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EVIDÊNCIAS DA AÇÃO ANTI-INFLAMATÓRIA DE *Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz EM MODELOS EXPERIMENTAIS *in vivo* E *in vitro*: UMA REVISÃO SISTEMÁTICA

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RESUMO

Introdução: O jucá ou pau-ferro [*Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz] é uma planta arbórea pertencente à família Fabaceae, amplamente encontrada nas regiões Norte e Nordeste do Brasil. Utilizada, popularmente, no tratamento de doenças como diarreia, anemia, diabetes, cicatrização entre outras apresentando também ação anti-inflamatória. Estudos em modelos animais são utilizados para verificação da ação anti-inflamatória de novos fármacos derivados de espécies vegetais, bem como para o conhecimento da segurança, eficácia e toxicidade destas plantas. **Objetivos:** Investigar as evidências da ação anti-inflamatória do jucá em experimentos *in vivo* e *in vitro*. **Metodologia:** Para a busca, a análise e a síntese das evidências de ação anti-inflamatória da *L. ferrea* em modelos *in vivo* e *in vitro* realizou-se uma Revisão Sistemática. Para a elaboração do protocolo segundo a ferramenta PRISMA apresentou a seguinte pergunta norteadora: Qual parte da planta de *L. ferrea* e que tipo de extrato tem os efeitos anti-inflamatórios mais evidentes nos modelos experimentais de inflamação aguda *in vivo* e *in vitro*?. As bases utilizadas para pesquisa foram PubMed, Science Direct, Scopus, LILACS e Web of Science. E na literatura cinzenta foram Google Scholar e ProQuest em fevereiro de 2020 e atualizada em março de 2021. Para análise do risco de viés utilizou-se a ferramenta SYRCLE para os estudos *in vivo*. Para análise da qualidade do estudo *in vitro* utilizou-se a ferramenta SciRAP e para os estudos *in vivo* a ferramenta GRADE para estudos *in vivo* e o GRADEpro. E para a descrição da toxicidade, aspectos botânicos, características fitoquímicas, propriedades etnofarmacológicas e estudos experimentais com o jucá elaborou-se uma Revisão Narrativa. **Resultados:** O protocolo foi registrado e aprovado no PROSPERO sob o número CRD42020159934. Na Revisão Sistemática foram identificados 609/504 (1ª busca/2ª busca) estudos, sendo 299/303 excluídos por não atenderem aos critérios de inclusão. 10/13 estudos foram incluídos para extração e análise dos dados, sendo 10 estudos *in vivo* e três, *in vitro*. A qualidade da evidência na maioria dos estudos foi considerada moderada. Na revisão Narrativa observou-se que tanto nos estudos *in vivo* quanto *in vitro* a toxicidade foi ausente. Dentre os modelos *in* vivo destacou-se o uso de ratos e camundongos. Os compostos fenólicos foram os mais identificados na planta. **Conclusões:** O jucá apresentou atividade anti-inflamatória em todos os estudos incluídos independente da parte da planta e tipo de extrato e/ou fração utilizada nos experimentos *in vivo* e *in vitro*, corroborando assim com os dados da literatura e utilização na medicina popular. Mais estudos relacionados à atividade anti-inflamatória desta planta devem ser realizados em modelos experimentais seguindo guias padronizados para a condução dos mesmos de forma a gerar maior nível de evidência.

Palavras-chave: *Libidibia ferrea*. Inflamação. Experimentação animal.

ABSTRACT

Introduction: Jucá or “pau-ferro” [*Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz] is a plant belonging to the Fabaceae family, widely found in the Northern and Northeastern regions in Brazil. Popularly used in the treatment of diseases such as diarrhea, anemia, diabetes, healing, among others, and presenting anti-inflammatory action. Animal model’s studies to verify the anti-inflammatory action of new drugs derived from plants are used, as well as to know the safety, efficacy and toxicity of these plants. **Aims:** Research the evidence of the anti-inflammatory action of the jucá in *in vivo* and *in vitro* experiments. **Methodology:** For the search, analysis and synthesis of evidence of the anti-inflammatory action of *L. ferrea* in *in vivo* and *in vitro* models, a Systematic Review was carried out. To preparation of the protocol according to PRISMA's tool the following guiding question were: Which part of the *L. ferrea* plant and what type of extract have the highest evidence of anti-inflammatory effects on acute inflammation using *in vivo* and *in vitro* experimental models?. The bases used for research were PubMed, Science Direct, Scopus, LILACS and Web of Science. And in the grey literature Google Scholar and ProQuest were used in February 2020 and an updated in March 2021. To analyze the risk of bias for *in vivo* studies the SYRCLE’s RoB tool was used. For the analysis of the quality of the *in vitro* study the SciRAP tool was used. And for the *in vivo* studies of the GRADE for *in vivo* studies and GRADEpro were used. And for the description of toxicity, botanical aspects, phytochemical characteristics, ethnopharmacological properties and experimental studies with jucá, a Narrative Review was elaborated. **Results:** The protocol was registered and approved in PROSPERO under the number CRD42020159934. At the Systematic Review, 609/504 articles were identified, of which 299/303 were excluded because they did not correspond to the inclusion criteria. 10/13 studies for data extraction and analysis were included. Thus, ten *in vivo* and three *in vitro* studies remained. In most studies, the quality of evidence was considered moderated. In the narrative review, both *in vivo* and *in vitro* studies the toxicity was absent. In use the *in vivo* models rats and mice were stood out. In the plant, phenolic compounds were the most identified. **Conclusions:** Jucá presents anti-inflammatory activity in all studies included regardless of plant part and type of extract and/or fraction used in *in vivo* and *in vitro* experiments. Thus, further studies following standardized guides should be conducted in experimental models related to the anti-inflammatory activity of this plant to generate a higher level of evidence.

Keywords: *Libidibia ferrea*. Inflammation. Animal experimentation.

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LISTA DE ABREVIATURAS, SIGLAS E SÍMBOLOS

%	porcentagem
μM	micromolar
3Rs	Substituição, refinamento e redução
AINES	Anti-inflamatórios Não Esteroidais
ARRIVE	*Animal Research: Reporting of In Vivo Experiments*
CAMARADES	*Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies*
COX	Cicloxigenase
DAMPs	Padrões Moleculares Associados ao Dano
GRADE	*Grading of Recommendations Assessment, Development and Evaluation*
GRADEpro GDT	*GRADE Guideline Development Tool*
GSPC	*Gold Standard Publication Checklist*
H	hora
IL	Interleucina
LPS	Lipopolissacarídeos
MDA	Malondialdeído
MeSH	*Medical Subject Headings*
mL	mililitros
mm^3	Milímetros cúbicos
MPO	Mieloperoxidase
nmol/μL	Nanomols por microlitro
PAMPs	Padrões Moleculares Associados aos Patógenos
PBS	*Phosphate Buffered Saline* (Tampão fosfato salino)
PCR	Proteína C-reativa
pg/mL	Picograma por mililitros
PMNL	Leucócitos polimorfonucleares
PRISMA	Transparent Reporting of Systematic Reviews and Meta-Analyses
PROSPERO	*International Prospective Register of Systematic Reviews*
RCT	*Randomized Clinical Trial*
RoB	*Risk of Bias*
ROS	Espécies Reativas de Oxigênio
RS	Revisão Sistemática
SciRAP	Science in Risk Assessment and Policy
SYRF	Systematic Review & Meta-analysis Facility
TLRs	Receptores do Tipo *Toll*
TNF-α	Fator de necrose tumoral tipo alfa
U/μL	Unidades por microlitros

APRESENTAÇÃO

Esta tese será apresentada na forma de texto com a abordagem geral sobre plantas medicinais, processos inflamatórios, modelos experimentais e os apêndices referentes às publicações obtidas nesta pesquisa.

Descrevem-se duas revisões realizadas sobre a planta *Libidibia ferrea* (jucá ou pau-ferro) na forma de capítulo o Capítulo I onde se elaborou uma Revisão Narrativa e o Capítulo II com a elaboração de uma Revisão Sistemática e seu respectivo Protocolo.

Boa leitura a todos!

SUMÁRIO

1 INTRODUÇÃO

A inflamação é um processo considerado benéfico dos organismos no intuito de resolver uma situação causada por injúria, trauma, agentes patogênicos, queimaduras e doenças (MEDZHITOV, 2008, 2010) e manifesta-se como primeira resposta frente a estas situações. Apresenta cinco sinais clássicos, denominados como cardinais, que são: dor, vermelhidão, calor, edema e perda de função (MEDZHITOV, 2010). Caracteriza-se como aguda (que tem duração de dias a semanas) e crônica (quando na permanência dos agentes causadores iniciais da inflamação levando a doenças como diabetes, autoimunes, entre outras) (GOLAN; TASHJIAN; ARMSTRONG, 2012).

O próprio organismo tenta resolver o processo inflamatório envolvendo componentes celulares, vasculares e substâncias solúveis (ABBAS; LICHTMAN; PILLAI, 2019). O uso de intervenções terapêuticas visa auxiliar neste processo seja ele na forma de fármaco ou tratamentos naturais como, por exemplo, uso de extratos vegetais, produzindo efeitos anti-inflamatórios (KATZUNG; MASTERS; TREVOR, 2014).

Devido aos efeitos colaterais dos fármacos anti-inflamatórios, têm-se visado outras formulações de anti-inflamatórios. O uso de plantas medicinais tem objetivado menos efeitos colaterais, maior eficácia na ação, melhor custo-benefício, maior acessibilidade. Além disso, modelo animal experimental tem sido bastante utilizado para esta forma de pesquisa (BHATTACHARYA, 2016; GHASEMIAN; OWLIA; OWLIA, 2016; RIBEIRO et al., 2018).

Uma destas plantas conhecidas de uso popular brasileiro é o jucá ou pau-ferro, cientificamente denominada *Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz. Pertence à família Fabaceae (STEHMANN; FARIA; BRAGIONI, 2019) e praticamente todas as partes (raiz, caule, folha, fruto, semente) desta planta são utilizadas para tratamento de alguma doença, como por exemplo, para tratamento contra amebíase utilizando as folhas (DI STASI; HIMURA-LIMA, 2002). Em virtude deste amplo uso na medicina popular, como por exemplo, na Mata Atlântica, utilizada contra problemas respiratórios, tosses e resfriados e na Região Amazônica, para problemas cardíacos na utilização do sumo das folhas (DI STATI; KIMURA, 2012). Assim, muitos estudos têm sido realizados demonstrando algumas destas propriedades medicinais como a ação antileishmania (COMANDOLLI-WYREPKOWSKII et al., 2017), anti-inflamatória

(CARVALHO et al., 1996; FALCÃO et al., 2019a, 2019b; PEREIRA et al., 2012), entre outras. Devido à variedade de estudos apresentando diferentes propriedades medicinais torna-se importante uma sumarização destes a fim de se analisar as evidências geradas nestes estudos através da realização de uma Revisão Sistemática.

Revisões Sistemáticas (RS) *tentam identificar, avaliar e sintetizar todas as evidências empíricas que atendam aos critérios de elegibilidade pré-especificados para responder a uma pergunta de pesquisa específica* (COCHRANE LIBRARY, 2020). São bastante utilizadas nas tomadas de decisão em saúde (HOOIJMANS et al., 2014).

A RS com estudos pré-clínicos é utilizada para elucidar sobre: a decisão dos 3Rs (substituição, refinamento e redução), futuros estudos pré-clínicos; buscar as diferenças que existem entre os estudos; esclarecer discrepâncias que existam nos resultados apresentados entre os ensaios pré-clínicos e clínicos; além de auxiliar na sumarização das evidências de diversos estudos com similaridade possibilitando estimar os efeitos de modo mais preciso (CAMARADES, 2021).

Assim, justifica-se a realização deste estudo mediante o volume de estudos associados a modelos animais experimentais utilizando extratos ou polissacarídeos do jucá, tornando importante a busca e a análise das evidências, sumarizando estas informações e avaliando-as através de uma Revisão Sistemática.

2 REFERENCIAL TEÓRICO

2.1 PLANTAS MEDICINAIS

Uma espécie vegetal, empregado com fins terapêuticos sendo cultivada ou não é a definição de uma planta medicinal segundo Formulário de Fitoterápicos da Farmacopeia Brasileira (ANVISA, 2018). Esta opção terapêutica tem sido utilizada na atenção básica em vários países desenvolvidos (BRASIL, 2016). Devido a sua grande importância, as plantas medicinais tornaram-se alvos de políticas públicas não só nacionais, mas também internacionais com iniciativa da Organização Mundial de Saúde (OMS) que criou nos anos 70, o Programa de Medicina Tradicional (BRASIL, 2012).

A maior diversidade de plantas medicinais do planeta encontra-se no Brasil (BRASIL, 2016) onde aproximadamente 45.000 espécies de plantas (20 a 22% do total do mundo) apresentam potencial farmacológico (RIBEIRO et al., 2018). São empregadas para a produção de fitoterápicos, medicamentos e para o uso na medicina tradicional, assim atuam como matéria-prima (BRASIL, 2016), ou seja, doenças têm sido tratadas com plantas medicinais, são alvo de pesquisas por apresentarem uma variedade de compostos fitoquímicos e diversas atividades biológicas e químicas consideradas promissoras (YANG et al., 2017; RIBEIRO et al., 2018).

Entre as diversas aplicabilidades das plantas medicinais, destaca-se a de ação anti-inflamatória, e dos compostos associados à esta ação, como por exemplo, flavonoides, terpenos e compostos fenólicos (RIBEIRO et al., 2018). Esta ampla diversidade de efeitos está vinculada a esta produção de metabólitos secundários (CLARK, 1996 apud DAR et al., 2017).

Além do seu amplo uso terapêutico, uma outra característica, das plantas medicinais, é possuir uma ampla aceitação por parte da população e, normalmente, apresentam um custo menor que os medicamentos sintéticos (CALIXTO, 2000). Dos medicamentos circulantes, aproximadamente 25%, são oriundos, de modo direto ou indireto, de plantas medicinais (BRASIL, 2012; BRASIL, 2016). A busca por novas substâncias de origem vegetal com ação anti-inflamatória tem sido realizada para o desenvolvimento de novos medicamentos (GHASEMIAN; OWLIA; OWLIA, 2016;

RIBEIRO et al., 2018) endossando a importância do conhecimento etnofarmacológico destas plantas (BHAT et al., 2015).

Na região Amazônica, o número de plantas medicinais consideradas úteis não só ao homem, mas também aos animais e ao meio ambiente corresponde em torno de 5 a 35 mil espécies (CARDENAS, 2017). Pode-se citar, por exemplo, o uso do breu branco, do cacau e do capim-santo como potente anti-inflamatório (infusão das folhas), no tratamento de infecções na garganta (secagem das folhas) e, contra febre e qualquer tipo de dor (chá das folhas) respectivamente (DI STASI; HIRUMA-LIMA, 2002).

A *Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz também está entre as plantas com potencial terapêutico, e entre suas atividades inclui-se sua identificação na ação anti-inflamatória, (BRASIL, 2006) da qual foi o alvo de investigação neste projeto de pesquisa.

2.2 *Libidibia ferrea* (Mart. ex Tul.) L.P. QUEIROZ

O material referente à descrição dos aspectos botânicos, propriedades etnofarmacológicas, características fitoquímicas, toxicidade e testes experimentais consta na Revisão Narrativa (Apêndice 1).

2.3 SISTEMA IMUNE INATO

A resposta do organismo frente a um patógeno ou lesão tecidual pode ocorrer de forma imediata ou tardia, sendo tradicionalmente classificada, esta imunidade, em Inata e Adaptativa, respectivamente, apresentando diferentes papéis e funções (MEDZHITOV; JANEWAY, 2000).

Cada uma destas imunidades apresenta características que as diferem em relação àquelas relacionadas à especificidade, memória, reatividade, diversidade de receptores (MEDZHITOV; JANEWAY, 2000) e componentes associados aos tipos celulares, proteínas sanguíneas e, às barreiras físicas, biológicas e químicas (MEDZHITOV; JANEWAY, 2000; CRUVINEL et al., 2010).

Na Imunidade Inata ocorre uma rápida resposta, sendo, portanto, a primeira linha de defesa, frente a uma diversidade de estímulos (CRUVINEL et al., 2010). Referente à especificidade, o sistema imune inato utiliza receptores para reconhecimento de estruturas moleculares, também denominadas padrões que são

denominados Padrões Moleculares Associados ao Patógenos (PAMPs) e Padrões Moleculares Associados ao Dano (DAMPs). Os primeiros estão relacionados às substâncias microbianas (MEDZHITOV; JANEWAY, 2000), que podem ser ácidos nucleicos, proteínas e lipopolissacarídeos (LPS), e os segundos (endógenos, liberados por células do hospedeiro) que podem ser produzidos como resultado de moléculas liberadas de células mortas ou produtos da cascata proteolítica derivados de um dano vascular ou produtos oriundos da matriz extracelular quando degradada (BASBAUM et al., 2009; MEDZHITOV 2010).

O sistema imune inato também utiliza moléculas solúveis no sangue/soro (sistema complemento) que atual de modo crucial frente aos agentes microbianos auxiliando no reconhecimento destes agentes. Além disso, diversas células efetoras deste sistema, principalmente, macrófagos, e células dendríticas, expressam estes receptores e possuem um papel indutor nas respostas inflamatória (MEDZHITOV; JANEWAY, 2000). Estas células são acionadas a partir do momento em que há o reconhecimento do patógeno, o que torna rápida a resposta inata (BORASCHI; ITALIANI, 2018).

Os neutrófilos são células leucocitárias de origem mieloide, classificados como granulócitos e denominadas como leucócitos polimorfonucleares (PMNL). Morfologicamente apresentam o núcleo multilobado (3-5 lóbulos ligados por pontes de cromatina) com diâmetro de 12 a 15 μM e citoplasma repleto de grânulos secundários/específicos (lisozimas e demais proteases) e primários/azurófilos [elastase e Mieloperoxidase (MPO)] (KIERSZENBAUM, 2008; JUNQUEIRA; CARNEIRO, 2011).

São considerados os mais abundantes (circulantes) no sangue (60-70% - 5.000/μL ou mm^3), sobrevivendo horas (6 a 7 h) ou poucos dias (aproximadamente 4 dias no tecido Conjuntivo). Sua função é a de fagocitose e realizar a digestão de microrganismos, eliminando bactérias opsonizadas ou auxiliando na limitação da reação inflamatória no tecido (KIERSZENBAUM, 2008; JUNQUEIRA; CARNEIRO, 2011).

Microrganismos induzem, os neutrófilos, a extrusão de seus conteúdos granulares e seu DNA formando redes extracelulares. Este conteúdo interno liberado é designado de rede extracelular de neutrófilos (NETs) (KUMAR et al., 2018).

Os macrófagos, são outros componentes celulares da imunidade inata que também realizam a função de fagocitose (fagócitos mononucleares) que quando circulantes no sangue (12 a 100 h) são denominados monócitos, e macrófagos, quando residentes em algum tecido em especial durante reações inflamatórias. São distribuídos na maioria dos órgãos recebendo denominações diversas e funções respectivas. Podem atuar como apresentadores de antígenos, além de removerem células mortas e no reparo de tecidos danificados (KIERSZENBAUM, 2008; JUNQUEIRA; CARNEIRO, 2011).

Normalmente, os macrófagos apresentam um núcleo oval ou reniforme, excêntrico a célula com diâmetro de 10-30 μm (KIERSZENBAUM, 2008; JUNQUEIRA; CARENIRO, 2011). Secretam citocinas [ex.: Fator de Necrose Tumoral tipo alfa (TNF-α), Interleucina tipo 1 (IL-1), quimiocinas] que auxiliam no recrutamento de mais leucócitos do sangue para os locais onde está ocorrendo a infecção. Estas citocinas atuam sobre células endoteliais (ABBAS; LICHTMAN; PILLAI, 2019). Juntamente com os mastócitos, os macrófagos produzem mediadores inflamatórios quando ocorre o reconhecimento inicial de infeções por estas células (MEDZHITOV, 2008).

Mastócitos são células que também podem participar da resposta imune. Estas células têm origem mieloide e normalmente são encontradas nos tecidos sendo, portanto, denominadas em: mastócitos de mucosas e mastócitos de tecido conjuntivo, que se diferenciam em número e tamanho entre as duas populações (JUNQUEIRA; CARNEIRO, 2011).

Atuam como sentinelas teciduais e possuem grânulos citoplasmáticos como fonte de mediadores (aminas) vasoativos (histamina e heparina) e quimiotáticos. (JUNQUEIRA; CARNEIRO, 2011). Alguns destes mediadores (ex. serotonina e histamina) estocados, pré-formados e liberados após a degranulação dos mastócitos. Os quimiotáticos recrutam neutrófilos, eosinófilos, monócitos, e secretam citocinas pró-inflamatórias (TNF-α) (MEDZHITOV, 2008) e mediadores lipídicos (ex. leucotrienos). Pela sua proximidade nos vasos sanguíneos, os mastócitos próximos aos vasos sanguíneos (perivasculares) liberam as substâncias acima citadas, sendo a histamina e os eicosanoides responsáveis pelo extravasamento de fluido e vasodilatação (NATHAN, 2002) sinais estes relacionados aos sinais cardinais da inflamação aguda que são calor e vermelhidão, e edema, respectivamente.

2.4 PROCESSO INFLAMATÓRIO E INFLAMAÇÃO AGUDA

A inflamação é um processo considerado importante para o organismo, haja vista atuar como resposta de defesa a agentes infecciosos, a respostas autoimunes, a injúrias ou a isquemia tecidual (LAVETI et al., 2013). Caracterizada como um processo no qual o sistema imune do hospedeiro realiza o recrutamento de leucócitos (principalmente: neutrófilos, monócitos e demais fagócitos) e proteínas plasmáticas (CHOVATIYA; MEDZHITOV, 2014; MEDZHITOV, 2008). Sendo, portanto, classicamente caracterizada como uma resposta à lesão ou à inflamação (OKIN; MEDZHITOV, 2012)

Deste modo, a inflamação pode ser iniciada por diferentes agentes etiológicos: infecciosos (ex. bactérias) e não infecciosos (ex. trauma, queimaduras, danos celulares) (CHEN et al., 2018), objetivando a restauração da homeostase do organismo (MEDZHITOV, 2008, 2010).

Cornelius Celsus descreveu quatro sinais clínicos ou cardinais da inflamação, que são: calor (atividade metabólica dos mediadores inflamação e aumento do fluxo sanguíneo), rubor (hiperemia), edema/tumor (vazamento das proteínas e aumento da permeabilidade vascular) e dor (modificações na perivasculatura e respectivas terminações nervosas) (VOGEL, 2002; LIBBY, 2007). Rudolf Virchow descreveu o quinto sinal, que é a perda de função (*function laesa*) (FERRERO-MILIANI et al., 2007; LIBBY, 2007). Este último sinal está associado a todo o processo inflamatório, e os quatro sinais descritos por Celsus são empregados na inflamação aguda (MEDZHITOV, 2010).

De modo geral, o processo inflamatório envolve quatro componentes: (1) indutores inflamatórios (ex. patógenos) que iniciam o processo inflamatório; (2) sensores (ex. TLRs) que identificam os indutores inflamatórios; (3) mediadores inflamatórios (ex. citocinas), estes são secretados pelas células especializadas (ex. macrófago); (4) tecidos alvos que são os locais de ação destes mediadores para resolução do processo inflamatório (MEDZHITOV, 2010).

A eliminação de um patógeno ou outro fator indutor da inflamação torna a inflamação um processo importante (ABDULKHALEQ et al., 2018). Em condições normais, esta é capaz de se autolimitar, contudo, se este processo permanece contínuo, podendo surgir processos inflamatórios crônicos (FERRERO-MILIANI et

al., 2007) resultando, muitas vezes, em dano ou falência de órgãos (ABDULKHALEQ et al., 2018).

A inflamação aguda é considerada benéfica, inicia-se de modo rápido (LAVETI et al., 2013), podendo os sintomas permanecerem por dias (PAHWA et al., 2021) e visa promover o reparo tecidual e impedir a infecção, por agentes oportunistas. Normalmente, este processo é finalizado quando o agente indutor da inflamação é eliminado e consequentemente, o tecido é reparado, retornando assim, o tecido, para a homeostasia (SERHAN; SAVILL, 2005; MEDZHITOV, 2010).

Eventos vasculares e celulares ocorrem na inflamação aguda. Logo após o dano tecidual, a fase vascular inicia-se através das células residentes no tecido (CRUVINEL et al., 2010). Ocorrem alterações, reversíveis, nos vasos sanguíneos dos tecidos que foram danificados ou infectados causando alterações no fluxo sanguíneo; permeabilidade a fluidos e às proteínas (tais como proteínas do complemento e anticorpos), aumento nas vênulas e nos capilares. Estas reações são induzidas por pequenas moléculas mediadoras e citocinas (ABBAS; LICHTMAN; PILLAI, 2019).

Nos eventos celulares, que ocorrem em paralelo aos vasculares, os neutrófilos são as primeiras células a serem recrutadas ao local da infecção seguidos dos monócitos (que ao adentrarem no tecido maturam e se passam a ser denominados de macrófagos), linfócitos, células *Natural Killers* e mastócitos (CHEN et al., 2018). Os leucócitos, por estarem no ambiente externo da região inflamada, necessitam adentrar ao local passando por uma série de etapas. As células residentes (ex.: macrófagos) liberam citocinas [ex. IL-1, e TNF-α) ativando as células endoteliais. Estas células, por sua vez, expressam selectinas (E-selectinas e P-selectinas), que aumentam a expressão de mediadores de adesão celular (CRUVINEL et al., 2010).

Em seguida, ocorre um processo de marginação dos leucócitos próximo ao local de revestimento dos vasos. As células endoteliais ligam-se às selectinas expressas, e através de um processo de rolamento na superfície endotelial, com subsequente transmigração dos leucócitos, atravessam o endotélio pelas junções destas células (CRUVINEL et al., 2010).

Neste processo de inflamação aguda, as células liberam mediadores inflamatórios, que atuam como marcadores que são citocinas e proteínas inflamatórias que podem agir como biomarcadores no diagnóstico ou preditores de

doenças inflamatórias (CHEN et al., 2018; FERRERO-MILIANI et al., 2007). As citocinas, portanto, são produzidas por diversos grupos celulares, como por exemplo macrófagos e apresentam um importante papel na resposta inflamatória, incluindo a ativação de leucócitos (MEDZHITOV, 2008).

Dentre as citocinas, destacam-se a TNF e as ILs. O TNF-α uma citocina pró-inflamatória que atua em vários tipos celulares e tem um importante papel no processo inflamatório (BAIZABAL-AGUIRRE et al., 2016). É produzida de forma abundante por vários tipos celulares, possui ação pleiotrópica atuando também na produção de citocinas e proliferação celular. Dentre as interleucinas destacam-se IL-1, -6, -8, -12 como pró-inflamatórias quando na ativação das células inflamatórias (ABBAS; LICHTMAN; PILLAI, 2019). A IL-1, -6 juntamente à TNF-α, também induzem alterações no endotélio o que favorece que entre as junções das células endoteliais ocorra a passagem de células associadas à resposta imune (VARELA et al., 2018).

Proteínas também atuam como marcadores como a proteína C reativa (PCR) que é uma proteína de fase aguda, produzida de forma ampla na resposta inflamatória pelo fígado; e enzimas antioxidantes como, por exemplo, a malondialdeído (MDA) e espécies reativas de oxigênio (ROS) (LAVETI et al., 2013; CHEN et al., 2018). Assim, dependendo do fator indutor da inflamação é que determinadas células e mediadores entram em ação (PUNCHARD; WHELAN; ADCOCK, 2004).

Conforme demonstrado, a resposta inflamatória envolve uma série de reações com o intuito de resolver a invasão de um patógeno ou um dano tecidual. Quando o mecanismo da resposta inflamatória aguda não resolve ou elimina o agente causador da inflamação, ou seja, este indutor permanece no organismo e, então, tem-se a inflamação crônica, como por exemplo, artrite reumatoide, aterosclerose (PUNCHARD; WHELAN; ADCOCK, 2004).

Órgãos como coração, fígado e outros sítios do organismo podem ser alvo de processos inflamatórios agudos ou crônicos causando injúria tecidual (CHEN et al., 2018). Fármacos com ação anti-inflamatória têm sido utilizados no tratamento destas reações imunes e os anti-inflamatórios não esteroidais (AINES) para amenizar os sinais cardinais da inflamação como dor e febre (MEEK; LAAR; VONKEMAN, 2010; ABD-ALLAH et al., 2018). Porém a descoberta de novos agentes anti-inflamatórios

provenientes de produtos naturais ainda é fundamental e necessária (ABDULKHALEQ et al., 2018).

Muitas destas pesquisas auxiliam não só a identificação de novas substâncias e fármacos, mas também na verificação de seu efeito anti-inflamatório sendo realizadas, na maioria das vezes, em modelos experimentais *in vivo* e *in vitro*.

2.5 ESTUDOS EM MODELO ANIMAL

A utilização de modelos animais de patologias humanas são ferramentas valiosas para se compreender a fisiopatologia de algumas doenças e se descobrir novas drogas e alvos terapêuticos. Algumas limitações existem como, por exemplo, a diferença entre humanos e animais, porém há muitas vantagens nos estudos pré-clínicos (FEDELE; GUALILLO; VECCHIONE, 2011).

O uso de animais ou a experimentação animal tem sido alvo de estudos no decorrer da história visando à investigação de mecanismos e a avaliação de terapias novas antes de serem aplicadas nos humanos e, esta investigação está associada à semelhança dos modelos experimentais com a anatomia e fisiologia humana (BARRÉ-SINOUSSI; MONTAGUTELLI, 2015; FRANCO, 2013).

A realização deste tipo de estudo (pré-clínicos) está relacionada à descoberta de novos medicamentos, além de estar também associado à análise toxicológica sendo esta, uma das exigências das instituições regulamentadoras antes dos testes em seres humanos (VANDAMME, 2014).

Dentre os animais utilizados nas pesquisas científicas, têm-se os roedores e dentre esses, os camundongos (*Mus musculus*). Estes últimos são de fácil manuseio por serem pequenos, além de apresentar: rápida taxa de reprodução, facilidade de mantê-los em laboratórios, vida curta (BAUMANS, 2007; FRANCO, 2013), existirem ofertas de muitas cepas de uma linhagem consanguínea (PERLMAN, 2016) e, assim, representarem os animais que predominam nas pesquisas biomédicas utilizados com o objetivo de descobrir curas e tratamentos quando utilizados como modelos de doenças humanas (HICKMAN et al., 2017).

Muitos estudos, com experimentação animal, têm sido realizados para a verificação do potencial da ação de plantas medicinais. Gamede e colaboradores (2018) demonstraram em ratos pré-diabéticos induzidos por dieta que a ação do ácido oleanólico, derivado de plantas, regula a homeostase da glicose. Outro

exemplo foi observado em um estudo com camundongos Swiss utilizando óleo essencial de *Renealmia alpinia* (Rottb.) que demonstrou ação analgésica (GÓMEZ-BETANCUR et al., 2019).

Na literatura, há a demonstraram da ação antimalárica *in vitro* e *in vivo* (BALB/c) do extrato das espécies *Picrolemma huberi* e *Picramnia latifólia* (BERTHI et al., 2018). E observou-se em um modelo experimental de inflamação intra-articular induzido por zimosan em ratos Wistar, a atividade anti-inflamatória do extrato aquoso de *Libidibia ferrea* (FALCÃO et al., 2019a).

Modelos bastante estudados são os de inflamação (*in vivo* e *in vitro*), contudo a busca de um medicamente seguro e eficaz torna-se desafiador e crítico, e nos últimos anos a descoberta e/ou utilização de fitoquímicos ou produtos naturais são considerados como fonte de drogas e consequentemente, candidatas à atuação na resposta anti-inflamatória. Estes modelos podem ser crônico ou agudo para *screening* da atividade anti-inflamatória, como por exemplo, o edema de pata induzido por carragenina (PATIL et al., 2019).

Além disso, para a utilização de animais, nestes experimentos, necessário se faz o uso da ética, através da análise nos Conselhos de Ética. O Conselho Nacional de Controle de Experimentação Animal (CONCEA) (MCTIC, 2016) estabelece resoluções e entre elas as que auxiliam na boa condução destes estudos.

2.6 MODELO DE INFLAMAÇÃO AGUDA EM ANIMAIS

Na busca de se desenvolver novas drogas anti-inflamatórias eficazes e seguras, o uso de testes em modelos animais ou pré-clínico é algo bastante observado e disponível na literatura. Estes modelos proporcionam a avaliação das propriedades anti-inflamatórias e dos mecanismos de ação das moléculas derivadas de plantas para teste de novas drogas. Alguns destes modelos, tanto para inflamação aguda quanto para crônica (PATIL et al., 2019).

Nos modelos de inflamação aguda, um dos estabelecidos é o de mensuração de edema de pata induzido por agentes flogísticos (ex. carragenina, histamina, bradicinina, dextrana) (PATIL et al., 2019). A inflamação ocorre em decorrência do processo inflamatório induzido por um agente flogístico (ou irritante) visando à redução ou cessação do edema (inchaço) causado pelo processo inflamatório. A forma como esta ação antiedematogênica acontece é observada através da

mensuração da pata dos animais teste em comparação com a pata dos animais controles, ou seja, a avaliação da variação deste edema em um determinado período (FEREIDONI et al., 2000; VOGEL, 2002; PATIL et al., 2019).

Além de ser considerado um modelo clássico de estudo, seletivos de COX (GONÇALVES et al., 2011) utilizado, desde a década de 60, como indutor de inflamação aguda também apresenta uma ótima reprodutibilidade (NANTEL et al., 1999; PATIL et al., 2019). É de simples execução e de curto período de realização e não causa danos ou injúria à pata inflamada do animal (ALQASOUMI et al., 2012; BAO et al., 2018).

Em 1969, Levy introduziu um modelo de edema de pata em camundongos (injeção subplantar de carragenina) que apresentava similaridade ao modelo em ratos já existente, para estudo não só de novas drogas anti-inflamatórias, mas também dos mediadores inflamatórios. Henriques et al. (1987) demonstraram que em camundongos ocorrem duas fases (bifásico) e que carragenina 1% seria o suficiente para a indução da inflamação na pata do camundongo (LEVY, 1969; WINTER; RIESLEY; NUSS, 1983).

A primeira fase ou fase inicial ocorre em até 90 min onde os primeiros mediadores detectados são histamina, bradicinina e serotonina geradas ou *in situ* ou pela infiltração de neutrófilos (como células predominantes), o que estaria relacionado ao aumento da permeabilidade vascular e a vasodilatação ocasionando o edema (EDDOUKS; CHATTOPADHYAY; ZEGGWAGH, 2012; SENGAR et al., 2014; BAO et al., 2018; LOPES et al., 2019). Esta primeira fase não sofre inibição pelos AINEs (NANTEL et al., 1999; NECAS; BARTOSIKOVA, 2013).

A segunda fase ou fase tardia ocorreria após os 90 min e seria caracterizada pela produção das prostaglandinas e citocinas (IL-1, IL-6 e TNF-α) (CUZZOCREA et al., 1998; NECAS; BARTOSIKOVA, 2013). Os leucócitos infiltram-se e são ativados no local da inflamação (NECAS; BARTOSIKOVA, 2013; POSADAS et al., 2004). Assim, a liberação de prostaglandinas é estimulada pela carragenina (WINTER; RIESLEY; NUSS, 1983).

Posadas e colaboradores (2004) demonstraram que em camundongos o edema de pata ocorria em duas fases (bifásico) e dependia da idade e do peso dos animais. Na fase inicial (0-6 h), os animais de 6 a 8 semanas apresentaram edema significativo enquanto os animais de 3 a 5 semanas, pesando menos, o edema foi

significativamente menor. Na segunda fase (24-96 h), não houve diferença de idade de 5 a 8 semanas no pico de indução do edema.

A carragenina é um polissacarídeo obtido de várias espécies pertencentes à família Rodophyceae (algas vermelhas), da espécie *Chondrus crispus*, e dos gêneros *Gigartia* e *Eucheuma*. Também pode ser conhecida como musgo de *carragen* e musgo irlandês ou *carraigin* na Inglaterra e Irlanda, respectivamente. É muito comum encontrá-la próximo à Grã-Betânia, América do Norte e Europa. Além do seu uso no campo experimental, ela tem sido empregada na área médica e farmacológica (NECAS; BARTOSIKOVA, 2013; TOBACMAN, 2001).

Além da carragenina, outros agentes flogísticos são utilizados para a indução da inflamação como, por exemplo, a dextrana e a formalina (FEREIDONI et al., 2000; VOGEL, 2002; PATIL et al., 2019) podendo também, ser empregados em outros modelos de inflamação.

2.7 MODELOS DE INFLAMAÇÃO *IN VITRO*

Alguns estudos com modelos para atividade anti-inflamatória *in vitro* foram descritas em um estudo de revisão (EZE et al., 2019), como a estimulação de células de macrófagos RAW 264.7 com lipopolissacarídeos (LPS) do qual se tem determinação de óxido nítrico. São células provenientes de camundongo (*Mus musculus*) da cepa BALB/c. A utilização de LPS estimula a lise dos eritrócitos (ATCC.ORG, 2021). Estas células, entre outras, em contato com o LPS estimulação a produção de citocinas, como por exemplo, IL-6 ou TNF-α (THERMOFISHER.COM, 2021).

As vantagens de se utilizar modelos *in vitro* são a confiabilidade, sensibilidade e reprodutibilidade. Além de possibilitar uma melhor compreensão dos mediadores inflamatórios envolvidos e do mecanismo de ação destes mediadores. Tendo como desvantagens, em especial, razões econômicas e a replicação dos mesmos resultados em estudos *in vivo* (EZE et al., 2019).

3 HIPÓTESE

Há diferença na ação anti-inflamatória entre os tipos de extrato e partes da planta *L. ferrea* utilizada em modelos animais e *in vitro*.

4 OBJETIVOS

4.1 GERAL

Identificar a parte da planta de *L. ferrea* e que tipo de extrato tem os efeitos anti-inflamatórios mais evidentes nos modelos experimentais de inflamação aguda *in vivo* e *in vitro*.

4.2 ESPECÍFICOS

- Executar uma Revisão Sistemática sobre a ação anti-inflamatória da *Libidibia ferrea* em modelos *in vivo* e *in vitro*.

- Revisar a literatura referente à inflamação aguda e estudos experimentais de modelos animais com a espécie *L. ferrea*.

- Fazer um levantamento na literatura acerca da *Libidibia ferrea* e seus aspectos gerais na forma de uma Revisão Narrativa.

CAPÍTULO I

APRESENTAÇÃO

O Capítulo I apresenta um referencial teórico do tipo Revisão Narrativa com a planta *Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz abordando os tópicos referentes às(aos):

- Aspectos botânicos do jucá;
- Informações das propriedades etnofarmacológicas;
- Características fitoquímicas;
- Informações sobre a toxicidade da planta; e
- Modelos experimentais utilizados relacionados ao uso do jucá

Esta revisão foi intitulada: *A narrative review of Libidibia ferrea: Botanical aspects, ethnopharmacological properties, phytochemical characteristics, toxicity, and experimental tests* e foi publicada na revista **European Journal of Medical Plants**.

REVISÃO NARRATIVA

5 ELABORAÇÃO DA REVISÃO NARRATIVA

Visando revisar a literatura acerca dos dados referentes à *Libidibia ferrea*, bem como seus aspectos botânicos, propriedades etnofarmacológicas, características fitoquímicas, toxicidade e testes experimentais realizou-se a Revisão Narrativa.

Este tipo de revisão difere da Revisão Sistemática uma vez que uma revisão narrativa se refere à uma busca na literatura onde não é preciso se esgotar as fontes de informação e não necessita da aplicação de uma estratégia de busca sofisticada (UNESP/CAMPUS BOTUCATU, 2015). Apresenta uma temática mais aberta não sendo utilizado um protocolo rigoroso nem parte de uma pergunta específica (CORDEIRO et al., 2007).

6 METODOLOGIA

Foi realizada uma busca eletrônica nas bases de dados eletrônicas, livros e referências dos artigos incluídos. As informações foram organizadas no Word™. O Excel™ foi utilizado para montar tabelas e figuras e o Power Point™ também para elaboração de figuras.

7 RESULTADOS

A revisão de literatura gerou um artigo a: **A narrative review of *Libidibia ferrea* (Mart. ex. Tul.) L.P. Queiroz: Botanical aspects, ethnopharmacological properties, toxicity, and experimental tests** publicado na revista **European Journal of Medicinal Plant** (Apêndice 1, na página 54), cujo link para acesso é: https://www.journalejmp.com/index.php/EJMP/article/view/30432.

CAPÍTULO II

APRESENTAÇÃO

Este capítulo apresentará uma breve introdução sobre Revisão Sistemática e a metodologia utilizada para a realização desta. Também demostrará as duas publicações realizadas:

Uma sobre o protocolo da Revisão Sistemática e a outra a revisão Sistemática propriamente dita.

O protocolo da Revisão Sistemática foi intitulado *An evidence-based review of Libidibia ferrea (jucá) anti-inflammatory action on in vivo and in vitro studies: Protocol*. Foi publicado na revista **Scientia Amazônia**.

A publicação da Revisão Sistemática na forma de artigo intitulada: *Libidibia ferrea (jucá) anti-inflammatory action: A systematic review of in vivo and in vitro studies* na revista **Plos One**.

REVISÃO SISTEMÁTICA

8. REFERENCIAL TEÓRICO

8.1 REVISÃO SISTEMÁTICA

A etapa mais importante da Revisão sistemática é o planejamento, sendo dividido em cinco passos: (1) Definição da equipe; (2) Escolha o tópico; (3) Busca de RSs anteriores no tópico escolhido; (4) Elaboração da pergunta da pesquisa; (5) Agrupamento das informações no protocolo (DE LUCA CANTO; RÉUS, 2020). Antes do início da primeira etapa deve-se elaborar a pergunta para verificação se, nas bases de dados de revisões sistemáticas, há alguma que apresente uma temática igual ou similar (CENTRE FOR REVIEWS AND DISSEMINATION, 2009), não só para evitar a duplicidade de informações, mas também para identificação de termos pertinentes à busca (PEREIRA; GALVÃO, 2014).

Alguns passos são utilizados para a execução de uma RS, que são a elaboração da pergunta referente ao tema a ser pesquisado; buscar os estudos/artigos na literatura em base de dados; selecionar os artigos em acordo com os critérios de elegibilidade estabelecidos; extrair os dados relacionados aos artigos incluídos. Em seguida, realiza-se a análise da qualidade destes dados extraídos, se os dados forem homogêneos, realiza-se a metanálise; avalia-se a qualidade da evidência dos dados e se elabora a redação e consequentemente, a publicação da RS (GALVÃO; PEREIRA, 2014).

Na etapa de elaboração da estratégia de busca e a busca nas bases de dados observa-se a existência dos vocábulos controlados ou “thesaurus” de cada base de dados, para poder montar a estratégia de busca. Após a escolha dos descritores da pergunta da Revisão Sistemática, a ser elaborada, utilizam-se os operadores booleanos (AND, OR ou NOT) no intuito de fazerem parte da estratégia de busca. Essa fase de elaboração da estratégia de busca bem como a da elaboração da pergunta é de extrema importância para o sucesso da RS (PEREIRA; GALVÃO, 2014).

Além disso, a inclusão de estudos relevantes está baseada no conhecimento prévio do conteúdo pelo pesquisador, o que pode colaborar com a introdução de

risco de viés. Visando a redução deste risco elabora-se um protocolo contendo as diferentes etapas da revisão sistemática (VRIES et al., 2014).

Contudo, a dificuldade de combinação das diferenças entre as populações estudadas, a definição dos desfechos, o viés de publicação, intervenções são apontados como principais limitações que influenciariam na confiabilidade dos resultados da revisão sistemática (BRASIL, 2021). Assim, a revisão sistemática não está livre de viés, contudo para redução deste o planejamento e transparência dos métodos utilizados é fundamental (SENA et al., 2014).

Normalmente, as revisões sistemáticas são voltadas para estudos na área da saúde e são práticas comuns, inclusive o próprio Ministério da Saúde (2021) estabeleceu as *Diretrizes Metodológicas para elaboração de revisão sistemática e metanálise de estudos observacionais comparativos sobre fatores de risco e prognóstico*. A Revisão Sistemática é sugerida para realização antes de se iniciar um financiamento ou uma pesquisa a fim de se identificar lacunas e se conhecer o que é existente (SOLIMAN; RICE; VOLLERT, 2020).

As RSs também são realizadas em estudos pré-clínicos com animais que são utilizados para gerar informações às pesquisas clínicas (KOREVAAR; HOOFT; TER RIET, 2011; LUIJK et al., 2014; VRIES et al., 2014).

8.2 REVISÃO SISTEMÁTICA COM ANIMAIS

As Revisões Sistemáticas com animais podem colaborar na questão dos 3R’s (substituição, refinamento e redução) (HOOIJMANS; RITSKES-HOITINGA, 2013; VRIES et al., 2014), orientar planejamento de experimentos novos, melhorar a qualidade metodológica da experimentação animal (VRIES et al., 2014), identificar lacunas no conhecimento, promover uma avaliação crítica da qualidade metodológica dos estudos experimentais, além de possíveis falhas metodológicas nos desenhos dos estudos publicados e colaborar com pesquisas clínicas baseando-se nas evidências obtidas nas RS de estudos pré-clínicos (CAMARADES, 2021).

As plataformas *Collaborative Approach to Meta-Analysis* and *Review of Animal Data from Experimental Studies* (CAMARADES) (SENA et al., 2010; SOLIMAN; RICE; VOLLERT, 2020), *Systematic Review & Meta-analysis Facility* (SYRF), *SYstematic Review Center for Laboratory animal Experimentation* (SYRCLE)

oferecem suporte e orientação para realização de Revisões Sistemáticas com estudos pré-clínicos (SOLIMAN; RICE; VOLLERT, 2020). Assim como em RS com estudos em humanos, nas RS de estudos pré-clínicos também é recomendada a submissão do protocolo em uma destas plataformas ou na *International Prospective Register of Systematic Reviews* (PROSPERO) (PROSPERO, 2021).

Os estudos experimentais com animais são modelos de imenso valor para compreensão de etiologia e alguns mecanismos das doenças que afetam os seres humanos (SENA et al., 2014). Contudo, RSs nesta área ainda são escassas (LUIJK et al., 2014; VRIES et al., 2014) e estas ainda são realizadas de modo retrospectivo, ou seja, após a execução dos ensaios clínicos (POUND; RITSKES-HOITINGA, 2020, PROSPERO, 2020).

Um guia de verificação de publicações de padrão ouro (GSPC – *gold standard publication checklist*) foi desenvolvido para estudo em animais visando à melhoria da qualidade dos estudos científicos publicados e consequentemente viabilizar a realização de Revisões Sistemáticas na área de Zoologia. O guia consiste em abordar aspectos na introdução, como por exemplo, o objetivo do estudo, descrição de lacunas no conhecimento, explicitar a hipótese ou a pergunta da pesquisa e a relevância clínica da pesquisa (HOOIJMANS; LEENAARS; RITSKES-HOITINGA; 2010).

Na parte da metodologia constam informações sobre: o desenho experimental, as características dos grupos testes e controles, as condições de alojamento, condições éticas, a intervenção e o desfecho; a descrição dos resultados e discussão dos principais resultados destacando a relevância clínica da pesquisa. É concluído que ao se utilizar este guia e disponibilizar os dados das pesquisas com animais isto auxiliaria na realização de estudos mais transparentes (HOOIJMANS; LEENAARS; RITSKES-HOITINGA, 2010).

Porém, uma preocupação ainda existe na pesquisa em relação à translocação de estudos de animais para humanos. Leenaars e colaboradores (2019) realizaram uma revisão de escopo apresentando que esta taxa de sucesso é algo imprevisível, que os estudos incluídos na pesquisa apresentavam um alto risco de viés, eram muito antigos, e que estudos mais aprofundados sobre esta questão são ainda fundamentais para análise das evidências probabilísticas.

Existem muitos desafios relacionados à translação dos estudos em animais para à clínica, tais como: Diferenças biológicas que existem entre a espécie e a cepa; a presença, nos experimentos com animais de uma baixa qualidade metodológica; ensaios clínicos normalmente se diferenciam dos desenhos de experimentação animal; viés de publicação; descrição insuficiente dos detalhes relacionados aos materiais, animais e métodos (HOOIJMANS; RITSKES-HOITINGA, 2013).

Na realização de uma RS com animais, faz-se necessária a adaptação de seu processo para as características dos estudos de intervenção em animais, haja vista, haver diferenças entre os estudos de ensaios clínicos em humanos (RCT) e estudos de intervenção em animais. Em RCT a doença ocorre naturalmente, visando, assim, demonstrar a eficácia clínica; além de se poder realizar o cegamento dos pacientes. Todavia, nos estudos de intervenção em animais, a doença normalmente é induzida; visando compreender o mecanismo da doença, além da toxidade, segurança e eficácia da intervenção; não há cegamento dos animais (HOOIJMANS et al., 2014).

As RSs, também, podem colaborar com a escolha do modelo animal que seria mais adequado para os estudos experimentais bem como no cálculo do tamanho amostral (NC3RS, 2020) reduzindo o ônus da pesquisa (HOOIJMANS; RITSKES-HOITINGA, 2013), o desperdício em estudos futuros, elucidar discrepâncias que possam ocorrer nos resultados dos ensaios clínicos *versus* pré-clínicos e investigar a heterogeneidade (CAMARADES, 2021).

Em uma revisão sistemática observou-se que o número de revisões sistemáticas e metanálise com estudos pré-clínicos que apresentavam potencial para serem aplicados em estudos com humanos praticamente duplicou, passando de 163 (2005 a 2010) para 246 (2009 a janeiro de 2013) (MUELLER et al., 2014), contudo a atenção à disseminação do risco de viés e a baixa qualidade metodológica continuavam necessitando de uma atenção especial (VESTERINEN et al., 2014).

Um guia contendo diretrizes, o *Animal Research: Reporting of in vivo experiments* (ARRIVE) foi criado visando otimizar o manuscrito, o desenho e a análise das experimentações com animal (KILKENNY et al., 2010). Logo, a síntese dos dados dos estudos pré-clínicos depende de uma alta qualidade metodológica (MUELLER et al., 2014). A listagem disponibiliza tópicos com recomendações

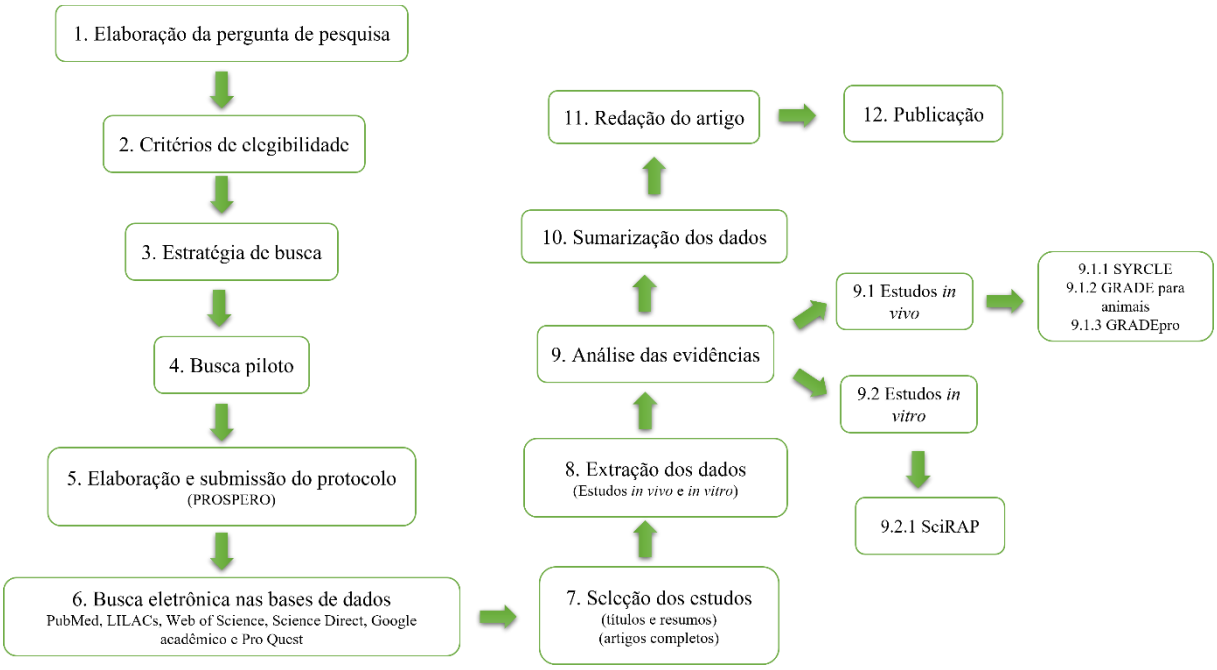
referentes ao título, resumo, introdução, métodos, resultados e discussão do trabalho com animais.

9 METODOLOGIA

9.1 ELABORAÇÃO DA REVISÃO SISTEMÁTICA

Abaixo (figura 1), está representado o fluxograma com as etapas (1 a 12) executadas para o desenvolvimento da Revisão Sistemática até a sua publicação.

Figura 1. Fluxograma referente às etapas realizadas na Revisão Sistemática.



Fonte: Própria autoria (2021).

9.1.1 Pergunta da pesquisa

No intuito de buscar evidências sobre a atividade anti-inflamatória do extrato de jucá em modelos animal e *in vitro*, foi elaborada uma Revisão Sistemática cuja pergunta norteadora era: **Qual parte da planta de *L. ferrea* e que tipo de extrato tem os efeitos anti-inflamatórios mais evidentes nos modelos experimentais de inflamação aguda *in vivo* e *in vitro*?**.

Para elaboração da pergunta de pesquisa e da estratégia de busca utilizou-se o acrônimo PICOS: **P (população)** - Animais (*Rattus novergicus* ou *Mus musculus*) ou células; **I (intervenção)** - Tratamento com extratos nos modelos *in vivo* e/ou *in vitro*

das diferentes partes da planta; **C (controle)** - Positivo (droga padrão) e negativo (salina ou PBS); **O (desfecho)** - efeito anti-inflamatório do extrato de jucá; **S (tipo de estudo)** - Modelos experimentais.

9.1.2 Registro Protocolo

Para possibilitar a transparência e que tanto a pergunta de pesquisa quanto o método utilizado sejam de ciência do mundo todo (Souza et al., 2020) elaborou-se o protocolo, onde se estruturou o título, autores, introdução, os objetivos, metodologia, abrangendo os critérios de elegibilidade, informações da pesquisa e estratégia de busca, além da adição nesta etapa dos registros de estudo e os dados dos artigos seguindo o PRISMA. O protocolo foi cadastro na plataforma on-line *International Prospective Register of Systematic Reviews* (PROSPERO) sob número CRD42020159934.

No intuito de proporcionar a transparência e redução de risco de subjetividade, (VRIES et al., 2014) buscou-se a publicação do protocolo da revisão sistemática em uma revista científica.

9.1.3 Critérios de elegibilidade

Os critérios de inclusão e exclusão estão descritos na tabela 2 seguir.

Tabela 1 - Demonstração dos critérios de inclusão e exclusão adotados para a Revisão Sistemática.

	Critérios de inclusão	Critérios de exclusão
Animais	Modelos animais de inflamação com rato (*Rattus novergicus*) ou camundongos (*Mus musculus*).	Estudos *in silico* ou *ex vivo*. Estudos com seres humanos, modelos de câncer, avaliação genética.
Intervenção	Testes anti-inflamatórios *in vivo* e *in vitro*. Tratamento com a *Libidibia ferrea* (*Caesalpinia ferrea*) com inclusão da dose do extrato, via de administração, esquema terapêutico, tipo do extrato, fração ou polissacarídeo.	Qualquer parte da planta que não seja a *Libidibia ferrea* (*Caesalpinia ferrea*). Estudos fitoquímicos, estudos de morfologia e anatomia, análise citogenética, estudo etnobotânico.
Comparador	Veículo (Salina ou PBS) ou droga padrão.	Sem grupo controle; Animais com doença sistemática prévia, condição autoimune, gravidez, ou outra condição inflamatória, como diabetes e obesidade.
Desenho do estudo	Estudos com grupo controle separados com média, desvio padrão em tabelas, gráficos e textos. Extração dos dados apenas referentes aos efeitos anti-inflamatória foram extraídos.	Estudos sem separação do grupo controle ou dados não mencionados.
Desfecho	Parâmetros de mensuração: volume da pata, número de células, citocinas, MPO e MDA, exsudato.	Dados histológicos, toxicidade, desfechos de viabilidade celular.

Legenda: Tampão fosfato-salina (PBS); Mieloperoxidase (MPO) e Malonaldeído (MDA)
Fonte: Própria autoria (2020).

Outros critérios de exclusão adotados foram: os estudos serem Revisões Sistemáticas, Revisões de Literatura, ou estudos que não atendiam aos padrões do comitê de ética de pesquisas com experimentação animal.

9.1.4 Estratégia de busca e busca nos bancos de dados

A busca foi realizada nas bases de dados de acordo com o tema estudado, abrangendo não só as bases de dados padrões, mas também a literatura cinzenta (ex. Google acadêmico e ProQuest) bem como uma busca manual, através da análise dos artigos que constavam na listagem dos artigos incluídos a fim de garantir a abrangência necessária para este tipo de estudo (PEREIRA; GALVÃO, 2014).

Em acordo com a pergunta de pesquisa utilizou-se os seguintes descritores e palavras-chave: “caesalpinia ferrea”, caesalpinia, “libidibia ferrea”, libidibia, jucá, pau ferro sendo combinadas entre si com o operador booleano OR, e estes foram associadas aos descritores “*anti-inflammatory activity*”, “*anti-inflammatory action*”,

“*anti-inflammatory effect*”, combinadas entre elas com o OR. Para que houvesse associação entre os termos referentes à planta e anti-inflamatório utilizou-se o operador booleano AND.

Realizou-se um teste piloto da estratégia de busca para sua viabilização conforme orientação do Centre for Reviews and Dissemination (2009) e Galvão; Pereira (2014) de elaboração da estratégia. Junto com esta busca houve a procura dos termos descritores no MeSH *terms* do PubMed (https://www.ncbi.nlm.nih.gov/mesh/) identificando apenas um descritor: “caesalpinia”, para o nome da planta (Tabela 2).

Tabela 2 – Estratégia de busca realizada em fevereiro/2020 no PubMed MeSH *terms*.

Estratégia de busca no PUBMED	MeSH *terms*
("Libidibia ferrea"[All Fields] OR Libidibia[All Fields] OR "Caesalpinia ferrea"[All Fields] OR ("caesalpinia"[MeSH Terms] OR "caesalpinia"[All Fields]) OR juca[All Fields] OR "pau-ferro"[All Fields]) AND ("anti-inflammatory activity"[All Fields] OR "anti-inflammatory activities"[All Fields] OR "anti-inflammatory property"[All Fields] OR "anti-inflammatory properties"[All Fields] OR "anti-inflammatory effect"[All Fields] OR "anti-inflammatory effects"[All Fields] OR "anti-inflammatory action"[All Fields] OR "anti-inflammatory actions"[All Fields])	- "caesalpinia"

Uma primeira busca (sem restrição de período e idioma) foi realizada em fevereiro de 2020, e uma atualização desta busca, realizada em março de 2021 (Apêndice 2).

A seguir, apresenta-se a estratégia de busca adaptada para cada base de dados:

I - Bases Principais

PubMed: ("libidibia ferrea" OR libidibia OR "caesalpinia ferrea" OR caesalpinia OR juca OR "pau-ferro") AND ("anti-inflammatory activity" OR "anti-inflammatory activities" OR "anti-inflammatory property" OR "anti-inflammatory properties" OR "anti-inflammatory effect" OR "anti-inflammatory effects" OR "anti-inflammatory action" OR "anti-inflammatory actions")

Science Direct: ("libidibia ferrea" OR libidibia OR "caesalpinia ferrea" OR caesalpinia OR juca OR "pau-ferro") AND ("anti-inflammatory activity" OR "anti-inflammatory property" OR "anti-inflammatory effect" OR "anti-inflammatory action")

Scopus: ("libidibia ferrea" OR libidibia OR "caesalpinia ferrea" OR caesalpinia OR juca OR "pau-ferro") AND ("anti-inflammatory activity" OR "anti-inflammatory property" OR "anti-inflammatory effect" OR "anti-inflammatory action")

LILACs:

1ª pesquisa: ("libidibia ferrea" OR libidibia OR "caesalpinia ferrea" OR caesalpinia OR juca OR "pau-ferro") AND ("anti-inflammatory activity" OR "atividade anti-inflamatória" OR "actividad antiinflamatoria")

2ª pesquisa: ("libidibia ferrea" OR libidibia OR "caesalpinia ferrea" OR caesalpinia OR juca OR "pau-ferro") AND ("anti-inflammatory property" OR "propriedade anti-inflamatória" OR "propriedad antiinflamatoria")

3ª pesquisa: ("libidibia ferrea" OR libidibia OR "caesalpinia ferrea" OR caesalpinia OR juca OR "pau-ferro") AND ("anti-inflammatory effect" OR "efeito anti-inflamatório" OR "efecto antiinflamatorio")

4ª. Pesquisa: ("libidibia ferrea" OR libidibia OR "caesalpinia ferrea" OR caesalpinia OR juca OR "pau-ferro") AND ("anti-inflammatory action" OR "ação anti-inflamatória" OR "acción antiinflamatoria")

Web of Science: ("libidibia ferrea" OR libidibia OR "caesalpinia ferrea" OR caesalpinia OR juca OR "pau-ferro") AND ("anti-inflammatory activity" OR "anti-inflammatory activities" OR "anti-inflammatory property" OR "anti-inflammatory properties" OR "anti-inflammatory effect" OR "anti-inflammatory effects" OR "anti-inflammatory action" OR "anti-inflammatory actions")

II. Literatura cinzenta

Google acadêmico: Para esta base foram selecionados os estudos até a página 11, na qual foram desmarcadas as opões de “incluir patentes” e “incluir citações”, sendo considerados, portanto, 110 estudos.

A estratégia de busca utilizada foi: ("libidibia ferrea" OR "caesalpinia ferrea" OR juca OR "pau-ferro") AND ("anti-inflammatory activity" OR "anti-inflammatory property" OR "anti-inflammatory effect" OR "anti-inflammatory action")

ProQuest: (libidibia NEAR/2 ferrea OR caesalpinia NEAR/2 ferrea OR juca OR pau-ferro) AND (anti-inflammatory NEAR/2 activity OR anti-inflammatory NEAR/2 property OR anti-inflammatory NEAR/2 effect OR anti-inflammatory NEAR/2 action)

Uma segunda busca (atualização) foi realizada em março de 2021. Apenas para a base de dados da Science Direct, foi necessário um ajuste da estratégia de busca devido ao espaço de inclusão da estratégia não comportar a pesquisa anterior. Assim, foi a realizada a busca com as seguintes palavras: "libidibia ferrea" OR "caesalpinia ferrea") AND ("antiinflammatory activity" OR "anti-inflammatory property" OR "anti-inflammatory effect" OR "antiinflammatory action").

A pesquisa de forma manual nas listagens de referências dos artigos incluídos na Revisão Sistemática também foi realizada.

Após esta etapa de busca, seguiu-se para o passo de seleção dos estudos encontrados.

9.1.5 Seleção dos estudos

A seleção dos estudos foi realizada em acordo com os critérios de inclusão e exclusão pré-estabelecidos no protocolo. Mas, primeiramente, as duplicadas foram removidas dos estudos selecionados. Em seguida, na fase I, através da leitura de títulos e resumos, a seleção dos estudos foi feita e em casos de discrepância, um terceiro autor colaborou para que houvesse um consenso. Após, na fase II, realizou-se a leitura completa dos artigos incluídos (CENTRE FOR REVIEWS AND DISSEMINATION, 2009).

Nesta RS, os estudos encontrados foram organizados em uma planilha do Excel™ do *software* Microsoft Office 365 organizados por bancos de dados. Em paralelo, as informações de títulos e resumos foram adicionadas no Word™ para posterior leitura destas (Fase I), após a remoção das duplicatas. Em seguida, uma nova planilha foi criada adicionando todos os estudos, organizando-os em ordem alfabética utilizando a ferramenta de classificar e foram identificados os estudos com nomes iguais (duplicatas). Seguiu-se, então, para a remoção das duplicatas.

Em uma nova planilha foi adicionada os estudos (sem as duplicatas) para a identificação de quais estudos seriam excluídos após a leitura dos títulos e resumos por dois autores cegos (NA e SF) classificando-os como “SIM”, “NÃO” e “TALVEZ”.

Em seguida, a leitura dos artigos completos foi realizada e, novamente, seguindo os critérios de inclusão e exclusão, apenas os que atendiam aos critérios de inclusão permaneceram. As discrepâncias foram resolvidas por dois autores (AC, EL). A fase seguinte foi a realização da extração dos dados.

9.1.6 Extração de dados

Os dados extraídos foram os que tentaram responder à pergunta de pesquisa. A tabela de coleta dos dados foi elaborada em uma planilha do Excel™ e a ordem dos estudos foi o ano de publicação. Os autores dos artigos incluídos foram contactados via e-mail para sanar informações faltantes ou não claras. Aguardou-se o retorno por 30 dias.

Esta etapa foi realizada por dois autores de forma independente. Os dados extraídos para os estudos foram: parte da planta utilizada; tipo de extrato, fração e polissacarídeo; modelos *in vivo* e *in vitro* de inflamação induzido por qualquer agente; número de animais por grupo e/ou células; esquema terapêutico; controle utilizado; avaliação dos parâmetros inflamatórios; espécie, idade, peso dos animais; métodos de indução da inflamação nos animais; dose, frequência, rota e tempo de administração; tipo de veículo; os dados dos desfechos referentes ao edema de pata (mL, μL), contagem do número de células (x10/mL), índices de citocinas (pg/mL), MPO e MDA (U/μL, nmol/μL), a permeabilidade vascular; Outros dados extraídos foram: autoria, ano de publicação, jornal, país de origem.

Esta etapa de extração dos dados foi seguida pela etapa da análise da qualidade dos estudos incluídos.

9.1.7 Análise dos riscos de viés e da qualidade metodológica dos estudos dos estudos *in vivo*

A identificação do risco de viés dos estudos incluídos na revisão sistemática foi feita através de instrumentos de avaliação de acordo com o tipo de delineamento (CENTRE FOR REVIEWS AND DISSEMINATION, 2009). No caso dos estudos com animais uma das ferramentas utilizadas foi o “SYstematic Review Center for Laboratory Animal Experimentation” (SYRCLE RoB *Tool*).

Esta ferramenta compreende a avaliação de seis riscos de vieses totalizando 10 entradas. Os riscos de vieses são apresentados abaixo bem como um exemplo de análise para cada tipo (HOOIJMANS et al., 2014):

- Viés de seleção: Está relacionado às entradas referentes à:

(1) geração da sequência de alocação dos animais, onde, por exemplo, utilizou-se uma sequência numérica gerada por computador;

(2) às características basais dos animais dos grupos experimentais e controle. Nesta, a pergunta de pesquisa definiu os tipos e o número de características basais;

(3) ocultação do processo de alocação dos animais, como por exemplo, no uso de envelopes lacrados;

- Viés de performance: consiste nas entradas relacionados à

(4) habitação aleatória em como os animais foram alojados,

(5) ao cegamento, onde o pesquisador que manipulou os animais não sabia qual era o grupo da intervenção, como foi realizado este cegamento em algum momento;

- Viés de detecção: refere-se às entradas

(6) se os resultados da pesquisa com os animais foram avaliados de forma aleatória;

(7) se houve cegamento no momento da análise dos resultados;

- Viés de atrito: entrada

(8) onde analisou-se se houve resultados incompletos, como por exemplo se algum animal foi perdido ao longo do experimento e/ou substituído;

- Viés de relato: corresponde à entrada

(9) sobre o relato seletivo dos resultados, onde, por exemplo, todos os métodos descritos no estudo foram relados em seus respectivos resultados;

- Risco de viés relacionado a outras fontes de viés: este tipo de viés depende da pergunta da pesquisa de acordo com o experimento, julgando assim, quais os itens que gerariam viés naquele tipo de estudo. Por exemplo, se houve ausência da influência de financiadores.

Para cada viés, perguntas são realizadas tendo como resposta as seguintes opções: SIM, NÃO e INCERTO (*unclear)*, que correspondem respectivamente a baixo, alto e incerto risco de viés.

Para a análise da qualidade dos estudos incluídos, as ferramentas utilizadas foram a *Grading of Recommendations, Assessment, Development, and Evaluation* (GRADE) para estudos pré-clínicos no contexto das intervenções terapêuticas focando na evidência indireta como um preditivo da translação dos estudos para humanos (HOOIJMANS et al., 2018) e o GRADEpro *Guideline Development Tool* (GRADEpro GDT) (GRADEpro GDT, 2020).

Elaborou-se uma tabela com planilhas referente ao viés de publicação, imprecisão, inconsistência e evidência indireta para a análise da evidência dos estudos pelo GRADE para estudos pré-clínicos (HOOIJMANS et al., 2018), haja vista o risco e viés ter sido realizado utilizando a ferramenta SYRCLE.

Para o viés de publicação observou se havia os seguintes dados nos estudos incluídos: financiamento (*funding*), agradecimentos, conflito de interesse e se foi publicado em uma revista científica;

Para o domínio de imprecisão observou-se quais foram os desfechos, tamanho da amostra (cálculo), se o método estatístico foi apropriado, a mensuração da variação e o poder do teste;

Para o domínio de inconsistência verificou-se o desfecho, as condições de teste (que poderiam influenciar o resultado): como habitação e criação, regime de alimentação e de água, e explicação do modelo estatístico;

Para o domínio evidência indireta observou-se em relação às características dos animais (idade, peso, espécie, cepa), animais com comorbidade, modelo apropriado, co-intervenção ou cotratamento/contaminação, se os critérios de inclusão e exclusão foram declarados, características da intervenção (duração, tempo, dose), janela de tempo, avaliação dos resultados, monitoramento das variáveis fisiológicas/normal, testado em múltiplas espécies, eficácia do tratamento testada em 2 ou mais espécies, intervenção terapêutica/tratamento, frequência de

tempo, rota de administração, homogeneidade entre as espécies, característica dos modelos de inflamação, métodos de diagnóstico.

Para a análise da certeza nas estimativas de efeito as certezas foram classificadas em alta, moderada e baixa aplicando-se o GRADEpro GDT (GRADEpro GDT, 2020). Para cada desfecho analisou-se o risco de viés, inconsistência, imprecisão. Para a sumarização dos resultados, fez-se o acesso no sítio da GRADEpro GDT (https://gradepro.org/). Após o login, na página inicial, marcou-se a opção de *novo projeto* adicionou-se o nome e em seguida selecionou-se a opção *perfil de evidências*.

A pergunta de pesquisa: **Qual parte da planta de *L. ferrea* e que tipo de extrato tem os efeitos anti-inflamatórios mais evidentes nos modelos experimentais de inflamação aguda *in vivo* e *in vitro*?**, foi adicionada. Os desfechos foram adicionados e analisados em cada domínio sendo cada um "julgado" como grave, não grave e muito grave. Ao final, automaticamente, a certeza da evidência foi classificada como muito baixa (⊕OOO), baixa (⊕⊕OO), moderada (⊕⊕⊕O) ou alta (⊕⊕⊕⊕). Nota-se que os desfechos são o alvo da avaliação (MARTINS; MELO; PORFÍRIO, 2020).

9.1.8 Redação da Revisão Sistemática

Para a redação da RS atualizou-se o PRISMA *check list* 2009 para o novo PRISMA *check list* 2021 (PAGE et al., 2021) que apresenta, além do *check list* anterior, um *check list* para o resumo (*abstract*) e um novo formato do fluxograma para expressar os dados dos estudos achados e incluídos na Revisão Sistemática.

9.1.9 Análise da qualidade metodológica dos estudos *in vitro*

Para a análise da qualidade dos estudos *in vitro* foi utilizada *a* ferramenta do *Science in Risk Assessment and Policy* (SciRAP) (SCIRAP.ORG, 2018). É um guia de instruções para avaliar a relevância de estudos de toxicidade *in vivo* e *in vitro*. A parte para reportar a qualidade metodológica foi adaptada para atender à Revisão Sistemática com os estudos *in vitro* incluídos.

No sítio (www.scirap.org) a opção de análise de toxicidade de estudos *in vitro toxicity studies* foi marcada e o relato da qualidade dos estudos foi realizado.

Quando necessário, uma adaptação nos critérios para melhor atender ao foco e desfechos dos estudos incluídos foi realizada.

A análise foi realizada classificando os desfechos em *fulfilled* (verde)*, partially fulfilled* (amarelo)*, not fulfilled* (vermelho) e não determinado (cinza). O resultado é exportado e, então, expresso na forma de uma planilha no Excel™ e um gráfico de barras com cores supra.

10 RESULTADOS

10.1 PROTOCOLO DA REVISÃO SISTEMÁTICA

O protocolo, em sua totalidade, pode ser acessado através do link: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020159934 e no apêndice 2.

Somado à submissão e aprovação do protocolo na plataforma da PROSPERO, este foi publicado na revista eletrônica **Scientia Amazonia** sendo intitulado: **An evidence-based review of *Libidibia ferrea* (jucá) anti-inflammatory action on *in vivo* and *in vitro* studies: Protocol** do qual pode ser acessado em: https://scientia-amazonia.org/wp-content/uploads/2021/12/v10-n3-CS57-CS63-2021.pdf.

Por motivo de direitos autorais, o artigo não se encontra disponível no apêndice.

10.2 REVISÃO SISTEMÁTICA

A Revisão Sistemática foi apresentada na forma de artigo científico publicado na revista **Plos One** intitulado ***Libidibia ferrea* (jucá) anti-inflammatory action: A systematic review of *in vivo* and *in vitro* studies** (Apêndice 4) e pode ser acessado através do endereço: https://doi.org/10.1371/journal.pone.0259545.

11 CONCLUSÕES

- O jucá apresenta efeito anti-inflamatório de acordo com os dados da literatura e esta ação foi observada em todas as partes da planta bem como em todos os tipos de extratos testados, porém mais estudos são necessários para melhor averiguar o efeito destes resultados e a sua possível translação para à clínica.
- Apesar de não ter sido realizada uma metanálise devido à heterogeneidade entre os desenhos e modelos experimentais e à baixa/média qualidade dos estudos incluídos, uma análise qualitativa foi realizada.
- Na maioria dos modelos foi utilizado o tratamento com o extrato via oral e foram aplicados antes da indução da inflamação. Situação em que nos humanos, a terapia normalmente é realizada após o processo inflamatório desenvolvido.
- Recomendam-se mais estudos utilizando o *Animal Research: Reporting of In Vivo Experiments* (ARRIVE) a fim de garantir maior qualidade nos relatos dos estudos primários para possibilitar melhor nível de evidência.

REFERÊNCIAS

ABBAS, A. K.; LICHTMAN, A. H.; PILLAI, S. **Imunologia Celular e Molecular.** Tradução da 9. edição. Editora GEN Guanabara Koogan, 576 páginas, 2019. ISBN 978-8535290745.

ABD-ALLAH, A. A. M.; EL-DEEN, N. A. M. N.; MOHAMED, W. .A.M.; NAGUIB, F. M. Mast cells and pro-inflammatory cytokines roles in assessment of grape seeds extract anti-inflammatory activity in rat model of carrageenan-induced paw edema. **Iranian Journal of Basic Medical Sciences**, v. 21, n. 1, p. 97–107, 2018. DOI: 10.22038/IJBMS.2017.25067.6219.

ABDULKHALEQ, L. A.; ASSI, M. A.; ABDULLAH, R.; ZAMRI-SAAD, M.; TAUFIQ-YAP, Y. H.; HEZMEE, M. N. M. The crucial roles of inflammatory mediators in inflammation: A review. **Veterinary World**, v. 11, n. 5, p. 627–635, 2018. DOI: 10.14202/vetworld.2018.627-635.

ALQASOUMI, S. I.; SOLIMA, G. A. E. H.; AWAADA, A. S.; DONIA, A. E. R. M. Anti-inflammatory activity, safety and protective effects of *Leptadenia pyrotechnica*, *Haloxylon salicornicum* and *Ochradenus baccatus* in ulcerative colitis. **Phytopharmacology**, v. 2, n. 1, p. 58–71, 2012. DOI: 10.3389/fvets.2020.00244.

ANVISA. **Formulário de Fitoterápicos, Farmacopeia Brasileira - Primeiro Suplemento**. Agência Nacional de Vigilância Sanitária, 1. ed., p. 160, 2018.

ATCC (American Type Culture Collection). Disponível em: https://www.atcc.org/products/tib-71. Acesso em: 20 out. 2021.

BAIZABAL-AGUIRRE, V.M.; ROSALES, C.; LÓPEZ-MACÍAS, C.; GÓMEZ, M.I. Control and Resolution Mechanisms of the Inflammatory Response. **Mediators of inflammation**, v. 2016, 2016:3591797, 2016. doi:10.1155/2016/3591797.

BAO, Y. et al. Therapeutic effects of *Smilax glabra* and *Bolbostemma paniculatum* on rheumatoid arthritis using a rat paw edema model. **Biomedicine & Pharmacotherapy**. v. 108, n. 2018, p. 309-315. https://doi.org/10.1016/j.biopha.2018.09.004.

BARRÉ-SINOUSSI, F.; MONTAGUTELLI, X. Animal models are essential to biological research: Issues and perspectives. **Future Science OA**, v. 1, n. 4, 2015. DOI: 10.4155/FSO.15.63.

BAUMANS, V. **The Welfare of Laboratory Rats: In: The Welfare of Laboratory Animals**. Filand: Springer, 2007. v. 2.

BASBAUM, A.I.; BAUTISTA, D. M.; CHERRER,G.; JULIUS, D. Cellular and Molecular Mechanisms of Pain. **Cell**, 139, October, 2009. DOI 10.1016/j.cell.2009.09.028.

BERTHI, W.; GONZÁLEZ, A.; RIOS, A.; BLAIR, S.; COGOLLO, A.; PABÓN, A. Anti-plasmodial effect of plant extracts from *Picrolemma huberi* and *Picramnia latifolia* . **Malaria Journal**, v. 17, p.1–12. https://doi.org/10.1186/s12936-018-2301-x.

BHAT, S. S.; HEGDE, K.; CHANDRASHEKHAR, S.; RAO, S. N.; MANIKKOTH, S. Preclinical screening of *Phyllanthus amarus* ethanolic extract for its analgesic and antimicrobial activity. **Pharmacognosy Research**, v. 7, n. 4, p. 378–384, 2015. DOI: 10.4103/0974-8490.159577.

BHATTACHARYA, S. Medicinal plants and natural products in amelioration of arsenic toxicity: A short review. **Pharmaceutical Biology**, v. 55, n. 1, p. 349–354, 2017. http://dx.doi.org/10.1080/13880209.2016.1235207.

BORASCHI, D.; ITALIANI, P. Innate Immune Memory: Time for adopting a correct terminology. **Frontiers in Immunology**. v.9, n. 799, 2018. DOI: 10.3389/fimmu.2018.00799.

BRASIL, **A fitoterapia no SUS e o Programa de Pesquisa de Plantas Medicinais da Central de Medicamentos**. Série B. Textos básicos de saúde, 1. ed. Brasília: Ministério da Saúde, Secretaria de Ciência, Tecnologia e Insumos Estratégicos, Departamento de Assistência Farmacêutica, 2006. ISBN 85-334-1187-1.

BRASIL, M. DA S. **Diretrizes metodológicas: elaboração de revisão sistemática e meta-análise de ensaios clínicos randomizados** [recurso eletrônico], Brasília: Secretaria de Ciência, Tecnologia, Inovação e Insumos Estratégicos em Saúde. Departamento de Gestão e Incorporação de Tecnologias em Saúde, 2021. ISBN 978-65-5993-011-1.

BRASIL, M. DA S. **Política e Programa Nacional de Plantas Medicinais e Fitoterápicos**. Brasília: Ministério da Saúde, Secretaria de Ciência, Tecnologia e Insumos Estratégicos, Departamento de Assistência Farmacêutica, 2016. ISBN 978-85-334-2399-2.

BRASIL, Ministério da Saúde. **Práticas integrativas e complementares: plantas medicinais e fitoterapia na Atenção Básica.** Ministério da Saúde. Série A. Normas e manuais técnicos. Caderno de Atenção B ásica; n. 31. Brasília-DF: Ministério da Saúde, 2012. ISBN 978-85-334-1912-4.

CALIXTO, J.B. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). **Brazilian Journal of Medical and Biological Research**, v. 33, pág. 179-189, 2000.

CAMARADES - PRECLINICAL SYSTEMATIC REVIEWS & META-ANALYSIS WIKI, (October, 2021), **CAMARADES** Berlin, QUEST-BIH Charité. Disponível em: https://www.CAMARADES.de. Acessado em: 29 out. 2021.

CARDENAS, J.D.R. **Plantas medicinais**, In.: GEEA: Grupo de Estudos Estratégicos Amazônicos/Organizadores: Geraldo Mendes dos Santos, Luiz Renato de França. Manaus; Editora INPA, 2017.v. 10. (Caderno de Debates), 116 p.: il. color.

CARVALHO, J. C. T. et al. Preliminary studies of analgesic and anti-inflammatory properties of *Caesalpinia ferrea* crude extract. **Journal of Ethnopharmacology**, v. 53, p. 175–178, 1996.

CENTRE FOR REVIEWS AND DISSEMINATION. **Systematic Reviews - CRD's guidance for undertaking reviews in health care**. University of York, Published by CRD. York Publishing Services Ltd, 2009. ISBN 978-1-900640-47-3.

CHEN, L. et al. Inflammatory responses and inflammation-associated diseases in organs. Review. **Oncotarget**, v. 9, n. 6, p. 7204-7218, 2018.

CHOVATIYA, R.; MEDZHITOV, R. Stress, inflammation, and Defense of Homeostasis. **Molecular Cell**, v. 54, 2014. http://dx.doi.org/10.1016/j.molcel.2014.03.030

COMANDOLLI-WYREPKOWSKII, C. D. et al. Antileishmanial activity of extracts from *Libidibia ferrea*: development of *in vitro* and *in vivo* tests. **Acta Amazonica**, v. 47, n. 4, p. 331–340, 21, 2017. http://dx.doi.org/10.1590/1809-4392201700871.

CORDEIRO, A.M.; OLIVEIRA, G.M. de; RENTERÍA, J.M.; GUIMARÃES, C.A.; GERS-Rio. Revisão Sistemática: Uma revisão narrativa. Comunicação científica. **Revista do Colégio Brasileiro de Cirurgiões**, vol. 34, n. 6, nov/dez. 2007.

CRUVINEL, W. DE M. et al. Sistema Imunitário – Parte I, Fundamentos da imunidade inata com ênfase nos mecanismos moleculares e celulares da resposta inflamatória. **Rev Bras Reumatol**, v. 50, n. 4, p. 434–61, 2010.

CUZZOCREA, S. et al. Antiinflammatory effects of mercaptoethylguanidine, a combined inhibitor of nitric oxide synthase and peroxynitrite scavenger, in carrageenan-induced models of inflammation. **Free Radical Biology & Medicine**, v. 24, n. 3, p. 450–459, 1998.

DAR, R. A. et al. General overview of medicinal plants: A review. **The Journal of Phytopharmacology**, v. 6, n. 6, p. 349–351, 2017.

DE LUCA CANTO, G.; RÉUS, J.C. **Mãos à obra¸** Capítulo 2. In.: DE LUCA CANTO. Revisões sistemáticas da literatura: guia prático. 1.ed., Curitiba: Brazil Publishing, 2020. ISBN 978-65-5016-352-5.

DI STASI, L. C.; HIMURA-LIMA, C. A. **Plantas medicinais na Amazônia e na Mata Atlântica**. 2. ed. rev. e ampl. São Paulo, editora UNESP, 2002. ISBN 85-7139-411-3.

EDDOUKS, M.; CHATTOPADHYAY, D.; ZEGGWAGH, N. A. Animal models as tools to investigate antidiabetic and anti-inflammatory plants. **Evidence-based Complementary and Alternative Medicine**, v. 2012, ID 142087, 14 pages, 2012. doi:10.1155/2012/142087.

EZE, F. I. et al. *In vitro* and *in vivo* models for anti-inflammation: An evaluative review. **INNOSC Theranostics and Pharmacological Sciences**, v. 2, n. 2, p. 3–15, 2019. DOI: 10.36922/itps.v2i2.775.

FACULDADE DE CIÊNCIAS AGRONÔMICAS, UNESP. Campus de Botucatu. **Tipos de revisão de litetaratura**. Biblioteca Prof. Paulo de Caralho Mattos, Botucatu, 2015. Disponíevl em: https://www.fca.unesp.br/Home/Biblioteca/tipos-de-evisao-de-literatura.pdf. Acessado em: 8 nov. 2021.

FALCÃO, T. R. et al. Crude extract from *Libidibia ferrea* (Mart. ex. Tul.) L.P. Queiroz leaves decreased intra articular inflammation induced by zymosan in rats. **BMC Complementary and Alternative Medicine**, v. 19, n. 1, p. 1–10, 2019a. https://doi.org/10.1186/s12906-019-2454-3.

FALCÃO, T. R. et al. *Libidibia ferrea* fruit crude extract and fractions show anti-inflammatory, antioxidant, and antinociceptive effect *in vivo* and increase cell viability *in vitro*. **Evidence-based Complementary and Alternative Medicine**, v. 2019, ID 6064805, 2019b. https://doi.org/10.1155/2019/6064805.

FEDELE, M.; GUALILLO, O.; VECHIONE, A. Animal models of human pathology. Editoral. **Journal of Biomedicine and Biotechnology**, v. 2011, ID 764618, 1 page. doi:10.1155/2011/764618.

FEREIDONI, M.; AHMADIANI, A.; SEMNANIAN, S.; JAVAN, M. An accurate and simple method for measurement of paw edema. **Journal of Pharmacological and Toxicological Methods**, v. 43, p. 11–14, 2000.

FERRERO-MILIANI, L.; NIELSEN, O. H.; ANDERSEN, P. S.; GIRARDIN, E.. Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1β generation. **Clinical and Experimental Immunology**, v. 147, p. 227–235, 2007. doi:10.1111/j.1365-2249.2006.03261.x.

FRANCO, N. H. Animal experiments in biomedical research: A historical perspective. **Animals**, v. 3, p. 238–273, 2013. DOI:10.3390/ani3010238.

GALVÃO, T. F.; PEREIRA, M. G. Revisões sistemáticas da literatura: passos para sua elaboração. **Epidemiologia e Serviços de Saúde**, v. 23, n. 1, p. 183–184, jan-mar 2014. DOI: 10.5123/S1679-49742014000100018.

GAMEDE, M.; MABUZA, L.; NGUBANE, P.; KHATHI, A. The effects of plant-derived oleanolic acid on selected parameters of glucose homeostasis in a diet-induced pre-diabetic rat model. **Molecules**, v. 23, n. 794, 2018. DOI:10.3390/molecules23040794.

GHASEMIAN, M.; OWLIA, S.; OWLIA, M. B. Review of Anti-Inflammatory Herbal Medicines. **Advances in Pharmacological Sciences**, v. 2016, ID 9130979, 11 pages, 2016. http://dx.doi.org/10.1155/2016/9130979.

GOLAN, D.E; TASHJIAN Jr., A.H.; ARMSTRONG, E. J.; ARMSTRONG, A.W.

Princípios de farmacologia: a base fisiopatológica da farmacoterapia. 2. ed. Rio de Janeiro, Guanabara Koogan, 2012.

GÓMEZ-BETANCUR, I.; BENJUMEA, D.; GÓMEZ, J. E.; MEJÍA, N.; LEÓN, J. F. Antinociceptive activity of essential oils from wild growing and micropropagated plants of *Renealmia alpinia* (Rottb.) *maas*. **Records of Natural Products**, v. 20XX,X:X, XX-XX, 2019. DOI: http://doi.org/10.25135/rnp.70.18.01.084.

GONÇALVES, D. O. et al. *In vivo* and *in vitro* anti-inflammatory and anti-nociceptive activities of lovastatin in rodents. **Brazilian Journal of Medical and Biological Research**, v. 44, n. 2, p. 173–181, 2011. DOI:10.1590/S0100-879X2011007500001.

GRADEpro Guideline Development Tool [Software]. McMaster University, 2020 (developed by Evidence Prime, Inc.). Available from gradepro.org.

HENRIQUES, M.G.M.O.; SILVA, P.M.R.; MARTINS, M.A.; FLORES, C.A.; CUNHA, F.Q.; ASSEUY-FILHO, J.; CORDEIRO, R.S.B. Mouse paw edema. A new model for inflammation?. **Brasilian J Med Biol Res**. 20: 243-249, 1987.

HICKMAN, D; JOHNSON, J.; VEMULAPALLI, T.H.; CRISLER, J. R.; SHEPHERD, R. Commonly used animals models, Chapter 7. In: **Principles of animal research for graduate and undergraduate students**. [s.l.], Elsevier Inc., 2017. v. 2507p. 117–175.

HOOIJMANS, C. R. et al. Facilitating healthcare decisions by assessing the certainty in the evidence from preclinical animal studies. **PLoS ONE**, v. 13, n. 1, p. 1–18, 2018. https://doi.org/10.1371/journal.pone.0187271.

HOOIJMANS, C. R. et al. SYRCLE's risk of bias tool for animal studies. **BMC Medical Research Methodology**, v. 14, n. 1, p. 1–9, 2014. http://www.biomedcentral.com/1471-2288/14/43.

HOOIJMANS, C. R.; LEENAARS, M.; RITSKES-HOITINGA, M. A gold standard publication checklist to improve the quality of animal studies, to fully integrate the three Rs, and to make systematic reviews more feasible. **ATLA - Alternatives to Laboratory Animals**, v. 38, p. 167–182, 2010.

HOOIJMANS, C. R.; RITSKES-HOITINGA, M. Progress in using systematic reviews of animal studies to improve translational research. **PLoS Medicine**, v. 10, n. 7, p. 1–4, 2013.

JUNQUEIRA, L.U.; CARNEIRO, J. **Histologia Básica**. Rio de Janeiro: Guanabara Koogan, 2011.

KATZUNG, B. G.; MASTERS, S.B.; TREVOR, A. J. **Farmacologia: Básica e Aplicada**. 12. ed., Porto Alegre: AMGH, seção VI. cap. 36, 2014.

KIERSZENBAUM, A.L. **Histologia e Biologia Celular: Uma introdução à Patologia**. Tradução da 2. ed., Rio de Janeiro: Elsevier, 2008.

KILKENNY, C.; BROWNE, W. J.; CUTHILL, I. C.; EMERSON, M.; ALTMAN, D. G. The ARRIVE guidelines: Animal Research: Reporting of *in vivo* experiments. Tradução Português (Brasil). Originally published in **PLoS Biology**, p. 1–2, June 2010.

KOREVAAR, D. A.; HOOFT, L.; TER RIET, G. Systematic reviews and meta-analyses of preclinical studies: Publication bias in laboratory animal experiments. **Laboratory Animals**, v. 45, n. 4, p. 225–230, 2011. DOI: 10.1258/la.2011.010121.

KUMAR, S.; GUPTA, E.; KAUSHIK, S; JYOTI, A. Neutrophil Extracelular Traps: Formation and Involvement in Disease Progression. **Iran Journal of Allergy Asthma and Immunology**, v. 17, n. 3, p. 208-220.

LAVETI, D. et al. Anti-inflammatory treatments for chronic diseases: A review. **Inflammation and Allergy - Drug Targets**, v. 12, n. 5, p. 349–361, 2013.

LEENAARS, C. H. C. et al. Animal to human translation: A systematic scoping review of reported concordance rates. **Journal of Translational Medicine**, v. 17, 223, p. 1–22, 2019. https://doi.org/10.1186/s12967-019-1976-2.

LEVY, L. Carrageenan paw edema in the mouse. **Life Sciences**, v. 8, Part I, p. 601–606, 1969.

LIBBY, P. Inflammatory Mechanisms: The Molecular Basis of Inflammation and Disease. **Nutrition Reviews**, v. 65, n. 12, p. S140–S146, 2007. DOI: 10.1301/nr.2007.dec.S140–S146.

LOPES, K. et al. Chemical composition, toxicity, antinociceptive, and anti-inflammatory activity of dry aqueous extract of *Varronia multispicata* (Cham.) *borhidi* (Cordiaceae) leaves. **Frontiers in Pharmacology**, v. 10, n. November, p. 1–15, 2019.

LUIJK, J. van; BAKKER, B.; ROVERS, M. M.; RISTSKES-HOITINHGA, M.; de VRIES, R. B. M.; LEENAARS, M. Systematic reviews of animal studies; missing link in translational research?. **Plos One**, v. 9, n. 3, p. 1–5, 2014. doi:10.1371/journal.pone.0089981.

MARTINS, C.C.; MELO, G.; PORFÍRIO, G.J.M. **Método GRADE para análise da certeza da evidência**, Capítulo 9. In.: DE LUCA CANTO, G. Revisões sistemáticas da literatura: guia prático. 1.ed., Curitiba: Brazil Publishing, 2020. ISBN 978-65-5016-352-5.

MEDZHITOV, R. Inflammation 2010: New adventures of an old flame. **Cell**, v. 140, n. 6, p. 771–776, 2010. DOI 10.1016/j.cell.2010.03.006.

MEDZHITOV, R.; JANEWAY Jr., C. Innate Immune. **The New England Journal of Medicine**, v.343, n. 5, p. 338-344, 2000. DOI: 10.1056/NEJM200008033430506.

MEDZHITOV, R. Origin and physiological roles of inflammation. **Nature**, v. 454, n. 7203, p. 428–435, July 2008. DOI:10.1038/nature07201.

MEEK, I. L.; LAAR, M. A. F. J. VAN DE; VONKEMAN, H. E. Non-Steroidal Anti-Inflammatory Drugs: An overview of cardiovascular risks. **Pharmaceuticals**, v. 3, p. 2146–2162, July, 2010. DOI:10.3390/ph3072146.

MINISTÉRIO DA CIÊNCIA, TECNOLOGIA, INOVAÇÕES E COMUNICAÇÕES. **Normativas do CONCEA para produção, manutenção de animais em atividades de ensino ou pesquisa científica: lei, decreto, portarias, resoluções, normativas e orientações técnicas**. 3. ed., Brasília, 2016.

MUELLER, K. F. et al. Dissemination bias in systematic reviews of animal research: A systematic review. **PLoS ONE**, v. 9, n. 12, p. 1–15, 2014. DOI:10.1371/journal.pone.0116016.

NANTEL, F. et al. Distribution and regulation of cyclooxygenase-2 in carrageenan-induced inflammation. **British Journal of Pharmacology**, v. 128, p. 853–859, 1999.

NATHAN, C. Points of control in inflammation. **Nature**, v. 420, 2002. DOI: 10.1038/nature01320

NATIONAL CENTRE FOR THE REPLACEMENT, REFINEMENT & REDUCTION OF ANIMALS IN RESEARCH (NC3Rs). Disponível em: https://www.nc3rs.org.uk/the-3rs. NECAS, J.; BARTOSIKOVA, L. **Carrageenan: a review**. v. 2013, n. 4, p. 187–205, 2013. Acessado em: 8 nov. 2021.

OKIN, D.; MEDZHITOV, R. Evolution of Inflammatory Diseases. **Current Biology**, v. 22, n. 17,p. R733-R740, 2012. doi:10.1016/j.cub.2012.07.029.

PAGE, M.J.; McKENZIE, J.E.; BOSSUYT, P.M.; BOUTRON, I.; HOFFMANN, T.C.; MULROW, C.D. et al. The PRISMA 2020 statement: Na update guideline for reporting sytematic reviews. **Plos Medicine**. 18(3):e1003583. https://doi.org/10.1371/journal.pmed.1003583.

PATIL, K. R. et al. Animal models of inflammation for screening of anti-inflammatory drugs: Implications for the discovery and development of phytopharmaceuticals. **International Journal of Molecular Sciences**, v. 20, 2019. DOI:10.3390/ijms20184367.

PAHWA,R.; GOYAL, A.; BANSAL, P.; JIALAL, I. **Chronic Inflmmation**. [Updated 2021 Sep 28]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from:https://www.ncbi.nlm.nih.gov/books/NBK493173/.

PEREIRA, L. D. P.; SILVA, R. O. da; BRINGEL, P. H. de S. F.; SILVA, K. E. S. da; ASSREUY, A. M. S.; PEREIRA, M. G. Polysaccharide fractions of *Caesalpinia ferrea* pods: Potential anti-inflammatory usage. **Journal of Ethnopharmacology**, v. 139, p. 642–648, 2012. DOI:10.1016/j.jep.2011.12.012.

PEREIRA, M. G.; GALVÃO, T. F. Etapas de busca e seleção de artigos em revisões sistemáticas da literatura. **Epidemiologia e Serviços de Saúde**, v. 23, n. 2, p. 369–371, 2014. DOI: 10.5123/S1679-49742014000200019.

PERLMAN, R. L. Mouse models of human disease: An evolutionary perspective. **Evolution, Medicine, and Public Health**, p. eow014, p. 170–176, 2016. doi:10.1093/emph/eow014.

POSADAS, I. et al. Carrageenan-induced mouse paw oedema is biphasic, age-weight dependent and displays differential nitric oxide cyclooxygenase-2 expression. **British Journal of Pharmacology**, v. 142, n. 2, p. 331–338, 2004.

POUND, P.; RITSKES-HOITINGA, M. Can prospective systematic reviews of animal studies improve clinical translation?. **Journal of Translational Medicine**, v. 18, n. 15, 6 pages, 2020. https://doi.org/10.1186/s12967-019-02205-x.

PROSPERO, **International Prospective register of systematic reviews**. National Institute for Health Research. Disponível em: https://www.crd.york.ac.uk/prospero/. Acessado em: jan. 2020.

PUNCHARD, N. A.; WHELAN, C. J.; ADCOCK, I. Journal of Inflammation. **Journal of Inflammation**, v. 4, p. 1–4, 2004. DOI: 10.1186/1476-9255-1-1.

RIBEIRO, V. P. ARRUDA, C.; EL-SALAM, M. A.; BASTOS, J. K. Brazilian medicinal plants with corroborated anti-inflammatory activities: a review. **Pharmaceutical Biology**, v. 56, n. 1, p. 253–268, 2018. DOI: 10.1080/13880209.2018.1454480.

SCIRAP.ORG. Instructions for evaluating reliability and relevance of *in vivo* and *in vitro* toxicity studies using the SciRAP tool. p. 1–5, 2018.

SENA, E.S.; van der WORP, H.B.; BATH, P.M.; HOWLLS, D.W.; MACLOAD, M.R. Publications bias in reports of animal stroke studies leads to major overstatement of efficacy. **Plos Biology**, v. 8, n. 3, e1000344, 2010. DOI:10.1371/journal.pbio.1000344.

SENA, E. S.; CURRIE, G. L.; McCANN, S. K.; MACLEOD, M.; HOWELLS, D. W. Systematic reviews and meta-analysis of preclinical studies: why perform them and how to appraise them critically. **Journal of Cerebral Blood Flow & Metabolism**, v. 34, p. 737–742, 2014. DOI:10.1038/jcbfm.2014.28.

SENGAR, N. et al. Anti-inflammatory, analgesic and anti-pyretic activities of standardized root extract of *Jasminum sambac*. **Journal of Ethnopharmacology**, v. 160, p. 140–148, 2015. http://dx.doi.org/10.1016/j.jep.2014.11.039.

SERHAN, C. N.; SAVILL, J. Resolution of inflammation: The beginning programs the end. **Nature Immunology**, v. 6, n. 12, p. 1191–1197, 2005. DOI:10.1038/ni1276.

SOLIMAN, N.; RICE, A. S. C.; VOLLERT, J. A pratical guide to preclinical systematic

review and meta-analysis. **Pain Journal**, v. 161, n. 9, p. 1949–1954, 2020. http://dx.doi.org/10.1097/j.pain.0000000000001974.

SOUZA, B.D.M.; CASETT, E.; POLMANN, H.; PORPORATTI, A.; DE CANTO LUCA, G. **Registro do protocolo**, Capítulo 3. In.: DE LUCA CANTO, G. Revisões sistemáticas da literatura: guia prático. 1. ed., Curitiba: Brazil Publishing, 2020. ISBN 978-65-5016-352-5.

STEHMANN, J. R.; FARIA, F. S.; BRAGIONI, T. **50 árvores do Museu**. Belo Horizonte: Formato Editora, p. 90, 2019. ISBN 978-85-62164-13-2.

THERMOFISHER. Disponível em: https://www.thermofisher.com/br/en/home/life-science/cell-analysis/cell-analysis-learning-center/immunology-at-work/immunology-protocols/immune-cell-stimulation-lps.html. Acessado em: 29 out. 2021.

TOBACMAN, J. K. Review of harmful gastrointestinal effects of carrageenan in animal experiments. **Environmental Health Perspectives**, v. 109, n. 10, p. 983–994, 2001.

VANDAMME, T.F. Use of rodents as models of human diseases. **Journal Pharmacy and Bioallied Sciences**. Jan-Mar; v.6, n.1, p. 2–9, 2014. DOI: 10.4103/0975-7406.124301, Disponível em: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3895289/. Acessado em: 8 nov. 2021.

VARELA, M. L.; MOGILDEA, M.; MORENO, I.; LOPES, A. Acute Inflammation and Metabolism. **Inflammation**, v. 41, n. 4, p. 1115–1127, 2018. DOI: 10.1007/s10753-018-0739-1.

VESTERINEN, H. M. et al. Meta-analysis of data from animal studies: A practical guide. **Journal of Neuroscience Methods**, v. 221, p. 92–102, 2014. http://dx.doi.org/10.1016/j.jneumeth.2013.09.010.

VOGEL, H. **Drug Discovery and Evaluation**. 2. ed. [s.l.] Springer-Verlag Berlin Heidelberg, New York, 2002. ISBN 3-540-42396-6.

VRIES, R. B. M. de; WEVER, K. E.; AVEY, M. T.; STEPHENS, M. L.; SENA, E. S.; LENNARS, M. The Usefulness of systematic reviews of aAnimal experiments for the design of preclinical and clinical studies. **Institute for Lboratory Animal Research (ILAR) Journal**, v. 55, n. 3, p. 427–437, 2014. DOI: 10.1093/ilar/ilu043.

WINTER, C. A.; RIESLEY, E. A.; NUSS, G. W. Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. **Proceedings of the Society for Experimental Biology and Medicine,** v.111, n.6, 1983.

YANG, R.; YUAN, Bo-CHUAN; MA, YONG-SHENG, ZHOU, S.; LIU, YING. **The anti-inflammatory activity of licorice, a widely used chinese herbPharmaceutical Biology**, v.55, n.1, 5–8, 2017. DOI: 10.1080/13880209.2016.1225775.

APÊNDICES

APÊNDICE 1

Artigo: Revisão Narrativa publicada na Revista European Journal of Medicinal Plants

A Narrative Review of *Libidibia ferrea*: Botanical Aspects, Ethnopharmacological Properties, Phytochemical Characteristics, Toxicity, and Experimental Tests

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A Narrative Review of *Libidibia ferrea*: Botanical Aspects, Ethnopharmacological Properties, Phytochemical Characteristics, Toxicity, and Experimental Tests

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Jucá or pau-ferro (*Libidibia ferrea*) is an arboreal plant from the Fabaceae family. It is commonly used in traditional medicine in the treatment of various diseases, including inflammatory process.
Aims: The objective of this narrative review is to present botanical aspects, ethnopharmacological properties, phytochemical characteristics, toxicity highlighting, and experimental models with *L. ferrea*.
Results: Botanical Aspects: Jucá has several uses such as in landscaping (stem and canopy), in arborization of urban areas. Ethnopharmacological Properties: It is used in the treatment of various diseases such as diabetes, flu, asthma and, inflammatory processes of which different parts are

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used (root, stem bark, leaves, fruits, seeds). Phytochemical Characteristics: Phenolic compounds, fatty acids, and terpenoids are among the compounds monthly used. Toxicity: *In vivo* models have been used to verify toxicity and in most studies the plant presented no toxicity in its use. Experimental studies: Animals, such as mice, dogs, rats, etc. and different models of studies to analyze the action of the plant were used.

Conclusions: Such low toxicity, associated with its widespread use in folk medicine and its various effects demonstrated in the studies included in this Review have corroborated for the continuity of the research with *L. ferrea*. New studies, however, ought to follow methodological guidelines, such as the Animal Research: reporting *in vivo* Experiments (ARRIVE) so that, a methodological design secures more homogeneous studies capable of quantifying the actual size of the effect in the plant may have in clinical studies.

GRAPHICAL ABSTRACT

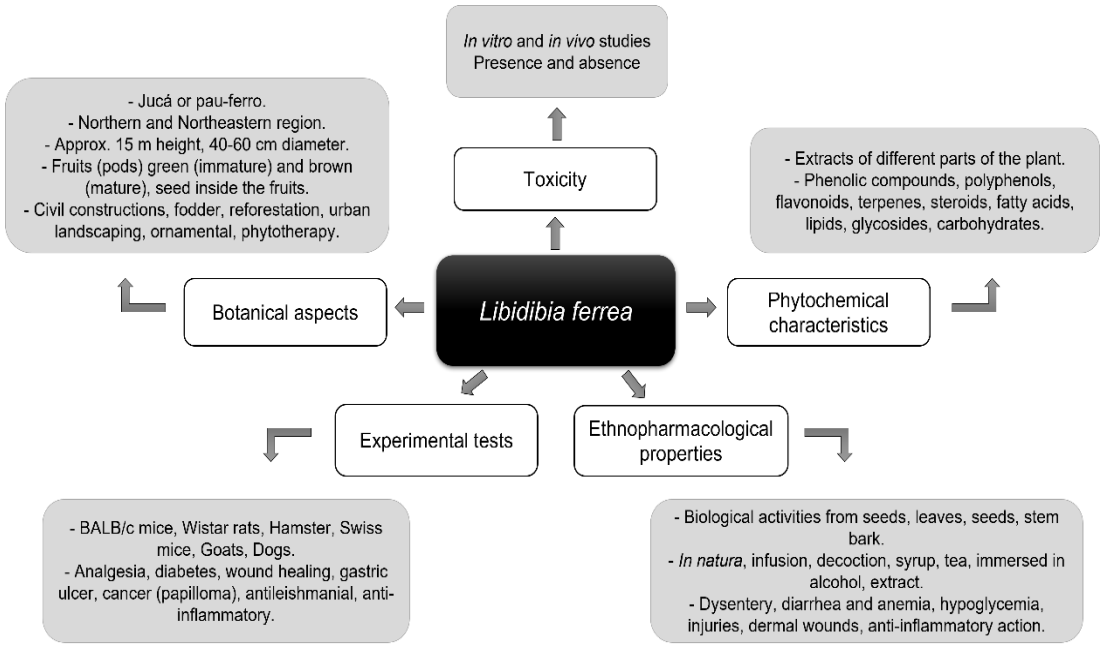

Fig. 1.

Keywords: Jucá; Medicinal plant; Animal experimentation.

1. INTRODUCTION

Brazil features a wide biodiversity of flora (20 to 22% of the world's total namely, 45 000 plants species) with pharmacological potential, but many of these plants have not yet been well studied to became targets of clinical studies [1].

According to the "Formulário de Fitoterápicos Farmacopeia Brasileira" [2], a medicinal plant is defined as a "plant species, cultivated or not, used for therapeutic purposes". Due to their great biological and chemical diversity, medicinal plants have been widely used for the treatment of various diseases, besides having a wide range of biological active compounds [1,3].

More than 35 000 species of medicinal plants can be found in the Amazon, of which approximately 5.000 have a great economic potential, not only for use in humans, but also in animals and environment. Popular knowledge observation about medicinal plants provides the opportunity to obtaining their active substances indicating the way to go with respect to biological activities [4]. In many developed countries the large part of the population, in primary care, depends on traditional medicine [5].

Approximately 25% of circulating drugs directly or indirectly derive from medicinal plants [6]. New substances of plant origin have been sought for the development of new medicines [1,7]. Among the plants associated with medicinal use, *Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz is one of them.

Libidibia ferrea (Mart. ex Tul.) L.P. Queiroz was included by the Ministry of Health (MS) in the National List of Medicinal Plants (SUS-RENISUS) in February 2009 [8,9]. Given the above, this study aims to fully review the literature on the species *Libidibia ferrea*.

2. BOTANICAL ASPECTS

Libidibia ferrea was designated by Car (Karl) Friedrich Philipp von Martius (Mart.) in 1828 as *Caesalpinia ferrea* (basionym) [10]. The genus named after the Italian botanist Andrea Caesalpinio, described by Carl Linnaeus [11] however, was described in 1844 by Louis René ('Edmon') Tulasne (Mart. ex Tul.) [10]. It suffered a taxonomic genus change from *Caesalpinia* to *Libidibia* by Lewis in 2005 and Luciano Paganucci de Queiroz [(Mart. ex Tul.) L.P. Queiroz], in 2009, allocated all variations of *Caesalpinia* to the genus *Libidibia*, named *Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz [12, 13] (Fig. 1).

Libidibia ferrea presents the following variations (var.): *ferrea* and *glabrescens*, with distribution in the Caatinga domain; *leiostachya* and *parvifolia* in the Atlantic Forest (Benth.) with differentiated distributions [12]. Regarding its taxonomic classification it belongs to the Plantae Kingdom, Magnoliophyta Phylum (Angiospermae), Magnoliopsida Class (Dicotyledonae), Fabales Order, Fabaceae Family, Caesalpiniaceae subfamily (Caesalpinoideae, Leguminosae), *Libidibia* genus, and *Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz species [10,14] (Fig. 2).

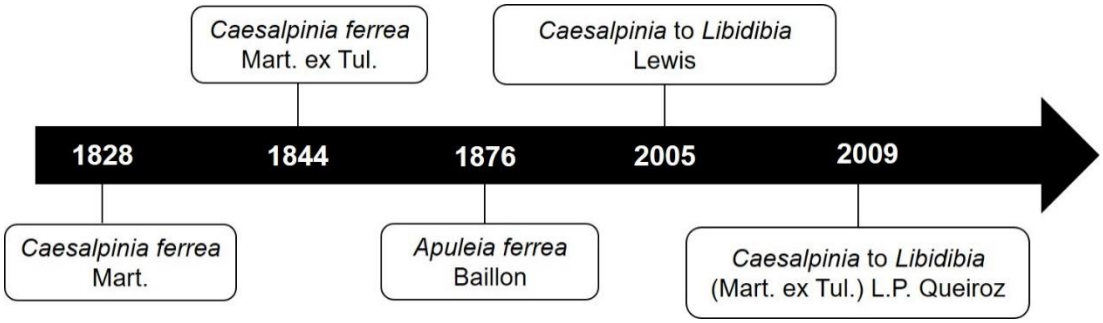

Fig. 1. *Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz timeline

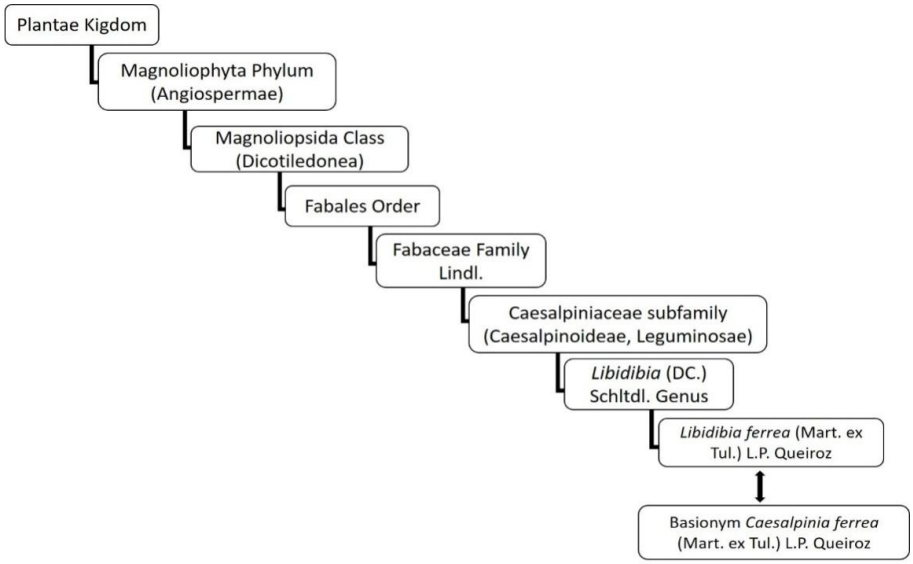

Fig. 2. *Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz taxonomic classification

Libidibia ferrea can be found in the phylogeographic domains of the Caatinga, Atlantic Forest, and Brazilian Cerrado [15]; in the Northern region [16] and in Northeastern region [17]. The name *ferrea* is related to the hardness existing in its wood [15]. It is known by popular names such as jucá, pau-ferro or true pau-ferro and by its indigenous names: imirá-itá, ibirá-obi [11, 18], muiré-itá, muirá-obi [11], jucaína [19].

Jucá is an arboreal plant, heliophile, native to Brazil that can reach up to 15 meters (m) in heigth [11]. It features a lush and wide canopy [15]. As for its morphology, this species has a hard core, smooth trunk [11] and squamous [15, 19], with 40-60 centimeters (cm) in diameter [18].

Galdino et al. (2007) have collected leaves, fruits, and seeds in the city of Manaus and in parallel planted the seeds for monitoring their germination (average of 14 days) and the jucá seedling. Through biometric measurements the leaf of the adult plant was estimates in 7-30 cm and the seedling in 3-5 cm [20].

The fruit presented the following dimensions 8.3 x 1.8 x 0.8 cm (length x width x thickness) and 5.27 g (3.55-7.30 g) of weight. The fruits could be green and brown in color when immature and mature, respectively. It is considered an indehiscent fruit since no seed is released when the fruit is ripe. The fruits base can be rounded to curved, and it has a protruding ventral suture and the apex is rounded with mucro (oval) [20].

The flowering period occurs towards the end of November [19] exhibiting small flowers in the form of yellowish brunches [20] the smallest petal has red stains [17], several fruits coming from the inflorescence [20].

The seeds are found in individual cavities inside the fruit evident in a transverse and uniseriate arrangement of 6-12 seeds per fruit. Seeds have an average size of 0.9 x 0.5 x 0.5 cm and 0.15 g weight, light green to yellowish color, firm in its consistency and slightly wrinkled integument, discoid to ovoid shape, with rounded apex and flattened base [20], which are responsible for the species propagation [21].

L. ferrea has several applications, such as civil construction [11] for its quite heavy and hard wood, but it is also popular as fodder for cattle [22], used in urban landscaping and reforestation [15, 18], planks, fences, firewood, in animal feed [23,24], in the fight against gastrointestinal parasites, such as sheep parasites [14,25], ornamental species (avenues and streets) [11,18] and its phytotherapy used [23,24].

In addition to the above applications studies presenting its ethnopharmacological characteristics are also developed.

3. ETHNOPHARMACOLOGICAL PROPERTIES

Herbs, leaves and roots ingestion to cure diseases and relieve illnesses is possibly the first form of plant use [27]. And for *L. ferrea* the biological activities have been researched and many authors have used aqueous extract to verify the biological activities of the fruits [26,28-34], leaves [35], stem bark [26,36,37] and seeds [38,39].

Libidibia ferrea is one of the species that standout in this use in folk medicine in the form of “garrafada” from stem bark, for the treatment of dysentery, diarrhea and anemia [40], in the use of leaf and fruit for the therapeutic treatment of hypoglycemia in the form of infusion and *in natura* [41], in the use of the left soaking for the treatment of fruit bronchitis and influenza [42].

In an ethnobotanical survey with residents around Serra da Capivara National Park in Piauí conducted by Reis et al. (2017) the use of *C. ferrea* leaves, pods, stem bark, root, and whole plant for the treatment of influenza, injuries, action on the liver, lungs, heart, throat, and as anti-inflammatory was reported [43].

Several parts of this plant are used in Amazon region. Leaves, in decoction, are used in the treatment of hemorrhoid (externa use), and in amebiasis and liver problems (internal use); treatment of tuberculosis with infusion of leaves with the fruits; in the form of syrup in the treatment of bronchitis and asthma [11]; use of the pods in the form of syrup, tea, and infusion for the treatment of gastric problems [44]. The use of fruits immersed in alcohol is used in the Lower Amazon in the treatment of several dermal wounds [45].

Investigation of the action from *L. ferrea* is carried out in the in the treatment of analgesic and inflammatory conditions [27,29,46]; cancer chemopreventive [47,48]; larvicidal activity against *Aedes aegypti* and presenting cellulosic, anticoagulant and amylase activity with the use

of the crude aqueous extract of the seed [38]; antimicrobial activity [33,36,49,50]; antiglicemic and the treatment of diabetes [37,51,52]; wound healing potential [45,53,54]; antioxidant and hepatoprotective, viral activity against the Herpes Simplex Virus e Poliovirus [55] and against the dengue virus (DENV-2) [56]; repellent action against species of the Calliphoridae family [57].

Other activities are performed by this plant: anthelmintic [25]; antileishmania action [58]; gastroprotective and antiulcerogenic [44]; cosmetic anti-whitening and anti-wrinkle potential effects as cosmetic [59]. In a study conducted with zebrafish (*Danio rerio*) that oral use of alcoholic extract can be used as an oral drug with an acceptable safety was observed [60]. Cutaneous treatment of wounds in goats was also observed in veterinary medicine with the use of stem bark as the basis for ointment production [61]. And there is a potential use for wound healing in dogs in a formulation containing 5% of jucá ethanol extract [45].

4. PHYTOCHEMICAL CHARACTERISTICS

Among various applications of medicinal plants extracts the anti-inflammatory action which has reports of some compounds, such as flavonoids, terpenes and, phenolic compounds [1] is highlight. In *L. ferrea* phenolic compounds the presence of gallic and ellagic acids, catechins and, epicatechins presence in aqueous extract from the stem bark [37] and bark [36].

Ueda et al. (2001) on analyzing the dried fruits of *C. ferrea* have observed the presence of ellagic acid and 2-(2,3,6-trihidroxy-4-carboxyphenyl) ellagic acid [62]. Identification of ellagic and gallic acids in fruits was also identified [63]. Studies have corroborated with the identification of steroids, phenolic compounds, saponins, coumarins, flavonoids and tannins in the hydroalcoholic extract from leaves and stem bark of *C. ferrea* [64]. Silva et al. (2013) have identified gallic acid and methylated gallate derivative from hydroalcoholic extract from the fruits [65]. Phenolic compounds, such as gallic acid and methyl gallate have been isolated from ethyl acetate extract from jucá fruits [48].

Frasson et al. (2003) upon analyzing the crude ethanolic extract of the stem of *C. ferrea* (Mart. ex Tul.) var. *leiostachya* Benth have observed the absence of saponins, low content of total tannin, presence of flavonoids in greater quantity in the ethyl acetate fraction, terpenes in petroleum ether and dichloromethane fractions [66].

Sawada et al. (2004) have observed the presence of different fatty acids such as palmitolenic, oleic, linoleic, linolenic, stearic, capric, palmitic in the lipidic portion seed [39]. Corroborating the data from Dias et al. (2013) who have identified by supercritical extract unsaturated fatty acid (52%), saturated fatty acids (26%) and terpenoids (13%) in pods of *L. ferrea* [53]. The presence of monosaccharide compounds (D-galactose e D-mannose) was observed at *C. ferrea* sulfated polysaccharide from seeds [55].

Comandolli-Wyrepkowski et al. (2017) have identified high levels of phenolic compounds and flavonoids from *L. ferrea* fruit and identified terpenoids in the plant [58]. Prazeres et al. (2019) have also identified phenolic compounds such as gallic acid and ellagic acid in the *Libidibia ferrea* dry extract from fruits [44]. Phenolic compounds, tannins and flavonoids have been identified in the lyophilized extract of the pods used as topical phytopharmaceutical [45]. Hassan et al. (2015) have identified carbohydrates and/or glycosides, tannins, and phenolic compounds in aqueous ethanolic extract of *C. ferrea* [51].

A study testing four different types of jucá extract have identified phenolic compounds, and carbohydrates (aqueous ethanol extract), lipids and predominance of organic acids (ethyl acetate extract), organic acids and predominance of lipids (chloroform extract), and alcohol and lipids (hexane extract) [67].

Table 1 presents the most compound observed in the articles included in this narrative review.

An observation about these compounds is that they vary according to the time of year, age of the plant, type of soil, climate, among others [66]. Among this diversity of existing compounds in medicinal plants it is known that they may or may not present toxicity. The evaluation of the toxicity degree is necessary since it may make the use of the plants impossible despite the plant showing a medicinal effect [68].

Table 1. *L. ferrea* compounds identified in the included studies

Compounds			References
Phenolic compounds	-----	-----	[51], [58], [64], [67]
	Hydrolysable tannins	gallic acid	[36], [37], [44], [48], [63], [65]
		ellagic acid	[37], [44], [62], [63]
		methyl gallate	[48], [65]
		2-(2,3,6,-trihydroxy-4-carboxyphenyl) ellagic acid	[62]
	Condensed tannins	catechin	[36], [37]
		epicatechin	[37]
		tannins	[45], [51], [64]
	Polyphenols	Total tannins	[66]
	Flavonoids	----	[45], [54], [66]
Terpenes	Triterpenoid	----	
		lupenone	[53]
Steroids and Lipds	----	----	[45] and [67]
Fatty acids	Unsaturated	linoleic acid	[39], [53]
		oleic acid	[39]
		palmitolenic acid	
		elaidic acid	[53]
		gamma-sitosterol	
	Saturated	palmitic acid	[39], [53]
		stearic acid	
		capric acid	[39]
Carbohydrates	----	----	[51], [67]
Glycosides	----	----	[51]
	saponin	----	[58], [64]
Monosaccharide	D-Galactose, D-Mannose	----	[55]
Coumarin	----	----	[64]
Organic acids	----	----	[67]
Alcohols	----	----	

5. TOXICITY

The degree of plant toxicity might be related to the method of its preparation as well as the part used and the rout of administration [41,69]. Few studies have been described regarding to the toxicity of *L. ferrea* [70].

Souza et al. (2006) have investigated the effects of aqueous extract of the fruits on red bone marrow using micronucleus model and chromosomal aberration in Wistar rats. Results have shown that there was no cytotoxic or clastogenic effect [71]. Reboredo et al. (2007) have demonstrated that the use of this extract had shown alteration only in the weight of the seminal vesicle but had not alter the weight of the other organs of the male reproductive system of male Wistar rats in the subacute toxicity tests, used at the dose of 300 mg/kg once a day intragastrically for five days [34].

Study in female Wistar rats (3 months, 160-190 g) during the blastocyst implementation period (5^{th} and 7^{th} days of pregnancy) 300 mg/kg by gavage of *C. ferrea* aqueous extract of the fruit was applied the presence of toxicity in rats or into blastocyst implantation [32]. Cavalheiro et al. (2009) have also tested acute toxicity in six male Swiss mice and found that the crude aqueous extract of the seeds applied intraperitoneally route at a dose of 0.3 mL/10 g showed no toxicity, weight loss, diarrhea, or behavioral changes [38].

Using 300 mg/kg of the aqueous extract applying 1 mL per gavage in a 52-day treatment caused no toxicity in Wistar rats [31]. In an acute oral toxicity test using aqueous extract and F80 fraction of *L. ferrea* var. *parvifolia* (Mart. ex Tul.) L.P. Queiroz at dose 2,500 mg/kg in female albino Swiss mice caused low toxicity [30].

No death of any animal has been recorded in the acute toxicity test of the ethanolic extract from pods. The test was performed in female Swiss mice with the application of a single dose of 2,000 mg/kg orally [46]. An *In vitro* toxicity test with macrophage RAW 264.7 cells using supercritical extract of raw fruits of jucá was carried out. And through the release of lactate dehydrogenase (LHD) it the induction of certain toxicity in these cells at the beginning of their release in the dressings was observed. However, there were no significant morphological changes in cells, led to the inference that the extract would not be toxic, but it had inhibited the adhesion of fibroblasts [53]. In the study with *C. ferrea* (Mart. ex. Tul.) var. *ferrea* using fruits/seeds low toxicity in HEp-2 ATCC CCL-23 cells was verified [55].

In 2015, a study using ethanolic aqueous extract from *C. ferrea* Martius leaves in Sprague-Dawley rats applying the dose of 1.500 mg/kg, no animal death or toxic reaction were observed, and much less mood change in animals until the end of the experiment [51]. Kobayashi et al. (2015) have tested oral toxicity at a dose of 5 g/kg using ethanol extract from *L. ferrea* (Mart. ex Tul.) var. *ferrea* fruits in 10 female Wistar rats and showed no acute toxicity [54].

Through the cell viability assay, at a concentration of 20 μg/mL of the dry extracts of the stem bark and pods, no cytotoxicity was significantly observed in normal human fibroblast cells [59]. Comandolli-Wyrepkowski et al. (2017) have observed low toxicity in J774 macrophages cells using methanol extract of fruits used to make gel in the topical treatment against infection with promastigotes and amastigote of *Leishmania* (*Leishmania*) *amazonensis* and *Leishmania* (*Viannia*) *guyanensis* in golden hamster (*Mesocricetus auratus*) [58].

Cunha et al. (2017) in a preparation of jucá seed gum containing polysaccharides (galactomannan) have observed, *in vitro*, through the LDH test in human leukocytes that there was no toxicity in the plasma membrane of neutrophils [72]. Guerra et al. (2017) using raw extract from the fruits of *L. ferrea* (Mar. ex Tul.) L.P. Queiroz var. *ferrea* tested cell viability in HT-29 and HEK-293 cells and have observed that there was no toxicity in HEK-293 in 40T, 60T and 80T extracts [73].

C. ferrea seed extract coated with silver nanoparticles (AgNP) presenting toxicity in L929 murine fibroblast cells exposed for 48h at the highest concentration (1,000 mg/mL), and this cytotoxicity was dose-dependent with the concentration of AgNP [74].

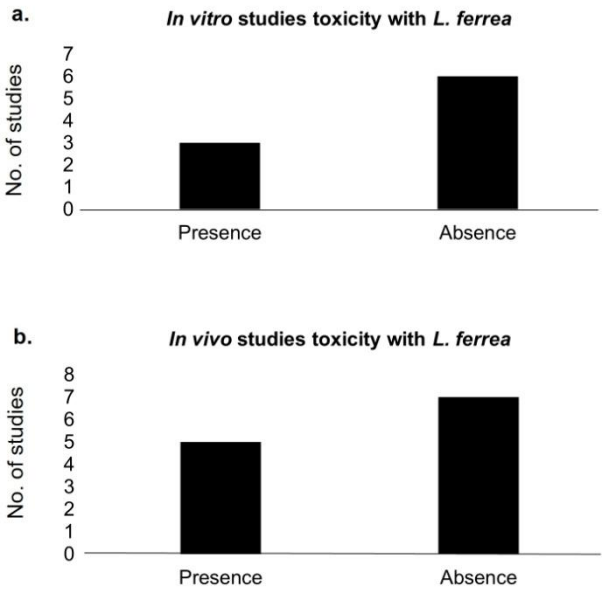

Fig. 3. *In vitro* and *in vivo* toxicity observed in articles with *L. ferrea*. a. Three *In vitro* studies presented toxicity action [55,58,74] and six not demonstrated toxicity action [29,53,59,67,72, 73]. b. Five *in vivo* studies presented toxicity action [30,34,60,75] and seven studies reported no toxicity [31,44,46,51,54,71].

A cell viability test with raw extract and hydroalcoholic, aqueous and ethyl acetate fractions of the fruits of *L. ferrea* (Mart. ex Tul.) L.P. Queiroz var. *ferrea* observed that there was an increase in cell viability [29]. Prazeres et al. (2019) have performed acute toxicity test in female Wistar rats orally treated with dry extract of the fruit of *L. ferrea* at a dose of 2,000 mg/kg. Results have shown that this dose caused no death, change in behavior, change in food consumption, or weight gain during the 14 days of treatment, only an increase in water consumption [44]. In a study with zebrafish (*Danio rerio*) with hydroalcoholic extract of the aerial parts with the fruits at a dose of 2 g/kg presented toxicity in the heart of concentration-dependent embryos have been observed. In adults significant histopathological changes in the gills were caused [60].

In the preparation of the *L. ferrea* (Mart. ex Tul.) L.P. Queiroz var. *ferrea* bark and seed hydroalcoholic extract, Pickler et al. (2019) have observed that both bark and seed extract were not proven to be safe when used in the gestational phase in Wistar rats [75]. Calandrini de Azevedo et al. (2020) have observed that only with the aqueous ethanolic extract from the jucá pods in the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test there was no change in cell survival in any concentration tested, nor did it present mutagenic or genotoxic effects [67]. Fig. 3 presents the survey about the toxicity effect and 21 studies were included.

In view of the above, to verify the effects and toxicological analysis, most experiments are carried out in animal models, especially in rodents.

6. EXPERIMENTAL TESTS

Many of the studies involving scientific research with *L. ferrea* are carried out in animal model, among which are rodents. Mice (*Mus musculus*) are easy to handle since they are small, have a rapid reproduction rate, ease of keeping them in laboratories, short life span [76, 77] and supply of many strains of blood-related [78] and, throughout history, has represented the *in vivo* model that predominates in biomedical research [76].

Studies, which have described experimental models with *L. ferrea*, identified in this research, will be described below in chronological (ascending) order.

Carvalho et al. (1996) used Swiss albino mice for analgesia testing and Wistar rats to verify anti-inflammatory action. Administration of the crude aqueous extract was oral, at concentrations of 10 and 20 mg/kg for analgesia and 100 mg/kg in the hot plate test observing analgesic property of the extract. And at a dose of 300 mg/kg of the extract administered orally inhibited paw edema [28].

Aqueous extract of the fruits of *Caesalpinia ferrea* showed a positive regulatory effect on myelopoiesis and may also act against opportunistic *Listeria monocytogenes* infection at concentrations of 500 and 1,000 mg/kg in oral BALB/c mice [79].

Female mice were used in two-stage cutaneous carcinogenesis experimentation and tested using gallic acid and methyl gallate isolated from the fruits of *Caesalpinia ferrea* Mart. demonstrating that there was a reduction in the number of papilloma [48].

Use of ellagic acid and ellagic acid 2-(2,3,6-trihydroxy-4-carboxyphenyl) (ASD) of *Caesalpinia ferrea* dried fruits *in vitro* in Diabetes-induced Wistar rats with streptozotocin (STZ) at doses of 50, 75, 100 mg/kg orally measuring sorbitol accumulation was tested by Ueda et al. (2004) suggesting that diabetic complications could be alleviated with the ingestion of fruits and vegetables containing ellagic acid [52].

There was no change in the blastocyst implementation process in three-month Wistar females in the use of 300 mg/kg via gavage of the crude aqueous extract of *C. ferrea* Mart fruits [32].

Stem bark aqueous extract used at doses of 450 mg/kg per day orally as a promising alternative in the treatment of diabetes was shown by Vasconcelos et al. (2011) in a model of streptozotocin-induced diabetes in Wistar rats [37].

Kobayashi et al. (2015) have topically tested ethanolic extract of jucá fruit and have observed that wound-healing at the dose of 12.5% in Wistar rats [54]. Topical use of hydrogel with methanol extract of fruits at a dose of 50 mg/day

have demonstrated antileishmania effect in hamster (*Mesocricetus auratus*), where there was a reduction in both volume and inflamed region and presented the lower parasitic load of *Leishmania* (*Leishmania*) *amazonensis* [58].

Galactomannan of the jucá seed at dose of 10 mg/kg was used in diabetic Wistar rats induced with streptozotocin suggesting its use as a potential functional food in the treatment of type 2 diabetes [72].

Dry extract of jucá pods used at doses of 50, 100, 200 and 400 mg/kg administered orally influenced the treatment of gastric ulcer caused by indomethacin in Wistar rats. Data have also shown that the extract presents antioxidant activity, which could be used as a prevention of various diseases associated with oxidative stress [44].

In addition to studies in rats, mice, and hamsters, two studies were also found using animal models, goats, and dogs. Oliveira et al. (2010) have observed that the application of an ointment based on the stem bark of *C. ferrea* in the cutaneous treatment in experimental wounds induced in male goats aided in the wound-healing process, reducing inflammatory exudate, edema, and hyperemia [61]. Another study was that of Américo et al. (2020) in which dermal wounds were induced in dogs and a 5% ethanolic extract of *L. ferrea* fruits and flowers of the adult plant were used topically (ointment). Extract presented a potential for wound healing, suggesting its veterinary use [45].

This study has shown the following limitations: diversity of animal models, plant parts, extract types and differentiated doses found in the articles, which precludes in-depth analysis of the plant's effect on investigated disease model. Another important limitation is related to survey in databases and the method of selection of studies which may have missed some relevant publications.

7. CONCLUSIONS

This research aimed to bring a state-of-the-art survey of what has been published on *Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz, since jucá has been described as a medicinal plant by popular belief and by RENISUS.

Demonstration that there is low, or no toxicity related to its use, as well as the positive and/or regulatory effect in various diseases, in addition to the use in folk medicine corroborate the use of this plant in future clinical research. However, it is observed that experimental studies require a standardized design such as (Guideline Animal Research: Reporting of *in Vivo* Experiments - ARRIVE) for the quantitative confirmation of pharmacological effects.

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CONSENT

It is not applicable.

ETHICS APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ribeiro VP, Arruda C, Abd El-Salam M, Bastos JK. Brazilian medicinal plants with corroborated anti-inflammatory activities: a review. Pharmaceutical Biology. 2018; 56(1):253-68. Available:https://doi.org/10.1080/13880209.2018.1454480.
2. ANVISA. Primeiro Suplemento - Formulário de Fitoterápicos Farmacopeia Brasileira. Agência Nacional de Vigilância Sanitária. 1ª ed. 2018;160. Portuguese.
3. Yang R, Yuan BC, Ma YS, Zhou S, Liu Y. The anti-inflammatory activity of licorice, a widely used Chinese herb. Pharmaceutical Biology. 2017;55(1):5-18. Available:https://doi.org/10.1080/13880209.2016.1225775.
4. Cardenas JDR. Diversidade botânica. In: dos Santos GM, França LR. GEEA: Grupo de Estudos Estratégicos Amazônicos, v. 10, (Cardeno de Debates). Editora INPA, Manaus; 2017.

ISBN 978-85-211-0168-0. Portuguese.
5. Brasil. Ministério da Saúde. Política e Programa Nacional de Plantas Medicinais e Fitoterápicos. Secretaria de Ciência, Tecnologia e Insumos Estratégicos, Departamento de Assistência Farmacêutica, Brasília: Ministério da Saúde; 2016.
ISBN 978-85-2399-2. Portuguese.
6. Brasil. Ministério da Saúde. Práticas integrativas e complementares: plantas medicinais e fitoterapia na Atenção Básica. Ministério da Saúde. Secretaria de Atenção à Saúde. Departamento de Atenção Básica. Série A. Normas e Manuais Técnicos. Brasília: Ministério da Saúde; 2012.
ISBN 978-85-334-1912-4. Portuguese.
7. Ghasemian M, Owlia S, Owlia MB. Review of Anti-inflammatory herbal medicines. Advances in Pharmacological Sciences; 2016.
Available:https://doi.org/10.1155/2016/9130979.
8. Brasil, Ministério da Saúde. A fitoterapia no SUS e o Programa de Pesquisa de Plantas Medicinais da Central de Medicamentos. Série B. T. Brasília: Ministério da Saúde, Secretaria de Ciência, Tecnologia e Insumos Estratégicos, Departamento de Assistência Farmacêutica; 2006.
ISBN 85-334-1187-1 Portuguese.
9. Brasil. Ministério da Saúde. Relação Nacional de Plantas Medicinais de Interesse ao SUS - RENISUS. DAF/SCTIE/MS. Fev/2009. Portuguese.
10. Tropicos. Missouri Botanical Garden. Accessed 29 Jul 2020.
Available:http://wwwtropicos.org.
11. Di Stasi LC, Himura-Lima CA. Plantas medicinais na Amazônia e na Mata Atlântica. 2ª ed. rev. e ampli. São Paulo: Editora UNESP; 2002.
ISBN 85-7139-411-3. Portuguese.
12. *Libidibia* in Lista de Espécies da Flora do Brasil. Jard Botânico Do Rio Janeiro. Accessed 01 August 2020
Available:http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB109828. Portuguese.
13. Queiroz LP. Leguminosas da Caatinga. Feira de Santana: Universidade Estadual de Feira de Santana; 2009. Portuguese.
14. Da Costa LM, Guilhon-Simplicio F, De Souza TP. *Libidibia ferrea* (Mart. Ex tul) L. P. Queiroz var. *ferrea*: Pharmacological, phytochemical and botanical aspects. International Journal of Pharmacy and Pharmaceutical Sciences. 2015;7(4):48-53.
15. Maia-Silva C, Silva CI da, Hrncir M, Queiroz RT de, Imperatriz-Fonseca VL. Jucazeiro. In: Guia de plantas visitadas por abelhas na caatinga, 1ª ed. Editora Fundação Brasil Cidadão, Fortaleza, CE. 2012;43.
ISBN 978-85-98564-05-0. Portuguese.
16. Silva MF da, Carreira LMM, Tavares AS, Ribeiro IC, Jardim MAG, Lobo M da GA, et al. As leguminosas da Amazônia brasileira: lista prévia. Acta Botanica Brasilica. 1989; 2(1):193-237. Portuguese.
17. Stehmann JR, Faria FS, Bragioni T. 50 árvores do Museu. Belo Horizonte: Formato Editora; 2019.
ISBN 978-85-62164-13-2 Portuguese.
18. Lorenzi H. Árvores Brasileiras: Manual de identificação e cultivo de plantas arbóreas nativas do Brasil. Nova Odessa, São Paulo: Plantarum. Portuguese; 1992.
19. Balbach A. Plantas que curam. 2ª ed. São Paulo: Missionária; 1972. Portuguese.
20. Galdino G, Mesquita MR, Ferraz IDK. Descrição morfológica da plântula e diásporos de *Caesalpinia ferrea* Mart – Nota científica. Revista Brasileira de Biociências, Porto Alegre. 2007;5(2):747-749. Portuguese.
21. Matos ACB, Ataíde G da M, Borges EE de L. Physiological, physical, and morpho-anatomical changes in *Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz) seeds after overcoming dormancy. Journal of Seed Science. 2015;37(1):26-32.
Available:https://doi.org/10.1590/2317-1545v37n1140433.
22. Sousa CC, Gomes SO, Lopes ACA, Gomes RLF, Brito FB, Lima PSC, et al. Comparison of methods to isolate DNA from *Caesalpinia ferrea* - Short Communication. Genetics and Molecular Research - Online Journal. 2014;13(2): 4486-4493.
Available:https://doi.org/10.4238/2014.June.16.7.
23. Carvalho SMC, Torres SB, Benedito CP, Nogueira NW, Souza AAT, Souza Neta ML de. Viability of *Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz var. *ferrea* seeds by tetrazolium test. Journal of Seed Science. 2017;39(1):7-12.

Available:https://doi.org/10.1590/2317-1545v39n1163784.

24. Santana JA da S, Ferreira L da S, Coelho RRP, Vieira F de A, Pacheco MV. Tecnologiais de baixo custo para superação de dormência em sementes de *Caesalpinia ferrea* var. *ferrea* Mart. ex. Tul. (pauferro). Revista Verde de Agroecologia e Desenvolvimento Sustentável. 2011; 6(1):225-229. Portuguese.
25. Salles HO, Braga ACL, do Nascimento MT dos SC, Sousa AMP, Lima AR, Vieira L da S, et al. Lectin, hemolysin and protease inhibitors in seed fractions with ovicidal activity against *Haemonchus contortus*. Brazilian Journal of Veterinary Parasitology. 2014;23(2):136-143. Available:https://doi.org/10.1590/S1984-29612014050.
26. Barros AO, De Souza RS, Aranha ESP, Da Costa LM, De Souza TP, De Vasconcellos MC, et al. Antioxidant and hepatoprotective activities of *Libidibia ferrea* bark and fruit extracts. International Journal of Pharmacy and Pharmaceutical Sciences. 2014;6(11):71-76.
27. Santos, SF dos, Santos, AS, Corpes, RS, Leão, NV. Aspectos dos cultivo *in vitro* de *Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz (Leguminosae-Caesalpinoideae) como fonte alternativa para produção de metabólitos secundários. Revista Espacios. 2018;39(37):17-24. Portuguese.
28. Carvalho JCT, Teixeira JRM, Souza PJC, Bastos JK, Dos Santos Filho D, Sarti SJ. Preliminary studies of analgesic and anti-inflammatory properties of *Caesalpinia ferrea* crude extract. Journal of Ethnopharmacology. 1996;53:175-178. Available:https://doi.org/10.1016/0378-8741(96)01441-9.
29. Falcão TR, Araújo AA De, Soares LAL, Farias IB De, Silva WAV Da, Ferreira MRA, et al. *Libidibia ferrea* fruit crude extract and fractions show anti-inflammatory, antioxidant, and antinociceptive effect *in vivo* and increase cell viability *in vitro*. Evidence-Based Complementary Alternative Medicine. 2019;2019(ID 6064805):14. Available:https://doi.org/10.1155/2019/6064805.
30. Freitas ACC, Ximenes NCA, Aguiar JS, Nascimento SC, Lins TUL, Magalhães LR, et al. Biological activities of *Libidibia* (*Caesalpinia*) *ferrea* var. *parvifolia* (Mart. ex Tul.) L. P. Queiroz pod preparations. Evidence-Based Complementary Alternative Medicine. 2012;2012(ID 514137):7. Available:https://doi.org/10.1155/2012/514134.
31. Lucinda LMF, Rocha CB, Reboredo MM, Faria VC, Sá RCS. Assessment of sperm production and reproductive organs of Wistar rats to long-term exposure of *Caesalpinia ferrea*. Anais da Academia Brasileira de Ciências. 2010;82(4):907-914. Available:https://doi.org/10.1590/s0001-37652010000400013.
32. Peters VM, Souza SO, Carvalho JCT, Borges L V., Guerra MO. Evaluation of reproductive toxicity of aqueous extract of the fruits from *Caesalpinia ferrea* Mart. in rats. Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas. 2008;7(5):268-272.
33. Sampaio FC, Pereira M do S V., Dias CS, Costa VCO, Conde NCO, Buzalaf MAR. *In vitro* antimicrobial activity of *Caesalpinia ferrea* Martius fruits against oral pathogens. Journal of Ethnopharmacology. 2009;124(2019):289-94. Available:https://doi.org/10.1016/j.jep.2009.04.034.
34. Reboredo M de M, Lucinda LMF, Rocha CB, Queiroz GT de, Faria VC de, Vieira V de A, et al. Avaliação da toxicidade do extrato aquoso de *Caesalpinia ferrea* em órgãos vitais, no sistema reprodutor e na produção de espermatozoides de ratos Wistar submetidos a tratamento subagudo. Boletim do Centro de Biologia da Reprodução. 2006;25:17-29. Portuguese.
35. Falcão TR, Rodrigues CAO, De Araújo AA, De Medeiros CACX, Soares LAL, Ferreira MRA, et al. Crude extract from *Libidibia ferrea* (Mart. ex. Tul.) L.P. Queiroz leaves decreased intra articular inflammation induced by zymosan in rats. BMC Complementary and Alternative Medicine. 2019;19(47):1-10. Available:https://doi.org/10.1186/s12906-019-2454-3.
36. De Araújo AA, Soares LAL, Assunção Ferreira MR, De Souza Neto MA, Da Silva GR, De Araújo RF, et al. Quantification of polyphenols and evaluation of anti-microbial, analgesic and anti-inflammatory activities of aqueous and acetone-water extracts of *Libidibia ferrea*, *Parapiptadenia*

rigida and *Psidium guajava*. Journal of Ethnopharmacology. 2014;156(2014):88-96.
Available:https://doi.org/10.1016/j.jep.2014.07.031.

37. Vasconcelos CFB, Maranhão HML, Batista TM, Carneiro EM, Ferreira F, Costa J, et al. Hypoglycaemic activity and molecular mechanisms of *Caesalpinia ferrea* Martius bark extract on streptozotocin-induced diabetes in Wistar rats. Journal of Ethnopharmacology. 2011;137(2011): 1533-1541.
Available:https://doi.org/10.1016/j.jep.2011.08.059.
38. Cavalheiro MG, Farias DF, Fernandes GS, Nunes EP, Cavalcanti FS, Vasconcelos IM, et al. Atividades biológicas e enzimáticas do extrato aquoso de sementes de *Caesalpinia ferrea* Mart., Leguminosae. Revista Brasileira de Farmacognosia. 2009;19(2B):586-591.
Available:https://doi.org/10.1590/S0102-695X2009000400014. Portuguese.
39. Sawada LA, Monteiro VSDC, Rabelo GR, Dias GB, Da Cunha M, Do Nascimento JLM, et al. *Libidibia ferrea* mature seeds promote antinociceptive effect by peripheral and central pathway: Possible involvement of opioid and cholinergic receptors. BioMed Research International. 2014;2014(ID 508725):10.
Available:https://doi.org/10.1155/2014/508725.
40. Agra M de F, Freitas PF de, Barbosa-Filho JM. Synopsis of the plants known as medicinal and poisonous in Northeast of Brazil. Revista Brasileira de Farmacognosia. 2007;17(1):114-140.
Available:https://doi.org/10.1590/S0102-695X2007000100021.
41. Santos KA dos, Vilanova CM. Estudo etnobotânico de plantas medicinais utilizadas como hipoglicemiantes por usuários do Programa de Fitoterapia da Universidade Federal do Maranhão, Brasil. Scientia Plena. 2017;13(03):1-12.
Available:https://doi.org/10.14808/sci.plena.2017.034501. Portuguese.
42. Gomes TMF, Lopes JB, Barros RFM de, Alencar NL. Plantas de uso terapêutico na comunidade rural Bezerro Morto, São João da Canabrava, Piauí, Brasil. Gaia Scientia. 2017;11(1):253-268.
Available:https://doi.org/10.22478/ufpb.1981-1268.2017v11n1.33683. Portuguese.
43. Reis CRM, Prereira AF de N, Cansanção IF. Levantamento etnobotânico de plantas medicinais utilizadas por moradores do entorno do Parque Nacional Serra da Capivara - PI. Biofarm. 2017;13(04):7-21.
Portuguese.
44. Prazeres LDKT, Aragão TP, Brito SA, Almeida CLF, Silva AD, De Paula MMF, et al. Antioxidant and antiulcerogenic activity of the dry extract of pods of *Libidibia ferrea* Mart. ex Tul. (Fabaceae). Oxidative Medicine and Cellular Longevity. 2019; 2019.
Available:https://doi.org/10.1155/2019/1983137.
45. Américo ÁVL dos S, Nunes KM, Assis FFV de, Dias SR, Passos CTS, Morini AC, et al. Efficacy of phytopharmaceuticals from the Amazonian plant *Libidibia ferrea* for wound healing in dogs. Frontiers in Veterinary Science. 2020;7:1-11.
Available:https://doi.org/10.3389/fvets.2020.00244.
46. Lima SMA, Araújo LCC, Sitônio MM, Freitas ACC, Moura SL, Correia MTS, et al. Anti-inflammatory and analgesic potential of *Caesalpinia ferrea*. Revista Brasileira de Farmacognosia. 2012;22(1): 169-175.
Available:https://doi.org/10.1590/S0102-695X2011005000197.
47. Nakamura ES, Kurosaki F, Arisawa M, Mukainaka T, Okuda M, Tokuda H, et al. Cancer chemopreventive effects of constituents of *Caesalpinia ferrea* and related compounds. Cancer Letters. 2002; 177(2002):119-124.
Available:https://doi.org/10.1016/S0304-3835(01)00708-X.
48. Nakamura ES, Kurosaki F, Arisawa M, Mukainaka T, Takayasu J, Okuda M, et al. Cancer chemopreventive effects of a Brazilian folk medicine, Juca, on *in vivo* two-stage skin carcinogenesis. Journal of Ethnopharmacology. 2002;81(2002):135-137.
Available:https://doi.org/10.1016/S0378-8741(02)00047-8.
49. Oliveira GP, Souza TP, Caetano SK, Farias KS, Venancio GN, Bandeira MFCL, et al. Atividade antimicrobiana in vitro de extratos da casca do caule e da vagem de *Libidibia ferrea* L. frente a microrganismos da cavidade bucal. Revista Fitos. 2013; 8(2):95-102.

Available:https://doi.org/10.5935/1808-9569.20130004. Portuguese.

50. Silva LCN da, Sandes JM, Paiva MM de, Araújo JM de, Figueiredo RCBQ de, Silva MV da, et al. Anti-*Staphylococcus aureus* action of three Caatinga fruits evaluated by electron microscopy. Natural Product Research: Formely Natural Products Letters. 2012;1-5. Available:https://doi.org/10.1080/14786419.2012.722090.
51. Hassan SK, El-Sammad NM, Mousa AM, Mohammed MH, Farrag A el RH, Hashim ANE, et al. Hypoglycemic and antioxidant activities of *Caesalpinia ferrea* Martius leaf extract in streptozotocin-induced diabetic rats. Asian Pacific Journal of Tropical Biomedicine. 2015;5(6):462-471. Available:https://doi.org/10.1016/j.apjtb.2015.03.004.
52. Ueda HU, Awanishi KK, Oriyasu MM. Effects of Ellagic Acid and 2-(2, 3, 6-trihydroxy-4-carboxyphenyl) ellagic Acid on Sorbitol Accumulation *in vitro* and *in vivo*. Biological and Pharmaceutical Bulletin. 2004;27(10):1584-1587.
53. Dias AMA, Rey-Rico A, Oliveira RA, Marceneiro S, Alvarez-Lorenzo C, Concheiro A, et al. Wound dressings loaded with an anti-inflammatory jucá (*Libidibia ferrea*) extract using supercritical carbon dioxide technology. The Journal of Supercritical Fluids. 2013;74(2014):34-45. Available:https://doi.org/10.1016/j.supflu.2012.12.007.
54. Kobayashi YT da S, Almeida VT de, Bandeira T, Alcântara BN de, Silva ASB da, Barbosa WLR, et al. Avaliação fitoquímica e potencial cicatrizante do extrato etanólico dos frutos de Jucá (*Libidibia ferrea*) em ratos Wistar. Brazilian Journal of Veterinary Research and Animal Science. 2015;52(1):34-40. Available:https://doi.org/10.11606/issn.1678-4456.v52i1p34-40. Portuguese.
55. Lopes N, Faccin-Galhardi LC, Espada SF, Pacheco AC, Ricardo NMPS, Linhares REC, et al. Sulfated polysaccharide of *Caesalpinia ferrea* inhibits herpes simplex virus and poliovirus. International Journal of Biological Macromolecules. 2013; 60(2013):93-99. Available:https://doi.org/10.1016/j.ijbiomac.2013.05.015.
56. Marques MMM, Morais SM De, Silva ARA Da, Barroso ND, Pontes Filho TR, Araujo FMDC, et al. Antiviral and Antioxidant activities of sulfated Galactomannans from plants of Caatinga Biome. Evidence-Based Complementary Alternative and Medicine. 2015;2015. Available:https://doi.org/10.1155/2015/591214.
57. Fernandes CPM, Machado C, Lopes TV, Cunha Filho N, Bretanha PR, Schons S, et al. Repellent Action of *Carapa guianensis* and *Caesalpinia ferrea* for flies species of Calliphoridae family. Ciência Rural. 2016; 46(5):867-870. Available:https://doi.org/10.1590/0103-8478cr20150727.
58. Comandolli-WyrepkowskiI CD, Jensen BB, Grafova I, Santos PA dos, Barros AMC, Soares FV, et al. Antileishmanial activity of extracts from *Libidibia ferrea*: development of *in vitro* and *in vivo* tests. Acta Amazonica. 2017;47(4):331-340. Available:https://doi.org/10.1590/1809-4392201700871.
59. Pedrosa T do N, Barros AO, Nogueira JR, Fruet AC, Rodrigues IC, Calcagno DQ, et al. Anti-wrinkle and anti-whitening effects of jucá (*Libidibia ferrea* Mart.) extracts. Archives of Dermatological Research; 2016. Available:https://doi.org/10.1007/s00403-016-1685-0.
60. Ferreira DQ, Ferraz TO, Araújo RS, Cruz RAS, Fernandes CP, Souza GC, et al. *Libidibia ferrea* (jucá), a traditional anti-inflammatory: A study of acute toxicity in adult and embryos zebrafish (*Danio rerio*). Pharmaceuticals. 2019;12(175):1-15. Available:https://doi.org/10.3390/ph12040175.
61. Oliveira AF, Batista JS, Paiva ES, Silva AE, Farias YJMD, Damasceno CAR, et al. Avaliação da atividade cicatrizante do jucá (*Caesalpinia ferrea* Mart. ex Tul. var. *ferrea*) em lesões cutâneas de caprinos. Revista Brasileira de Plantas Medicinais. 2010;12(3):302-310. Available:https://doi.org/10.1590/s1516-05722010000300007. Portuguese.
62. Ueda H, Tachibana Y, Moriyasu M, Kawanishi K, Alves SM. Aldose reductase inhibitors from the fruits of *Caesalpinia ferrea* Mart. Phytomedicine. 2001;8(5): 377-381. Available:https://doi.org/10.1078/0944-7113-00043.

63. Ferreira MA, Fernandes MM, da Silva W V, Bezerra IF, de Souza T, Pimentel M, et al. Chromatographic and spectrophotometric analysis of phenolic compounds from fruits of *Libidibia ferrea* Martius. Pharmacognosy Magazine. 2016;0:0. Available:https://doi.org/10.4103/0973-1296.179665.
64. Gonzalez FG. Estudo farmacognóstico e farmacológico de *Caesalpinia ferrea* Martius. Universidade de São Paulo, Doctoral's Thesis; 2005. Portuguese.
65. Silva LCN, Miranda R de CM de, Gomes E de B, Macedo AJ, Araújo JM de, Figueiredo RCBQ de, et al. Evaluation of combinatory effects of *Anadenanthera colubrina*, *Libidibia ferrea* and *Pityrocarpa moniliformis* fruits extracts and erythromycin against *Staphylococcus aureus*. Journal of Medicinal Plants Research. 2013;7(32):2358-2364. Available:https://doi.org/10.5897/JMPR2013.2597.
66. Frasson APZ, Bittencourt CF, Heinzmann BM. Caracterização físico-química e biológica do caule de *Caesalpinia ferrea* Mart. Revista Brasileira de Farmacognosia. 2003;13(1):35-39. Available:https://doi.org/10.1590/s0102-695x2003000100004. Portuguese.
67. Calandrini de Azevedo LF, Alves Ferreira TA, Melo KM, Porfírio Dias CL, Bastos CEMC, Santos SF, et al. Aqueous ethanol extract of *Libidibia ferrea* (Mart. ex Tul) L.P. Queiroz (juca) exhibits antioxidant and migration-inhibiting activity in human gastric adenocarcinoma (ACP02) cells. PLoS One. 2020;15(1):e0226979. Available:https://doi.org/https://doi.org/10.1371/journal.pone.0226979.
68. Pereira VS, Saraiva CRN, Rocha JE, Lopes J da C, Silva MK do N, Bandeira SMF, et al. Estudo químico, toxicidade e atividade antimicrobiana do óleo essencial de *Ocimum gratissimum*. Revista Interfaces: Saúde, Humanas e Tecnologia. 2014;2(Número especial): 2014. Portuguese.
69. Negri G. Diabetes melito: Plantas e princípios ativos naturais hipoglicemiantes. Revista Brasileira de Ciências Farmacêuticas. 2005;41(2):121-42. Available:https://doi.org/10.1590/S1516-93322005000200002. Portuguese.
70. Ferreira MRA, Soares LAL. *Libidibia ferrea* (Mart. ex Tul.) L. P. Queiroz: A review of the biological activities and phytochemical composition. Journal of Medicinal Plants Research. 2015;9(2):140-150. Available:https://doi.org/10.5897/jmpr2014.5706.
71. Souza AB de, Mara L, Souza S, Carlos J, Carvalho T, Maistro EL. No clastogenic activity of *Caesalpinia ferrea* Mart. (Leguminosae) extract on bone marrow cells of Wistar rats. Genetics and Molecular Biology. 2006;29(2):380-383.
72. Cunha AP, Ribeiro ACB, Ricardo NMPS, Oliveira AC, Dávila LSP, Cardoso JHL, et al. Polysaccharides from *Caesalpinia ferrea* seeds - Chemical characterization and anti-diabetic effects in Wistar rats. Food Hydrocolloids. 2017;65:68-76. Available:https://doi.org/10.1016/j.foodhyd.2016.10.039.
73. Guerra ACV de A, Soares LAL, Ferreira MRA, Araújo AA de, Rocha HA de O, Medeiros JS de, et al. *Libidibia ferrea* presents antiproliferative, apoptotic and antioxidant effects in a colorectal cancer cell line. Biomedicine and Pharmacotherapy. 2017;92:696-706. Available:https://doi.org/10.1016/j.biopha.2017.05.123.
74. Soares MRPS, Corrêa RO, Stroppa PHF, Marques FC, Andrade GFS, Corrêa CC, et al. Biosynthesis of silver nanoparticles using *Caesalpinia ferrea* (Tul.) Martius extract: Physicochemical characterization, antifungal activity and cytotoxicity. PeerJ. 2018;2018:1-16. Available:https://doi.org/10.7717/peerj.4361.
75. Pickler TB, Lopes KP, Magalhães SA, Krueger CMA, Martins MM, Filho VC, et al. Effect of *Libidibia ferrea* bark and seed in maternal reproductive and biochemical outcomes and fetal anomaly in rats. Birth Defects Research. 2019;111:863-871. Available:https://doi.org/10.1002/bdr2.1520.
76. Franco NH. Animal experiments in biomedical research: A historical perspective. Animals. 2013;3:238-273. Available:https://doi.org/10.3390/ani3010238.
77. Baumans V. The welfare of laboratory rats: In: The welfare of laboratory animals. vol. 2. Springer; 2007.

Available:https://doi.org/10.1007/978-1-4020-2271-5.

78. Perlman RL. Mouse Models of Human Disease: An Evolutionary Perspective. Evolution, Medicine & Public Health. 2016; eow014:170-176.
Available:https://doi.org/10.1093/emph/eow014.

79. Queiroz MLS, Justo GZ, Valadares MC, Pereira-da-Silva FRR. Evaluation of *Caesalpinia ferrea* extract on bone marrow hematopoiesis in the murine models of listeriosis and Ehrlich ascites tumor. Immunopharmacology and Immunotoxicology. 2001;23(3):367-382.
Available:https://doi.org/10.1081/IPH-100107337

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/77369

APÊNDICE 2

Protocolo da Revisão Sistemática na plataforma da PROSPERO

Animal review

1. * Review title.

Give the working title of the review. This must be in English. The title should have the interventions or exposures being reviewed and the associated health or social problems.

AN EVIDENCE-BASED REVIEW OF Libidibia ferrea (JUCÁ) ANTI-INFLAMMATORY ACTION ON IN VIVO AND IN VITRO STUDIES: PROTOCOL

2. Original language title.

For reviews in languages other than English, this field should be used to enter the title in the language of the review. This will be displayed together with the English language title.

UMA REVISÃO BASEADA EM EVIDÊNCIAS DA AÇÃO ANTI-INFLAMATÓRIA DA ESPÉCIE Libidibia ferrea (JUCÁ) DE ESTUDOS IN VIVO E IN VITRO: PROTOCOLO

3. * Anticipated or actual start date.

Give the date when the systematic review commenced, or is expected to commence.

01/02/2020

4. * Anticipated completion date.

Give the date by which the review is expected to be completed.

03/08/2020

5. * Stage of review at time of this submission.

Indicate the stage of progress of the review by ticking the relevant Started and Completed boxes. Additional information may be added in the free text box provided.
Please note: Reviews that have progressed beyond the point of completing data extraction at the time of initial registration are not eligible for inclusion in PROSPERO. Should evidence of incorrect status and/or completion date being supplied at the time of submission come to light, the content of the PROSPERO record will be removed leaving only the title and named contact details and a statement that inaccuracies in the stage of the review date had been identified.
This field should be updated when any amendments are made to a published record and on completion and publication of the review.

The review has not yet started: No

Review stage	Started	Completed
Preliminary searches	Yes	No
Piloting of the study selection process	Yes	No
Formal screening of search results against eligibility criteria	No	No
Data extraction	No	No
Risk of bias (quality) assessment	No	No
Data analysis	No	No

Provide any other relevant information about the stage of the review here (e.g. Funded proposal, protocol not yet finalised).

Review started with preliminary searches, like describes above, however, no data extraction was performed yet.

Review started with preliminary searches, like describes above, however, no data extraction was performed yet.

6. * Named contact.

The named contact acts as the guarantor for the accuracy of the information presented in the register record.

Nayanne Cristina Oliveira da Silva Almeida

Email salutation (e.g. "Dr Smith" or "Joanne") for correspondence:

Mrs Almeida

7. * Named contact email.

Enter the electronic mail address of the named contact.

nayannebiologia@yahoo.com.br

8. * Named contact address.

Enter the full postal address for the named contact.

General Rodrigo Octavio Jordao Ramos Avenue, number 1200, Coroado I, Manaus, Amazonas, Brasil.

69067-005

9. Named contact phone number

Enter the telephone number for the named contact, including international dialling code.

+ 55 92 995356141

10. * Organisational affiliation of the review.

Full title of the organisational affiliations for this review and website address if available. This field may be completed as ‘none’ if the review is not affiliated to any organisation.

UFAM (Federal University of Amazonas)

Organisation web address:

11. * Review team members and their organisational affiliations.

Give the personal details and the organisational affiliations of each member of the review team. Affiliation refers to groups or organisations to which review team members belong. **NOTE: email and country are now mandatory fields for each person.**

Mrs Nayanne Cristina Oliveira da Silva Almeida. UFAM (Universidade Federal do Amazonas)
Dr Silvania da Conceição Furtado. UFAM
Dr José Fernando Marques Barcellos. UFAM
Dr Emerson Silva Lima. UFAM
Mr Felipe Rodolfo Pereira da Silva. UFAM
Mrs Ana Lúcia Basílio Carneiro. UFPB (Universidade Federal da Paraíba)

12. * Funding sources/sponsors.

Give details of the individuals, organisations, groups or other legal entities who take responsibility for initiating, managing, sponsoring and/or financing the review. Any unique identification numbers assigned to the review by the individuals or bodies listed should be included.

None.

Grant number(s)

13. * Conflicts of interest.

List any conditions that could lead to actual or perceived undue influence on judgements concerning the main topic investigated in the review.

None

14. Collaborators.

Give the name, affiliation and role of any individuals or organisations who are working on the review but who are not listed as review team members.

15. * Review question.

Give details of the question to be addressed by the review, clearly and precisely.

Which part of the *L. ferrea* plant and what type of extract have the most evident anti-inflammatory effects in *in vivo* and *in vitro* experimental models of acute inflammation?

Context and rationale

Provide a brief description of the context and rationale of the review, including information on the relevance of your review for human health (max 250 words).

Inflammation is a process considered a natural phenomenon where cell recruitment occurs (eg monocytes, phagocytes and neutrophils), plasma proteins and cytokines in extra vascular tissues and may also act locally and in lesions. Cardinal signs are the result of this inflammatory process. They are: pain, heat, edema, flushing and loss of function. However, some damage may be caused by this process, such as rheumatoid arthritis. Inflammation can be classified into: acute inflammation and chronic inflammation, with

their respective marker and cellular characteristics (LAVETI et al., 2013; KATZUNG, MASTERS & TREVOR, 2014; ABBAS & LICHTMAN, 2015; MINISTRY OF HEALTH, 2016).

Studies have indicated that *L. ferra* extracts have antiulcerogenic action (BACCHI et al., 1995), antiinflammatory and antinociceptive activity (CARVALHO et al., 1996), cancer chemopreventive activity (NAKAMURA et al., 2002; NAKAMURA et al. , 2002), antioxidant and hepatoprotective action (BARROS et al., 2014), anti-wrinkle and anti-whitening effect (PEDROSA et al., 2016) and anti-leishmania action (COMANDOLI-VIEIRA et al., 2017). Several studies have been performed to prove the medicinal properties attributed to this plant (KOBAYASHI et al., 2015).

Although the pharmacological market represents a wide diversity of anti-inflammatory drugs, a search for new herbal products that mimic adverse effects still promotes research in this area (GHASEMIAN et al. 2016; RIBEIRO et al., 2018). Nevertheless, an *L. ferra* species was registered (2008) in the National List of Medicinal Plants of Interest to the Unified Health System (RENISUS) and since 2011, in the Form of Herbal Medicines of the Brazilian Pharmacopoeia (FNF). This demonstra governmental interest in this issue. In addition, it is a plant used in folk medicine (COSTA, GUILHON-SIMPLICIO and SOUZA, 2015) and focus of research from anti-inflammatory action by its various parts, and what is needed to provide an overview of how available to date, which probable will allows the reduce waste and optimize time in future searches.

In addition, this systematic review results may help researchers make the decision for more effective experimental designs that prioritize the reduction of animal and prescribe the sustainability principle.

16. * Searches.

Give details of the sources to be searched, and any restrictions (e.g. language or publication period). The full search strategy is not required, but may be supplied as a link or attachment.

An unrestricted electronic search will be conducted in February 2020 on PubMed, ScienceDirect, Web of Science, LILACS, Scopus, and in Gray literature: Google Scholar and ProQuest for articles published before 03, February 2020.

17. URL to search strategy.

Give a link to the search strategy or an example of a search strategy for a specific database if available (including the keywords that will be used in the search strategies).

https://www.crd.york.ac.uk/PROSPEROFILES/159934_STRATEGY_20191125.pdf

Alternatively, upload your search strategy to CRD in pdf format. Please note that by doing so you are consenting to the file being made publicly accessible.

Do not make this file publicly available until the review is complete

18. * Human disease modelled.

Give a short description of the disease, condition or healthcare domain being modelled.

Acute inflammation tends to be localized to the site of the lesion and the treatment of these acute

inflammatory conditions aims to provide the organism with no progression to chronic inflammation. Some situations such as trauma, contusions, injuries (involving tendons, muscles and ligament), other traumatic inflammation, tendonitis and other types of soft tissue rheumatism and mild and localized arthritis, such as leg, osteoarthritis, post traumatic pain and postoperatively, are inflammatory conditions in which drugs are used to alleviate or reduce the symptoms of inflammation, such as pain and edema.

19. * Animals/population.

Give summary criteria for the animals being studied by the review, e.g. species, sex, details of disease model. Please include details of both inclusion and exclusion criteria.

Inclusion criteria:

To be included in this systematic review, the studies should be perfomed in animal models by use of rats or mice (*Mus musculus* and *Rattus novergicus*) both sex. The disease model evaluated should be any characteristic inflammatory process such as paw edema, peritonitis, near edema, inflammatory bowel disease, other inflammatory models of the plant *L. ferrea*.

Exclusion criteria:

Studies performed *in silico* or *ex vivo* models should be excluded from this systematic review. In addition, studies in human being, genetic evaluation studies or cancer model studies also should not be included in this research.

20. * Intervention(s), exposure(s).

Give full and clear descriptions of the nature of the interventions or the exposures to be reviewed (e.g. dosage, timing, frequency). Please include details of both inclusion and exclusion criteria.

Inclusion criteria:

Test for anti-inflammatory action in in vivo and in vitro experimental models.

Treatment with extract plant from any part of *Libidibia ferrea* (*Caesalpinia ferrea*). The studies also should include the exact extract dose, administration route, as well as therapeutic scheme. Studies with results of in vitro that tested anti-inflammatory action of the species Libidibia ferrea or Caesalpinia ferrea regardless of the plant part (fruit, leaf and bark) used and type of extract, fraction and polysaccharides.

Exclusion criteria:

Treatment with any plant except from *L. ferrea* (*C. ferrea*) or based in interventions with the plant in noninflammatory processes. Phytochemical studies; morphological and anatomical studies; cytogenetic analysis; ethnobotanical studies

21. * Comparator(s)/control.

Where relevant, give details of the type(s) of control interventions against which the experimental condition(s) will be compared (e.g. another intervention or a non-exposed control group). Please include details of both inclusion and exclusion criteria.

Inclusion criteria:

Vehicle-treated control animals (salina or PBS) or standard drug for the based-inflammatory process used in the studies.

Exclusion criteria:

Studies without control group. Animals with previous systematic disease, auto-immune condictions, or any other condictions that may interfer the inflammatory model disease evaluated such as obesity, diabetes or pregnancy.

22. * Study designs to be included.

Give details of the study designs eligible for inclusion in the review. If there are no restrictions on the types of study design eligible for inclusion, or certain study types are excluded, this should be stated. Please include details of both inclusion and exclusion criteria.

Inclusion criteria:

Controlled studies with a separated control group which the results are represented by mean and SD values in tables, text or graphics.

Studies analyzing properties other than anti-inflammatory effect in the same study will only have data on anti-inflammatory action extracted.

Exclusion criteria:

Studies without a separated control group or with unavailable data mentioned in the studies.

23. Other selection criteria or limitations applied.

Give details of any other inclusion and exclusion criteria, e.g. publication types (reviews, conference abstracts), publication date, or language restrictions.

Literature reviews, systematic reviews or studies that did not respect the standards of Ethics Committee.

24. * Outcome measure(s).

Give detail of the outcome measures to be considered for inclusion in the review. Please include details of both inclusion and exclusion criteria.

Inclusion criteria:

Parameters of measures of inflammation: paw volume (mL, uL), cell number (leukocytes), MPO and MDA activity, cytokines (TNF-a e IL-1beta - pg/mL), exudate (cell number/mL).

Exclusion criteria:

Toxicity, cell viability outcomes, histological data.

25. N/A.

This question does not apply to systematic reviews of animal studies for human health submissions.

26. * Study selection and data extraction.

Procedure for study selection

Give the procedure for selecting studies for the review, including the screening phases (title and/or title-abstract and/or full-text), the number of researchers involved, and how discrepancies will be resolved.

Title and abstract screening will be performed blindly by these two authors, classifying studies as "yes", "no" or "perhaps" based on the information provided by the title and abstract. The full text will be collected for all articles rated "yes" or "perhaps", and additional researches where no abstract was available or the information available was inconclusive to reach a decision. The inclusion and exclusion criteria will be strictly applied to full text articles and where questions remain, efforts will be made to contact the study authors.

During screening, duplicates and articles that do not match with the survey question will be removed.

If there is a discrepancy we will rephrase the question or we will change the Systematic Review objectives.

Prioritise the exclusion criteria

Multiple exclusion criteria may apply to an abstract/paper, which can cause discrepancies between reviewers in the reason for exclusion recorded. To avoid this, it is helpful to prioritize the exclusion criteria (e.g. 1) not an animal study; 2) not a myocardial infarction model, etc.) and record the highest ranking applicable criterion as the reason for exclusion. Please sort the exclusion criteria defined in questions 19 to 24. If applicable, do so for each screening phase.

Title-abstract screening:

1. Studies performed in silico or ex vivo models
2. Studies in human being, genetic evaluation studies or cancer model studies
3. Treatment with any plant except from *L. ferrea* (*C. ferrea*)
4. Studies based in interventions with the plant *L. ferrea* in noninflammatory processes
5. Phytochemical studies; morphological and anatomical studies; cytogenetic analysis; ethnobotanical studies
6. Literature reviews, systematic reviews or studies that did not respect the standards of Ethics Committee

Full text-screening:

As above, with the addition of:

7. Studies without control group

8. Animals with previous systematic disease, auto-immune condictions, or any other condictions that may interfer the inflammatory model disease evaluated such as obesity, diabetes or pregnancy

9. Studies without a separated control group or with unavailable data mentioned in the studies

10. Toxicity, cell viability outcomes, histological data

Methods for data extraction

Describe methods for data extraction, including the number of reviewers performing data extraction, extraction of data from text and/or graphs, whether and how authors of eligible studies will be contacted to provide missing or additional data, etc.

Study selection and data collection will be performed by two independent blind authors (NA and SF), with discrepancies being resolved by discussion with the latter author (FB). Title and abstract screening will be performed blindly by these two authors, classifying studies as "YES", "NO" or "MAYBE" based on the information provided by the title and abstract.

Data will be taken from texts, tables, figures and supplementary data. And in case of doubt about the published results, details of the studies will be requested from the authors of the articles that will be contacted via email and the answers will be awaited up to 30 days.

Data to be extracted: study design

Specify the data to be extracted related to characteristics of the study design, e.g. controlled versus cross-over, number of experimental groups, etc.

In vivo and in vitro model and inflammation inducing by any agent, number of animals per group and/or cell type studies, Therapeutic scheme, Control used in the studies, Evaluated inflammatories parameters.

Data to be extracted: animal model

Specify the data to be extracted related to characteristics of the animal model, e.g. species, sex of the animals, etc.

The following data will be extracted: species, sex, age, weight, methods of inducing inflammation.

Data to be extracted: intervention of interest

Specify the data to be extracted related to characteristics of the intervention of interest, e.g. dose, timing, etc.

Dose extract and frequency of the administration, route of administration, timing of administration, vehicle.

Data to be extracted: primary outcome(s)

Define the primary outcome measure(s). For each outcome measure, specify in which format data will be extracted, including the eligible units of measurement, and data type (continuous/dichotomous). A description of any other manipulation or transformation of the extracted data that is planned may be included.

Measure from paw edema (mL, uL), Blood parameters (mg/dL). cell number count (x10/mL), MPO and MDA (U/uL; nmol/uL), cytokines (pg/mL).

Data to be extracted: secondary outcome(s)

Define the secondary outcome measure(s). For each outcome measure, specify in which format data will be extracted, including the eligible units of measurement, and data type (continuous/dichotomous). A description of any other manipulation or transformation of the extracted data that is planned may be included.

Weight of some organ / limb that was induced to edema (mg); Vascular permeability (nm)

Data to be extracted: other

Specify any other data or study characteristics to be extracted, e.g. bibliographical details, such as author, year and language.

Author, year of publication, journal, country of origin.

27. * Risk of bias and/or quality assessment.

State whether and how risk of bias and/or study quality will be assessed. Assessment tools specific for pre-clinical animal studies include SYRCLE's risk of bias tool and the CAMARADES checklist for study quality

No risk of bias and/or quality assessment planned

No

By use of SYRCLE's risk of bias tool

Yes

By use of SYRCLE's risk of bias tool adapted as follows:

No

By use of the CAMARADES checklist for study quality

Yes

By use of the CAMARADES checklist for study quality, adapted as follows:

No

Other criteria, namely

Yes

Evaluation of data (aggregated and individual animal data).

Studies feature.

Quantitative synthesis (effect measures, method of combining results, statistical heterogeneity, possibility of publication bias).

Method for risk of bias and/or quality assessment

Give the procedure for the risk of bias and/or quality assessment, including the number of reviewers involved, their contribution, and how discrepancies will be resolved.

Two calibrated authors will performe the bias assessment which any disagree will be solved by a third author.

28. * Strategy for data synthesis.

Planned approach

For each outcome measure, specify whether a quantitative or narrative synthesis is planned and how this decision will be made.

From the included studies, a narrative synthesis will be provided organized into the type of inflammation model, extract type and dose, part of the plant used in the experiment, route of administration of the extract and the inflammation-inducing agent, type of induced inflammation and form of measurement of the results of the inflammatory model.

In this study, in principle, we will perform the qualitative analysis. If the included (three at least) studies present sufficiently homogeneous and standardized data, a quantitative analysis will be performed by meta-analysis.

When applicable, for dichotomous results the risk ratio will be calculated or when for continuous results the mean difference will be calculated. If with the extracted data it is possible to perform a meta-analysis, the risk of bias with the other tests relevant to this methodology will be calculated.

If a meta-analysis is planned, please specify the following:

Effect measure

For each outcome measure, specify the effect measure to be used (e.g. mean difference, odds ratio etc.).

The data will be extracted and composed by continuous data represented as mean and SD values of variables measured in included studies. Therefore, to perform the comparisons will be used Mean Difference statistical measure validated by Z test with p0.05 as level of significance.

Effect models

For each outcome measure, specify the statistical model of analysis (e.g. random-effects or fixed-effect model).

Based on the level and significance of heterogeneity, the authors will use the Random-effects statistical model based on the calculations proposed by Dersimonian and Laird (1986) which the data will treated by the same weight in the calculations. On the other hand, we may use the Fixed-effect statistical model when heterogeneity be non-significant. This statistical model was proposed by Mantel and Haenszel (1959) and

treat the data as different weight being more trustworthy. All these calculations will be performed to validate the Mean Difference value observed with standardized deviation with p0.05 as significance level.

Heterogeneity

Specify the statistical methods to assess heterogeneity (e.g. I^2, Q). For further guidance please refer to the introduction and practical guide to pre-clinical meta-analysis.

The presence or absence of Heterogeneity (I^2) will be evaluated by the Cochran's X^2 test of the $?^2$ Q-based statistical test and evaluated by Higgins and Thompson (2002). I^2 will classified on increased I^2 (value 75%, p0.05), moderated I^2 (value between 50% and 75%, p0.05) and decreased I^2 (value 50%, p0.05). The I^2 also will be evaluated by visualization of funnel plot graphic for heterogeneity.

Other

Specify other details of the meta-analysis methodology (e.g. correction for multiple testing, correction for multiple use of control group).

Some studies performed by the same authors may bring data from the same control group. We will try to select one of the intervtion group and exclude the other, or we will combine al the relevant intervention groups in a single pair-wise comparison. Based in this situation, possibly found in the included studies, we will evaluate the better strategy suggested by Cochrane Handbook for solve this issue.

29. * Analysis of subgroups or subsets.

Subgroup analyses

Give any planned exploration of subgroups or subsets within the review. ‘None planned’ is a valid response if no subgroup analyses are planned.

No analysis of subgroups or subsets is planned at this time. After extracting the data, we will verify the possibility of it in relation to the species used in the animal model, the quantity and weight of the animals; regarding intervention: dose and plant part, route of administration; animal para volume measurement, cell count after treatment / intervention application. We will make a qualitative synthesis of the results for a better description of them.

Sensitivity

For each outcome measure, specify any sensitivity analyses you propose to perform.

An sensitivity analysis will be performed to verify the impact of each study in the pooled Mean Difference calcuation. To performe this evaluation, one single study will be excluded at the time to evaluate the influence of the study in the calculation.

Publication bias

Specify whether an assessment of publication bias is planned. If applicable, specify the method for assessment of publication bias.

The publication bias will be evaluated by means of Egger's linear regression test and Begg's test with p0.05.

In addition, the analyse of asymmetry in funnel plot graphic will be performe to verify the presence of

absence of publication bias.

30. * Review type.

Type of review

Animal model review
Yes

Experimental animal exposure review
No

Pre-clinical animal intervention review
No

31. Language.

Select each country individually to add it to the list below, use the bin icon to remove any added in error.
English

There is not an English language summary

32. * Country.

Select the country in which the review is being carried out from the drop down list. For multi-national collaborations select all the countries involved.

Brazil

33. Other registration details.

List other places where the systematic review protocol is registered. The name of the organisation and any unique identification number assigned to the review by that organisation should be included.

This systematic review will be registered only in PROSPERO database and will be part of the partial results

from a thesis project.

34. Reference and/or URL for published protocol.

Give the citation and link for the published protocol, if there is one.

No applicable

Give the link to the published protocol.

Alternatively, upload your published protocol to CRD in pdf format. Please note that by doing so you are consenting to the file being made publicly accessible.

No I do not make this file publicly available until the review is complete

Please note that the information required in the PROSPERO registration form must be completed in full even if access to a protocol is given.

35. Dissemination plans.

Give brief details of plans for communicating essential messages from the review to the appropriate

audiences.

After the possible registration of the protocol in the PROSPERO database, the systematic review steps will be continued and after its completion, a journal will be chosen, according to the developed theme, for the complete publication of the manuscript. In addition, the full article will be added to the results of the thesis project of the first author of this protocol. If the data can generate information that helps in reducing the number of animals, or replacing them in research will be generated a letter to the editor of SYRCLE as a suggestion to be analyzed this possibility.

Do you intend to publish the review on completion?

Yes

36. * Keywords.

Give words or phrases that best describe the review. Separate keywords with a semicolon or new line.

Anti-inflammatory

In vivo

In vitro

Systematic review

37. Details of any existing review of the same topic by the same authors.

Give details of earlier versions of the systematic review if an update of an existing review is being registered, including full bibliographic reference if possible.

38. * Current review status.

Review status should be updated when the review is completed and when it is published.
Please provide anticipated publication date

Review_Ongoing

39. Any additional information.

Provide any further information the review team consider relevant to the registration of the review.

This review is being conducted as part of the design of a doctorate project that aims verify the potential therapeutic use of *L. ferrea* in animals.

40. Details of final report/publication(s) or preprints if available.

This field should be left empty until details of the completed review are available OR you have a link to a preprint. Give the full citation for the preprint or final report or publication of the systematic review.

Give the link to the published review.

APÊNDICE 3

Quantitativo de artigos selecionados em cada etapa da realização da revisão Sistemática

BASE DE DADOS	PALAVRAS-CHAVE	09.2019 (piloto)	PALAVRAS-CHAVE	02.2020 (busca)	PALAVRAS-CHAVE	03.2021 (atualização)
PUBMED	("libidibia ferrea" OR libidibia OR "caesalpinia ferrea" OR caesalpinia OR juca OR "pau-ferro") AND ("anti-inflammatory activity" OR "anti-inflammatory activities" OR "anti-inflammatory property" OR "anti-inflammatory properties" OR "anti-inflammatory effect" OR "anti-inflammatory effects" OR "anti-inflammatory action" OR "anti-inflammatory actions")	**40**	("libidibia ferrea" OR libidibia OR "caesalpinia ferrea" OR caesalpinia OR juca OR "pau-ferro") AND ("anti-inflammatory activity" OR "anti-inflammatory activities" OR "anti-inflammatory property" OR "anti-inflammatory properties" OR "anti-inflammatory effect" OR "anti-inflammatory effects" OR "anti-inflammatory action" OR "anti-inflammatory actions")	**41**	("libidibia ferrea" OR libidibia OR "caesalpinia ferrea" OR caesalpinia OR juca OR "pau-ferro") AND ("anti-inflammatory activity" OR "anti-inflammatory activities" OR "anti-inflammatory property" OR "anti-inflammatory properties" OR "anti-inflammatory effect" OR "anti-inflammatory effects" OR "anti-inflammatory action" OR "anti-inflammatory actions")	**43**
SCIENCE DIRECT (via periódico da CAPES)	("libidibia ferrea" OR libidibia OR "caesalpinia ferrea" OR caesalpinia OR juca OR "pau-ferro") AND ("anti-inflammatory activity" OR "anti-inflammatory property" OR "anti-inflammatory effect" OR "anti-inflammatory action")	**240,** sendo 50 artigos de revisão, 2 enciclopédias, 15 capítulos de livro e **138 artigos**	("libidibia ferrea" OR libidibia OR "caesalpinia ferrea" OR caesalpinia OR juca OR "pau-ferro") AND ("anti-inflammatory activity" OR "anti-inflammatory property" OR "anti-inflammatory effect" OR "anti-inflammatory action")	**258,** *Encyclopedia (2); Book chapters (16); Conference abstracts (6); Correspondence (1); Mini reviews(3); Short communications (8); Other (19);* Review articles (58) e **145 artigos**	("libidibia ferrea" OR "caesalpinia ferrea") AND ("anti-inflammatory activity" OR "anti-inflammatory property" OR "anti-inflammatory effect" OR "anti-inflammatory action")	**39,** Review article (6), Short communication (3), Other (1) e **Research article (29)**
WEB OF SCIENCE (via periódico da CAPES)	("libidibia ferrea" OR libidibia OR "caesalpinia ferrea" OR caesalpinia OR juca OR "pau-ferro") AND ("anti-inflammatory activity" OR "anti-inflammatory activities" OR "anti-inflammatory property" OR "anti-inflammatory properties" OR "anti-inflammatory effect" OR "anti-inflammatory effects" OR "anti-inflammatory action" OR "anti-inflammatory actions")	**54**	("libidibia ferrea" OR libidibia OR "caesalpinia ferrea" OR caesalpinia OR juca OR "pau-ferro") AND ("anti-inflammatory activity" OR "anti-inflammatory activities" OR "anti-inflammatory property" OR "anti-inflammatory properties" OR "anti-inflammatory effect" OR "anti-inflammatory effects" OR "anti-inflammatory action" OR "anti-inflammatory actions")	**55**, sendo review (3); meeting abstract (2); e **50 artigos**	("libidibia ferrea" OR libidibia OR "caesalpinia ferrea" OR caesalpinia OR juca OR "pau-ferro") AND ("anti-inflammatory activity" OR "anti-inflammatory activities" OR "anti-inflammatory property" OR "anti-inflammatory properties" OR "anti-inflammatory effect" OR "anti-inflammatory effects" OR "anti-inflammatory action" OR "anti-inflammatory actions")	**56**, Review (3), Meeting abstract (2), **Article (51)**
LILACs	1ª pesquisa: ("libidibia ferrea" OR libidibia OR "caesalpinia ferrea" OR caesalpinia OR juca OR "pau-ferro") AND ("anti-inflammatory activity" OR "atividade anti-inflamatória" OR "actividad antiinflamatoria") – 23 artigos 2ª pesquisa: ("libidibia ferrea" OR libidibia OR "caesalpinia ferrea" OR caesalpinia OR juca OR "pau-ferro") AND ("anti-inflammatory property" OR "propriedade anti-inflamatória" OR "propriedad antiinflamatoria") – 1 artigo 3ª pesquisa: ("libidibia ferrea" OR libidibia OR "caesalpinia ferrea" OR caesalpinia OR juca OR "pau-ferro") AND ("anti-inflammatory effect" OR "efeito anti-inflamatório" OR "efecto antiinflamatorio") – 7 artigos 4ª. Pesquisa: ("libidibia ferrea" OR libidibia OR "caesalpinia ferrea" OR caesalpinia OR juca OR "pau-ferro") AND ("anti-inflammatory action" OR "ação anti-inflamatória" OR "acción antiinflamatoria") – 2 artigos	**33**	1ª pesquisa: ("libidibia ferrea" OR libidibia OR "caesalpinia ferrea" OR caesalpinia OR juca OR "pau-ferro") AND ("anti-inflammatory activity" OR "atividade anti-inflamatória" OR "actividad antiinflamatoria") – 24 artigos 2ª pesquisa: ("libidibia ferrea" OR libidibia OR "caesalpinia ferrea" OR caesalpinia OR juca OR "pau-ferro") AND ("anti-inflammatory property" OR "propriedade anti-inflamatória" OR "propriedad antiinflamatoria") – 1 artigo 3ª pesquisa: ("libidibia ferrea" OR libidibia OR "caesalpinia ferrea" OR caesalpinia OR juca OR "pau-ferro") AND ("anti-inflammatory effect" OR "efeito anti-inflamatório" OR "efecto antiinflamatorio") – 7 artigos 4ª. Pesquisa: ("libidibia ferrea" OR libidibia OR "caesalpinia ferrea" OR caesalpinia OR juca OR "pau-ferro") AND ("anti-inflammatory action" OR "ação anti-inflamatória" OR "acción antiinflamatoria") – 2 artigos	**34**	1ª pesquisa: ("libidibia ferrea" OR libidibia OR "caesalpinia ferrea" OR caesalpinia OR juca OR "pau-ferro") AND ("anti-inflammatory activity" OR "atividade anti-inflamatória" OR "actividad antiinflamatoria") – 24 artigos 2ª pesquisa: ("libidibia ferrea" OR libidibia OR "caesalpinia ferrea" OR caesalpinia OR juca OR "pau-ferro") AND ("anti-inflammatory property" OR "propriedade anti-inflamatória" OR "propriedad antiinflamatoria") – 1 artigo 3ª pesquisa: ("libidibia ferrea" OR libidibia OR "caesalpinia ferrea" OR caesalpinia OR juca OR "pau-ferro") AND ("anti-inflammatory effect" OR "efeito anti-inflamatório" OR "efecto antiinflamatorio") – 7 artigos 4ª. Pesquisa: ("libidibia ferrea" OR libidibia OR "caesalpinia ferrea" OR caesalpinia OR juca OR "pau-ferro") AND ("anti-inflammatory action" OR "ação anti-inflamatória" OR "acción antiinflamatoria") – 2 artigos	**35 (25 + 1 + 7 + 2)**
SCOPUS (via periódico da CAPES)	("libidibia ferrea" OR libidibia OR "caesalpinia ferrea" OR caesalpinia OR juca OR "pau-ferro") AND ("anti-inflammatory activity" OR "anti-inflammatory property" OR "anti-inflammatory effect" OR "anti-inflammatory action")	**74**	("libidibia ferrea" OR libidibia OR "caesalpinia ferrea" OR caesalpinia OR juca OR "pau-ferro") AND ("anti-inflammatory activity" OR "anti-inflammatory property" OR "anti-inflammatory effect" OR "anti-inflammatory action")	**76** Review Show preview for (6); Book Chapter Show preview for (1) Conference Paper (1), **68 artigos**	("libidibia ferrea" OR libidibia OR "caesalpinia ferrea" OR caesalpinia OR juca OR "pau-ferro") AND ("anti-inflammatory activity" OR "anti-inflammatory property" OR "anti-inflammatory effect" OR "anti-inflammatory action")	**76**, Review (6), Booke chapter (1), Conference paper (1), **Article (68)**
GOOGLE SCHOLAR	("libidibia ferrea" OR libidibia OR "caesalpinia ferrea" OR caesalpinia OR juca OR "pau-ferro") AND ("anti-inflammatory activity" OR "anti-inflammatory activities" OR "anti-inflammatory property" OR "anti-inflammatory properties" OR "anti-inflammatory effect" OR "anti-inflammatory effects" OR "anti-inflammatory action" OR "anti-inflammatory actions")	5.390 resultados (considerar até a 5ª página) **50 referências**	("libidibia ferrea" OR "caesalpinia ferrea" OR juca OR "pau-ferro") AND ("anti-inflammatory activity" OR "anti-inflammatory property" OR "anti-inflammatory effect" OR "anti-inflammatory action")	447 resultados **110 artigos** (até página 11)	("libidibia ferrea" OR "caesalpinia ferrea" OR juca OR "pau-ferro") AND ("anti-inflammatory activity" OR "anti-inflammatory property" OR "anti-inflammatory effect" OR "anti-inflammatory action")	502 resultados **110 artigos** (até a página 11)
PROQUEST	("libidibia ferrea" OR libidibia OR "caesalpinia ferrea" OR caesalpinia OR juca OR "pau-ferro") AND ("anti-inflammatory activity" OR "anti-inflammatory activities" OR "anti-inflammatory property" OR "anti-inflammatory properties" OR "anti-inflammatory effect" OR "anti-inflammatory effects" OR "anti-inflammatory action" OR "anti-inflammatory actions")	**124**	(libidibia NEAR/2 ferrea OR caesalpinia NEAR/2 ferrea OR juca OR pau-ferro) AND (anti-inflammatory NEAR/2 activity OR anti-inflammatory NEAR/2 property OR anti-inflammatory NEAR/2 effect OR anti-inflammatory NEAR/2 action)	**35**	(libidibia NEAR/2 ferrea OR caesalpinia NEAR/2 ferrea OR juca OR pau-ferro) AND (anti-inflammatory NEAR/2 activity OR anti-inflammatory NEAR/2 property OR anti-inflammatory NEAR/2 effect OR anti-inflammatory NEAR/2 action)	**145**
		513		**483 (609 sem remoção)**		**481 (504 sem remoção)**

APÊNDICE 4

Artigo: Revisão Sistemática publicado na PloS One

Libidibia ferrea (jucá) anti-inflammatory action: A systematic review of *in vivo* and *in vitro* studies

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RESEARCH ARTICLE

Libidibia ferrea (jucá) anti-inflammatory action: A systematic review of *in vivo* and *in vitro* studies

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Abstract

Libidibia ferrea (Mart. ex Tul.) L. P. Queiroz (jucá) is a plant extensively used in the Brazilian folk medicine for the treatment of the inflammatory process. Primary studies have focused on the verification of these biological activities, highlighting the role of this plant in inflammatory conditions. This systematic review aimed to critically establish which part of the plant and what type of plant extract present the highest evidence of anti-inflammatory activity as *in vivo* and *in vitro* experimental models. This study has followed the recommendations by PRISMA and was registered in the PROSPERO database under number CRD42020159934. The literature review was carried out in several medical and scientific databases (Google Scholar, LILACS, ProQuest, PubMed, ScienceDirect, Scopus and Web of Science) in studies published up to February 2020 and updated on March 2021. No language restriction was made to this search. Eligibility criteria were adopted instead. The risk of bias was evaluated through SYRCLE's RoB tool for the *in vivo* studies. 609 studies were initiated to identify the whole and the subsequent steps of screening. 13 studies remained in the results (10 *in vivo* and 3 *in vitro*). In most studies the risk of bias was low or unclear. The high risk of bias was related to the risk of attrition and reporting bias. The fruit and the aqueous extract were identified as the most used in the studies carried out on the qualitative analysis and the results of the *in vivo* and *in vitro* studies were conducive to the anti-inflammatory action, a meta-analysis could not be performed due to heterogeneity between studies and the potential risk of bias to estimate the side effects. Therefore, the implementation of *in vivo* studies following the international guidelines could collaborate with analyses of the anti-inflammatory effect of jucá.

Introduction

Libidibia ferrea (Mart. ex Tul.) L. P. Queiroz, popularly referred to as pau-ferro (Brazil) or jucá (Amazon region) [1], belongs to the Fabaceae family [2]. This is a native arboreal plant

Competing interests: The authors have declared that no competing interests exist.

occurring throughout the North [3] and Northeast [2, 4, 5] of Brazil widely used as a medicinal plant.

Several published studies have demonstrated the medicinal properties assigned to *L. ferrea* basis related to the extensive use of this plant in folk medicine [6], cancer chemopreventive [4, 7], hepatoprotective and antioxidant [8], anti-whitening and antiwrinkle effects [9], antileishmanial activity [10], healing, gastroprotective, antioxidant and antiulcerogenic [11] as well as analgesic and anti-inflammatory properties [12].

As described above, many studies have been conducted with *L. ferrea* in order to verify and confirm its biological properties. Among these studies, some have been performed in *in vivo* models [9, 12] and *in vitro* models [4]. Thus, aiming at implementing future research with less waste of resources and more optimization of time, retrospective, and systematic research help in providing the methodologies employed and results obtained.

This systematic review intends to organize and analyze scientific evidence of anti-inflammatory actions by *Libidibia ferrea* or *Caesalpinia ferrea* developing *in vivo* and *in vitro* studies. This systematic review was carried out to find answers to the following questions: Which part of the *L. ferrea* plant and what type of extract have the highest evidence of anti-inflammatory effects on acute inflammation using *in vivo* and *in vitro* experimental models?

Which part of the *L. ferrea* plant and what type of extract have the most evident anti-inflammatory effects *in vivo* and *in vitro* experimental models of acute inflammation?

Methods

This Systematic Review followed the recommendations by Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) [S1 and S2 Tables] and was registered in the Prospective Registry of Systematic Reviews (PROSPERO) database under protocol number CRD42020159934 (https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=159934).

Search strategy

A search strategy was first performed on February 3, 2020, and updated on March 12, 2021 in the following databases: Google, Scholar, ProQuest, LILACS, PubMed, ScienceDirect, Scopus and Web of Science. The manual research was carried out in the articles included identifying a possible article that was not screened in the electronic search.

The descriptor used was divided into two groups 1. *Libidibia ferrea* OR *Caesalpinia ferrea* (intervention group) and 2. anti-inflammatory effect OR anti-inflammatory action OR anti-inflammatory properties OR anti-inflammatory. Boolean operators (AND and OR) were used to make the combinations (Search strategy) [S1 Appendix]. There was no language restriction in the systematic search from which all the references of the included studies were screened for identifying potential additional study. References were organized in Microsoft Excel™ and the duplicates were removed in the same program.

Study selection

Screening based on the information in titles and abstracts were performed by two independents blind authors classified in "yes", "no" or "maybe". Titles and abstracts were first read, and then, the full article. Both steps were screened applying the eligibility criteria.

Two authors (NCOSA, SCF), independently, selected the studies and collected the data. Studies showing discrepancies were settled in discussions with two other authors (ALBC, ESL).

Eligibility criteria

PICOS criteria were established as 1. Population: Animals (*Rattus novergicus* or *Mus musculus*) or *in vitro* test; 2. Intervention: Treatment with extracts from different parts of the plant in *in vivo* and/or *in vitro* models; 3. Control: negative (saline or PBS) and positive (standard drug) controls; 4. Outcome: anti-inflammatory action; 5. Study type: experimental studies.

The inclusion criteria were published articles with non-restricted time or language; articles with titles and abstracts accorded to the research questions; *In vivo* and *in vitro* studies, which tested the anti-inflammatory action of *L. ferrea* or *Caesalpinia ferrea*, regardless of the tested part of the plant and the extract type. In studies, which analyzed other effects, in addition to the anti-inflammatory activity, only such data were extracted: studies that described mean and standard derivation in tables, graphs, or embedded in the texts.

The exclusion criteria for title-abstract screening were:

1. Literature reviews, systematic reviews or studies, which have not complied with the standards of Ethics Committee;
2. Studies in human beings, genetic evaluation studies or cancer model studies;
3. Phytochemical studies; morphological and anatomical studies; cytogenetic analysis; ethnobotanical studies;
4. Studies performed *in silico* or *ex vivo* models;
5. Treatment with any plant except from the *L. ferrea* (*C. ferrea*);
6. Studies based on interventions with the plant *L. ferrea* in non-inflammatory processes;
7. Animals with previous systematic disease, auto-immune conditions, or any other conditions, which might interfere in the inflammatory model disease evaluated such as obesity, diabetes, or pregnancy;
8. Studies without control group;
9. Toxicity, cell viability outcomes, histological data;
10. Studies without a separated control group or with unavailable data mentioned in the studies.

Besides, book chapters; encyclopedias; literature reviews; systematic reviews; conference abstracts; short communications were excluded.

Regarding the criteria related to the animal population, studies, which used mice or rats of both sexes were included. With respect to the acute inflammation model those related to paw and/or ear edema, peritonitis, vascular permeability, formally-induced paw licking, zymosan-induced arthritis, excisional wound, and wound dressing were included.

Data collection process

Data were collected, using customized data extraction in Microsoft Excel™ with the following data: First author; Year of publication; Publishing journal; Country of origin/ collection location/ or period of the year; Plant part; Extract type; Extract dose and route of administration; Type of inflammation model or type of assay; *In vivo* or *in vitro* model; Number of animals for group and cell type; Therapeutic scheme; Control used; Evaluated parameters; Results.

The variables analyzed for the two models (*in vivo* and *in vitro*) were plant collection location; plant part; extract type; inflammatory cytokines levels (TNF-α, IL-1); nitrate. Data such as mean, standard deviation and percentage were also collected.

The variables analyzed for *in vivo* model were: extract dose; route of administration; animal model (rat or mice); the number of animals for group and number of groups; paw edema volume; area under the curve (paw edema); edema ear weight; polymorphonuclear leukocyte count (PMNL); myeloperoxidase levels (MPO); malondialdehyde levels (MDA); glutathione levels; Release of vasoactive amines; peripherical inflammatory pain; plasm leakage; mast cells counting; prostaglandin E_2 (PGE_2); wound diameter / ulcerated area.

The variables analyzed for *in vitro* model were extract concentration; type of cell; cell assay type, control group, treatment.

Risk of bias in individual studies

Risk of bias was conducted and evaluated by two reviewers (NCOSA, SCF). The Systematic Review Center for Laboratory animal Experimentation (SYRCLE) containing 10 entries related to six types of bias to analyze the methodological quality was used. These entries were selection bias (sequence generation, baseline characteristics, and allocation concealment); performance bias (random housing and blinding); detection bias (random outcome assessment and blinding); attrition bias (incomplete outcome data), reporting bias (selective outcome reporting) and other biases [13]. Bias information was organized in an Excel spreadsheet with the related judgments: "yes" indicates a low risk of bias, "no" indicates a high risk of bias and "unclear" indicates not sufficient information reported.

Synthesis methods

Studies, which attended the eligibility criteria were included for narrative synthesis, thus a summarization of the collected data and descriptive analysis of the results. The data synthesis is presented at the results session. Some authors were contacted to supply some unclear or missing data.

In addition to the use of SYRCLE as described above, indirectness domain was also used to analyze the quality of evidence, following the GRADE for *in vivo* studies [14]. In addition, Grades of Recommendation, Assessment, Development and Evaluation Working Group Guideline Development Tool (GRADEpro GDT) [15] was used.

Extraction and summarized data from *in vitro* studies were described in Tables 4 and 5. To the best of our knowledge, no checklist to analyze the risk of bias validated to *in vitro* studies exists [16, 17]. Thus, there is an evaluation tool to assess the *in vitro* toxicity studies using the Science in Risk Assessment and Policy (SCIRAP tool) [18].

Results

Study selection

Exactly 609 studies were screened in the initial electronic search, and, after a previous screening 126 reports were excluded: encyclopedia (n = 2), book chapter (n = 16), mini reviews (n = 3), short communications (n = 8), conference abstract (n = 6), correspondence (n = 1), review article (n = 58), review (3), meeting abstract (2), review show preview for (n = 6), book chapter show preview for (n = 1), conference paper (n = 1), other (n = 19) were excluded. After this, 483 studies were considered eligible to follow up on the systematic review. From those 338 studies were from the database and 145 from grey literature. Duplicates were also removed and, after reading titles and abstracts, 17 studies were considered for full-text screening. Ten studies were considered eligible according to the eligibility criteria after the consensus by the reviewers (Fig 1).

However, this Systematic Review was actualized using the criteria described above. With this update, the research recovered 504 articles, of which 23 were excluded, there remaining 481 studies. After the removal of duplicates, 318 followed the first stage (title and abstract screening). Then, 15 remain for full-text screening. Of these 15, 10 have already been identified in the first search (February 2020). And were identified and included three more different studies (one *in vivo* and two *in vitro*) were identified and included. Two studies were excluded, in a total of 13 studies for the quantitative analysis (Fig 1).

At the first search, seven articles were included in the second phase (full article screening) and seven studies were excluded because: one presents the same genus, but it was a different species (*Caesalpinia sapan*) (reason 1), another study was a thesis which the article had already been included for data extraction and analysis (reason 2). Another study referred to a chemical characterization of *L. ferrea* (reason 3). Two reports were an ethnobotanical study (reason 4), one study presents the hypoglycemic activity of the plant (reason 5), and one study used the powder for the anti-inflammatory tests and not the extract (reason 6). The last two articles were the same that appeared at the update carried out on March, 2021 and they were also excluded (Fig 1).

Study characteristics

The year of publication of the 13 articles ranged from 1996 to 2020 (Table 1). And in all studies, Brazil was the country where the plant was collected. Eleven of the studies were written in English and two in Portuguese.

Concerning studied part of the plant it was noted that: six authors used fruits [12, 23–26, 28], one used the bark [19], three used the stem bark [20, 21, 29], two used leaves [22, 30] and one used seeds [27] (Table 1).

Therefore, as regards the type of extract: the aqueous extract was performed in five studies [12, 19, 22, 25, 26], one used ethanolic extract [23], another acetonic extract [19] two rich-polysaccharides extracts [20, 21], one used four different fractions from hydroalcoholic extract CE20, CE40, CE60 e CE80% [25], one used hydroalcoholic extract [29], and one used dry extract [30]. Polysaccharides fractions [24], lipid portion of acetone extract [27], fraction 80 (F80) [26], ethyl acetate and aqueous fraction [25] and supercritical fluid [28] (Table 1).

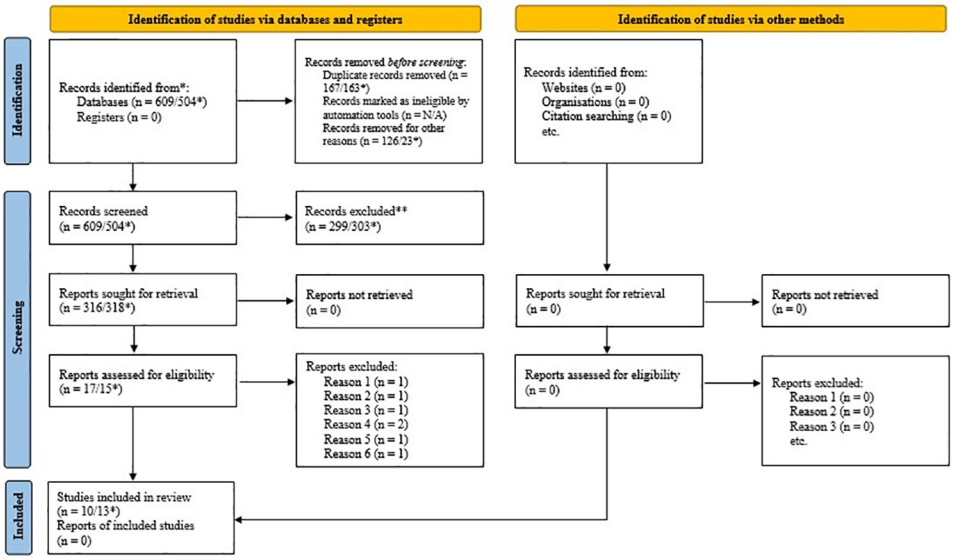

Fig 1. Flow diagram describing the study selections from literature searching. *Update values.

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Table 1. Plant part and type of extract from *L. ferrea* overview used *in vivo* and *in vitro* studies.

Plant part	Type of extract	Reference	Study type
Bark	Aqueous	[19]	*In vivo*
	Acetone-water		
Stem bark	Polysaccharide-rich (TPL-Cf)	[20]	
	Rich-polysaccharide	[21]	
Leaves	Crude aqueous	[22]	
Pods (peels and seeds)	Ethanol	[23]	
Pods (devoid of seeds)	TPL, FI, FII e FIII	[24]	
Fruits (var. *ferrea*)	Aqueous Crude	[25]	
	CE20, CE40, CE60 e CE80		
	Ethyl acetate fraction (EAF)		
	Aqueous fraction (AqF)		
Pods (var. *parvifolia*)	Crude aqueous	[26]	
	F80		
Fruits	Crude aqueous	[12]	
Mature seeds	Lipidic portion of *Libidibia ferrea*	[27]	
Fruits	Supercritical fluid extraction	[28]	*In vitro*
Stem bark	Hydroalcoholic	[29]	
Leaves	Dry (ELFLF)	[30]	

TPL-Cf (Total polysaccharides of *C. ferrea* barks); TPL (Total polysaccharides); FI to FIII (major polysaccharide fractions); CE 20.0–80.0% (Hydroalcoholic fractions of 20.0–80.0% ethanol); F80 (partially purified fraction); ELFLF (Lyophilizes extract from *L. ferrea* leaves).

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It was observed that of the 10 *in vivo* studies included, the animals used in the experiments were Swiss mice (n = 6) [19, 21, 23, 25–27] and Wistar rats (n = 4) [12, 20, 22, 24]. Regarding the inflammation model used in the studies, there was a variety of these, and three studies performed more than one inflammation model [21, 23, 24] to verify the anti-inflammatory action of *L. ferrea* (Table 2).

The most used route of administration for treatment was the orally (n = 8) [12, 19, 21–23, 25–27]. Other types of routes of administration present in the other studies were intravenous (n = 2) [21, 24], topical [20]. In all preclinical experimental models, anti-inflammatory activity was suggested independently of the plant and it was independent of the animal model, part of the plant and the type of extraction or fraction used in the studies. This potential action was observed through a reduction/inhibition of paw edema volume [12, 21, 24], reducing/migration from the number of PMNL [19, 21–26], reduction of ear edema [23], inhibition of vascular permeability [20, 23], reduction in the number of licks [27], reduction of wound area [20], evaluation of inflammatory mediators [20–22, 25] (Table 3).

In the *in vitro* studies, the predominant cell type was the RAW cells 264.7 macrophages [28, 29], Balb/3T3 clone A31 fibroblasts [28], BV2 microglial cell [30], monocytes of human peripheral blood [29] (Table 4). The identification of anti-inflammatory action was verified by identifying inflammatory mediators (Table 5).

Risk of bias in individual studies

The outcomes evaluate the risk of bias in *in vivo* studies. Therefore, when there was a similarity between the studies, the analysis was executed once, and when there was any different outcome, this was separably analyzed (Table 6).

Following the SYRCLE's RoB tool, the following risk of bias presents: eight studies with unclear selection bias risk [12, 19–24, 26] since they only described that they were divided into

Table 2. Data from *in vivo* studies included.

Author / Year	Country of Origin / Collection Country / Year Period	Route	Dose	Control group	Animal	Sex	Weight (g)	Age (days)	n. / group	Group
Carvalho et al., 1996 [12]	Brazil / Icoaracy- Belém (PA) / Mar-1988	Oral	300 mg/kg	Indomethacin	Wistar	Both	140–170	?	13	3
Freitas et al., 2012 [26]	Brazil / Ibimirim (PE) / Aug-2006	Oral	100 mg/kg	Saline, dexamethasone, indomethacin, piroxicam	Swiss	Both	approx. 25	approx. 50	6	6
Lima et al., 2012 [23]	Brazil / Barbalha (CE) / Jun-2007	Oral	12.5, 25, 50 mg/kg	Saline, indomethacin	Swiss	Male	25 ± 5	50	7	5, 3, 3
Pereira et al., 2012 [24]	Brazil / District of Custódio-Quixadá (CE)	Intravenous	0.01, 0.1, 1 mg/kg	Saline, indomethacin	Wistar	?	150–200	?	6	?
				Saline						
				Saline, methisergide, indomethacin, L-NAME						
De Araújo et al., 2014 [19]	Brazil / PE	Oral	50, 100, 200 mg/kg	DMSO, indomethacin	Swiss	Female	25–35	?	6	11
Sawada et al., 2014 [27]	Brazil / Joanes, Salvaterra, Marajó Island (PA) / 2011	Oral	10 mg/kg	Saline, indomethacin	Swiss	Male	30–35	56	10	5
Pereira et al., 2016 [20]	Brazil / Custódio-Quixadá District (CE) / May-2013 and Feb-2014	Topic	0.025–0.1%	Saline, collagenase ointment	Wistar	Male	180–200	61	16	6
Falcão et al., 2019 [22]	Brazil / Caatinga Biome in Recife (PE) / Sept-2014	Oral	100, 200, 300 mg/kg	Normal (without zymosan treated with 50 mg saline 0.9%) / zymosan + salina) / diclofenac (100 mg/kg)	Wistar	Male	150 ± 250	?	6	6
Falcão et al., 2019 [25]	Brazil / Limoeiro (PE)	Oral	50, 100, 200 mg/kg	Saline, diclofenac	Swiss	Male	40 ± 2.0	60	6	9
Holanda et al., 2020 [21]	Brazil / District of Custódio (Quixadá/CE)	Intravenous	0.001, 0.01, 0.1 and 1 mg/kg	NaCl and zymosan	Swiss	Female	25–35	?	8	?
		Oral	1 mg/kg	Ascorbic acid, zymosan						

?: data not found; mg/kg: milligram/kilo; g: gram; NaCl: sodium chloride; approx.: approximately.

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groups, not stating whether they have been randomized or not. The other two studies described that the animals have been randomized but have not informed the method used to take such step [25, 27]. They were, then, judged as having a low risk of bias (1).

All *in vivo* studies present a low risk of bias regarding baseline characteristics, in other words, the animals were induced to the inflammatory condition after which, they were given treatment [12, 19, 21–26] or induced to wound [20] before treatment application (2). As to allocation concealment the risk was considered unclear for all *in vivo* studies for lack of sufficient information with respect such concealment (3).

Concerning the risk of performance bias, all studies have been categorized as low risk of bias. This type of bias refers to random housing as they have been maintained in baseline conditions before the beginning of the experiment, such as the provision of water and food (4). Yet, as to blinding (5) there was no evidence as to whether the researchers who manipulated the animals had any knowledge of what group was the control or the treatment group.

Regarding detection bias, both the random evaluation of the outcome (6) and blinding (7) were described as uncertain, since it was not mentioned in the primary studies whether the analysis of the outcomes was performed randomly or whether those who analyzed the outcomes were random. In the analysis of the risk of frictional bias (8), it has been observed that

Table 3. Data from outcomes of *in vivo* studies included.

Author / Year	Anti-inflammatory model	Measure parameter	Anti-inflammatory activity results	Measurement (Mean ± S.E.M. / S.D.)	Measurement (%)	
Carvalho et al., 1996 [12]	Carrageenan-induced paw edema	Paw volume (mL)	Paw edema reduction in the 2nd and 3rd hours	?	Extract: 36.3% (2nd hour) / 23.0% (3rd hour)	Indomethacin: 61.0% (2nd hour) / 64.6% (3rd hour)
Freitas et al., 2012 [26]	Carrageenan-induced peritonitis	PMNLs counting (polymorphonuclears leucocytes) ($x10^6$/mL)	Exudate inflammatory reduction in number of PMNL	(PMNL/mL exudate ± S.E.M) CE: 5.8 ± 0.3 x 10^6 / F80: 38.6.0 ± 0.1 x 10^6	CE: 40.9% /F80: 38.2%.	Indomethacin: 72.2%; Piroxicam: 46.7%; Dexamethasone: 68.1%.
Lima et al., 2012 [23]	Thioglycolate induced peritonitis	Total number of leukocytes ($x10^6$)	Inhibition inflammatory response	(S.D.) 25 mg/kg: 4.14 ± 0.5 x 10^6 / 50 mg/kg: 3.69 ± 0.5 x 10^6	25 mg/kg: 68.4% / 50 mg/kg: 71.8%	
	Xylene-induced ear edema	Ear weight (mg)	Ear edema reduction	(S.D.) 50 mg/kg: 5.5 + 1.2 mg	50 mg/kg: 66.6%	Indomethacin: 83.9%
	Vascular permeability induced by acetic acid	Release of vasoactive amines and formation of edema (nm)	Inhibition vascular permeability	?	50 mg/kg: 66.1%	
Pereira et al., 2012 [24]	Carrageenan-induced paw edema	Paw volume difference of displacement (mL) and area under curves—AUC (arbitrary units %); Plasma leakage (mg Evans's blue/g).	Inhibition of paw edema, antiedematogenic activity	(S.E.M.) TPL 1 mg/kg: 60–180 min (23 ± 2.86 AUC) / 180–300 min (6 ± 2.14 AUC) / FIII 1 mg/kg: 60 min—0.28 ± 0.06 mL	TPL 1mg/kg: 60.0% (max. inhibition) / 48.0% (initial) / 76% (late) / FIII: 60 min—53.0% 300 min—85.0%	Indomethacin: 64.0% (initial) / 53.0% (late)
	Paw edema induced by dextran			(S.E.M.) FIII 1 mg/kg: 30 min—38.45 ± 8.66 AUC / 180 min: 0.08 ± 0.03 mL	FIII 1 mg/kg: 30 min– 53.0%, 180 min—70.0%	
	Paw edema induced by PGE2, L-arginine			?	PGE2—FIII: 63.0% L-arginine—FIII: 61.0%	
	Paw edema induced by Histamine, Serotonin, Bradykinin, 48/80 compound			?	Bradykinin—FIII: 60.0%. Histamine—FIII: 65.0%; 48/80 Compound—FIII: 36.0%; Serotonin—FIII: 62.0%	
	Carrageenan-induced peritonitis	Count of total and differential leukocytes (neutrophils, eosinophils, mast cells, mononuclear) ($\times10^3$/mL) and proteins (mg/mL) in peritoneal fluid.	Inhibition of leucocyte counting	(S.E.M.) FIII 1 mg/kg: 2.24 ± 0.03 x 10^3 carrageenan: 6.23 ± 0.07 x 10^3	FIII: 64.0%	
	Peritonitis induced by fMLP			FIII 1 mg/kg: 0.54 ± 0.04 x 10^3 fMLP: 4.39 + 0.49 x 10^3	FIII: 88.0% fMLP: 84.0%	
	Anti-inflammatory activity evaluation	Mast count	Degranulated mast cells (late phase)	240 min—FIII: 0,56 ± 0,05 mL / 300 min—FIII: 0,43 ± 0,05 mL	L-NAME: 84.0% Methisergide: 84.0%	
De Araújo et al., 2014 [19]	Carrageenan-induced peritonitis	Total leukocyte count (total number of cells per peritoneal cavity)	Leukocyte migration reduction	?	?	
Sawada et al., 2014 [27]	Formalin-induce licking (inflammatory pain)	Number of licks induced with formalin/ Evaluation of the mechanism of action LPLF seeds	Licks quantitative reduction	?	74 ± 2% in early phase, 100% late phase—maximal inhibition	Indomethacin: 76 ± 7% in late phase
Pereira et al., 2016 [20]	Wound model	Wound area (mm^2), (wound closure %), Vascular permeability vascular (nm), Inflammatory mediators (IL-1β, PGE2, TNF-α; MPO, Nitrate, MDA)	Wound area reduction, leukocyte infiltration and vascular permeability	(S.E.M.) TPL-Cf 0.1%: 38.99 ±1.9 mm^2 in area reduction and increase on the wound at the 2nd day. TPL-Cf—IL-1: reduction of 2.20 ± 0.03 pg/mL, at 2nd day/ PGE_2: 0.0062 ± 0.001 reduction at 7th day. / Nitrite: 26.86 ± 9.5 μM increase at 5th day / MPO: 41.28 ± 4.66 U/mg tissue (2nd day) and 19.67 ± 8.18 U/mg tissue (5th day) reduction / MDA: 937.6 ± 72.36 μM/g tissue reduction at 5th day / Vascular permeability: 29.08 ± 4.18 (2nd day) and 26.44 ± 4.18 mg Evans Blue/g tissue (5th day) reduction. Collagenase - 2nd day 38.27 ± 1.3 / 5th day 29.22 ± 1.9 / 7th 7.08 ± 0.8 mm^2	TPL-Cf: 29.0% (2nd day) and 26.0% (5th day) reduction of polymorphonuclear infiltration / IL-1: reduction 42.0% at 2nd day / PGE_2: reduction 73.0% at 7th day / MPO: 53.0% (2nd day) and 63.0% (5th day) reduction / MDA: 38.0% / Vascular permeability reduction: 48.0% (2nd day) and 52.0% (5th day)	

(*Continued*)

Table 3. (Continued)

Author / Year	Anti-inflammatory model	Measure parameter	Anti-inflammatory activity results	Measurement (Mean ± S.E.M. / S.D.)		Measurement (%)	
Falcão et al., 2019 [22]	Zymosan-induced arthritis	Cellular influx (global leukocyte counting (mm^3), MPO (U/mL), MDA (nmol/mL), Glutathione (nmol/mL), Inflammatory cytokines [IL-1β (pg/mL) / TNF-α (pg/mL)]	Leukocyte influx reduction from synovial fluid, reduction of the levels from IL-1β, TNF-α, MPO, MDA, glutathione increase	?		(S.E.M.) Extract reduced leukocyte influx in 76 ± 2% at the 100, 200 and 300 mg/kg doses / MPO: reduction in approx. 85% + 7% / Glutathione levels increased: 41 nmol/mL / MDA levels reduced: 60.0% (200 e 300 mg/kg)	Diclofenac: 56%
Falcão et al., 2019 [25]	Carrageenan-induced peritonitis	MPO (U/μL.), MDA (nmol/μL) and glutathione total levels (nmol/μL) / leukocyte numbers ($x10^3$)	Leucocyte migration reduced in all preparations, Reduction in MPO and MDA levels, increase in glutathione levels	?		?	
Holanda et al., 2020 [21]	Zymosan induced paw edema	Paw volume (mL) or area under the curve -AUC / MPO (U/mg tissue)	Paw edema inhibition, reduction in MPO levels	PE-Cf 1 mg/kg 58 ± 9 mL (4h), 52 ± 10 mL (5h) / 1-3h - PE-Cf 0.1 mg/kg: 220 ± 5 AUC, PE-Cf 1 mg/kg: 140 ± 16 AUC / 3–6 h—PE-Cf 0.01 mg/kg: 580 ± 15 AUC, PE-Cf 0.1 mg/kg: 331 ± 15 AUC, PE-Cf 1 mg/kg: 182 ± 18 AUC; MPO—PE-Cf 1 mg/kg: 17 + 1 U/mg		PE-Cf 1 mg/kg 71.0% (4h), 74.0% (5h) / 1-3h - PE-Cf 0.1 mg/kg: 39.0%, PE-Cf 1 mg/kg: 61.0% / 3–6 h—PE-Cf 0.01 mg/kg: 43.0%, PE-Cf 0.1 mg/kg: 36.0%, PE-Cf 1 mg/kg: 69.0% / MPO—PE-Cf 1 mg/kg: 43.0%	
	Peritonitis induced by zymosan (i.v.)	Leukocyte migration (total leukocyte, neutrophil, mononuclear) (mm^3) / GSH (μmol/mL–A_{412} nm) / GPx (U/mg proteins–A_{340} nm) / Nitrate (mM–A_{540} nm) / MDA (U/mL–A_{535} nm)	Leukocytes and neutrophils reduction. Increase in GSH e GPx levels, reduction n NO_2./No_{3-}, MDA levels	PE-Cf 1mg/kg—Leukocytes 1.063 ± 130 mm^3, neutrophils 432 ± 45 mm^3 / GSH: 736 ± 65 μmol/mL / GPx: 0.037 ± 0.007 U/mg protein / NO_2./NO_3.: 0.131 ± 0.033 mL e MDA: 98 ± 10 U/mL		PE-Cf 1mg/kg—Leucocytes 69.0% / neutrophils 84.0% / GSH: 65.0% / GPx: 72.0% / NO_2./NO_3.: 73.0% / MDA: 37.0%	
	Peritonitis induced by zymosan (p.o.)	Leukocyte migration (mm^3)	Inhibition of leukocyte and neutrophils migration	PE-Cf 1mg/kg–Leucocytes: 2.143 ± 123 mm^3, neutrophils: 742 ± 75 mm^3	Zymosan: 3.149 ± 23/ mm^3	PE-Cf 1mg/kg–Leucocytes: 41.0%, neutrophils 76.0%	

? (Data not demonstrated); S.E.M. (standard error of the mean); S.D. (standard deviation); min (minute); mL (milliliter); CE (Crude aqueous extract); F80 (partially purified fraction); TPL (Total polysaccharides); FI-FIII (major polysaccharide fractions); LPLF (Lipidic portion from *Libidibia ferrea*); TPL-Cf (Total polysaccharides of *C. ferrea* barks); g (gram); CE20-CE80 (Hydroalcoholic fractions of 20.0–80.0% ethanol); fMLP (N-formyl-methionyl-leucyl-phenylalanine); LfAE (Crude aqueous extract of *L. ferrea*); mg (milligram); kg (kilo) PGE2 (Prostaglandin E_2); PMNL (polymorphonuclears leucocytes); h (hour); AUC (area under curve); p/v (weight/volume); i.p. (intraperitoneal); *p.o.* (per oral); COX-2 (cyclocoxygenase-2); nm (nanometer); ng (nanogram); μg (microgram); μL (microliter); U/μL (units/microliter); nmol/μL (nanomole/microliter); MPO (myeloperoxidase); MDA (malondialdehyde); TNF-α (Tumor necrosis factor alpha); IL-1 (Interleukin 1); mm^3 (cubic millimeters), NaCl (sodium chloride); PE-Cf (Rich-polysaccharides extract of *Caesalpinia ferrea* stem bark); GHS (Reduced glutathione); GPx (Glutathione peroxidase); ~ (about).

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Table 4. General characteristics of *in vitro* studies.

Author / Year	Country of Origin / Collection Country / Year Period	Extract concentration	Control group	Cellular type	Assay type
DIAS et al., 2013 [28]	Portugal / Belém do Pará (Brazil)	30 mg/mL (vol 5 μL)	Negative: without LPS or sample / Positive: com LPS	RAW 264.7 macrophage and Balb/3T3 clone A31 fibroblasts (ATCC, Manassas) $1x10^5$ / 2 mL	LPS-induced inflammation
NETO, 2018 [30]	Brazil / Pici Campus—Fortaleza (CE) / Mar, 2017	1 mg/mL (150 μL)	Control: 100 μL Griess reagent	BV2 microglial cells from rats' brain, retrovirus transformed (1 x 10^6 cells/mL)	Nitrite determination / LPS induced neuroinflammation
LINS, 2020 [29]	Brazil / AM	7.5% (w/v) (1.56; 3.12; 6.25; 12.5; 25; 50; 100 μg/mL)	Negative: DMEM / Positive: LPS from *E. coli* 1 μg/mL / Standard drug: Dexamethasone	RAW 264.7 macrophages (10^6 cells/mL)	Nitrite quantification / LPS from *Escherichia coli*
			Negative: RPMI 1640/ Positive: LPS de *E. coli* 1 μg/mL / Standard drug: Dexamethasone	Peripherical human blood monocytes ($2x10^6$ cells/mL)	

mg/mL (milligram/milliliter); ATCC (*American Type Culture Collection*); LPS (lipopolysaccharides).

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no study has reported an animal loss during the experiment. Carvalho et al. (1996) described the division of two groups of animals in the methodology, however, in the results, they presented three groups, that is, they included a negative control group [12].

Nine studies reporting bias [12, 20–27] described all outcomes related to the reporting bias risk (9). However, De Araújo et al. (2014) related acetonic and aqueous extract of *L. ferrea* extract on the discussion without apparent description of the anti-inflammatory action of this results in isolation [19].

Table 5. Outcome description from the *in vitro* studies.

Autor / Year	Treatment	Parameter evaluated	Results
DIAS et al., 2013 [28]	Cell culture in DMEM-F12 HAM medium with phenol red medium in 24-well plate and were pre-incubated with samples of each dressing (approximately $1cm^2$) without load or extract, after 20 mL of LPS was added to the medium. 2, 6, 24, and 72 h collection of an aliquot of 500 mL.	Quantification of the amount of extract loaded/released (gravitationally) / cytocompatibility / Production of IL-1α and TNF-α (ELISA) / Nitric Oxide Concentration (quantification curve 0–15 mM); LDH cytosolic enzyme released in the culture medium	LDH test: demonstrated low cell viability after 72 h / Levels of TNF-α increases progressively as a function of time from 2 to 24 hours, while IL-1α levels increase in two hours.
NETO, 2018 [30]	Cell suspension incubated in 96 well plates for 24 h. ELFLF extract was added. After 1 h was challenge with LPS. 100 μL of Griess reactive was added.	Nitrite quantification (NO) (standard curve 15 μM a 1000 μM)	NO levels formation was significative reduced by 50 μg/mL. $p < 0.05$
LINS, 2020 [29]	RAW 264.7 macrophage was sanded in DMEM medium in 96 well plates. Culture medium was removed, and the cells was challenged with 1 μg/mL– 50 μg/well of LPS. Cells was treated with *L. ferrea* extract (1.56, 3.125, 6.25, 12.5, 25, 50 and 100 μL/well). Cells with LPS was incubate for 24h. Three experiments were made with triplicates.	Nitrite determination (standard curve)	Compared to dexamethasone and LPS, 50 e 100 μg/mL better reduced the NO levels. $p < 0.05$
	Human monocytes were sanded in RPMI medium in 96 well plates. Same procedure of RAW 264.7 macrophage.		All concentration inhibition the NO levels, although 50 and 100 μg/mL were better than the other concentrations. $p < 0.05$

LPS (lipopolysaccharides); cm^2 (square centimeters); mL (milliliters); mM (milimolar); LDH (lactate dehydrogenase); h (hour); TNF-α (Tumor necrosis factor alpha); IL (interleukin); ELISA (Enzymatic immunoadsorption assay); μg/mL (microgram per mL); NO (nitric oxide); ATCC (American Type Culture Collection); DMEM-F12 (Dulbecco's Modified Eagle Medium: Nutrient mixture F-12); RPMI (Roswell Park Memorial Institute).

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Table 6. Risk of bias *in vivo* studies according to SYRCLE's RoB tool of the ten studies included in the systematic review.

Study	Inflammatory model	Selection bias							Performance bias		Detection bias
									Attrition bias	Reporting bias	Other
1	2	3	4	5	6	7	8	9	10		
Carvalho et al., 1996	Paw edema (carrageenan)	?	Y	?	Y	?	?	?	N	Y	Y
Freitas et al., 2012	Peritonitis (carrageenan)	?	Y	?	Y	?	?	?	Y	Y	Y
Lima et al., 2012	Peritonitis (thioglycolate)	?	Y	?	Y	?	?	?	Y	N	Y
	Ear edema (xylene)	?	Y	?	Y	?	?	?	Y	Y	Y
	Vascular permeability	?	Y	?	Y	?	?	?	Y	Y	Y
Pereira et al., 2012	Paw edema (carrageenan)	?	Y	?	Y	?	?	?	Y	Y	Y
	Paw edema (dextran)	?	Y	?	Y	?	?	?	Y	Y	Y
	Paw edema (histamine)	?	Y	?	Y	?	?	?	Y	N	Y
	Paw edema (serotonin)	?	Y	?	Y	?	?	?	Y	N	Y
	Paw edema (48/80 compound)	?	Y	?	Y	?	?	?	Y	N	Y
	Paw edema (bradykinin)	?	Y	?	Y	?	?	?	Y	N	Y
	Paw edema (PGE-2)	?	Y	?	Y	?	?	?	Y	N	Y
	Paw edema (L-arginine)	?	Y	?	Y	?	?	?	Y	N	Y
	Peritonitis (carrageenan)	?	Y	?	Y	?	?	?	Y	N	Y
	Peritonitis (fMLP)	?	Y	?	Y	?	?	?	Y	N	Y
	Inflammatory evaluated	?	Y	?	Y	?	?	?	Y	Y	Y
De Araújo et al., 2014	Peritonitis (carrageenan)	?	Y	?	Y	?	?	?	Y	N	Y
Sawada et al., 2014	Licking	Y	Y	?	Y	?	?	?	Y	Y	Y
Pereira et al., 2016	Wound	?	Y	?	Y	?	?	?	Y	Y	Y
Falcão et al., 2019	Arthritis (zymosan)	?	Y	?	Y	?	?	?	Y	Y	Y
Falcão et al., 2019	Peritonitis (carrageenan)	Y	Y	?	Y	?	?	?	Y	Y	Y
Holanda, 2019	Paw edema (zymosan)	?	Y	?	Y	?	?	?	Y	Y	Y
	Peritonitis (i.v.) (zymosan)	?	Y	?	Y	?	?	?	Y	Y	Y
	Peritonitis (p.o.) (zymosan)	?	Y	?	Y	?	?	?	Y	Y	Y

Y (YES) = low risk of bias; N (NO) = high risk of bias,? = Unclear bias. Sequence generation (1), Baseline characteristics (2), Allocation concealment (3), Random housing (4), Blinding (5), Random outcome assessment (6), Blinding (7), Incomplete outcome (8), Selective outcome reporting (9) and others (10).
Note: Scale was adapted according to the use of different *in vivo* experimental models of inflammation.

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At the peritonitis experiment [23] the ethanolic extract dose (12,5 mg/kg) received more description than the other doses (25 e 50 mg/kg). About other sources of bias (10) all studies were classified as low risk of bias. Although, two studies have not shown the ethics committee number [12, 27].

Certainty of evidence

The analysis of the uncertainty of inconsistency, publication bias, inaccuracy and *in vivo* studies were presented in a narrative description:

Imprecision: It was observed that there is a heterogeneity in the studies, such as the size of the samples and amounts of groups used by experiments; the metrics of variation, in most studies, was through mean ± SEM [20–22, 24–28], mean ± standard deviation [23] and the expression of volume difference [12]. In all *in vivo* studies the calculation of the sample size was not detected. Even with these inconsistencies the studies tended to present the same direction of the effect, that is, *L. ferrea* anti-inflammatory activity, so the certainty of the evidence would not downgrade [S2 Appendix].

Publication bias related to the included studies: only one study [23] described in the topic of funding by agencies, which have supported the work. This topic was not requested in the journal in the other studies. Therefore, many added this funding information in the acknowledgement, and none presented to be funded by any industry. In four studies [12, 24, 26, 27] the topic of conflict of interest was not required in the journal. In the other six studies [20–23, 25, 28] the topic was dealt with, and a conflict of interest was identified. With this information it can be considered that the publication bias was apparently undetected, given the existing level of uncertainty. All *in vivo* studies were published in a scientific journal [S2 Appendix].

Since the meta-analysis was not performed, the inconsistency was not required to be taken into account. Considering the conditions, which could affect the outcome, apparently all performed the housing and apply water and food regimes *ad libitum*. In all studies were identified that the animals were acclimatization, describing at least the temperature, only in one study was not detected this information [27] [S2 Appendix].

Indirectness: As to the research question it was observed that the part of the plant most frequently used in the experiments was the fruit and the mostly used extract was the aqueous extract. As all studies presented anti-inflammatory activity, it can be inferred that those are the ones that showed the greatest evidence of this action, regardless of the experimental model used. Usually, teas/infusions are administered after the appearance of some inflammatory process in humans. However, excepted one study [20] almost all the other studies have induced the inflammatory process after plant administration. Thus, the certainty of evidence should be downgraded [S2 Appendix].

Based on the GRADE criteria the certainty of evidence for *in vivo* studies was also evaluated. Only one outcome was considered high [20], others were considered with moderate certainty [21–27] and low certainty [12, 19, 24]. Further information can be found at Table 7.

In vitro studies: SciRAP [18] was used with adaptations as a tool for the evaluation of the quality of reports. Five aspects (test compound and controls, test system, administration of test compound and data collection and analysis) were presented, with 23 topics on the whole. Items related to the compound used chemical (item 1), purity of the compound (item 2), solubility of the test compound (item 3) (test compound and controls); system source (item 7), metabolic competition (item 8) were removed since these items are related to the toxicity of the compound (test system); effect of the compound test on cytotoxicity (item 19) since this was not the focus of the study (data collection and analysis).

With respect to test and control compound, studies have been analyzed under the items associated to the description of the vehicle, and to the untreated control or the vehicle if they were analyzed as fulfilled [28, 29] and partially fulfilled [30]. As to the item test system, the identification of the cell line/cell type in which all studies presented this information (fulfilled) were analyzed. Apparently, only one study has described the days in which cell passages to one of the cell line [29] have taken place. In the other studies no identification was possible. Information on the screening of contamination was not identified in the studies. They were presented as undetermined [28–30] and not fulfilled [29] (Fig 2A–2D).

In the item administration of test compounds concentrations or doses, cell densities and number of replicates have been described in all studies (completed). The duration of the treatment was considered as fulfilled [28, 29] and partially fulfilled [29, 30] (Fig 2A–2D).

Data collection and analysis, if the tests and/or analytic methods were sufficient to describe the results, the criterion was considered as fulfilled [28, 29], partially fulfilled [30]. Time point for the data was considered fulfilled [28–30], partially fulfilled [29]. It was observed that all studies have demonstrated the results. Except in one study [28], all statistical methods were described (Fig 2A–2D).

Table 7. Certainty of evidence from *in vivo* studies.

Outcome	Certainty assessment					Certainty
	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	
Inflammation inhibition (paw volume) [12]	serious[a]	not serious	serious	not serious	none	⊕⊕◯◯ low
Cellular migration reduction (PMNL counting) [26]	not serious	not serious	serious	not serious	none	⊕⊕⊕◯ moderate
Inhibition of cellular migration [23]	not serious	not serious	serious[b]	not serious	none	⊕⊕⊕◯ moderate
Ear edema reduction [23]	not serious	not serious	serious	not serious	none	⊕⊕⊕◯ moderate
Vascular permeability inhibition [23]	not serious	not serious	serious	not serious	none	⊕⊕⊕◯ moderate
Paw edema inhibition (carrageenan; dextran) [24]	not serious	not serious	serious	not serious	none	⊕⊕⊕◯ moderate
Paw edema inhibition (histamine; serotonin; bradykinin, PGE-2; L-arginine; compound 48/80) [24]	serious[b]	not serious	serious	not serious	none	⊕⊕◯◯ low
Peritonitis (carrageenan; fMLP) [24]	serious[b]	not serious	serious	not serious	none	⊕⊕◯◯ low
Inflammatory evaluated [24]	serious[b]	not serious	serious	not serious	none	⊕⊕◯◯ low
Total leukocyte count [19]	serious[b]	not serious	serious	not serious	none	⊕⊕◯◯ low
Number of licks induced with formalin [27]	not serious	not serious	serious	not serious	none	⊕⊕⊕◯ moderate
Wound area reduction [20]	not serious	not serious	not serious	not serious	none	⊕⊕⊕⊕ high
Cellular migration reduction [22]	not serious	not serious	serious	not serious	none	⊕⊕⊕◯ moderate
Reduction of cell influx [25]	not serious	not serious	serious	not serious	none	⊕⊕⊕◯ moderate
Paw edema inhibition [21]	not serious	not serious	serious	not serious	none	⊕⊕⊕◯ moderate
Leukocytes and neutrophils reduction [21]	not serious	not serious	serious	not serious	none	⊕⊕⊕◯ moderate
Inhibition of leukocyte and neutrophils migration [21]	not serious	not serious	serious	not serious	none	⊕⊕⊕◯ moderate

[a.] Most domains presented uncertain risk of bias; It was not detected the ethics committee number or if the animals were randomized.

[b.] It was not detected the animal randomization. Most domains presented uncertain of bias.

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With respect to financing and competing interests, in the source of funding criteria, two studies were considered as fulfilled [28, 29] and one as partially completed study [30]. None of the studies apparently showed any conflict of interest (Fig 2A–2D).

Updates

Throughout the systematic review, some amendments were required to be made. We have, thus, included this topic concerning PRISMA 2020. One of these amendments was the update of the systematic review, given that data from one year had passed from the data to the first search (February 2020); Search strategy that follows in this search is the date of the first search and the update together in the flow diagram; No data was extracted as one of the criteria for analysis of the outcome of anti-inflammatory action of the plant/extract; More information on data extraction from *in vitro* studies has been added; Two authors resolving the discrepancies when arising.

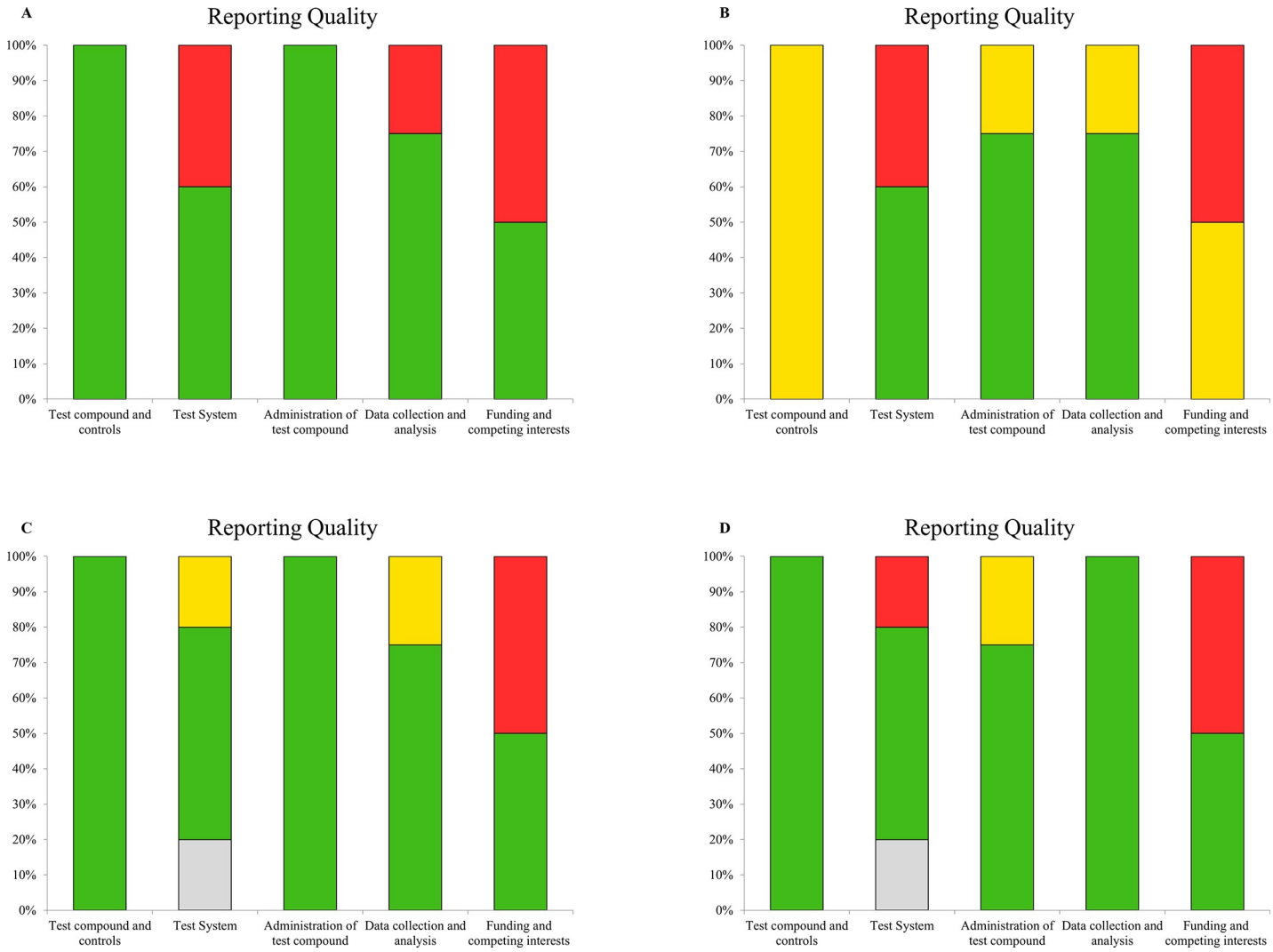

Fig 2. Reporting quality in *in vitro* studies. A. *L. ferrea* fruits quality reporting on *in vitro* study. B. *L. ferrea* leaves quality reporting on *in vitro* study. C and D. *L. ferrea* stem bark quality reporting on *in vitro* study. Grey: not determined; green bar: fulfilled; yellow bar: partially fulfilled; red bar: not fulfilled.

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Discussion

In view of the systematic organization and analysis of scientific evidence of the anti-inflammatory effects of *L. ferrea* or *Caesalpinia ferrea* on *in vivo and* in *vitro* studies, we have sought to answer that part of the *L. ferrea* plant, and which type of extract has the most evident anti-inflammatory effects in the experimental models of acute inflammation on *in vivo* and *in vitro* studies.

Although the electronic research has identified one systematic review entitled Natural Antimicrobials and Oral Microorganisms: A Systematic Review on Herbal Interventions for the Eradication of Multispecies Oral Biofilms [31], that provide antimicrobial data from various medicinal plants, including *Caesalpinia ferrea*, the anti-inflammatory activity data were not described in said study. The originality of this study is, therefore, ratified. This would be the main strength of this research.

Nine studies [19–26, 30] have obtained the plants in the Northeastern region in Brazil, and four [12, 27, 28, 29] have obtained them in the Northern region which corroborates the

literature data, which have demonstrated the wide distribution of this plant throughout Brazil, occurring in Caatinga, Atlantic Forest, and Cerrado domains especially in this area [3] and Northern region (AM, AP, PA, RO, RR) [32].

In this context, the Amazon region stands out, with a great diversity of plant species, where about 5,000 of the 35,000 plant species have great economic potential, either by the production of waxes, essential oils or by other constituents considered useful not only to humans, but also to the environment, animals and plants [33]. Brazil is the country with the greatest biodiversity on the planet (around 15% to 20%), of which, as plants are subsidies in the manufacture of medicines [34]. Among these plants, *L. ferrea* stands out and is the focus of research in this systematic review.

We have analyzed the methodological design of the ten *in vivo* studies and data described from the *in vitro* studies; it has been observed that the most used extract was aqueous extract. This has been found by Agra; Freitas; Barbosa-Filho (2007) whose study aimed to conduct a survey of plants and their modes of use for therapeutic purposes in northeastern Brazil. It has been demonstrated that the *L. ferrea* stem bark was used by decoction method or as an admixture solution [35].

In addition, the use of fruits left "soaking" and used for the treatment of influenza and bronchitis [36] has also been demonstrated. The study by Santos; Vilanova (2017) and Vásquez; Vásquez; de Mendonça; Noda (2014) has also demonstrated the use of leaf and fruit in the form of infusion and *in natura;* and the use of leaf and fruit in the preparation of tea, syrup, and macerated for the treatment of sore inflammation, sore throat, respectively [37, 38]. Infusion of leaves and fruits has also been demonstrated in the treatment of tuberculosis and liver inflammations in the Amazon region [1].

Regarding the anti-inflammatory effect, all the studies included in this systematic review have observed the existence of the anti-inflammatory activity of the plant, possibly independently of the part and/or type/fraction of the extract used. This is probably related to the fact that medicinal plants present some compounds (e.g., phenolic compounds) enabling anti-inflammatory action among various biological activities [39]. The presence of these and other compounds can be verified in fruits where gallic acid [4, 25], methyl gallate [4] and fatty acids [27, 28], have already been identified. For example, gallic acid regulates pro-inflammatory pathways, as the signaling pathway of nuclear factor kappa B (NF-κB) [40].

In addition, in the process of acute inflammation, inflammatory mediators are released. Mediators as cytokines and inflammatory proteins would act as biomarkers or predictors in the diagnosis and inflammatory diseases, respectively [41]. This has been observed in the modulation of TNF-α, IL-1β, NO and TGF-β controlling the inflammatory phase and also attenuating hypernociception in the wound healing study [20]. Anti-inflammatory activity could also occur via negative modulation, e.g., in carrageenan-induced paw edema, using the following mediators: bradykinin, nitric oxide, histamine, serotonin, and PGE_2 [24].

This diversity in the several uses of the *L. ferrea* (extract and parts of the plant) as well the use of a great diversity of experimental models of inflammation, genus, species, animal number, and the number of animals by groups may cause difficulty in grouping the results by the similarity that makes impossible to demonstrate the sizes of the effect.

Exception by Pereira et al. (2016) who induced wounds on the animals and then administrated dressing contain the plant extract; all other *in vivo* studies have performed the treatment before inducing inflammation with the flogistic agent challenged to verify the anti-inflammatory action [20]. This conduct in the experimental designs differs from that applied in humans since the treatment is administrated after the onset of the disease. This is described as one of the challenges of the successful translations from animal models to the clinical environment in humans [42].

The principal limitations observed in the studies, object of this this systematic review (in accordance with the "unclear" risk of bias) were related to the risks of bias having to do with the concealment of the allocation, in addition to blinding of both the animals (induction of inflammation) and those, which they referred. The results have failed to indicate the groups to which they referred. Data on whether the animals had been properly randomized or not, and which method had been used were not provided in articles. Both this information and the execution of the blind assessment and the allocation concealment have helped reduce the impact of the bias on the experiments. These have enabled a reduction in the threats to the internal validity of the studies [43].

Limitations of this research are those inherent to systematic reviews of animal studies, such as the difficulty in the extraction of data, which are often presented in different ways in studies, especially when analyzing designs with high or unclear risk of bias. The authors of this research may have insufficiently interpreted the results presented in the included studies; the difficulty in collecting some data have not been taken into account, not all journals rely on some information, such as funding. Thus, in addition to the limitations inherent to preclinical studies, we still have these other limitations.

In vitro studies have been identified [28–30] ratifying the use of this type of experimental design to try to explain the mechanism of the action of anti-inflammatory drugs [44]. These studies could be translated into biomedical research when analyzed in more complex organisms [45]. However, it may be difficult to reflect the same results in terms of *in vivo* pharmacodynamics and pharmacokinetics studies [44].

Furthermore, quality analysis in preclinical studies without metanalysis is more challenging due to the subjectivity of the analyses. In addition, reporting the quality of *in vitro* studies followed the same principle of subjectivity in the analysis of the studies.

Conclusions

Jucá (*L. ferrea*) appears to demonstrate anti-inflammatory activity regardless of the part of the plant and type of extract used in the experimental models and presents itself as a promising species in non-clinical research, thus corroborating its use in folk medicine for the treatment of inflammations. Although the evidence is considered as moderate by GRADEpro, a careful analysis of the results is important, given the presence of methodological bias. And the certainty of evidence is still insufficient to recommend the use of this plant in research.

For this reason, it is suggested preclinical studies in models of inflammation with greater methodological rigor based on standardized tools be designed for a more detailed evaluation of the effects of this plant of traditional use.

Supporting information

S1 Table. Prisma check list.
(DOCX)

S2 Table. Prisma abstract check list.
(DOCX)

S1 Appendix. Search strategy.
(DOCX)

S2 Appendix. Certainty of evidence in *in vivo*.
(XLSX)

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References

1. Di Stasi LC, Himura-Lima CA (2002) *Caesalpinia ferrea*. In: Plantas medicinais na Amazônia e na Mata Atlântica, 2 ed. rev. e ampl. Editora UNESP, São Paulo, ISBN 85-7139-411-3. pp. 279–280
2. Stehmann JR, Faria FS, Bragioni T (2019) Pau-ferro. In: 50 árvores do Museu. Formato Editora, Belo Horizonte, ISBN 978-85-62164-13-2. pp. 90
3. Maia-Silva C, Silva CI da, Hrncir M, Queiroz RT de, Imperatriz-Fonseca VL (2012) Jucazeiro. In: Guia de plantas visitadas por abelhas na caatinga, 1 ed. Editora Fundação Brasil Cidadão, Fortaleza, CE, ISBN 978-85-98564-05-0. pp. 43
4. Nakamura ES, Kurosaki F, Arisawa M, Mukainaka T, Okuda M, Tokuda H, et al (2002) Cancer chemopreventive effects of constituents of *Caesalpinia ferrea* and related compounds. Cancer Lett 177:119–124 https://doi.org/10.1016/s0304-3835(01)00708-x PMID: 11825658
5. Ferreira MRA, Soares LAL (2015) *Libidibia ferrea* (Mart. ex Tul.) L. P. Queiroz: A review of the biological activities and phytochemical composition. J Med Plants Res 9:140–150. https://doi.org/10.5897/JMPR2014.5706
6. Kobayashi YT da S, Almeida VT de, Bandeira T, Alcântara BN de, Silva ASB da, Barbosa WLR, et al (2015) Avaliação fitoquímica e potencial cicatrizante do extrato etanólico dos frutos de Jucá (*Libidibia ferrea*) em ratos Wistar. Brazilian J Vet Res Anim Sci 52:34–40. https://doi.org/10.11606/issn.1678-4456.v52i1p34-40
7. Nakamura ES, Kurosaki F, Arisawa M, Mukainaka T, Takayasu J, Okuda M, et al (2002) Cancer chemopreventive effects of a Brazilian folk medicine, Juca, on *in vivo* two-stage skin carcinogenesis. J Ethnopharmacol 81:135–137 https://doi.org/10.1016/s0378-8741(02)00047-8 PMID: 12020938
8. Barros AO, De Souza RS, Aranha ESP, Da Costa LM, De Souza TP, De Vasconcellos MC, et al. (2014) Antioxidant and hepatoprotective activities of *Libidibia ferrea* bark and fruit extracts. Int J Pharm Pharm Sci 6:71–76. ISSN-0975-1491
9. Pedrosa T do N, Barros AO, Nogueira JR, Fruet AC, Rodrigues IC, Calcagno DQ, et al (2016) Anti-wrinkle and anti-whitening effects of jucá (*Libidibia ferrea* Mart.) extracts. Arch Dermatol Res 308:643–654. https://doi.org/10.1007/s00403-016-1685-0 PMID: 27623800
10. Comandolli-Wyrepkowskil CD, Jensen BB, Grafova I, Santos PA dos, Barros AMC, Soares FV, et al (2017) Antileishmanial activity of extracts from *Libidibia ferrea*: development of *in vitro* and *in vivo* tests. Acta Amaz 47:331–340. https://doi.org/10.1590/1809-4392201700871
11. Prazeres LDKT, Aragão TP, Brito SA, Almeida CLF, Silva AD, de Paula MMF, et al (2019) Antioxidant and antiulcerogenic activity of the dry extract of pods of *Libidibia ferrea* Mart. ex Tul. (Fabaceae). Oxid Med Cell Longev. https://doi.org/10.1155/2019/1983137 PMID: 31827669
12. Carvalho JCT, Teixeira JRM, Souza PJC, Bastos JK, Dos Santos Filho D, Sarti SJ (1996) Preliminary studies of analgesic and anti-inflammatory properties of *Caesalpinia ferrea* crude extract. J Ethnopharmacol 53:175–178 https://doi.org/10.1016/0378-8741(96)01441-9 PMID: 8887026

13. Hooijmans CR, Rovers MM, De Vries RBM, Leenaars M, Ritskes-Hoitinga M, Langendam MW (2014) SYRCLE's risk of bias tool for animal studies. BMC Med Res Methodol 14:1–9. https://doi.org/10.1186/1471-2288-14-1 PMID: 24383436

14. Hooijmans CR, De Vries RBM, Ritskes-Hoitinga M, Rovers MM, Leeflang MM, IntHout J, et al (2018) Facilitating healthcare decisions by assessing the certainty in the evidence from preclinical animal studies. PLoS One 13:1–18. https://doi.org/10.1371/journal.pone.0187271 PMID: 29324741

15. GRADEpro GDT: GRADEpro Guideline Development Tool [Software]. McMaster University, 2020 (developed by Evidence Prime, Inc.). Available from gradepro.org.

16. Lima CNF, de Lima LF, Correia DB, Machado ST de S, de Sousa JP, et al (2020) Systematic review: Medicinal use and scientific elucidation of the *Piper genus* for the treatment of symptoms and inflammatory diseases. J Med Plants Res 14:62–72. https://doi.org/10.5897/JMPR2019.6855

17. Duchman KR, Lemmex DB, Patel SH, Ledbetter L, Garrigues GE, Riboh JC (2019) The Effect of nonsteroidal anti-inflammatory drugs on tendon-to-bone healing: a systematic review with subgroup meta-analysis. Iowa Orthop J 39:107–119 PMID: 31413684

18. SciRAP.org (2018) Instructions for evaluating reliability and relevance of *in vivo* and *in vitro* toxicity studies using the SciRAP tool. 1–5

19. De Araújo AA, Soares LAL, Ferreira MRA, Souza Neto MA de, Silva GR da, Araújo RF de Jr, et al (2014) Quantification of polyphenols and evaluation of antimicrobial, analgesic and anti-inflammatory activities of aqueous and acetone-water extracts of *Libidibia ferrea*, *Parapiptadenia rigida* and *Psidium guajava*. J Ethnopharmacol 156:88–96. https://doi.org/10.1016/j.jep.2014.07.031 PMID: 25124277

20. Pereira LDP, Mota MRL, Brizeno LAC, Nogueira FC, Ferreira EGM, Pereira MG, et al (2016) Modulator effect of a polysaccharide-rich extract from *Caesalpinia ferrea* stem barks in rat cutaneous wound healing: Role of TNF-α, IL-1β, NO, TGF-β. J Ethnopharmacol 187:213–223. https://doi.org/10.1016/j.jep.2016.04.043 PMID: 27125588

21. Holanda BF, Freitas de Araujo D, da Silva JNR, Pereira MG, de Freitas Pires A, Sampaio Assreuy AM (2020) Polysaccaride-rich extract of *Caesalpina ferrea* stem barks attenuates mice acute inflammation induced by zymosan: Oxidative stress modulation. J Ethnopharmacol. https://doi.org/10.1016/j.jep.2020.113501 PMID: 33122121

22. Falcão TR, Rodrigues CAO, De Araújo AA, De Medeiros CACX, Soares LAL, Ferreira MRA, et al (2019) Crude extract from *Libidibia ferrea* (Mart. ex. Tul.) L.P. Queiroz leaves decreased intra articular inflammation induced by zymosan in rats. BMC Complement Altern Med 19:1–10. https://doi.org/10.1186/s12906-018-2420-5 PMID: 30606178

23. Lima SMA, Araújo LCC, Sitônio MM, Freitas ACC, Moura SL, Correia MTS, et al (2012) Anti-inflammatory and analgesic potential of *Caesalpinia ferrea*. Brazilian J Pharmacogn 22:169–175. https://doi.org/10.1590/S0102-695X2011005000197

24. Pereira LDP, Silva RO da, Bringel PHDSF, Silva KES da, Assreuy AMS, et al (2012) Polysaccharide fractions of *Caesalpinia ferrea* pods: Potential anti-inflammatory usage. J Ethnopharmacol 139:642–648. https://doi.org/10.1016/j.jep.2011.12.012 PMID: 22178173

25. Falcão TR, Araújo AA de, Soares LAL, Farias IB de, Silva WAV da, Ferreira MRA, et al (2019) *Libidibia ferrea* fruit crude extract and fractions show anti-inflammatory, antioxidant, and antinociceptive effect *in vivo* and increase cell viability *in vitro*. Evidence-based Complement Altern Med. https://doi.org/10.1155/2019/6064805 PMID: 30915148

26. Freitas ACC, Ximenes NCA, Aguiar JS, Nascimento SC, Lins TUL, Magalhães LR, et al (2012) Biological activities of *Libidibia* (*Caesalpinia*) *ferrea* var. *parvifolia* (Mart. ex Tul.) L. P. Queiroz pod preparations. Evidence-based Complement Altern Med. https://doi.org/10.1155/2012/514134 PMID: 22675382

27. Sawada LA, Monteiro VSDC, Rabelo GR, Dias GB, Da Cunha M, Nascimento JLM do, et al (2014) *Libidibia ferrea* mature seeds promote antinociceptive effect by peripheral and central pathway: Possible involvement of opioid and cholinergic receptors. Biomed Res Int. https://doi.org/10.1155/2014/508725 PMID: 24860820

28. Dias AMA, Rey-Rico A, Oliveira RA, Marceneiro S, Alvarez-Lorenzo C, Concheiro A, et al (2013) Wound dressings loaded with an anti-inflammatory jucá (*Libidibia ferrea*) extract using supercritical carbon dioxide technology. J Supercrit Fluids 74:34–45. https://doi.org/10.1016/j.supflu.2012.12.007

29. Lins MA (2020) Citotoxicidade e ação anti-inflamatória in vitro do extrato hidroalcoólico da Libidibia ferrea L. M. Sc. Thesis. Universidade Federal do Amazonas. Available from: https://tede.ufam.edu.br/handle/tede/7950

30. Neto FC das C (2018) Desenvolvimento do extrato seco de Libidibia ferrea (jucá) como alimento funcional: caracterização química e avaliação das atividades antioxidante e anti-inflamatória em modelos de neuroinflamação. M. Sc. Thesis. Universidade Federal do Ceará. Available from: http://www.repositorio.ufc.br/handle/riufc/30226

31. Karygianni L, Al-Ahmad A, Argyropoulou A, Hellwig E, Anderson AC, Skaltsounis AL (2016) Natural antimicrobials and oral microorganisms: A systematic review on herbal interventions for the eradication of multispecies oral biofilms. Front Microbiol 6:1–17. https://doi.org/10.3389/fmicb.2015.01529 PMID: 26834707

32. Silva MF da, Carreira LMM, Tavares AS, Ribeiro IC, Jardim MAG, Lobo M da GA, et al (1989) As leguminosas da Amazônia brasileira: lista prévia. Acta Bot Brasilica 2:193–237

33. Cardenas JDR (2017) Diversidade botânica. In: dos Santos GM, França LR. GEEA: Grupo de Estudos Estratégicos Amazônicos, v. 10. INPA, Manaus. pp. 59

34. Brasil M da S (2016) Política e Programa Nacional de Plantas Medicinais e Fitoterápicos. Ministério da Saúde, Secretaria de Ciência, Tecnologia e Insumos Estratégicos, Departamento de Assistência Farmacêutica, Brasília. ISBN 978-85-334-2399-2

35. Agra M de F, Freitas PF de, Barbosa-Filho JM(2007) Synopsis of the plants known as medicinal and poisonous in Northeast of Brazil. Brazilian J Pharmacogn 17:114–140. ISSN 0102-695X

36. Gomes TMF, Lopes JB, Barros RFM de, Alencar NL(2017) Plantas de uso terapêutico na comunidade rural Bezerro Morto, São João da Canabrava, Piauí, Brasil. Gaia Sci 11:253–268. https://doi.org/10.21707/gs.v11.n01a17

37. Santos KA dos, Vilanova CM (2017) Estudo etnobotânico de plantas medicinais utilizadas como hipoglicemiantes por usuários do Programa de Fitoterapia da Universidade Federal do Maranhão, Brasil. Sci Plena 13:1–12. https://doi.org/10.14808/sci.plena.2017.034501

38. Vásquez SPF, de Mendonça MS, Noda S do N (2014) Etnobotânica de plantas medicinais em comunidades ribeirinhas do município de Manacapuru, Amazonas, Brasil. Acta Amaz 44:457–472. https://doi.org/10.1590/1809-4392201400423

39. Ribeiro VP, Arruda C, Abd El-Salam M, Bastos JK (2018) Brazilian medicinal plants with corroborated anti-inflammatory activities: a review. Pharm Biol 56:253–268. https://doi.org/10.1080/13880209.2018.1454480 PMID: 29648503

40. Kahkeshani N, Farzaei F, Fotouhi M, Alavi SS, Bahramsoltani R, Nazeri R, et al (2019) Pharmacological effects of gallic acid in health and disease: A mechanistic review. Iran J Basic Med Sci 22:225–237. https://doi.org/10.22038/ijbms.2019.32806.7897 PMID: 31156781

41. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, et al (2018) Inflammatory responses and inflammation-associated diseases in organs. Oncotarget. https://doi.org/10.18632/oncotarget.23208 PMID: 29467962

42. Hooijmans CR, Ritskes-Hoitinga M (2013) Progress in using systematic reviews of animal studies to improve translational research. PLoS Med 10:1–4. https://doi.org/10.1371/journal.pmed.1001482 PMID: 23874162

43. Sena ES, Currie GL, Mccann SK, Macleod MR, Howells DW (2014) Systematic reviews and meta-analysis of preclinical studies: why perform them and how to appraise them critically. J Cereb Blood Flow & Metab 34:737–742. https://doi.org/10.1038/jcbfm.2014.28 PMID: 24549183

44. Eze FI, Uzor PF, Ikechukwu P, Obi BC, Osadebe PO (2019) *In vitro* and *in vivo* models for anti-inflammation: An evaluative review. INNOSC Theranostics Pharmacol Sci 2:3–15. https://doi.org/10.36922/itps.v2i2.775

45. Fedele M, Gualillo O, Vecchione A (2011) Animal models of human pathology. J Biomed Biotechnol 2011:1. https://doi.org/10.1155/2011/764618 PMID: 21776191