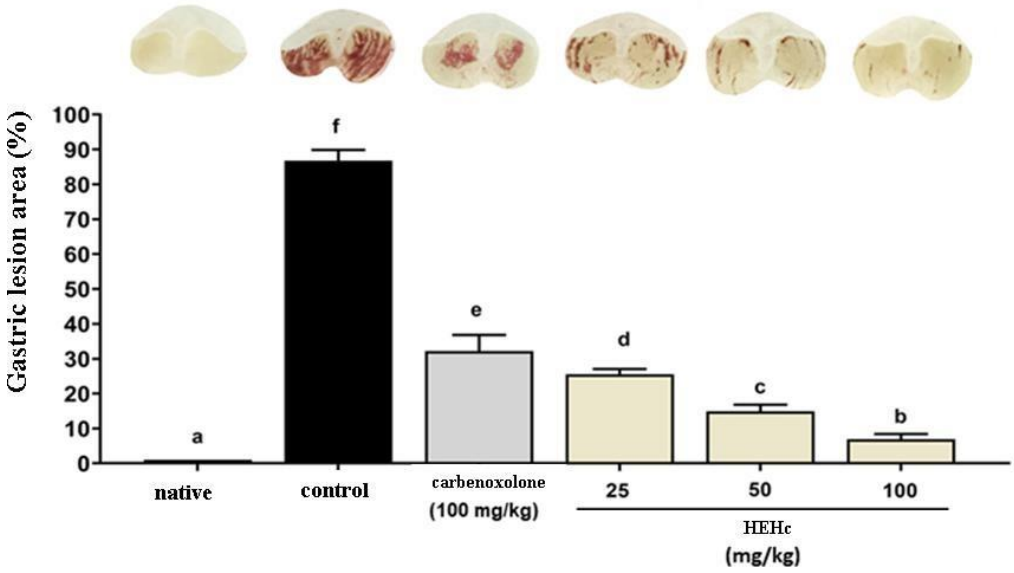
All results were expressed as mean ± standard deviation (SD) (n=6). Means superscripted by different lowercase letters are significantly different($p<0.05$) by the Bonferroni test. Control (saline, 0.9% (w/v)).

3.7. Gastroprotective activity

In the present study, tissue damage was assessed by testing gastric lesions induced by ethanol alone and lesions induced by the combination of ethanol and hydrochloric acid (HCl). Ethanol injury is induced reliably and quickly, as it is already possible to observe hemorrhagic erosions in the gastric mucosa between 1-2 hours after administration. Among the mechanisms induced by ethanol times: the reduction of mucus production, for example. It is noteworthy that the association of ethanol to an acid solution intensifies the harmful effects of alcohol (GLAVIN, Gary B.; SZABO, Sandor, 1992).

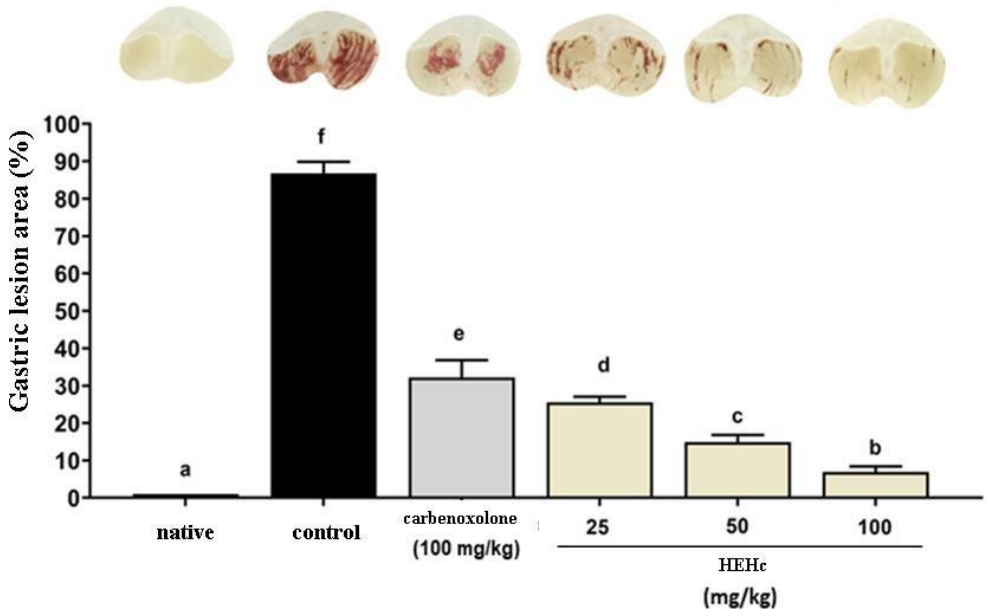
As evidenced in figures 4 and 5, it was shown that groups of individuals treated with HEHc, in all possible concentrations, showed a good gastroprotective activity, as good as carbenoxolone. In both methodologies used to evaluate tissue damage (ethanol and ethanol/HCl), the lowest tested dose of the extract obtained a degree of prevention of damage to the stomach mucosa tissue similar to the group treated with an anti-inflammatory and cytoprotective drug that is a reference of use for the treatment of ulcerations in the GI tract, carbenoxolone. There are already studies in the literature that showthe antiulcerogenic effect of gallic acid, the main metabolite present in HEHc. There are reports that this phytochemical compound provided a reversal of ethanol-induced tissue ulceration by up to 91%, at relatively low doses (50- 100mg/kg). In accordance with these facts, the hydroalcoholic extract of *Hymenaeacangaceira* was able to significantly reduce the gastric lesion area, obtaining, at concentrations of 50 and 100mg/kg, gastroprotection levels of approximately 63-72%.

Figure3. Effects of the hydroalcoholic extract of the leaves of*Hymenaeacangaceira* (HEHc) and carbenoxolone in ethanol-induced gastric lesions in mice.



All results were expressed as mean ± standard deviation (SD) (n=6).Means overwritten by different lowercase letters in the column are significantly different ($p<0.05$) by the Bonferroni test.

Figure4. Effects of the hydroalcoholic extract of the leaves of*Hymenaeacangaceira* (HEHc) and carbenoxolone inethanol-induced acidified gastric lesions in mice.



All results were expressed as mean ± standard deviation (SD) (n=6). Means overwritten by different lowercase letters in the column are significantly different ($p<0.05$) by the Bonferroni test.

3.8. Mechanism of action

To investigate the characteristic mechanisms of action of HEHc in gastroprotection, we examined the importance of different ways of maintaining gastric integrity, such as the production of PGs from the conversion of arachidonic acid and catalyzed by COX, the release of NO in the gastrointestinal microcirculation, the increase of non-protein sulfhydryl groups (GSH) and the activation of ATP Dependent Potassium Channels (K-ATP) for the exchange of hydrogen and potassium ions in the parietal cell membrane, instrumented by the H+ , K+ -ATPase (the protons). The respective blockers of these pathways were previously applied in order to study the effect on the gastroprotection mechanism induced by HEHc against ethanol-induced gastric lesions.

Under pretreatment with N-ethylmaleimide (NEM), known to block sulfhydryl groups, the gastroprotective potential of HEHc and carbenoxolone in ethanol-induced gastric lesions was evaluated. It is demonstrated that the protection conferred p does not significantly interfere in the mechanism of actionfor gastroprotection of the studied extract, but the inhibition of this pathway significantly reversed the effect of carbenoxolone. Thus, it is possible to affirm that the gastroprotective effect of HEHc is independent of the production or presence of GSHs compounds.

Table 5 also shows the gastroprotective action of HEHc under the effect of the L-NAME blocker, in which there was an increase in the area of the gastric lesion induced in mice compared to the control, when HEHc is applied underthe effect of no inhibitor.

As shown in Table 5, these two pathways were found to be the main mechanisms of the gastroprotective and antiulcerogenic action of the hydroalcoholic extract of *Hymenaeacangaceira*. Results displayed in percentage of gastric mucosal area injured and percentage of gastroprotection in relation to the reduction of injured area.

Table 5. Evaluation of the mechanisms of action of the hydroalcoholic extract of leaves of Hymenaeacangaceira (HEHc) against ethanol-inducedgastric lesions in mice.

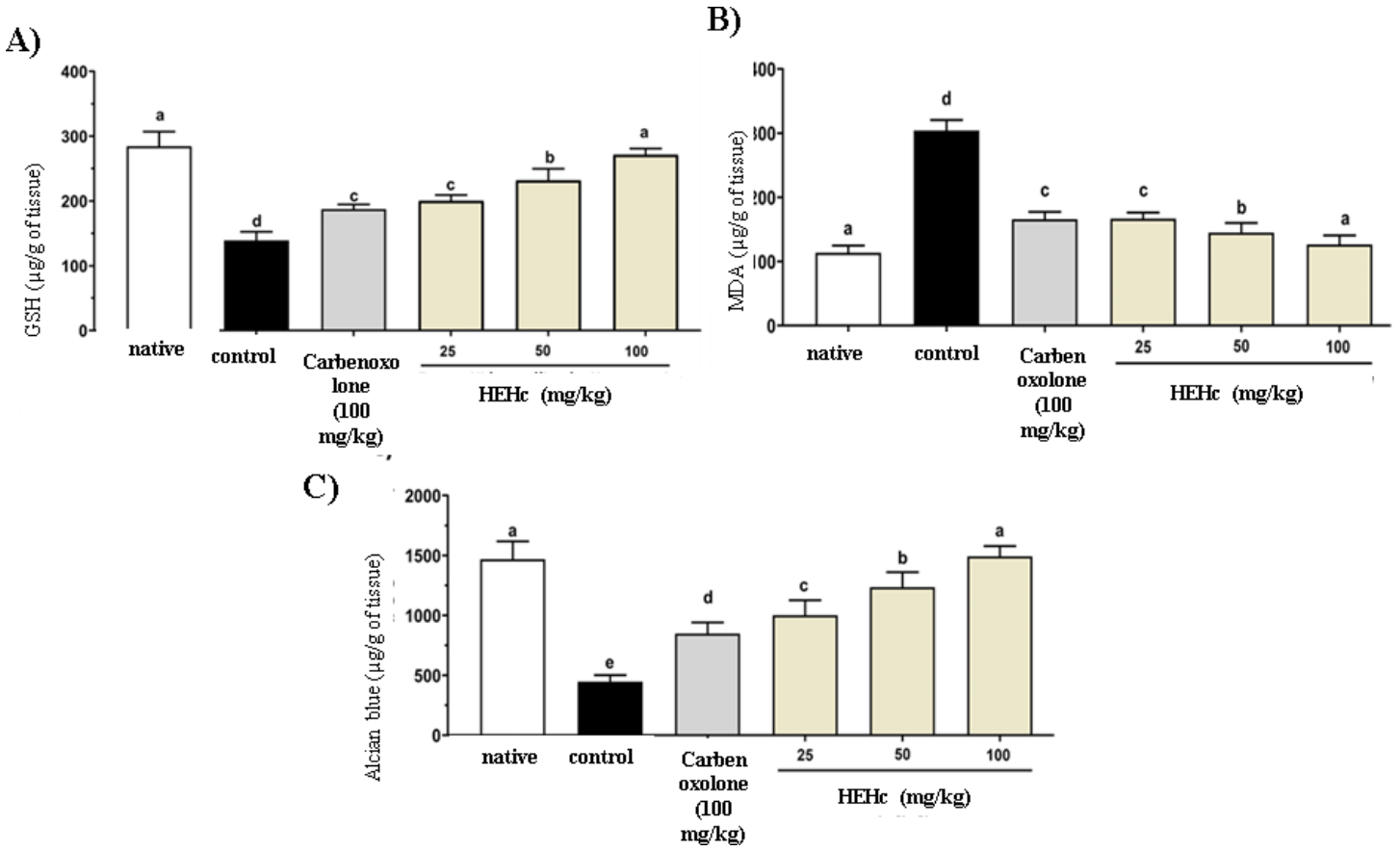
Mechanism	Pre-treatment	Dose (mg/kg; ip)	treatment	Dose (mg/kg; p.o.)	Area of Gastric Injury (%)	Gastroprotection (%
Control	Saline	–	Saline	–	72.47±6.12^{f}	–
	Saline	–	HEHc	100	4.13±1.89^{a}	94.30^{a}
	Saline	–	carbenoxolone	100	32.45±3.27^{c}	55.22^{c}
Sulphydryl Compounds (-SH)	NOR	10	Saline	–	87.53±5.17^{g}	–
	NOR	10	HEHc	100	8.12±3.21^{a}	90.72^{a}
	NOR	10	carbenoxolone	100	55.48±2.78^{e}	36.61^{d}
Nitric Oxide (NO)	L-NAME	10	Saline	–	87.46±4.02^{g}	–
	L-NAME	10	HEHc	100	68.21±5.45^{f}	22.01
	L-NAME	10	carbenoxolone	100	72.24±2.98	17.40^{e}
ATP Dependent Potassium Channels (K-ATP)	glibenclamide	10	Saline	–	82.25±2.91^{g}	–
	glibenclamide	10	HEHc	100	25.12±3.69^{b}	69.45^{b}
	glibenclamide	10	carbenoxolone	100	47.12±2.85^{d}	42.71^{d}
PGE2 synthesis	indomethacin	10	Saline	–	78.87±4.68^{f}	–
	indomethacin	10	HEHc	100	47.12±2.90^{d}	40.25^{d}
	indomethacin	10	carbenoxolone	100	45.48±2.78^{d}	42.33^{d}

All results were expressed as mean ± standard deviation (SD) (n=6). Means superscripted by different lowercase letters are significantly different($p<0.05$) by the Bonferroni test.

3.9. Protein dosage and oxidative stress

Thus, measurement of malondialdehyde (MDA) and reduced glutathione (GSH) content has long been used as a marker of lipid peroxidation in studies related to oxidative stress. As shown in Figure 6, analyzing the effect of HEHcon the content of these molecules together with the mucin secretion content, allowed us to corroborate the gastroprotective action of this extract, even in exposure to oxidative stress and stimuli that can unbalance and inhibit mucosal defense mechanisms stomach, being able to restore them.

Figure5. Effect of the hydroalcoholic extract of the leaves of *Hymenaeacangaceira* (HEHc) on reduced glutathione (GSH), malondialdehyde(MDA), and gastric mucus levels in ethanol-induced gastric ulcer in mice.



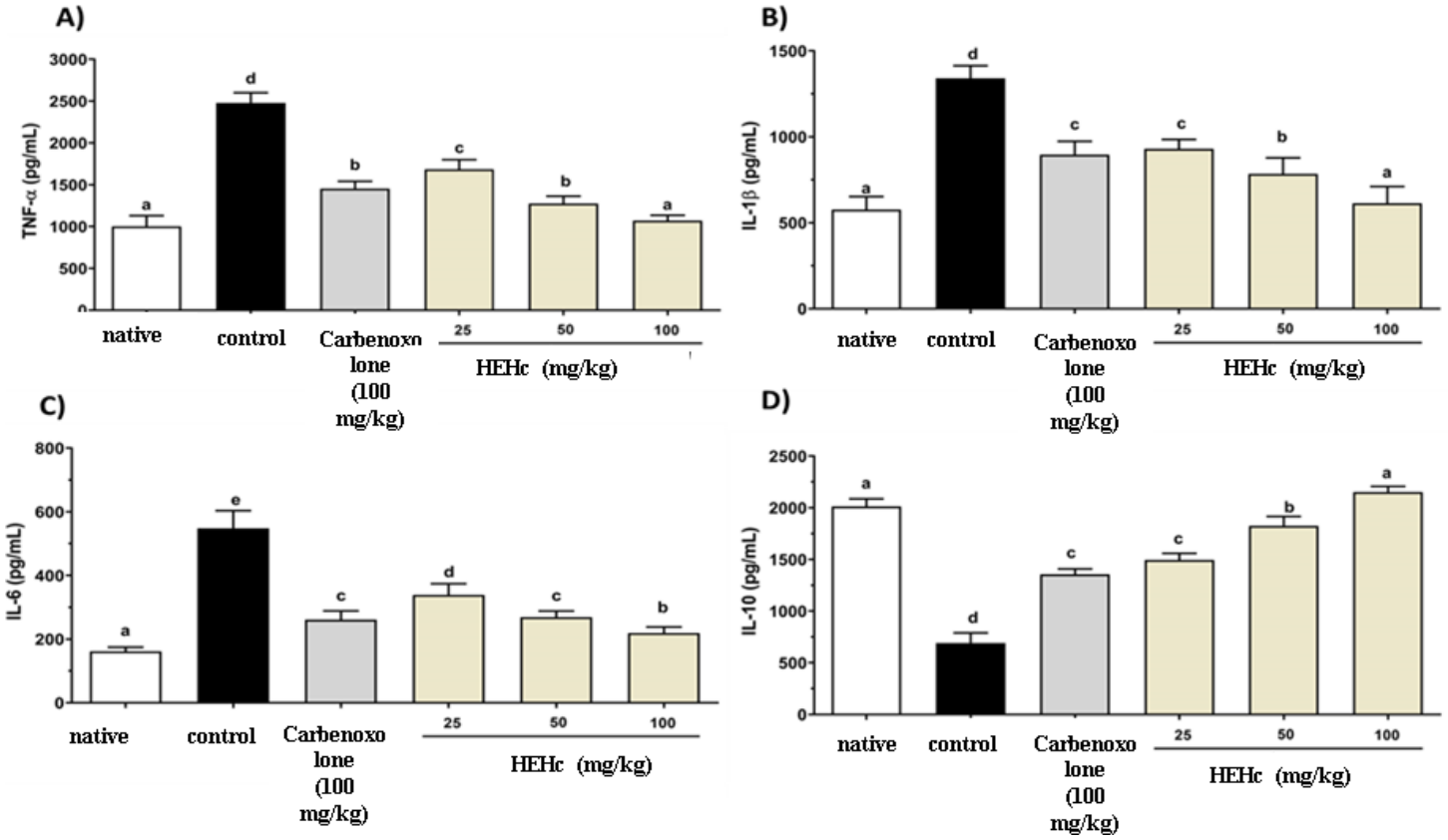
All results were expressed as mean ± standard deviation (SD) (n=6). Means superscripted by different lowercase letters are significantly different($p<0.05$) by the Bonferroni t

3.10. Cytokine dosage

Thus, it is also important to elucidate the effect of the hydroalcoholic extract of *Hymenaeacangaceira* on the levels of some main cytokines. Using the appropriate methodology, the cytokines IL-1, IL-6, IL-10 and TNF-α were chosen for measurement, evaluating the effect of HEHc on their concentrations. A strong potential for inhibiting signaling by these interleukins was observed under the action of HEHc at a concentration of 100mg/kg, being comparable or even superior to the effect of the control drug carbenoxolone. However, there was an exception in the case of Interleukin-10, where the dosage of 25mg/kg was the most efficient, although with less effectiveness than the control, and it decreased with the increase in the dose, reaching a reverse effect on the dosage of 100mg/kg, having increased the levels of IL-10 measured.

Knowing these signaling molecules, their aspects and mechanisms, the action of the hydroalcoholic extract of *Hymeaneacangaceira* is interpreted as very promising, since in not very high concentrations. HEHc was able to attenuate the levels of pro-inflammatory cytokines IL-1β, IL-6 and TNF-α, which can have many benefits for immunopathologies and disturbances in homeostasis caused by an inflammatory exacerbation, such as peptic ulcer. Furthermore, the extract of *H. cangaceira* had a different relationship with the levels of the cytokine IL-10, with an increase in this cytokine directly proportional to the concentration of the extract used. With a greater degree of increase than the control drug carbenoxolone, HEHc proved to be potentially effective against pathological inflammation. However, a greater depth is desirable to elucidate the final effect on the signaling cascade of these molecules, going beyond purely their concentration found.

Figure6. Effect of the hydroalcoholic extract of the leaves of *Hymenaeacangaceira* (HEHc)on the levels of (A), IL-1β (B), IL-6 (C)and IL-10 (D) in ethanol-induced gastric ulcer in mice.



All results were expressed as mean ± standard deviation (SD) (n=6). Means superscripted by different lowercase letters aresignificantly different ($p<0.05$) by the Bonferroni test.

4. DISCUSSON

The hydroalcoholic extract using by solvent maceration of the leaves of *Hymenaeacangaceira* obtained a yield of approximately 4% (300g of plant material yielding 12g of extract), a good yield making a comparative analysis with other methods of extraction such as the suspension in essential oil of *MyrciaeximiaDC*that obtained a yield of 0.36%(FERREIRA et al., 2020). The processing of HEHc also obtained a better yield than extracts taken from other plant species through the same method as the hydroalcoholic extract of the species *Eclipta alba*, which presented a yield of 2.5%(ACHARYA et al., 2020).

The main phenolic compound found in HEHcthrough of the HPLC analysiswas gallicacid (or 3,4,5-trihydroxy acidbenzoic) (Table 1 and Figure 2), which is an organic acid commonly found in walnuts, oak bark and plants such as *Hamamelis* and *RhusCoriaria*(KAUR; MUTHURAMAN, 2018).A phenolic widely known in the literature for its powerful antioxidant capacity is gallic acid itself, modulating the presence of reactive oxygen species and the intensity of oxidative stress, having valuable therapeutic effects already demonstrated, such as hearing protection against cisplatin-induced ototoxicity(KILIC et al., 2019). Also found in other plant species, such as the fruit of *Mangiferaindica*, popularly called 'ataulfo' mango, gallic acid also has antiproliferative activities in cancer cells, such as the LS180 lineage, arising from colorectal adenocarcinoma. It was found that some polyphenols that had a greater amount of gallic acid caused a lower proliferation rate when in contact with cells. Such effects may be due to mechanisms of cellular apoptosis dependent on reactive oxygen species(VELDERRAIN-RODRÍGUEZ et al., 2018).

The oral toxicity of this phenolic compound was systematically studied by Shi et al. (2013), who in a review reported that for acute oral ingestion, there were no evident adverse effects at 5g/kg/pvgallic acid for Swiss albino mice and 1g/kg/pv for mongrel rats, respectively. In this study, the researchers evaluated in detail the metabolic responses of the mice's system by analyzing the animals' liver, urine, plasma and feces after administration of gallic acid. Although notoxic effects were found, a significant influence of the compound at high doses on metabolism was reported, involving glycolysis,glycogenolysis, citric acid cycle, metabolism of amino acids, purines and pyrimidines, in addition to gut microbiota functions (SHI et al., 2013). This suggests that despite the

therapeutic doses proven in research being far from a possible toxic dose, bioactive plant extracts involving higher concentrations of gallic acid in their composition should be studied more carefully regarding their potential for severe metabolic alteration and long-term toxicity.

Thymoquinone, the major constituent of *Nigella sativa* EO, is the main responsible for its therapeutic action and has a Median Lethal Dose (LD_{50}) of only 57.5 mg/kg by intraperitoneal injection and 794.3 mg/kg orally, showing considerable toxicity. Other constituent compounds of EOs also show higher than desirable levels of toxic effects, such as epoxycarvone which, despite having reports of few clinical manifestations of adversities, has an LD_{50} lower than 1g/kg, suggesting a risk of present toxicity, although low. Anethole, on the other hand, is a phytocompound that has already shown clinical manifestations of toxicity in rats, with changes in liver function from doses of only 500 mg/kg (DE ASSIS OLIVEIRA et al., 2014).

Although there are cases of toxicity, plant extracts are still a very promising group for the treatment of inflammatory diseases, and many of the new extracts studied do not demonstrate toxicity at the therapeutic doses used. Pioneering and current research, such as the use of *Baccharistrimera* (carqueja) for the treatment of gastric lesions, provide very encouraging results, reducing the degree of tissue involvement by up to 65%. Although the toxicity of
B. trimera essential oil has not been properly investigated to date, intraperitoneal or oral injection of its hydroalcoholic extract did not show signs oftoxic effect in rats.

Like carqueja, the hydroalcoholic extract of *Hymenaeacangaceira* did not present acute oral toxicity even at very high doses (Table 2), evidencing the safety and potential of its therapeutic use for the treatment of gastrointestinal ulcerations(BUENO et al., 2021).

Antioxidant and radical scavenger compounds are precious dietary supplements due to the protection of human health against various disorders,as they play a crucial role in restoring physiological oxidative balance and modulating biological pathways and membrane function (Lee et al., 2002; Wei et al., 2007; Hammami et al., 2015). Studies indicate that there is no single method capable of perform quantitatively and precisely evaluating the

antioxidant properties of a given compound, requiring the use of different techniques (Ornano et al., 2013).

When compared to other leaf extraction methodologiesof *H. cangaceira*, such as the essential oil extraction performed by OLIVEIRA DE VERAS et al. (2019) that reached an IC_{50} value of 0.01 µg/mL and 0.11 µg/mL for $DPPH^+$ and $ABTS^+$, respectively, a considerable maintenance of the oxidative potential of the compounds present in both extraction methodologies is seen. Although the extraction of oil from the leaves preserves a greater antioxidant activity, the hydroalcoholic extraction has advantages because it presents an easier preparation and more applicable to the population, precisely because it does not have many processing steps and requires simple machinery when compared to essential oil extraction. .

This antioxidant activity can be attributed to the gallic acid molecule.Intermediate products of phenolic compounds act as radical scavengers and sometimes as metal chelators, and complexes formed arerelatively stable due to the aromatic ring resonance exhibited by these substances.Gallic acid, for example, found in the leaves of *H. cangaceira*, is investigated for possessing antioxidant properties of polyphenols, has beenexplored in the inhibition of fibrillar protein deposits that lead to disorders such as Alzheimer's and Parkinson's disease (PAL, SINGH MANISH, 2018).

Gallic acid has also been tested in the composition of grafts for delivery of pilocarpine in the treatment of glaucoma, and it was noted that the increase in AG in the constitution of these grafts increased the antioxidant level and decreased the level of nitrite in the aqueous humor (CHOU; LUO; LAI, 2016). Some authors also studied the combination of GA with other antioxidant agents for nutrition, such as curcumin (or saffron), ascorbic acid and xanthine, and it was seen that the AG-curcumin combination is the best form of consumption, with preservation and increased antioxidant action of both (NAKSURIYA; OKONOGI, 2015). Other investigators have reported the different vascular protective effects of gallic acid, and when tested in saline solutions, AG wasable to generate superoxide anions, decrease H_2O_2 levels and activate cyclooxygenase and activation of muscle K^+ channels (PRISCILLA; PRINCE, 2009).

The encoding and processing of noxious environmental, physiological or pathological stimuli that result in pain is a neural process called nociception, which occurs through a complex cascade of interactions and occurrences from the periphery to the higher structures of the central nervous system(ALVES et al., 2017). Pain can be described as an unpleasant subjective experience associated with actual or potential tissue damage, among other factors. As a complex and multifactorial mechanism, it has sensory, emotional, social, behavioral and cognitive components. Pain and nociception act as a defense mechanism, since they are involved in the recognition of threats and damage, alerting the occurrence of something potentially harmful in the body (WILLIAMS;CRAIG, 2016).

Despite being a necessary and important mechanism in the body, the perception of harmful stimuli and pain, when it becomes a chronic and recurrent element, leads to a decrease in the quality of life. Therefore, it is important to establish safe, effective and non-toxic antinociceptive therapies to improve the quality of life of those affected by disorders related to chronic pain(DINAKAR; STILLMAN, 2016). Some compounds with high oxidizing power, such as reactive oxygen species (ROS), when present in significant concentrations, can lead to the activation of nociceptor pathways through their aggressivestimulation of oxidation. Gallic acid, in addition to having already evidenced antioxidant activity, is capable of blocking nociceptors present in the peripheral circulation and can also cross the blood-brain barrier and act directly on the central nervous system, in brain regions responsible for processing nociceptive stimuli(FARBOOD et al., 2013). It was seen that gallic acid reduced calcium influx mediated by transient ankyrin receptor 1 (TRPA1) activation, the latter being identified as a relevant target for the development of new analgesics. Furthermore, oral administration of AG decreased spontaneous nociception triggered by allylisothiocyanate, cinnamaldehyde and H_2O_2 (TREVISAN, GABRIELA et al., 2014).

The abdominal writhing model induced by acetic acid, despite not having such a high specificity, is a valid method due to its high sensitivity to the use of anti-inflammatory and antinociceptive drugs (GRIPP et al., 2020). It is suggested that acetic acid injection may trigger the release of a variety of endogenous mediators, particularly histamine, bradykinin, serotonin and prostaglandins,

mainly prostanoids, resulting in increased levels of PGE2 and PGF2α in the peritoneal fluid and other products of the lipoxygenase pathway, where acetic acid activity involves the process or release of arachidonic acid metabolites (GRIPP et al., 2020).

In this study, the test demonstrated a significant reduction ($p < 0.01$) in the number of writhes due to the hydroalcoholic extract of *Hymenaeacangaceira*, reducing the harmful stimulus at the concentrations of 25, 50 and 100 mg/kg tested (Figure 3). As a standard antinociceptive drug, Indomethacin (20mg/kg) showed a lower degree of reduction than the optimal concentration of HEHc (100mg/kg), with an effect close to that of HEHc at a concentration of 50mg/kg, and morphine (10 mg/kg) showed the highest inhibitory degree, reducing the number of acetic acid-induced abdominal twitching.

For the prevention and treatment of gastric lesions and pathologies suchas peptic ulcer, a key point is the control of the local inflammation that is established. Inflammatory cells, which perform functions of defense and maintenance of the organism, when intensely and continuously stimulated, end up amplifying tissue damage. This generates a cascade effect in which the damage caused by the immune system itself generates molecular patterns that are detected and result in pro-inflammatory signals, contributing to exacerbated inflammation and tissue aggression, which can lead to the worsening of the condition and complications. In view of this, a very common treatment route for gastrointestinal disorders is immunomodulatory pharmacotherapy (KÖHLER et al., 2014).

Inflammation inhibitor drugs are already known and widely used in the treatment of numerous different pathologies. These drugs are effective both in improving symptoms caused by a possible exacerbated response to pathogens such as headache, body aches and fever, as well as in enabling the resolutionof tissue damage associated with an established chronic inflammatory process. A study carried out in 2014 even points to an application of treatment with anti-inflammatory drugs for depressive symptoms. This research reports on how the use of these drugs, particularly the selective cyclooxygenase-2 (COX-2) class of inhibitors, resulted in an improvement in neurological symptoms for people with diagnosed depression, through an as yet unspecified mechanism(KÖHLER et al., 2014).

Despite having high versatility and efficacy, anti-inflammatories have a major disadvantage; their continuous and prolonged use is marked by frequent manifestations of toxicity. Among the most common adverse effects caused by this class of drugs are dyspepsia and nausea, however, toxicity can progress to more serious side effects ranging from lesions to the gastrointestinal mucosa, to problems with glomerular filtration and electrolyte balance caused by failure acute or chronic kidney disease. This toxicity evidenced in the literature occurs mainly through the same mechanism of action of the therapeutic effect of these drugs, the inhibition of cyclooxygenase enzymes. These enzymes are involved in the arachidonic acid cycle, transforming it into inflammatory molecules such as prostaglandins and prostacyclins, thus, its inhibition is effective as an anti-inflammatory therapy. However, COX inhibition also generates metabolic imbalances related to a deficit in cellular integrity and renal filtration, in addition to causing cardiogenic events(HARIRFOROOSH; ASGHAR; JAMALI, 2013).

Therefore, it is also important to search for therapies that can attenuate local inflammation for diseases such as gastric ulcer. Ulcerations in the GI tract are usually accompanied or even caused by a chronic inflammatory process established in the tissue, which can be recurrent. Therefore, it is of interest that the anti-inflammatory therapies used are safe and their prolonged use does not cause aggravating adverse effects that impair the patient's quality of life in the long term. Plant extracts are part of a very promising field of study for the treatment of various pathologies. This is done through molecules isolated from plants that have proven beneficial effects, being easier to metabolize and having less relative toxicity. A field in particular in phytotherapy is the regulation of the immune system, being present in popular culture, especially in small villages, reports of teas and plant preparations that strengthen immunity, as well as those that control it to improve allergies and irritations (BAG et al., 2013).

As a proof of concept, several studies have already characterized plant species with medicinal properties for the treatment of immunopathies. To this end, a team of Indian researchers collected the fruits of the *Terminaliachebula* plant, separated and crushed the seeds, from which they extracted the active ingredient using 70% ethanol as a solvent. After filtration and concentration by rotary evaporator, the hydroalcoholic extract of *T. chebula* had its anti- inflammatory capacity evaluated in mice following the methodology of paw

edema induced by carrageenan. This test uses a suspension of carrageenan,an inflammatory agent that activates the prostaglandin pathway, which is injected directly into the animals' hind paw joint. With the inflammation that is established, swelling occurs in the region, making it possible to assess the intensity of the inflammatory process relating it to the level of localized edema. (BAG et al., 2013).

There are reports in the literature of the use of plant extracts to combat immunopathies, with very promising inhibition percentage results, such as the *T. chebula*extract, which in optimal concentration, offered a 69.96% decrease in local inflammation, in addition to others. plants such as *Rosa canina*, which was studied by another group of researchers and obtained similar results(LATTANZIO et al., 2011).

The chemical characterization of the extract further corroborates these results, since gallic acid, the main phytochemical component of HEHc, is known in the literature for its potent anti-inflammatory effect through several mechanisms. Corroborating the findings of the present study, it was seen that carrageenan-induced edema was largely reduced by pretreatment with gallic acid.

Since demonstrated in the HEHc, the inhibition of the synthesis of prostaglandins and nitric oxide are part of the mechanism of action of this metabolite, consistent with its biochemical characterization that presents gallic acid as the predominant compound. However, other mediators of this anti- inflammatory action mentioned in the literature are the inhibition of nuclear transcription factors such as NF-kβ and the signal transducer and transcription activator 3 (STAT-3), also inhibiting the production of pro-inflammatory products of these pathways such as TNF-α and caspases 3 and 9 (apoptosis-related proteins). The inhibition of neutrophil and macrophage infiltration was also presented in the literature as a mechanism of action for the anti-inflammatory effect of AG, in addition to the decrease in the levels of several cytokines and molecules that cause inflammatory perpetuation and intensification,such as COX-2 and the interleukins IL-1 β, IL-6, IL-17, IL-21 and IL-23(KAHKESHANI et al., 2019).

The compound L-NAME is a specific inhibitor of the enzyme NO synthetase, which catalyzes the production of nitric oxide (NO) from L-arginine. In the smooth muscle of the stomach lumen, NO is responsible for the increase in blood flow in the gastric mucosa and consequent stimulus in the production ofmucus, in addition to inhibiting the adhesion of neutrophils recruited by the local inflammatory response, thus preventing an aggravation of inflammation and an increase in the oxidative stress at the injured site(SÁNCHEZ-MENDOZA et al., 2020). In evaluating the action of HEHc under the exposed conditions, it was seen that the gastroprotective effect of this extract is dependent on the oxide pathway.

The importance of K+ -ATP channels in the regulation of gastroprotection conferred on HEHc through ethanol induction was examined. This activation pathway mediates the release of some prostaglandins and NO, promoting gastroprotection(ROFAEIL; GABER, 2019). With the use of the K+ -ATP channel blocker, glibenclamide, it was possible to perceive that this signaling pathway has an influence on the gastroprotective potential of the extract, but in a small way when compared to other models studied.

In addition to nitric oxide synthesis, another important mechanism of action investigated was the involvement of prostaglandins in the progression of the induced injury. As the main mechanisms that mediate the therapeutic effects of gallic acid, both NO and prostaglandins help to maintain the integrityof the gastric mucosa, with PGs being responsible for increasing the production and secretion of bicarbonate and mucus, in addition to modulating the releaseof acid. hydrochloric acid and inflammatory modulators released by mast cells. Like nitric oxide, PG molecules also play a role in maintaining blood flow. Other bioactive extracts from species that have a phytochemical profile similar to *Hymenaeacangaceira*, with a predominance of phenolic compounds, have also been studied to determine the mechanisms involved in their physiometabolic effects. In a study with the plant *Tephrosiaegregia*, for example, which has a phytochemical predominance of phenolic compounds, it also produces gastroprotective effects through the synthesis of nitric oxide and prostaglandins, proving the importance and performance of these metabolic pathways in helping the extracts to achieve the cure of a ulcerative lesion in the gastrointestinal mucosa(FERREIRA ROGÉRIO, 2017).

To investigate the entire process between gastroprotection and ulceration, an important analysis to be made is regarding the integrity of the intrinsic defense system of the stomach tissue. As is well known, the gastrointestinal system has certain ways of protecting its own cells from the aggressive stimulus related to its function. Among the defense mechanisms, mucus secretion is the first and most important, as it forms an adherent gel to the mucosal surface that, in combination with the HCO_3^- ion, forms a mucus/bicarbonate complex that provides a physical-chemical barrier against aggression of enzymes and stomach acid (HCl).

Reduced glutathione (GSH) is an important antioxidant found in most mammalian cells and is essential for maintaining the integrity of the gastric mucosa. GSH protects cells from damage induced by free radicals by protecting against lipid peroxidation or by protecting sulfhydryl groups from becoming irreversibly oxidized after oxidative injury (DEVI et al., 2007). Malondialdehyde (MDA) is a small and reactive organic molecule formed by three carbon atoms with two aldehyde groups on carbons 1 and 3. MDA exists in different forms in aqueous solutions due to its pH-dependent tautomeric chemical property. At pH higher than its pKa of 4.46, the dominant form is the enol anion, which has low chemical reactivity. However, at lower pH (expected under oxidative stress conditions), MDA appears in equilibrium between its protonated enol (ab- unsaturated carbonyl) aldehyde forms and the dialdehyde form. These tautomers produced at acidic pH are chemically reactive(MORALES; MUnne- BOSCH, 2019).

After the analyses, it was possible to evidence the restorative action of gastric defense promoted by the hydroalcoholic extract of *Hymenaeacangaceira*, given the measurement of reduced glutathione and gastric mucus that returned to levels comparable to normal physiological levels after administration of the most efficient therapeutic dose (100 mg/kg). In line with the oxidation protection test, in Figure 6, it is possible to observe the antioxidant activity of the extract, since in addition to increasing the levels of reduced glutathione; it also provides a reduction in the levels of malondialdehyde (MDA), a molecule that characterizes the presence of low pH and oxidative stress. Furthermore, in all tests performed, the lowest therapeutic

dose of HEHc tested had an effect comparable to that of carbenoxolone, whichis an anti-ulcerogenic drug used as a reference in the market.

The results of the investigation of the mechanisms and physiometabolic activities of the extract make it possible to clarify the gastroprotection process promoted by gallic acid, the active principle of HEHc. The extract is capable of producing antioxidant activity by several mechanisms, which may involve nitric oxide, prostaglandia and reduced glutathione. In convergent action, the increase in mucus production and anti-inflammatory activity amplify the antiulcerogenic action of the compound, being evidenced in the gastric ethanol lesion test as a significant reduction of the lesion area (around 70%). In addition, the potential use of *Hymenaeacangaceira* extract for the treatment of ulcerative gastroenteritis is very promising, as the major compound in the solution (gallic acid) was also able to reduce the signaling of the nociceptive pathway, which would contribute to reducing pain and patient discomfort during the treatment of tissue injury.

Cytokines are proteins produced and secreted by almost every cell type in the human body. This nomenclature groups a large number of similar molecules that perform immune regulatory functions. They can be called interleukins, growth factors, chemokines, among other names, they are proteins of small size (<40kDa) that have pleiotropic characteristics, in the sense that they interact on different types of cells and that the effect of each cytokine depends on the cell with which the connection and communication is taking place. These molecules participate in a very complex and extremely important network of interactions for the organism, because, as is well known, efficient immune activity depends on a fine balance between defense against possible threats and unnecessary cellular damage. Cytokines are usually produced locally in response to region-specific stimuli to induce or inhibit the inflammatory process. In addition, they have a short half-life and work with a kind of chain reaction, since their release stimulates the secretion of more signaling molecules, generally with similar function, which act synergistically(BERRAONDO et al., 2019).

The Interleukin-1 family comprises some protein subtypes, such as IL-1α and IL-1β, which are encoded by different genes but are translated into a larger precursor protein that is cleaved and separated into two different but interacting

amino acid sequences, however interacting with the same type of receptor (IL- 1R). IL-1α has more affinity for receptor subtype 1 (IL-1R1) and is normally found within several cell types such as hepatocytes, endothelium, nephrotic and gastrointestinal epithelia. This protein is characterized as an alarmin (immune system alert proteins), since it is released into the extracellular environment only in cases of necrosis, as it tightly binds to chromatin in the nucleus during the apoptotic process. Being an alarmin, IL-1α triggers a pro-inflammatory signaling, stimulating release of other cytokines such as IL-6 and TNF-α in peripheral blood mononuclear cells (PMBCs). The IL-1α cascade of action stimulates an increase in membrane permeability in cells, initiating a process called inflammation-induced apoptosis, through an inflammasome mechanism dependent on the Caspase-1 protein(KANY; VOLLRATH; RELJA, 2019).

The β subtype of this interleukin has more affinity with the soluble IL-1R2 receptor, which is present in the plasma and is found mainly in hematopoietic tissue cells such as monocytes, microglia and other types such as dendritic cells activated by the binding of a receptor. recognition pattern (PRR) with a Pathogen-Associated Molecular Pattern (PAMP) or Damage-Associated Molecular Pattern (DAMP). This subtype of Interleukin-1 may be an interesting study target as it has a possible relationship with diseases such as diabetes, obesity and cardiovascular disorders (KANY; VOLLRATH; RELJA, 2019).

Interleukin-6 is a glycopeptide with a sequence of 184 amino acids secreted by a wide range of tissues in the human body, influencing from adipocytes and keratinocytes to fibroblasts, endothelial cells and leukocytes. This cytokine plays a key role in immunological communication, acting in the maturation and differentiation of human cells, mainly B Lymphocytes. Also known as Interferon-β2 (IFN-β2), it belongs to the class of pro-inflammatory cytokines and induces the expression of several proteins. involved in the process of acute inflammation. An important characteristic of IL-6 is its pleiotropism, being involved in several processes related to immune regulation and physiological processes, in addition to the induction of acute phase proteins, such as antigen-specific immune responses, hematopoiesis,tissue metabolism and programmed cell death (UCIECHOWSKI; DEMPKE, 2020).

In contrast, Interleukin-10 is an anti-inflammatory agent that protects the

body from a potentially harmful exacerbated immune response. This protein is involved in pathophysiological processes such as wound healing, autoimmunity and cancer. The main cells producing Interleukin-10 are blood cells of both myeloid and lymphoid lineages, being an important immunological mediator, it is also present in non-blood tissues such as epithelial tissue, appearing even in neoplastic cells, a fact that is correlated with its ability to cause immunosuppression. Upon activation of the IL-10 receptor (IL-10R), signal transduction cascades are triggered and result in immunosuppression of macrophages and other antigen-presenting cells (APCs),as well as in the inhibition of the synthesis of several other proteins necessary for the immunological synapse and activation of the immune response, such as class II major histocompatibility complex (MHC), costimulatory and adhesion molecules (SARAIVA et al., 2020).

Finally, Tumor Necrosis Factor Alpha (TNF-α) is a cytokine that stimulates the inflammatory process by recruiting immune cells to the site of secretion. Being mainly produced by leukocytes, it is related to an amplification of the immune response and formation of granulomas(SINAGA; AMIR, 2021). TNF-αis one of the most important pro-inflammatory cytokines, it has strong pleiotropism, exerting a regulatory role in pathophysiological processes that influence several aspects of the immune response. Normal levels of this cytokine are relatively low and its increase is related to various disorders such as rheumatoid arthritis, psoriasis and cancer(FILIK; AVAN, 2020). There are two bioactive forms of Tumor Necrosis Factor Alpha, a plasma membrane- associated transmembrane configuration and a free circulating form, which can be derived from cleavage of its transmembrane form by a disintegrin. Both forms interact with TNF receptors (TNFR1 and TNFR2) and perform distinct functions(MIAO et al., 2020).

5. CONCLUSION

According to the results obtained in this work, it was possible to obtain the hydroalcoholic extract of *Hymenaeacangaceira*, with good yield, and it was possible to obtain the chemical composition, verifying the presence of the constituents, with gallic acid being the major component. With the characterization of the extract, it was possible to observe that there was no acute oral toxicity. In all applied methodologies, the therapeutic doses remained very far from the maximum dose tested for toxic effects. In pharmacological tests involving nociceptive, anti-

inflammatory, antioxidant and gastroprotective models, HEHc showed potent and dose-dependent activities. The profile of inflammatory cytokines and the oxidative parameters corroborate the results of this study. Thus, the present study was able to verify that HEHc has potent pharmacological activity mediated mainly by mechanisms involving nitric oxide (NO) and prostaglandin synthesis (PGE_2), while it does not cause any acute toxic effects, even at very high doses in in relation to the therapeutic dose.

The gastric mucosa is a highly complex and specialized tissue, having as one of its functions the protection of the stomach wall against the aggression of gastric acid and the elements that pass through the stomach. For this, the superficial cells of the mucosa secrete a transparent and viscous substance, composed of 95% water and 5% proteins, called mucus(RIBEIRO, 2017).

There is a balance between aggressive agents of the gastric epithelium and the defensive factors of the mucosa. When this balance fails, with mucosal defenses being reduced, injury to the stomach epithelium can occur, which compromises the function of the area and makes the local epithelium even more vulnerable to the erosive action of gastric acid itself, for example (SILVA et al., 2018).

Once the lesion remains and is not properly cared for and healed, the case can worsen, leading to the appearance of ulceration in the stomach mucosa. By definition, gastric ulcer is a severe to open lesion in the lining layer of the stomach, which may be restricted to the mucosa and submucosa, as well as reaching the deeper layers of the stomach wall (muscular and serous) and is characterized by tissue loss, which may involve have problems such as bleeding (ALMEIDA et al., 2021).

In the present study, tissue damage was assessed by testing gastric lesions induced by ethanol alone and lesions induced by the combination of ethanol and hydrochloric acid (HCl).

As evidenced in figures 4 and 5, it was shown that groups of individuals treated with HEHc, in all possible concentrations, showed a good gastroprotective activity, as good as carbenoxolone. In both methodologies used to evaluate tissue damage (ethanol and ethanol/HCl), the lowest tested dose of the extract obtained a degree of prevention of damage to the stomach mucosa tissue similar to the group

treated with an anti-inflammatory and cytoprotective drug that is a reference of use for the treatment of ulcerations in the GI tract, carbenoxolone. There are already studies in the literature that showthe antiulcerogenic effect of gallic acid, the main metabolite present in HEHc. There are reports that this phytochemical compound provided a reversal of ethanol-induced tissue ulceration by up to 91%, at relatively low doses (50- 100mg/kg). In accordance with these facts, the hydroalcoholic extract of *Hymenaeacangaceira* was able to significantly reduce the gastric lesion area, obtaining, at concentrations of 50 and 100mg/kg, gastroprotection levels of approximately 63-72%. According to the literature, it is suggested that continuing the treatment for more days is still beneficial for the tissue, amplifying gastric protection and offering some reversal of the inflammatory and harmful condition(RAMOS DOS SANTOS MEDEIROS, 2018).

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5.CONCLUSÕES

De acordo com os resultados obtidos nesse trabalho foi possível obter com um bom rendimento o EHHc e determinar a sua composição química, tendo como componente majoritário o ácido gálico. Os testes farmacológicos mostraram que EHHc apresentou excelente atividade, dose dependente, para os modelos nociceptivos, anti-inflamatório, antioxidante e gastroprotetor. Frenteao perfil de citocinas inflamatórias foi visto que o presente extrato foi capaz de atenuar os níveis das citocinas pró-inflamatórias IL-1β, IL-6 e TNF-α. Já em relação aos parâmetros oxidativos (*níveis de malondialdeído (MDA), glutationa reduzida (GSH) e muco gástrico)* permitiu corroborar a ação gastroprotetora desse extrato, mesmo em exposição a estresse oxidativo e estímulos que podem desequilibrar e inibir mecanismos de defesa da mucosa gástrica, sendo capaz de restaurá-los.O mecanismo de ação de EHHC envolve o óxido nítrico (NO) e síntese de prostaglandinas (PGE_2), enquanto não provoca nenhum efeito de toxicidade aguda, mesmo em doses muito elevadas em relação à dose terapêutica.

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