UNIVERSIDADE FEDERAL DO PAMPA
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOQUÍMICA

TESE DE DOUTORADO

RISCOS ASSOCIADOS AO USO MEDICINAL DO EXTRATO DA CASCA DE IPÊ-AMARELO *Handroanthus chrysotrichus* E SUA AÇÃO PROTETORA EM CASOS DE ENVENENAMENTOS PELA SERPENTE *Philodryas patagoniensis*: AVALIAÇÃO *IN SILICO, IN VITRO* E *IN VIVO*

MÁRCIO TAVARES COSTA

ORIENTADOR: VANDERLEI FOLMER
COORIENTADOR: ROBSON PUNTEL

Uruguaiana - RS, Brasil
2021

MÁRCIO TAVARES COSTA

RISCOS ASSOCIADOS AO USO MEDICINAL DO EXTRATO DA CASCA DE IPÊ-AMARELO *Handroanthus chrysotrichus* E SUA AÇÃO PROTETORA EM CASOS DE ENVENENAMENTOS PELA SERPENTE *Philodryas patagoniensis*: AVALIAÇÃO *IN SILICO, IN VITRO* E *IN VIVO*

Tese apresentada ao Programa de Pós-Graduação em Bioquímica *Stricto sensu* da Universidade Federal do Pampa, como requisito parcial para obtenção do título de Doutor em Bioquímica.

Orientador: Prof. Dr. Vanderlei Folmer
Coorientador: Prof. Dr. Robson Puntel

Uruguaiana - RS, Brasil
2021

Ficha catalográfica elaborada automaticamente com os dados fornecidos
pelo(a) autor(a) através do Módulo de Biblioteca do
Sistema GURI (Gestão Unificada de Recursos Institucionais) .

C837r Costa, Márcio Tavares
Riscos associados ao uso medicinal do extrato da casca de ipê-amarelo Handroanthus chrysotrichus e sua ação protetora em casos de envenenamentos pela serpente Philodryas patagoniensis: avaliação in silico, in vitro e in vivo / Márcio Tavares Costa.
129 p.

Tese(Doutorado)-- Universidade Federal do Pampa, DOUTORADO EM BIOQUÍMICA, 2021.
"Orientação: Vanderlei Folmer".

1. Etnobotânica. 2. Farmacologia. 3. Ofidismo. 4. Tabebuia. 5. Toxicologia. I. Título.

MÁRCIO TAVARES COSTA

RISCOS ASSOCIADOS AO USO MEDICINAL DO EXTRATO DA CASCA DE IPÊ-AMARELO *Handroanthus chrysotrichus* E SUA AÇÃO PROTETORA EM CASOS DE ENVENENAMENTOS PELA SERPENTE *Philodryas patagoniensis*: AVALIAÇÃO *IN SILICO*, *IN VITRO* E *IN VIVO*

Tese apresentada ao Programa de Pós-Graduação em Bioquímica da Universidade Federal do Pampa, como requisito parcial para obtenção do Título de Doutor em Bioquímica.

Tese defendida e aprovada em: 03 de setembro de 2021.

Banca examinadora:

Prof. Dr. Vanderlei Folmer

Orientador

(UNIPAMPA)

Prof. Dr. Daniel Mendes Pereira Ardisson de Araújo

(UNB)

Prof.ª Dra. Silvana Peterini Boeira

(UNIPAMPA)

Prof. Dr. Leonardo Magno Rambo

(UNIPAMPA)

Prof. Dr. Favero Reisdorfer Paula

(UNIPAMPA)

Assinado eletronicamente por **LEONARDO MAGNO RAMBO**, **Coordenador(a) de Curso**, em 12/09/2021, às 18:00, conforme horário oficial de Brasília, de acordo com as normativas legais aplicáveis.

Assinado eletronicamente por **VANDERLEI FOLMER**, **PROFESSOR DO MAGISTERIO SUPERIOR**, em 13/09/2021, às 07:16, conforme horário oficial de Brasília, de acordo com as normativas legais aplicáveis.

Assinado eletronicamente por **SILVANA PETERINI BOEIRA**, **PROFESSOR DO MAGISTERIO SUPERIOR**, em 13/09/2021, às 18:36, conforme horário oficial de Brasília, de acordo com as normativas legais aplicáveis.

Assinado eletronicamente por **FAVERO REISDORFER PAULA**, **PROFESSOR DO MAGISTERIO SUPERIOR**, em 30/09/2021, às 14:10, conforme horário oficial de Brasília, de acordo com as normativas legais aplicáveis.

A autenticidade deste documento pode ser conferida no site https://sei.unipampa.edu.br/sei/controlador_externo.php?acao=documento_conferir&id_orgao_acesso_externo=0, informando o código verificador **0608821** e o código CRC **8EF9D463**.

In memoriam...
Amélia Maria Tavares Costa e Paulo Roberto Costa;
Pais, amigos e mestres.

AGRADECIMENTOS

Primeiramente, destaco que este trabalho não teria se concretizado caso a UNIPAMPA não fosse uma instituição pública. O amparo de diversos laboratórios de excelência, com suas infraestruturas e recursos humanos, em especial os Laboratórios de Biologia e Diversidade Animal, de Bioquímica e Toxicologia de Produtos Naturais e Sintéticos e de Virologia também marcaram esta caminhada. Desta forma, agradeço a Instituição, ao PPG Bioquímica e aos professores que contribuíram para que eu possa estar aqui. Além das agências de fomento: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior; Conselho Nacional de Desenvolvimento Científico e Tecnológico; e a Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul.

Sou grato aos meus pais, Paulo e Amélia, pelo amor, ensinamentos e valores passados que me guiam e me guiarão durante minha vida. Obrigado por acreditarem nos estudos e me fazerem estudar!

Aos meus dindos, Sonia e Paulo, que me acolheram e incentivaram de forma única.

À minha eterna namorada, Luciana, pelo amor, carinho e apoio. À sua paciência, que foi pré-requisito em inúmeros momentos ao longo desses anos. À Alanis, minha enteada, pela tolerância que teve neste período. E à Helena, que passou a ser um estímulo para grandes conquistas. Amo vocês!

Ao meu irmão Cleine e minhas irmãs Ana e Zeti, que mesmo de longe, sempre me apoiaram. Me deram a força e estímulo que somente a família é capaz de dar. Além daquela garra que apenas um membro do Quarteto Fantástico reconhece! Sem esquecer, é claro, da minha sobrinha Jú e meus sobrinhos Gabriel e Pedro!

A todos colegas e amigos que tive a oportunidade de trabalhar no PPG, dentro e fora do laboratório, e que me acolheram e ajudaram de forma fantástica. Em especial à Aline da Silva, Kellen Rocha, Andréia Salgueiro, Hemerson Rosa, Morgana Duarte e Diogo Bicca; pois este trabalho tem um pouco de todos que ajudaram nas experimentações, na escrita ou discutindo resultados e possibilidades. Obrigado!

Aos corajosos que me ajudaram nas coletas das serpentes em campo, acreditando no trabalho: Giancarlo Bilo e Edward Pessano; têm minha gratidão.

E, um agradecimento especial ao meu coorientador, Prof. Dr. Robson Puntel, e ao meu orientador, Prof. Dr. Vanderlei Folmer, que me acolhem desde 2014. E me proporcionaram esta chance de crescer, como profissional, como discente, e como pessoa.

Não realizamos nada sozinhos. A tod@s, meus mais sinceros agradecimentos!

Podemos facilmente perdoar uma criança que tem medo do escuro; a real tragédia da vida é quando os homens têm medo da luz."
Platão

RESUMO

Desde a antiguidade, o uso de plantas medicinais está entre as práticas populares para tratar envenenamentos causados por diversos animais. Atualmente, em casos de envenenamentos por serpentes, extratos vegetais são utilizados como terapia alternativa ou como coadjuvantes à soroterapia. O tratamento com soro antiofídico é o procedimento adotado nestes casos, mas é limitado tanto em sua distribuição quanto eficácia. Neste contexto, diferentes extratos do ipê-amarelo *Handroanthus chrysotrichus* são empregados empiricamente em casos de envenenamentos ofídicos. Trata-se de uma árvore pertencente à família Bignoniaceae e com ampla distribuição no Brasil. Assim, esta pesquisa teve como objetivos: i) investigar o perfil fitoquímico do extrato hidroetanólico da casca do *H. chrysotrichus* e seu potencial farmacológico e toxicológico *in silico, in vitro* e *in vivo;* ii) avaliar o potencial terapêutico do extrato frente aos danos induzidos pela peçonha da serpente *Philodryas patagoniensis* em camundongos. Para isso, o extrato hidroetanólico da casca obtido por percolação foi liofilizado e, no momento dos testes, solubilizado em solução salina (0,9%) estéril. O extrato foi testado no modelo de *Artemia salina*, indicando a presença de compostos bioativos por meio de uma DL_{50} de 276 μg. mL^{-1} (R^2 = 0,9912). Em seguida, o extrato foi analisado por métodos colorimétricos e GC-MS, os quais apontaram altos níveis de polifenóis e a ocorrência de α-curcumeno, β-bisaboleno, 4-(4-metilfenil) pentanal, ácido pentanóico e acetato de isoamila, sendo os dois primeiros os compostos majoritários. Predições das atividades biológicas *in silico* dos compostos identificados estão de acordo com seu uso tradicional, e apresentam baixa probabilidade de toxicidade. As propriedades antioxidantes (TAC, $DPPH^{\bullet}$ e $ABTS^{\bullet+}$ *scavenger*, FRAP, teste de degradação da desoxirribose e quelação de Fe^{++}) do extrato, juntamente com a ausência de citotoxicidade e genotoxicidade *in vitro,* complementam as predições *in silico.* E alicerçam o uso do ipê-amarelo pela medicina popular **(Artigo 1)**. No entanto, *in vivo,* a exposição aguda ao extrato hidroetanólico causou danos oxidativos nos tecidos cerebrais, hepáticos e renais de camundongos a partir da concentração de 50 mg. kg^{-1}, como constatado nas análises de oxidação lipídica e proteica desses tecidos. Além disso, houve um aumento significativo nos níveis de creatinina do grupo exposto a 100 mg. Kg^{-1} e leucopenia ($p<0,05$) em todas as concentrações testadas (10 – 100 mg. Kg^{-1}). Ainda, a exposição subcrônica à concentração mais alta do extrato (100 mg. kg^{-1}) alterou o comportamento dos animais ao diminuir o número de cruzamentos no teste de campo aberto e aumentar o tempo desprendido com autolimpeza. Nessa exposição, os tecidos cerebrais e hepáticos dos camundongos também demonstraram aumento significativo dos níveis de proteínas carboniladas em todas as

concentrações administradas **(Artigo 2)**. Para avaliar o potencial terapêutico do extrato em casos de envenenamento, propôs-se o emprego da peçonha da *P. patagoniensis* como agente tóxico. Esta peçonha apresenta potencial miotóxico, edematogênico, nociceptivo e hiperalgésico. E, embora seja uma espécie cujos acidentes sejam negligenciados, sua peçonha detém atividades similares às do gênero *Bothrops* **(Manuscrito 1).** Assim, ao comparar testes comportamentais do grupo de camundongos injetados com a peçonha e tratados com o extrato, com o grupo não tratado, nota-se uma diminuição significativa da nocicepção e hiperalgesia nos primeiros. O efeito protetor do extrato é verificado também nas mensurações do edema e temperatura induzidos pela peçonha de *P. patagoniensis* na pata traseira direita dos camundongos ao longo dos dias. Danos sistêmicos, avaliados pelos níveis de creatinina no sangue e danos oxidativos em leucócitos (genotoxicidade) e nos tecidos cerebrais, hepáticos e renais (oxidação lipídica e proteica), foram minimizados no grupo tratado com o ipê-amarelo **(Manuscrito 2).** Os resultados aqui apresentados permitem concluir que o extrato hidroetanólico da casca de *H. chrysotrichus*, utilizado na medicina tradicional para tratar envenenamentos por serpentes, tem ação protetora contra os danos induzidos pela peçonha de *P. patagoniensis*, principalmente contra os sinais cardinais da inflamação e o estresse oxidativo sistêmico desencadeado pela mesma. Em conjunto, os dados levantam a possibilidade de o extrato apresentar efeitos adversos, os quais exigem precauções no seu uso. Neste contexto, demonstra-se os potenciais benefícios do tratamento com o ipê-amarelo e abrem-se perspectivas para investigações futuras do uso desta planta como terapia complementar ao tratamento clínico tradicional.

Palavras-chave: Etnobotânica. Farmacologia. Ofidismo. Tabebuia. Toxicologia.

ABSTRACT

Since ancient times, the use of medicinal plants has been among the popular practices to treat envenoming caused by various animals. Currently, in cases of snake envenoming, plant extracts are used as alternative therapy or as an adjunct to serum therapy. Treatment with antivenom is the procedure adopted in these cases, but it is limited both in its distribution and efficacy. In this context, different extracts of the Golden trumpet *Handroanthus chrysotrichus* are used in cases of ophidism. This is a tree belonging to the Bignoniaceae family and with wide distribution in Brazil. Thus, this study aimed: i) to investigate the phytochemical profile of the hydroethanolic extract of *H. chrysotrichus* bark and its pharmacological and toxicological potential *in silico, in vitro,* and *in vivo*; ii) to evaluate the therapeutic potential of the extract against the damage induced by the *Philodryas patagoniensis* snake venom in mice. For this, the hydroethanolic extract of the bark obtained by percolation was lyophilized and, at the time of the tests, it was solubilized in sterile saline solution (0.9%). The extract was tested in the model *Artemia salina*, indicating the presence of bioactive compounds through an LD_{50} of 276 μg. mL^{-1} ($R^2 = 0.9912$). Then, the extract was analyzed by colorimetric methods and GC-MS, which indicated high levels of polyphenols and the occurrence of α-curcumene, β-bisabolene, 4-(4-methylphenyl) pentanal, pentanoic acid and isoamyl acetate. Predictions of the *in silico* biological activities are in line with their traditional use and have a low probability of toxicity. The antioxidant properties (TAC, $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ scavenger, FRAP, Deoxyribose degradation test, and Fe^{++} chelation) of the extract, together with absence of *in vitro* cytotoxicity and genotoxicity, complement the *in silico* predictions. Data favorable to its use in traditional medicine **(Article 1)**. However, acute exposure to the hydroethanolic extract *in vivo* caused oxidative damage in the brain, liver, and kidney tissues of mice from the concentration of 50 mg. kg^{-1}. In addition, there was a significant increase in creatinine of the group exposed to 100 mg. kg^{-1} and leukopenia ($p<0.05$) at all concentrations tested (10 – 100 mg. kg^{-1}). Furthermore, sub-chronic exposure to the highest concentration of the extract (100 mg. kg^{-1}) altered the behavior of the animals by decreasing the number of crossings in the open field test, and increasing the time spent on self-grooming. In this exposure, the brain and liver tissues of the mice also showed a significant increase in carbonylated proteins levels at all concentrations administered **(Article 2)**. In order to evaluate the therapeutic potential of the extract in cases of envenoming, we proposed the use of *P. patagoniensis* venom as a toxic agent. This venom has a myotoxic, edematogenic, nociceptive, and hyperalgesic potential. Although it is a species whose accidents are neglected, its venom has activities similar to those of the genus *Bothrops*

(Manuscript 1). Thus, behavioral tests of the animal group injected with the venom and treated with the extract, compared to the untreated group, showed a significant decrease in nociception and hyperalgesia. The protective effect of the extract is also verified in the measurements of edema and temperature induced by *P. patagoniensis* venom in the right hind paw of the mice over the days. Systemic damages, assessed by blood creatinine levels and oxidative damage in leukocytes and brain, liver, and kidney tissues, was minimized in the Golden trumpet treated group **(Manuscript 2)**. In summary, the results presented here allow us to conclude that the hydroethanolic extract of *H. chrysotrichus* bark, used in traditional medicine to treat snake envenoming, has a protective action against the damage induced by *P. patagoniensis* venom. Mainly against the cardinal signs of inflammation and the systemic oxidative stress triggered by envenoming. Together, the data raise the possibility that the extract has adverse effects, which require precautions in its use. In this context, perspectives to future investigations on the use of this plant as a complementary therapy to traditional clinical treatment are open.

Keywords: Ethnobotany. Pharmacology. Ophidism. Tabebuia. Toxicology.

LISTA DE FIGURAS

LISTA DE ABREVIATURAS E SIGLAS

ABTS$^{\bullet+}$: 2,2′-Azino-bis (-6-sulfónico ácido 3-etilbenzotiazolina) Diamonio Sal

ALT: Alanina aminotransferase

ANOVA: Análise de variância

ANVISA: Agência Nacional de Vigilância Sanitária

AST: Aspartato aminotransferase

CA: Células totais analisadas

COX: Ciclooxigenase

CRISPs: Proteínas secretadas ricas em cisteínas

DI: Índice de dano

DL_{50}: Dose letal mediana

DNPH: 2,4-dinitrophenylhydrazine

DPPH$^{\bullet}$: 2,2-difenil-1-picrilhidrazil

DTNB: Regente de Ellman

EDTA: Solução de ácido etilenodiamino tetra-acético

EROs: Espécies reativas de oxigênio

FRAP: Determinação do poder de redução do íon ferro (do inglês, Ferric Reducing Antioxidant Power)

FST: Teste de nado forçado

GC-MS: Cromatografia Gasosa acoplada a um Espectro de Massas

GSH: Glutationa

HCBE: Extrato da casca de *Handroanthus chrysotrichus* (do inglês, *Handroanthus chrysotrichus* Bark Extract)

HCCE: Extrato bruto da *Handroanthus chrysotrichus* (do inglês, *Handroanthus chrysotrichus* Crude Extract)

IC_{50}: Concentração inibitória média

IL:	Interleucina
LD_{50}:	Dose letal mediana (do inglês Lethal Dose)
MDA:	Malondialdeído
ND:	Risco não-detectado
NPSH:	Níveis de tióis não proteicos
OFT:	Teste de campo aberto
Pa:	Atividade provável
PBMC:	Células mononucleares do sangue periférico
PG:	Prostaglandina
Pi:	Inatividade provável
PLA2:	Fosfolipase A2
PM:	PubMed
PpV:	Peçonha da *Philodryas patagoniensis*
p.o.:	Administração oral
Sc:	Scopus
S1:	Fração do sobrenatante
SD:	Desvio padrão
SOD:	Superóxido dismutase (SOD)
SVMPs:	Metaloproteinases derivadas da peçonha das serpentes
TAC:	Capacidade antioxidante total
TBA-RS:	Substâncias reativas ao ácido tiobarbitúrico
TCA:	Ácido tricloroacético
TFK:	Tampão fosfato
TNF-α:	Fator de necrose tumoral do tipo alfa
TST:	Teste de suspenção de cauda
WoS:	Web of Science

SUMÁRIO

1. APRESENTAÇÃO

Esta Tese está constituída da seguinte forma: Introdução, Revisão Bibliográfica, Justificativa, Objetivos, Resultados, Discussão Geral, Conclusões e Referências Bibliográficas.

Os **Resultados** são apresentados sob a forma de **02 Artigos Científicos** e **02 Manuscritos Científicos**. Assim, cada documento contém sua Introdução, Metodologia, Resultados, Discussão e suas respectivas Referências. A formatação dos Artigos atende às normas próprias dos periódicos onde foram publicados, ou, no caso dos Manuscritos, aos periódicos pretendidos para submissão.

Sempre que foi necessário, os trabalhos tiveram seus protocolos aprovados pela Comissão de Ética no Uso de Animais (CEUA) institucional para realização dos ensaios experimentais (Anexos 1 – 3).

Por fim, as **Referências Bibliográficas**, presentes ao final do trabalho, consideram apenas as citações apresentadas na estrutura da Tese – **Introdução, Revisão Bibliográfica** e **Discussão Geral** – uma vez que os Artigos e Manuscritos têm suas Referências apresentadas na própria composição.

2. INTRODUÇÃO

Extratos de plantas medicinais têm sido utilizados ao longo dos séculos para tratar as mais diversas afecções, entre elas os acidentes ofídicos. Espécies vegetais utilizadas com a finalidade de inibir a ação do veneno das serpentes são administradas por via oral a partir de infusão, maceração hidroalcóolica ou mascando-se a planta, bem como utilizam-se banhos externos ou cataplasmas em casos de maior gravidade (OTERO et al., 2000a).

Entre as famílias botânicas destacadas na medicina popular está a Bignoniaceae, grupo dos ipês dos gêneros *Tabebuia* e *Handroanthus*. Os ipês são árvores ornamentais empregadas tanto em ambientes urbanos e parques quanto em reflorestamentos. Também são utilizadas como plantas medicinais para obtenção de efeitos analgésico, vulnerário, antitumoral, antifúngico, anti-inflamatório, antipirético e contra úlceras pépticas, diabetes e anemia (OGA; SEKINO, 1969; RAI et al., 2012).

De forma geral, diversos extratos como de erva-cidreira, erva-mate e macela proporcionam efeitos benéficos conhecidos, como neuroproteção (MARTINS et al., 2012) e ações antioxidante e antimutagênico (BRACESCO et al., 2018; COLPO et al., 2016; SALGUEIRO et al., 2016a). Entretanto, nos tratamentos em casos de exposição a ofídios, os ipês têm recebido destaque. No Pará, a decocção a partir das folhas de *Handroanthus barbatus* é utilizada para tratar as vítimas de envenenamentos ofídicos (de MOURA et al., 2015). Na América Central a *Tabebuia rosea* tem suas flores, folhas e raízes utilizadas para o mesmo fim (HOUGHTON; OSIBOGUN, 1993), inclusive com seu potencial antiofídico descrito na literatura (NÚÑEZ et al., 2004).

Nos casos de exposição a ofídios, o tratamento com soro antiofídico específico é o procedimento padrão adotado pelo Ministério da Saúde e administrado em ambiente hospitalar (BRASIL, 2021). Porém, o uso de plantas na terapia primária ou complementar à medicamentosa faz-se relevante, uma vez que o soro não está disponível a todos, principalmente aos trabalhadores rurais. E, mesmo ocorrendo soroterapia, a composição da peçonha pode variar entre populações de uma mesma espécie de serpente, reduzindo a eficácia do soro antiofídico (THEAKSTON; WARRELL; GRIFFITHS, 2003). Ao considerar que em 2018 foram registrados pelo Sistema de Informação de Agravos de Notificação – Sinan 28.961 acidentes ofídicos no Brasil, com 105 mortes, além de elevados custos para o tratamento e reabilitação dos sobreviventes (CRODA et al., 2020), a medicina popular intensifica-se como terapia alternativa.

Dentre os principais sintomas após estas exposições ofídicas estão edema, sangramento, equimose, bolhas, alterações na coagulação sanguínea e cefaleia (MISE; LIRA-DA-SILVA; CARVALHO, 2007). Biomarcadores de estresse oxidativo (aumento da produção de radicais livres e/ou redução das defesas antioxidantes) são encontrados nos tecidos sanguíneos das vítimas e chegam a persistir por 30 dias, mesmo após o tratamento com soro antiofídico. Assim, antioxidantes naturais poderiam ser uma opção, em conjunto com o soro antiofídico, para o tratamento nos casos de envenenamento (STRAPAZZON et al., 2015).

Parte destas manifestações, como dor, sangramento breve, eritema e edema – são compartilhadas por envenenamentos causados por *Philodryas patagoniensis* (de MEDEIROS et al., 2010), cuja peçonha também apresenta compostos neuro e miotóxicos, e atividades biológicas similares às peçonhas do gênero *Bothrops* (COSTA et al., 2008; ROCHA; FURTADO, 2007). *P. patagoniensis*, conhecida popularmente como papa-pinto, é uma serpente habitual no Bioma Pampa (LOEBMANN; QUINTELA, 2009) e utilizada neste trabalho. Neste sentido, nosso grupo de estudo padronizou diferentes ensaios em camundongos, injetando a peçonha obtida a partir da *P. patagoniensis* em roedores. Em especial, padronizou-se os ensaios de nocicepção, hiperalgesia, edema e genotoxicidade (dados ainda não publicados).

Mesmo que o ipê-amarelo *H. chrysotrichus* seja uma espécie utilizada na medicina popular para tratar envenenamentos por serpentes (BOLSON et al., 2015), apenas uma análise de seu potencial analgésico foi realizada por Grazziotin et al. (1992) utilizando extrato de seu caule; sem quaisquer estudos de sua toxicidade, ou da eficácia de seu uso em casos de envenenamento. Desta forma, o presente trabalho tem como proposta principal responder aos seguintes problemas de pesquisa:

a) A composição fitoquímica do extrato hidroalcóolico da casca do ipê-amarelo e suas análises, *in silico* ou *in vitro,* apoiam seu uso na medicina popular?
b) O uso desse extrato pode ocasionar toxicidade *in vivo*?
c) O extrato do ipê-amarelo em questão apresenta potencial terapêutico frente à peçonha de *P. patagoniensis*?

3. REVISÃO BIBLIOGRÁFICA

3.1. Plantas medicinais

As plantas medicinais constituem-se de espécies vegetais, cultivadas ou não, utilizadas com propósitos terapêuticos. Chamam-se plantas frescas aquelas coletadas no momento de uso e plantas secas as que foram precedidas de secagem e estabilização, equivalendo à droga vegetal. Estas plantas são amplamente reconhecidas como a principal fonte da maioria dos novos medicamentos, sendo historicamente utilizadas no tratamento e prevenção de doenças (BRANDELLI, 2017a).

Desde a antiguidade, o uso de plantas medicinais está entre as práticas populares para tratar todo tipo de envenenamento. No Brasil, a herança indígena contribuiu para a utilização destes recursos, os quais são utilizados em larga escala devido ao processo de colonização, onde ocorreram trocas de conhecimentos. A atividade essencialmente rural do país até a primeira metade do século XX e a união entre diferentes culturas também influenciaram na dispersão desses saberes. Fatores que, num país com a maior biodiversidade de árvores do mundo, garantem a propagação do conhecimento das plantas locais e de seus usos (BEECH et al., 2017; LORENZI; MATOS, 2002; PEREIRA; JACCOUD; MORS, 1996; SILVA; WESLLING; GABRIEL, 2019)

Em especial nos casos de envenenamentos por serpentes, os extratos são utilizados atualmente como coadjuvantes à soroterapia ou como terapia alternativa aplicado na falta de recursos soroterápicos (OTERO; FONNEGRA E JIMÉNEZ, 2000). Estudos etnobotânicos ampliam a gama de espécies medicinais identificadas por meio de consultas às comunidades negras, ribeirinhas, indígenas, entre outras (BENÍTEZ; VALOIS, 2004; BOOM, 1996; RIBEIRO et al., 2017). No entanto, não existem muitos dados para a maioria destas plantas referente as suas ações e validação do seu uso.

A ausência de estudos pode conduzir a população a uma falsa cura. Um exemplo é a tintura *Específico Pessoa*, extraída de raízes e folhas de ervas medicinais e muito conhecida no Brasil. Elaborada com base no tubérculo chamado cabeça-de-negro ou raiz-de-cobra, esta "garrafada" é recomendada em casos de envenenamentos por serpentes, aranhas ou escorpiões há décadas (CARDOSO, 2009). Sem registro na Agência Nacional de Vigilância Sanitária - ANVISA, é vendido pela internet ou em feiras, principalmente no Norte e Nordeste do Brasil. Contudo, o produto não possui atividade antioxidante, além de ser potencialmente citotóxico e desencadear episódios de vômitos (CASTOLDI et al., 2020; SACHETT et al., 2020; SANTOS; PRUDENCIO, 2019).

Por outro lado, muitas plantas utilizadas popularmente como antídotos para tratar envenenamentos por serpentes devem ter essas propriedades, devido ao grande número de compostos ativos que contêm (MORS et al., 2000). Inclusive, algumas destas espécies vegetais (*Brosimum guianense*, *Bauhinia forficata*, *Tabebuia aurea*, *Tamarindus indica*) foram testadas em laboratório e indicaram atividades antiofídicas, pois neutralizam a letalidade das peçonhas de diferentes víboras, bem como os efeitos inflamatórios, hemorrágicos, miotóxicos, edematogênicos, coagulatórios e nociceptivas desencadeados pelas mesmas (BITTENCOURT et al., 2014; OLIVEIRA et al., 2005; REIS et al., 2014; USHANANDINI et al., 2006). Todas com enorme potencial para gerar produtos inovadores e proporcionar benefícios sociais.

Mas diante de tantas informações controversas, o desafio nos dias atuais é garantir o acesso e o uso racional das plantas medicinais e medicamentos fitoterápicos – resultado da industrialização da planta medicinal a partir de uma formulação específica (BRANDELLI, 2017b). Acredita-se que cerca de 80% da população de países em desenvolvimento faz uso da medicina tradicional para sua atenção primária, principalmente à base de plantas (BRASIL, 2006a). No Brasil, este percentual pode ser ainda maior (VEIGA JUNIOR, 2008).

Assim, para que a população tenha segurança, eficácia e qualidade no tratamento adotado, o país estabeleceu em 2006 a "Política Nacional de Plantas Medicinais e Fitoterápicos", seguido em 2008 pelo "Programa Nacional de Plantas Medicinais e Fitoterápicos" (BRASIL, 2006a, 2009). Ambos fomentam o conhecimento acerca dos saberes e práticas da medicina popular e apoiam pesquisas que investiguem a eficácia e segurança destas práticas. No entanto, o controle dos produtos à base de ervas medicinais é feito pela ANVISA.

Por fim, o Sistema Único de Saúde – SUS realiza a conexão exigida, facilitando o acesso da população às plantas medicinais e aos seus derivados, bem como às informações quanto ao manejo e uso correto das plantas medicinais e fitoterápicos (BRASIL, 2006b). Caracterizando a fusão do saber popular com o saber técnico.

3.2. *Handroanthus chrysotrichus*

A família Bignoniaceae apresenta distribuição pantropical, com aproximadamente 120 gêneros e 800 espécies. Entre estas espécies, 100 são conhecidas popularmente como ipês, incluídas atualmente nos gêneros *Handroanthus* e *Tabebuia* (GROSE; OLMSTEAD, 2007; SOUZA; LORENZI, 2005). Gêneros amplamente utilizados na ornamentação urbana devido à beleza de sua floração, podendo ser branca, amarela, rosa e roxa. Também por este motivo, o

ipê-amarelo é a flor-símbolo do Brasil. Destaca-se que o termo Ipê se originou do tupi e significa “árvore cascuda”. (LORENZI, 2002).

O ipê-amarelo *Handroanthus chrysotrichus* (Figura 1), anteriormente denominada como *Tabebuia chrysotricha*, é uma árvore que pode atingir 10 m de altura e 40 cm de diâmetro do tronco (LORENZI, 2002). Suas cascas e caule são macerados e utilizados para conter processos alérgicos e como depurativos por ribeirinhos mato-grossenses (RIBEIRO et al., 2017). No Paraná, utiliza-se a decocção de suas flores e caule contra males do sistema cardiovascular, distúrbios imunológicos e envenenamentos ofídicos (BOLSON et al., 2015).

Os ipês (*T. rosea*, *H. ochraceus* e *H. impetiginosus*) apresentam efeitos antioxidantes em seus extratos oriundos da casca (OSPINA et al., 2013; PARK et al., 2003). Característica esta que ampara o uso medicinal de diferentes extratos. E, nos casos de envenenamentos, ensaios utilizando agentes antioxidantes exógenos como melatonina indicam redução dos danos oxidativos gerados pela peçonha da serpente *Naja haje* (MONEIM et al., 2015). Além disso, tratamentos com antioxidantes como ácido ascórbico e complexos vitamínicos protegem contra o envenenamento induzido por espécies do gênero *Bothrops* em modelos experimentais (OLIVEIRA; SIMÃO; MARCUSSI, 2016).

Somado aos efeitos antioxidantes, alguns ipês demonstram potencial no tratamento de acidentes ofídicos específicos. O ipê-rosa *T. rosea* foi efetivo em neutralizar a peçonha da jararaca *Bothrops atrox* (OTERO et al., 2000b, 2000c). E o extrato da casca do ipê-amarelo *T. aurea* possui efeitos anti-inflamatórios e diminui a algesia, a miotoxicidade e os episódios hemorrágicos induzidos pela peçonha do gênero *Bothrops* (MALANGE et al., 2019; REIS et al., 2014).

Contudo, pouco se conhece sobre *H. chrysotrichus*, além da comprovada ação analgésica do extrato de seu caule em camundongos (GRAZZIOTIN et al., 1992). Embora haja déficit de estudos toxicológicos, ao considerar sua família e estudos etnobotânicos, é presumível que seu extrato apresente potencial para tratar ou amenizar os danos ocasionados por exposição ofídica.

Figura 1. Exemplar de ipê-amarelo *Handroanthus chrysotrichus* encontrado na Universidade Federal do Pampa – Campus Uruguaiana, espécime fonte de recursos para obtenção do extrato desta pesquisa. Acervo pessoal. Foto: Márcio Tavares Costa.

3.3. Procedimento padrão adotado em casos de envenenamentos por serpentes

Estudos epidemiológicos indicam que o número de acidentes por animais peçonhentos vem crescendo nos últimos anos e que a chance de morte por acidente ofídico é maior (GONÇALVES et al., 2020; TAVARES et al., 2020). Ao todo, o Brasil registrou 30.482 casos de exposição a serpentes em 2019, com 147 mortes. A região Norte do país foi a mais afetada, com 34% dos acidentes. O grupo botrópico representou 68,5% dos casos. No entanto, as espécies não peçonhentas e ignoradas foram 20% (SINAN, 2020).

Envenenamentos por serpentes são tratados com soro antiofídico específicos para cada grupo de serpentes com importância médica (Figura 2) – Viperidae (gêneros *Bothrops, Crotalus* e *Lachesis*) e Elapidae (cobras-corais do gênero *Micrurus*). As imunoglobulinas são produzidas pelo fracionamento do plasma, geralmente obtido de grandes animais domésticos hiperimunizados. Estes soros, quando injetados em pacientes envenenados, pode neutralizar qualquer uma das peçonhas usadas em sua produção e, em alguns casos, também neutralizará

os efeitos do envenenamento de espécies intimamente relacionadas (WHO, 2008). Apesar de existirem soros específicos para diferentes gêneros de serpentes, o processo de produção é similar, como apontado no Manual de Diagnóstico e Tratamento de Acidentes por Animais Peçonhentos (FUNASA, 2001).

No Brasil, a soroterapia com antídoto é um procedimento ambulatorial oferecido em Unidades Básicas de Saúde, constituindo polos regionais de atendimento. Este tratamento tem reduzido o número de óbitos das vítimas de acidentes ofídicos (CITELI et al., 2018; FAN; CARDOSO, 1995). Em envenenamentos por *Bothrops* (ex.: cruzeiras e jararacas), gênero responsável por cerca de 90% dos casos de exposição a serpentes no Brasil, o tratamento com antiveneno botrópico é capaz de reverter os efeitos sistêmicos (FUNASA, 2001; ROSENFELD, 1971). Por outro lado, pacientes que recebem o soro podem apresentar reações imediatas como febre, palidez, náuseas, vômitos, taquicardia, hipotensão, dispneia e broncoespasmo (MISE; LIRA-DA-SILVA; CARVALHO, 2007). E, mesmo ocorrendo o tratamento padrão, a neutralização dos efeitos locais dificilmente é obtida. A evolução do quadro local pode acarretar na perda do membro afetado (ROSENFELD, 1971).

Outra limitante é a composição da peçonha, que pode variar entre populações de uma mesma espécie de serpente, reduzindo a eficácia do soro antiofídico. Por fim, o soro não está disponível a todos, principalmente aos trabalhadores rurais (THEAKSTON; WARRELL; GRIFFITHS, 2003). Tais restrições aumentam a necessidade de se pesquisar e estabelecer alternativas que possam auxiliar no tratamento das vítimas de acidentes ofídicos.

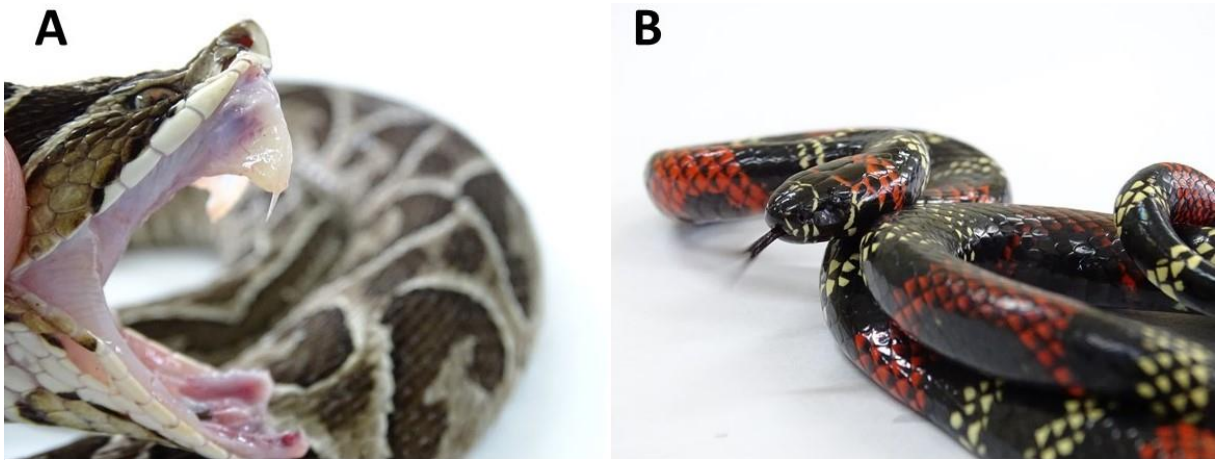


Figura 2. Espécimes com importância médica. A) *Bothrops alternatus*, conhecida popularmente como cruzeira, pertence a família Viperidae. B) Coral-verdadeira *Micrurus altirostris*, espécie da família Elapidae. Acervo pessoal. Fotos: Márcio Tavares Costa.

3.4. *Philodryas patagoniensis* e sua peçonha

A papa-pinto *P. patagoniensis* (Figura 3A) é uma serpente comum em áreas abertas do Bioma Pampa (LOEBMANN; QUINTELA, 2009). Espécie diurna, sua coloração amarronzada confere uma vantagem para camuflagem. São fundamentalmente terrícolas, embora possam abranger hábitos arborícolas ao forragear (HARTMANN; MARQUES, 2005).

Essa serpente pertence à família Dipsadidae, a qual é responsável por cerca de 20 a 40% dos acidentes ofídicos no Brasil (CARVALHO; NOGUEIRA, 1998; LOEBMANN; QUINTELA, 2009; ROSENFELD, 1971; SALOMÃO; ALBOLEA; ALMEIDA-SANTOS, 2003; SANTOS-COSTA et al., 2001; SILVEIRA; NISHIOKA, 1992). Como consequência dessas exposições, as vítimas deste grupo apresentam dor, edemas, hematomas, hemorragia, necrose muscular, além de efeitos sistêmicos, como tonturas e vômitos (ARAÚJO; SANTOS, 1997; ASSAKURA et al., 1992; DE MEDEIROS et al., 2010; PRADO-FRANCESCHI et al., 1996, 1998; RIBEIRO; PUORTO; JORGE, 1999). Especificamente a espécie *Philodryas olfersii* é responsável por envenenamentos graves e fatais em humanos (MACKESSY, 2002).

Esse potencial toxicológico se deve a peçonha produzida pela glândula Duvernoy. Com dentição opistóglifa (Figura 3B), a peçonha da *P. patagoniensis* é liberada sob estímulo mecânico e apresenta uma constituição de aproximadamente 90% de proteína, principalmente metaloproteinases derivadas da peçonha das serpentes (*snake venom metalloproteinases* – SVMPs). Desta forma, a peçonha pode desencadear efeitos edematogênicos, hemorrágicos, nociceptivos e de necrose sobre a área afetada (ROCHA; FURTADO, 2007; SERAPICOS; MERUSSE, 2006).

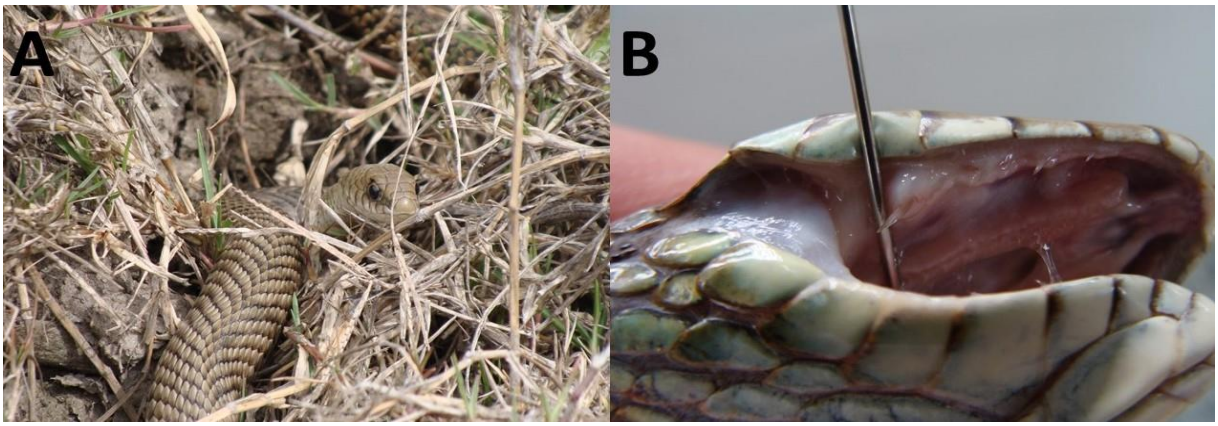


Figura 3. *Philodryas patagoniensis*. A) Espécime encontrado na Universidade Federal do Pampa - Campus Uruguaiana; B) Dentição opistóglifa da espécie. Acervo pessoal. Fotos: Márcio Tavares Costa.

Até o momento, registrou-se para a peçonha de *P. patagoniensis* atividade da fosfolipase A2 (PLA2), embora esta atividade tenha sido considerada baixa (ZELANIS et al.,

2010). Duas proteínas foram isoladas: a SVMP α-fibrinogenase patagonfibrase e a proteína rica em cisteína patagonina. A patagonfibrase tem suas atividades aumentadas na presença do íon cálcio e é capaz de hidrolisar fibrinogênio e azocaseína. E está envolvida com efeitos hemorrágicos, miotóxicos, e inibição da agregação plaquetária. Enquanto que a patagonina tem atividade miotóxica (PEICHOTO et al., 2007, 2009).

Com potencial para ocasionar mionecrose (PRADO-FRANCESCHI et al., 1998), as atividades proteolíticas verificadas para o gênero *Philodryas* superaram as da peçonha de *Bothrops alternatus* (GAY et al., 2005) e de *B. jararaca* (ROCHA et al., 2006). Para Rocha e Furtado (2007), a peçonha da *P. patagoniensis* possui atividades biológicas similares às de *Bothrops*. Assim, em casos de exposição a serpentes do gênero *Philodryas*, incorpora-se soro antiofídico botrópico à terapia, além dos métodos usuais para o tratamento, como administração de analgésicos, anti-inflamatórios e, nos casos de infecções, antibiótico (BUCARETCHI et al., 1993; NISHIOKA; SILVEIRA, 1994).

Estudos em diferentes modelos animais demonstraram que, em envenenamentos induzidos por espécies do gênero *Bothrops*, o uso do anti-inflamatório Dexametasona isolado, ou associado à soroterapia, tem efeito benéfico ao reduzir o edema e evitar potenciais danos musculares (ARAÚJO et al., 2007; CARVALHO et al., 2018; PATRÃO-NETO et al., 2013). Com envenenamentos causados por *P. patagoniensis* não é diferente, sugerindo o envolvimento de eicosanóides como principais mediadores do edema e nocicepção nos casos de exposição a estes ofídios (LOPES et al., 2017).

3.5. Danos relacionados às peçonhas

3.5.1. Estresse oxidativo

Organismos estão expostos continuamente às ações de agentes reativos. Formados a partir do metabolismo do oxigênio, as principais espécies reativas de oxigênio (EROs) radicalares são hidroxila (HO^-), superóxido ($O2^-$), peroxila (ROO^-) e alcoxila (RO^-); e entre as não-radicalares estão o oxigênio, peróxido de hidrogênio e o ácido hipocloroso. A reatividade destes EROs no organismo são variáveis, enquanto alguns atacam lipídios, proteínas, DNA, outros reagem apenas com lipídios (BARREIROS; DAVID; DAVID, 2006).

Embora sejam parte integrante dos organismos ao longo da vida, as células desenvolveram mecanismos de defesa antioxidantes, enzimáticos e não enzimáticos, para limitar os níveis intracelulares de EROs e controlar os danos decorrentes (BIANCHI; ANTUNES, 1999). Havendo desequilíbrio entre a geração de moléculas oxidantes e o sistema de defesa antioxidante, estabelece-se ali o estresse oxidativo. Processo que conduz à oxidação

de biomoléculas, com possíveis perdas de funções biológicas e desequilíbrio homeostático, cuja manifestação é o dano oxidativo potencial contra células e tecidos. Se não combatido, o estresse oxidativo pode ser letal (BARBOSA et al., 2010; HALLIWELL; WHITEMAN, 2004).

Lesões originárias de envenenamento por serpentes são danos geradores de estresse oxidativo (STRAPAZZON et al., 2015). O fenômeno de estresse resulta na formação de radicais livres a partir do choque, danos ao tecido no local da mordida e hemorragia (ALAM; GOMES, 1998). No local afetado pelo envenenamento ocorre o acúmulo de leucócitos, acarretando em uma demasiada geração de EROs, incluindo a produção endógena aumentada de peróxido de hidrogênio (ZAMUNER et al., 2001). O processo hemorrágico resulta em hemólise dos glóbulos vermelhos (liberação de íons de ferro) e queda da atividade da enzima superóxido dismutase – SOD (FERREIRA; MATSUBARA, 1997). Além disso, toxinas específicas das peçonhas induzem a liberação de mediadores pró-inflamatórios e, assim, aumento do estresse oxidativo (GIRISH; KEMPARAJU, 2011).

Em vítimas de envenenamento, toxinas oriundas da peçonha perturbam e podem danificar a membrana celular, bem como romper a membrana mitocondrial, e causar consequente aumento de EROs. O fígado e os eritrócitos estão sempre ameaçados por estas espécies reativas, que podem induzir a perfusão capilar resultando em lise celular. No entanto, estes eventos tendem a ser equilibrados por um sistema antioxidante enzimático e não enzimático em indivíduos saudáveis (SEBASTIN SANTHOSH et al., 2013).

O estresse oxidativo não é reconhecido na patologia dos acidentes por mordidas de cobra, mas é capaz de gerar trombocitopenia, coagulopatia e hemorragia sistêmica. Situações que produzem complicações secundárias de longo prazo. O estresse oxidativo induzido por envenenamentos também pode causar toxicidade renal, hipopituitarismo e infertilidade (SUNITHA et al., 2015).

Neste sentido, estudos com agentes antioxidantes apresentam relevância. A melatonina, bem como, o ácido ascórbico e um complexo vitamínico – ácido ascórbico, vitamina E, e todas vitaminas do complexo B – foram testadas como terapias alternativas e demonstraram efeito protetor contra envenenamento ofídico (MONEIM et al., 2015; OLIVEIRA; SIMÃO; MARCUSSI, 2016). Portanto, o tratamento com antioxidantes apresenta potencial de, junto à terapia com soro antiofídico, ser a primeira linha de defesa em casos de acidentes com estes animais, e assim, reduzir a incidência de complicações associadas ao estresse oxidativo (Figura 4).

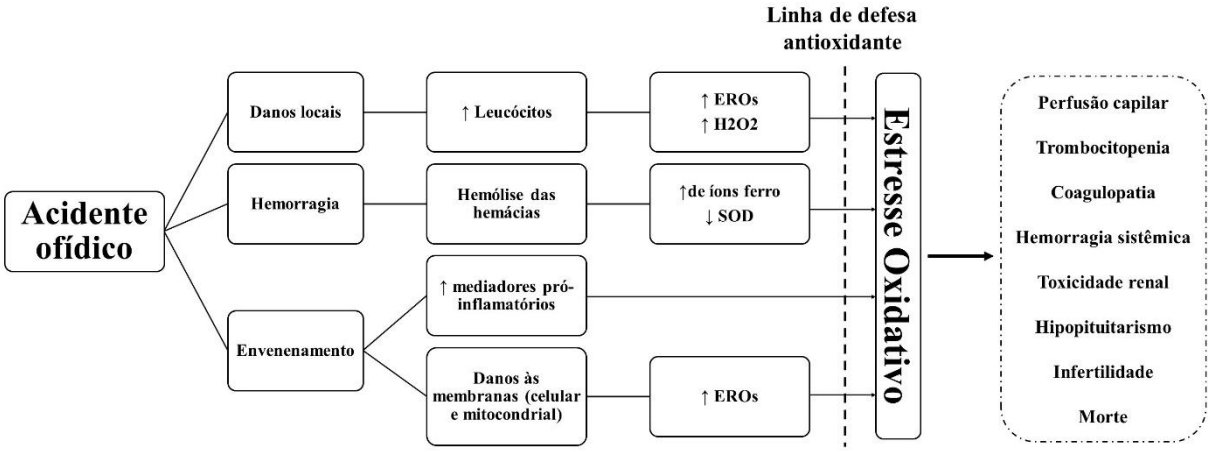


Figura 4. Representação esquemática da importância do processo de estresse oxidativo nos envenenamentos por serpentes e da incorporação de uma linha de defesa antioxidante. Fonte: autor.

3.5.2. Processo inflamatório

Na ocorrência de uma lesão tecidual, o organismo aciona mecanismos imunes de controle com o propósito de limitar os danos e auxiliar a regeneração. Estes mecanismos fazem parte da resposta inflamatória, caracterizada por quatro sinais cardinais: dor, rubor, calor, edema e, em alguns casos, culminando com perda parcial ou total da função (SCHMID-SCHÖNBEIN, 2006).

Neste contexto, uma exposição ofídica causa lesão tecidual por vários mecanismos (Figura 5), mesmo o trauma mecânico direto pode desencadear uma resposta inflamatória. O envenenamento subsequente agrava o quadro para uma inflamação sistêmica, ao induzir estresse oxidativo sistêmico, recrutar macrófagos e estimular a liberação de mediadores pró-inflamatórios, sendo esses, efeitos característicos de fenômenos multifatoriais (HARRIS et al., 1992; SEBASTIN SANTHOSH et al., 2013).

Esta inflamação no estágio inicial, ou agudo, é mediada principalmente pela ativação das células do sistema imunitário e seu sistema complemento. No local afetado, os primeiros mediadores químicos liberados são a histamina e a serotonina, os quais são produzidos por mastócitos, basófilos e plaquetas sanguíneas (FRANCISCHETTI et al., 2010). A histamina provoca vasodilatação e aumento da permeabilidade vascular, os quais resultam no extravasamento de líquido destes capilares, gerando eritema e edema típicos do processo. A serotonina, por sua vez, ativa os monócitos e impede a sua apoptose, além de modular a produção de citocinas e quimiocinas nos mesmos (CAMPBELL et al., 2010; SOGA et al., 2007).

A partir de então, com a vasodilatação possibilitando o aumento do fluxo sanguíneo e da permeabilidade vascular no local, começa a migração celular de leucócitos. E, de forma concomitante, ocorre o aumento da expressão de moléculas de adesão, prejuízo tecidual por atividade de proteases e EROs, necrose e apoptose, além da liberação de inúmeros mediadores pró-inflamatórios como as citocinas fator de necrose tumoral do tipo alfa (TNF-α) e interleucina 1 beta (IL-1β) (HUERRE; GOUNON, 1996; VIVIER; MALISSEN, 2005).

Além disso, a inflamação envolve outros sistemas, como o de coagulação e a cascata do ácido araquidônico. Este último originará as prostaglandinas (PGs) pela ação das ciclooxigenases (COX 1 e 2), leucotrienos e tromboxanos (pela ação de lipooxigenases), todos mediadores envolvidos no processo inflamatório (RANG; DALE, 2007). Diversos desses mediadores inflamatórios, quando liberados, ativam os nociceptores (fibras nervosas tipos Aδ e C) e facilitam a transmissão dolorosa, caracterizando o quadro de hiperalgesia (WRIGHT, 1999). A bradicinina, a PG E_2, o fator de crescimento nervoso e as IL pró-inflamatórias parecem exercer papel fundamental na nocicepção periférica (CHUANG et al., 2001).

Determinadas SVMPs são capazes de exercer quimiotaxia sobre os neutrófilos por meio do sistema complemento (FARSKY et al., 2000); enquanto outras, como a jararagina – toxina isolada da *Bothrops jararaca* – pode estimular diretamente a expressão do TNF-α, IL-1β e IL-6 nos macrófagos (CLISSA et al., 2001), agravando então, o processo inflamatório.

Estudos mostram que o uso de anti-inflamatórios clínicos associados a soroterapia aliviam os sinais cardinais da inflamação causadas por envenenamentos ofídicos. O anti-inflamatório esteroidal Dexametasona (inibidor de PLA2) inibe a resposta inflamatória, a miotoxicidade e o edema induzidos por envenenamentos do gênero *Bothrops*. Indometacina (inibidor da COX), um anti-inflamatório não esteroidal, também age amenizando o edema causado pelo grupo (ARAÚJO et al., 2007; PATRÃO-NETO et al., 2013).

Vítimas de acidentes causados por cobras da família Dipsadidae, frequentemente apresentam edema proeminente e hiperalgesia (PRADO-FRANCESCHI; HYSLOP, 2002). A peçonha de *P. patagoniensis* é um exemplo, pois provoca uma rápida resposta inflamatória com estes sintomas (de MEDEIROS et al., 2010). Resultados de testes realizados pelo nosso grupo de estudo apontam que o edema e a hiperalgesia podem perdurar entre seis e oito dias (dados não publicados). Destaca-se que a patagonfibrase, uma das toxinas isolada da *P. patagoniensis*, apresenta atividades alfa-fibrinolíticas, hemorrágicas e induz resposta inflamatória em camundongos (PEICHOTO et al., 2011).

Nesta espécie, testes utilizando a Dexametasona e a Indometacina diminuíram o edema causado pela sua peçonha, mas somente a Indometacina agiu aliviando a dor no local do

ferimento. Como não há alteração pelo tratamento com Celecoxibe (inibidor da COX-2) em nenhum dos casos, os derivados do ácido araquidônico parecem ser os principais mediadores do edema, enquanto que a dor está diretamente relacionada a COX-1 (LOPES et al., 2017). No entanto, os efeitos adversos de vários anti-inflamatórios utilizados na clínica demandam pesquisas por novos compostos com ação analgésica e que bloqueiem os processos inflamatórios.

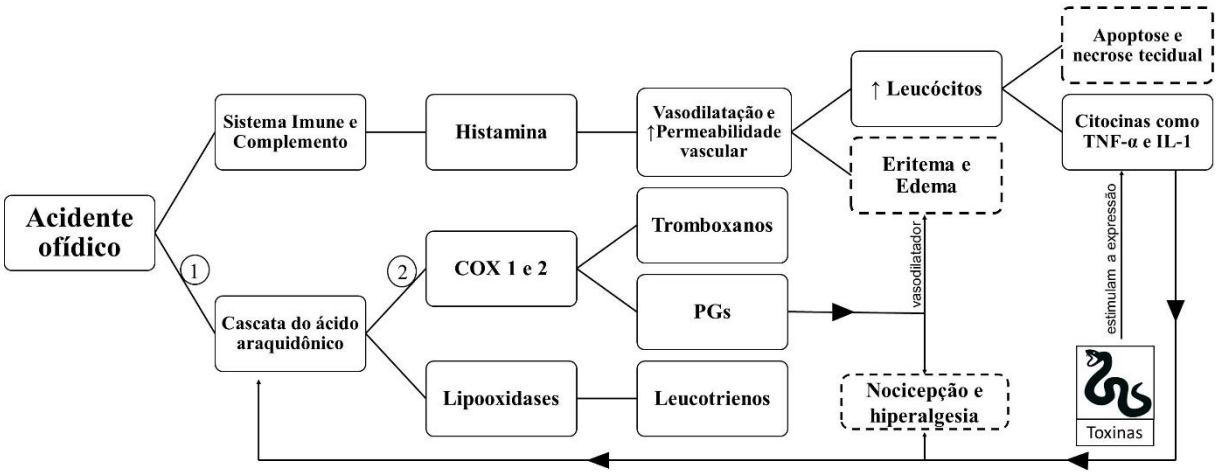


Figura 5. Simplificação do processo inflamatório desencadeado nos acidentes com serpentes *in situ*. Anti-inflamatórios como o Dexametasona (1) e Indometacina (2) atuam amenizando sintomas (linhas pontilhadas). Fonte: autor.

4. JUSTIFICATIVA

Diferentes espécies do gênero *Handroanthus* são extensivamente utilizadas pela medicina popular para tratar envenenamentos diversos. No entanto, o uso é empírico, e estudos que investiguem o perfil toxicológico e farmacológico destas plantas são necessários. Para que assim a população tenha segurança, eficácia e qualidade no tratamento, e também para disponibilizar uma possível fonte de compostos biologicamente ativos de interesse farmacêutico.

Esta proposta apresenta o ipê-amarelo *H. chrysotrichus*, espécie presente do Nordeste ao Sul do Brasil, principalmente nos Biomas Cerrado, Mata Atlântica e Pampa. *H. chrysotrichus* é utilizada em casos de exposição a serpentes por pessoas, as quais podem estar impossibilitadas de receber o procedimento adotado nas Unidades Básicas de Saúde. E quando tratadas com o antiveneno, o mesmo não garante plena recuperação.

Contudo, assim como em outros casos, o potencial toxicológico desta planta medicinal não é conhecido, bem como, seu potencial contra os efeitos lesivos ocasionados pelas peçonhas ofídicas. Considerando o elevado índice de acidentes envolvendo serpentes no Brasil, e todos os danos teciduais resultantes dos mesmos, estudos que buscam terapias alternativas para auxiliar na redução destes efeitos adversos são relevantes.

O fato de envenenamentos ocasionados pelas serpentes do gênero *Philodryas* serem tratados com soro antiofídico demonstra o potencial toxicológico deste grupo. Mas a escolha da serpente *P. patagoniensis* para obtenção de peçonha para este trabalho deve-se também: a) os efeitos induzidos pela peçonha são similares aos gerados pelas peçonhas do gênero *Bothrops*; b) estipula-se que sua família, Dipsadidae, seja responsável por cerca de 20 a 40% dos acidentes ofídicos registrados; c) a alta frequência com que esta espécie é encontrada no Bioma Pampa.

Por fim, o trabalho fomenta pesquisas na região do Bioma Pampa – fonte da matéria prima estudada. Incorpora a peçonha da *P. patagoniensis* como um recurso possível para trabalhos futuros. E, considerando a ausência de estudos descrevendo o perfil bioquímico, toxicológico e/ou farmacológico do extrato da casca do ipê-amarelo *H. chrysotrichus* na literatura científica, este trabalho proporciona perspectivas para estudos futuros complementares em áreas correlatas e transversais.

5. OBJETIVOS

Geral

Investigar o potencial do extrato da casca de ipê-amarelo *Handroanthus chrysotrichus* como agente terapêutico em casos de envenenamentos por serpentes

Específicos

- Analisar a composição química do extrato hidroetanólico da casca do ipê-amarelo **(Artigo 1)**;
- Avaliar *in silico* as predições farmacológicas e toxicológicas dos principais componentes químicos identificados do extrato **(Artigo 1)**;
- Determinar *in vitro* as atividades antioxidantes, citotoxicológicas e genotoxicológicas, bem como, a dose letal mediana em *Artemia salina* do extrato **(Artigo 1)**;
- Verificar a toxicidade *in vivo* do extrato da casca de *H. chrysotrichus* em casos de exposição aguda e subcrônica **(Artigo 2)**;
- Analisar o estado da arte acerca da peçonha de *Philodryas patagoniensis* e a possibilidade de sua utilização em testes toxicológicos e farmacológicos **(Manuscrito 1)**;
- Investigar a ação do extrato da casca de ipê-amarelo como atenuante dos principais sintomas de envenenamento causado por *P. patagoniensis* em camundongos **(Manuscrito 2)**.

6. RESULTADOS

Os resultados aqui apresentados estão sob a forma de dois Artigos Científicos e dois Manuscritos Científicos. O primeiro Artigo Científico foi publicado no ano de 2020 na revista "*Research, Society and Development*", e o segundo foi publicado em 2021 na revista "*Toxicology Research*". Os Manuscritos serão submetidos para avaliação e publicação em revistas especializadas com Qualis CAPES.

Desta forma, este capítulo inclui na íntegra as produções que avaliaram *in silico, in vitro* e *in vivo* o extrato alvo. E propõe, por meio dos Manuscritos, finalizar a hipótese desta Tese. O Quadro abaixo relaciona os objetivos de cada trabalho, tipo de pesquisa realizada e os resultados alcançados.

Objetivo específico	Metodologia	Resultados	Situação
Investigar a composição, potencial antioxidante, farmacológico e toxicológico do extrato hidroalcóolico da casca do ipê-amarelo *Handroanthus chrysotrichus*	Qualitativa e quantitativa, com testes *in silico* e *in vitro*	**Artigo 1** Antioxidant and toxicological potential of the Golden trumpet hydroalcoholic stem bark extract	Publicado na revista *Research, Society and Development* em 2020
Avaliar a toxicidade do extrato hidroalcóolico da casca de *Handroanthus chrysotrichus* em camundongos	Pesquisa quantitativa. Verificou-se os efeitos *in vivo* das exposições aguda e subcrônica ao extrato	**Artigo 2** *In vivo* effects of exposure to Golden trumpet *Handroanthus chrysotrichus* in mice	Publicado na revista *Toxicology Research* em 2021
Verificar o estado da arte acerca da peçonha da *Philodryas patagoniensis* e levantar a possibilidade de sua utilização nos campos Toxicológico e Farmacológico	Pesquisa qualitativa descritiva. Levantou-se artigos de três bases de dados, os quais foram fonte das informações	**Manuscrito 1** *Philodryas patagoniensis* venom: status of the art and its potential use in toxicological and pharmacological fields	A ser submetido ao *Journal of Venomous Animals and Toxins including Tropical Diseases* após as considerações da banca examinadora
Avaliar o efeito protetor do extrato hidroetanólico da casca de *H. chrysotrichus* frente aos principais danos desencadeados pelo envenenamento por *P. patagoniensis* em camundongos	Pesquisa quantitativa. Avaliou-se alterações comportamentais, além dos danos locais e sistêmicos induzidos pela peçonha	**Manuscrito 2** Analgesic and antioxidant effects of the Golden trumpet on snake-envenomed mice	A ser submetido ao *The Journal of Pharmacology and Experimental Therapeutics* após as considerações da banca examinadora

6.1. Artigo científico 1

Research, Society and Development, v. 9, n. 4, e122942936, 2020
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i4.2936

Potencial antioxidante e toxicológico do extrato hidroalcoólico da casca do Ipê-amarelo

Antioxidant and toxicological potential of the Golden trumpet hydroalcoholic stem bark extract

Potencial antioxidante y toxicológico del extracto hidroalcohólico de corteza de Lapacho amarillo

Recebido: 28/02/2020 | Revisado: 03/03/2020 | Aceito: 11/03/2020 | Publicado: 20/03/2020

Márcio Tavares Costa
ORCID: https://orcid.org/0000-0003-4940-3567
Federal University of Pampa, Brazil
E-mail: marciocosta@unipampa.edu.br
Aline da Silva Goulart
ORCID: https://orcid.org/0000-0002-3222-5816
Federal University of Pampa, Brazil
E-mail: alinefsgoulart@gmail.com
Jefferson de Jesus Soares
ORCID: https://orcid.org/0000-0003-4497-6761
Federal University of Pampa, Brazil
E-mail: jefferjsoares@hotmail.com
Andréia Caroline Fernandes Salgueiro
ORCID: https://orcid.org/0000-0003-4770-2379
Federal University of Pampa, Brazil
E-mail: acfsalgueiro@gmail.com
Hemerson Silva da Rosa
ORCID: https://orcid.org/0000-0002-8474-5100
Federal University of São Paulo, Brazil
E-mail: hemerdarosa@gmail.com
Elton Luis Gasparotto Denardin
ORCID: https://orcid.org/0000-0003-4768-2216
Federal University of Pampa, Brazil
E-mail: eltondenardin@gmail.com
Clésio Soldateli Paim
ORCID: https://orcid.org/0000-0001-7789-0492
Federal University of Pampa, Brazil

E-mail: csoldatelli30@hotmail.com

Robson Luiz Puntel

ORCID: https://orcid.org/0000-0001-9047-2906

Federal University of Pampa, Brazil

E-mail: robsonunipampa@gmail.com

Vanderlei Folmer

ORCID: https://orcid.org/0000-0001-6940-9080

Federal University of Pampa, Brazil

E-mail: Vandfolmer@gmail.com

Resumo

Handroanthus chrysotrichus é uma árvore da família Bignoniaceae, conhecida como ipê-amarelo e distribui-se pelo Nordeste, Sudeste e Sul do Brasil. Suas flores, caule e casca são usadas para fins medicinais no tratamento de doenças relacionadas ao sistema cardiovascular e imunológico. Esse estudo tem por objetivos avaliar o perfil fitoquímico, espectro de atividade biológica, capacidade antioxidante e potencial toxicológico do extrato da casca de *H. chrysotrichus*. O extrato hidroetanólico foi obtido por percolação e liofilizado. Os compostos presentes no extrato foram analisados por métodos colorimétricos e GC-MS. A avaliação do espectro de atividade biológica foi realizada *in silico*. O poder antioxidante foi determinado pela investigação da capacidade antioxidante total, capacidade quelante de ferro, ensaios $DPPH^{\bullet}$ e $ABTS^{\bullet+}$, e teste de degradação da desoxirribose. A capacidade de inibição da lipoperoxidação induzida por Fe^{+} foi avaliada em cérebros e fígados de camundongos. Náuplios de *Artemia salina* foram utilizados para avaliação da dose letal mediana. A toxicidade foi avaliada por simulação computacional e *in vitro* em linfócitos humanos. Como resultados, os métodos colorimétricos sugerem altos níveis de polifenóis e os dados de GC-MS indicaram a ocorrência de α-curcumeno, β-bisaboleno, 4- (4-metilfenil) pentanal, ácido pentanóico e acetato de isoamil no extrato da casca. Simulações computacionais apontaram atividades biológicas que estão de acordo com seu uso tradicional. A casca do extrato exibiu atividade antioxidante em diversos ensaios e foi efetiva em proteger cérebros e fígados de camundongos da lipoperoxidação induzida por Fe^{+}. A casca de *H. chrysotrichus* demonstrou uma toxicidade média em *A. salina* com potencial presença de compostos bioativos. Em geral, os compostos apresentaram baixa probabilidade de toxicidade nas previsões *in silico*. Não houve citotoxicidade e genotoxicidade nos ensaios realizados com linfócitos humanos. Os resultados indicam que a casca de *H. chrysotrichus* possui compostos com espectro de atividade biológica e baixo potencial

toxicológico. Além disso, mostra capacidade antioxidante e ação protetora contra a peroxidação lipídica. Os dados apresentados apoiam o uso medicinal do ipê-amarelo e apontam o mesmo como um extrato promissor para avaliações *in vivo*.

Palavras-chave: Fitoquímica; Terpenos; Cromatografia; Medicina tradicional; Tabebuia.

Abstract

Handroanthus chrysotrichus is a tree of the Bignoniaceae family known as golden trumpet that is distributed throughout Northeast, Southeast and South Brazil. Its flowers, stem and bark are used for medicinal purposes in the treatment of cardiovascular and immune system diseases. This study aims to evaluate the phytochemical profile, biological activity spectrum, antioxidant capacity and toxicological potential of *H. chrysotrichus* stem bark extract. Hydroethanolic extract was obtained by percolation and lyophilized. Compounds present in the extract were analyzed by colorimetric methods and by GC-MS. Evaluation of the biological activity spectrum was performed *in silico*. Antioxidant power was determined by investigation of total antioxidant capacity, iron chelating capacity, $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ assays, and deoxyribose degradation test. The ability to inhibit Fe^{+} induced lipoperoxidation was evaluated in mouse brains and livers. Nauplii of *Artemia salina* were used to evaluate the median lethal dose. Toxicity was assessed by computer simulation, and in vitro in human lymphocytes. As a result, colorimetric methods suggest high levels of polyphenols and GC-MS data indicated the occurrence of α-curcumene, β-bisabolene, 4- (4-methylphenyl) pentanal, pentanoic acid and isoamyl acetate. Computer simulations have pointed biological activities that are in accordance with their traditional use. The *H. chrysotrichus* stem bark extract exhibited antioxidant activity in several assays and was effective in protecting mouse brains and livers from Fe^{+} induced lipoperoxidation. *H. chrysotrichus* stem bark extract showed medium toxicity in *A. salina* with potential presence of bioactive compounds. In general, the compounds showed low probability of toxicity *in silico* predictions. There was no cytotoxicity and genotoxicity in human lymphocyte evaluation. The results indicate that *H. chrysotrichus* stem bark extract has compounds with biological activity spectrum and low toxicological potential. It also shows antioxidant capacity and protective action against lipid peroxidation. The data presented support the medicinal use of golden trumpet and point to it as a promising extract for *in vivo* evaluations.

Keywords: Phytochemistry; Terpenes; Chromatography; Traditional medicine; Tabebuia.

Resumen

Handroanthus chrysotrichus es un árbol de la familia Bignoniaceae conocido como lapacho amarillo (ipe-amarillo en Brasil) y se distribuye por todo el noreste, sudeste y sur de Brasil. Sus flores, tallo y corteza se utilizan con fines medicinales en el tratamiento de enfermedades relacionadas con el sistema cardiovascular y sistema inmune. Este estudio tiene como objetivo evaluar el perfil fitoquímico, el espectro de actividad biológica, la capacidad antioxidante y el potencial toxicológico del extracto de

corteza de *H. chrysotrichus*. El extracto hidroetanólico se obtuvo por percolación y se liofilizó. Los compuestos presentes en el extracto se analizaron por métodos colorimétricos y GC-MS. La evaluación del espectro de actividad biológica se realizó *in silico*. El poder antioxidante se determinó mediante la investigación de la capacidad antioxidante total, la capacidad quelante de hierro, los ensayos $DPPH^{\cdot}$ y $ABTS^{\cdot+}$, y la prueba de degradación de desoxirribosa. La capacidad de inhibir la lipoperoxidación inducida por Fe^{+} se evaluó en cerebros e hígados de ratones. Se utilizaron nauplios de *Artemia salina* para evaluar la dosis letal media (DL_{50}). La toxicidad se evaluó mediante simulación por computadora y también in vitro en linfocitos humanos. Como resultados los métodos colorimétricos sugieren altos niveles de polifenoles y los datos de GC-MS indicaron la presencia de α-curcumeno, β-bisaboleno, ácido 4- (4-metilfenil) pentanal, ácido pentanoico y acetato de isoamilo en el extracto. Las simulaciones por computadora han señalado actividades biológicas que están de acuerdo con su uso tradicional. El extracto exhibió actividad antioxidante en varios ensayos y fue eficaz para proteger los cerebros e hígados de ratones de la lipoperoxidación inducida por Fe^{+}. El extracto de la corteza de *H. chrysotrichus* mostró una toxicidad media en *Artemia* con posible presencia de compuestos bioactivos. En general, los compuestos mostraron baja probabilidad de toxicidad en predicciones *in silico*. No hubo citotoxicidad ni genotoxicidad en los ensayos con linfocitos humanos. Los resultados indican que el extracto de la corteza de *H. chrysotrichus* tiene compuestos con espectro de actividad biológica y bajo potencial toxicológico. También muestra capacidad antioxidante y acción protectora contra la peroxidación lipídica. Los datos presentados respaldan el uso medicinal del lapacho amarillo y lo señalan como un extracto prometedor para evaluaciones *in vivo*.

Palabras clave: Fitoquímica; Terpenos; Cromatografía; Medicina tradicional; Tabebuia.

1. Introduction

The family Bignoniaceae is the major group of Angiosperms plants and has been used for its beneficial health properties. Several parts of Bignoniaceae species as leaves, fruits, roots, sap, flowers and bark are traditionally used for treatment of diabetes, high blood pressure, asthma, cancer, uterine infection and others diseases. In all these uses, different extractive methods are employed including decoction, maceration, infusion, poultice, syrup and tincture (Bolson et al., 2015; Ribeiro et al., 2017).

In Brazil, *Handroanthus chrysotrichus* (Mart. ex DC.) Mattos, known as golden trumpet, is a native tree of Bignoniaceae family and occurs in Northeast, Southeast and South of country (Jardim Botânico do Rio de Janeiro, 2018). Its flowers, stem and bark are used in popular medicine to treat cardiovascular and immunological system diseases, allergic process and poisoning by insect bite and snakebite (Bolson et al., 2015; Ribeiro et al., 2017). *H.*

chrysotrichus was previously identified as *Tabebuia chrysotricha*. However, according to "The Plant List" (www.theplantlist.org), currently both names are synonymous.

In this way, the scientific literature reports many therapeutics properties for *Handroanthus*. For example, species *H. impetiginosus* showed analgesic, anti-inflammatory and antiulcerogenic effects in animal models (Lee et al., 2012; Twardowschy et al., 2008). *H. impetiginosus* has presented antibacterial and antioxidant activity (Park et al., 2003; Park et al., 2006). *H. chrysanthus* demonstrated immunostimulant activity (Perez et al., 2004) and *T. aurea* decreases inflammatory, myotoxic and hemorrhagic activities induced by snake venom (Reis et al., 2014). These studies point confirmations that family Bignoniaceae is a promising group with pharmacological activities.

Thus, considering the *H. chrysotrichus* empirical use, this study aimed: a) to quantify the total polyphenols and flavonoids contents; b) to identify the phytochemical major compounds in bark extract; c) to evaluate the biological activity spectrum of identified major compounds; d) to determine the antioxidant capacity of extract; d) to verify the median lethal dose, cytotoxicity and genotoxicity for crude extract and major compounds.

2. Materials and Methods

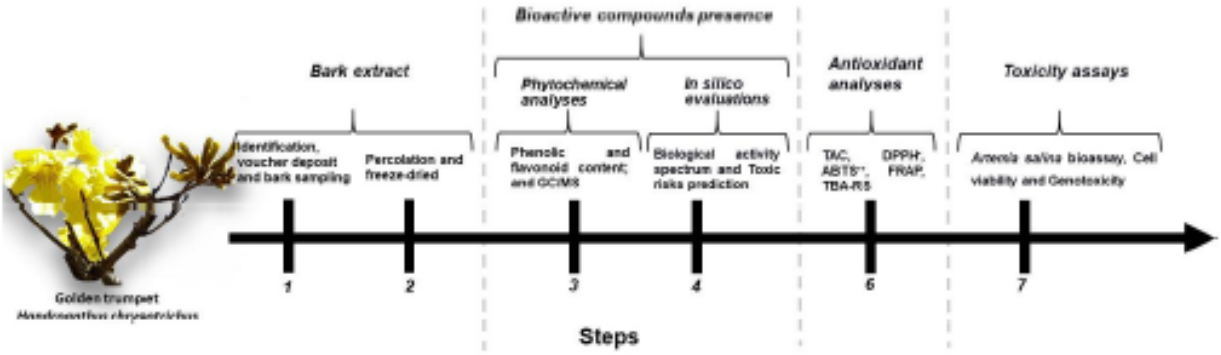
Chemicals

Ethanol, methanol, acetic acid, ascorbic acid, gallic acid, iron (III) chloride anhydrous and ferrous sulfate were purchased from Merck (Darmstadt, Germany). Quercetin, 6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid (trolox), 2-deoxy-D-ribose, 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2′-Azino-bis (3-ethylbenzothiazoline-6- sulfonic acid) diammonium salt (ABTS), and histopaque® were acquired from Sigma Chemical Co. (St. Louis, MO, USA).

Plant Material and Extract preparation

H. chrysotrichus bark was collected at 29°49'49.2"S 57°06'07.1"W geographical coordinates in summer 2017. After botanical identification, a voucher specimen was deposited at institutional herbarium (protocol number 142/2017). The plant material was subjected to drying at 40°C for five days and reduced to powder. Subsequently, samples of the powdered material were used in the percolation technique. Percolation was carried out by two hours in a glass column using hydroethanolic solution (70%) in a ratio of 1:10 (w/v). After that, the *H. chrysotrichus* bark extract (HCBE) was freeze-dried for later use. The experimental design is shown in Figure 1.

Figure 1. Experimental design of this study.



Phytochemical Evaluation

Total phenolic and flavonoid content of HCBE was measured according Nurmi et al. (1996) and Choi et al. (2002), respectively. A standard curve of gallic acid and quercetin was used to determine the polyphenols and flavonoids equivalents content. GC-MS analyses were performed according Soares et al. (2017), with some modifications. Briefly, the oven temperature program was as follows: initial oven temperature was held at 50°C for 5 min, and then increased to 150°C at a rate of 10°C. min^{-1} for 10 min, after was increased to 200°C at a rate of 10°C. min^{-1} and detained for 01 min and finally increased to 280°C at a rate of 10°C. min^{-1} and held for 10 min. Ion source and transfer line temperatures were 280°C. Compounds were identified by comparing mass spectra with data from the NIST library that are available in the instrument.

In silico Evaluations of GC-MS Identified Compounds

Biological activity spectrum

The computational Pass (Prediction of Activity Spectra for Substances, available in http://www.pharmaexpert.ru/PASSonline/predict.php) was applied on five major compounds identified in HCBE (Drwal & Griffith, 2013) to search potential pharmacological actions. Results were expressed in percentage of probable activity (Pa) and probable inactivity (Pi). Pa and Pi values vary from 0.000 to 1.000 and it was considered significant activity with Pa > Pi and Pa > 0.700.

Toxic risks prediction

A computational simulation experiment was performed to estimate possible toxicity risks of five major compounds from HCBE. For this five online computer program were

employed: ACD/Labs (Toronto, Canada), admetSAR server (Cheng et al., 2012), pkCSM platform (Pires et al., 2015), PreADMET web-based (https://preadmet.bmdrc.kr/) and OSIRIS Properties Explorer (http://www.organic-chemistry.org/prog/peo/). The toxic risks assessed were expressed in a flexible manner: (+) low potential, (++) medium risk, (+++) high risk and non-detected risk (ND).

HCBE Antioxidant Analyses

Total antioxidant capacity (TAC), DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ scavenger assay

TAC of HCBE was measured through spectrophotometric method proposed by Prieto et al. (1999). HCBE activity against DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radicals were performed in accordance with Choi et al. (2002) and Re et al. (1999), respectively. Results were expressed as inhibitory concentration of 50% (IC_{50}), based on percentage of radical inhibition in relation to the control without extract.

Ferric reducing antioxidant power (FRAP) assay

FRAP assay was measured for HCBE by spectrophotometric method (Benzie & Strain, 1996) and determined by plotting in a standard curve produced by the addition of ferrous sulfate to the FRAP reagent. Results were expressed as IC_{50}, based on the extract ability to reduce Fe^{3+} to Fe^{2+}.

Thiobarbituric acid reactive substances (TBA-RS) assay

TBA-RS was measured according Ohkawa et al. (1979) and used as an index of 2-deoxy-D-ribose degradation, and lipid peroxidation marker in brain and liver of Swiss albino mice, as described below.

a) Deoxyribose degradation assay

The deoxyribose degradation assay was performed according Puntel et al. (2005) with modifications. Briefly, the reaction medium was prepared containing: HCBE (concentrations 0 – 1000 μg. mL^{-1}), 2mM deoxyribose, 0.05mM and $FeSO_4$. After incubation at 37°C for 60 min, the reaction was stopped with 2% of trichloroacetic acid (TCA). Color reaction started with addition of 0.4mL of TBA and allowed to incubate for 30 min at 100°C. Standard curves of malondialdehyde (MDA) were performed to determine the MDA generated by the deoxyribose degradation, and the values were expressed as percentage of control (blank).

b) Analyses with brain and liver of Swiss albino mice

These biological tissues were donated from another projects where animals were maintained and used in accordance with guidelines of the Committee on Care and Use

of Experimental Animal Resources (009/2016). Mouse of controls groups (3 months, 30-35g) were euthanized by decapitation and the brain and liver were quickly removed, homogenized in NaCl (150 mM) and kept in ice. After homogenization, samples were centrifuged at 2000g at 4°C for 10 min to yield a low speed supernatant fraction (S1). The obtained S1 was mixed with ferrous sulfate ($FeSO_4$) in concentrations of 0.01mM, with or without HCBE (concentrations 0 – 1000 µg. mL^{-1}). A standard curve of MDA was constructed to determine TBA-RS content. Results are expressed as IC_{50}, based on the extract ability to inhibit lipid peroxidation of tissue, corrected by mg of tissue.

Median Lethal Dose Determination (LD50) and Toxicity assays

HCBE LD_{50} was evaluated according Meyer et al. (1982), with some modifications. Briefly, *A. salina* cysts were induced to hatch in aerating solution (saline water 3%) for 24 h. The nauplii were collected and transferred individually to a 96-well plate containing different HCBE concentrations (50, 100, 300, 600 and 900 µg. mL^{-1}) or control saline solution. The mortality was analyzed after 24 h. Assays were performed thrice in triplicate, with n=180 nauplii in each assay. The median lethal dose (LD_{50}) was the required concentration to kill 50% of nauplii. It was considered $LD_{50} < 1000$ µg. mL^{-1} as toxic and a possible presence of bioactive compounds.

For toxicity evaluation, bioassays in peripheral blood mononuclear cells (PBMC) were performed. Briefly, samples of human blood (10mL) were collected from healthy adult volunteers by venous puncture in heparinized tubes and incubated for four hours with HCBE (10 – 500 µg. ml^{-1} concentrations) or hydrogen peroxide (H_2O_2 5 mM, positive control). After incubation time, PBMC were separated with histopaque® (1:1) and submitted the following analyzes:

a) Cell viability

Cell viability was tested with trypan blue (0.2%) dye exclusion test. Peripheral blood mononuclear cells (PBMC) were counted in a Neubauer chamber. Stained cells and cells that have undergone the balloon effect were considered dead.

b) Genotoxicity

Genotoxicity was evaluated in PBMC through comet assay according to Singh et al. (1988). After mounting slides and performing electrophoresis, the lengths of tails were measured under fluorescence microscopy after adding ethidium bromide on the slides. One hundred cells from each of the three replicate slides were analyzed. Cells were visually scored according to tail length and receive scores from 0 (no migration) to 4

(maximal migration). Analyses was based on the reading damage index, which represented the sum of cells identified in each class multiplied by the class.

Statistical Analyses

Data were analyzed by two-way ANOVA followed by Tukey's multiple comparisons Test. Cell viability assays were interpreted by one-way ANOVA followed by Bonferroni's *post hoc* Test. Genotoxicity were analyzed by nonparametric Kruskal-Wallis with Dunn's Test. Values of $p < 0.05$ were considered significant. Data are presented as mean and standard deviation (SD). IC_{50} and LD_{50} determinations were performed using a logarithmic regression curve.

3. Results

Phytochemical Evaluation

Data showed that HCBE presents high levels of polyphenols (154.3 mg. g^{-1} gallic acid equivalents), and flavonoids (0.5 mg. g^{-1} of quercetin equivalents). Furthermore, GC/MS analyses identified the five most representative volatile compounds: α-curcumene, β-bisabolene, 4-(4-Methylphenyl) pentanal, pentanoic acid and isoamyl acetate respectively (Figure 2 and Table 1).

Figure 2. GC/MS analyses from *Handroanthus chrysotrichus* bark extract. Five identified volatile compounds are (1) pentanoic acid, (2) isoamyl acetate, (3) 4-(4-methylphenyl) pentanal, (4) α-curcumene, and (5) β-bisabolene.

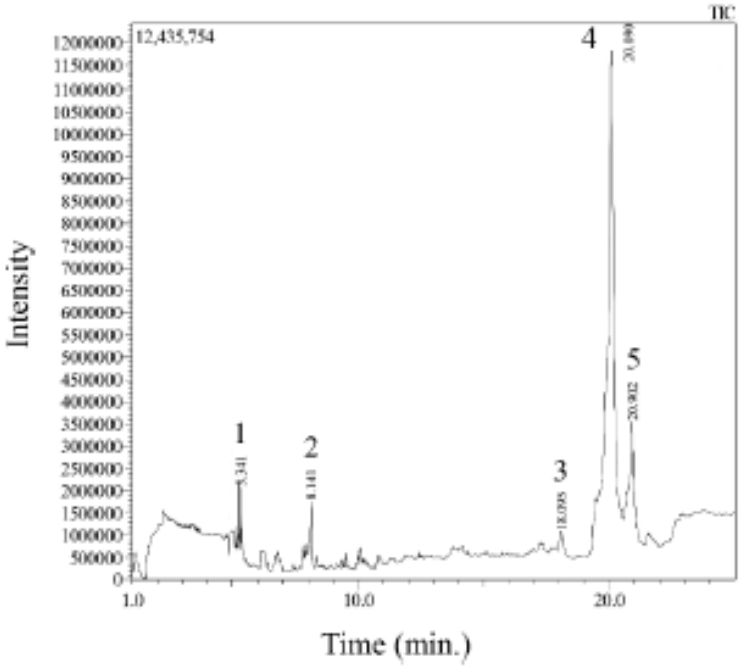


Table 1. Volatile compounds identified from *Handroanthus chrysotrichus* hydroethanolic extract.

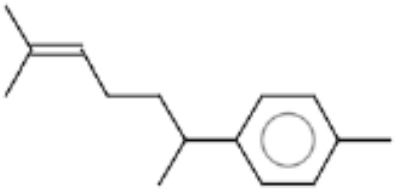
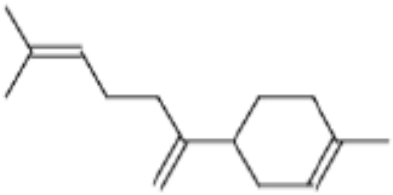
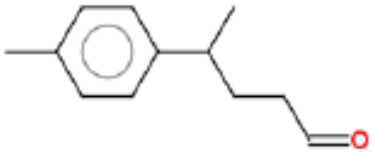
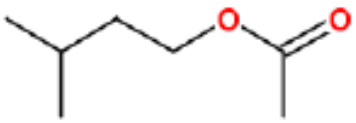
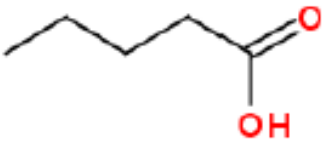
Volatile compounds	RT*	RI**	Area	
			(.10^5)	%
Pentanoic acid	5.341	875	72	2.67
Isoamyl acetate	8.141	820	58	2.14
4-(4-methylphenyl) pentanal	18.095	1429	75	2.79
α-curcumene	20.090	1524	2007	73.74
β-bisabolene	20.902	1500	508	18.66

* Retention Time; ** Retention Index

In silico Evaluations HCBE Major Compounds

The possible biological activity spectrum related to the five HCBE major compounds was evaluated by online platform PASS (Table 2). We selected the predicted properties with values of Pa > 0.7. Our data indicate that α-curcumene, β-bisabolene, 4-(4-methylphenyl) pentanal, pentanoic acid and isoamyl acetate, respectively, presented properties in common as mucobembranous protector and antieczematic. Others frequently properties in compounds are its carminative and fibrinolytic potential.

Table 2. Pharmacological activities predicted for *Handroanthus chrisotrichus* compounds.

Phytoconstituents	*Main predicted properties by PASS online*	*Pa**	*Pi**
alpha-Curcumene 	Mucomembranous protector	0.942	0.004
	Antieczematic	0.872	0.007
	Carminative	0.783	0.004
	Fibrinolytic	0.728	0.014
	Gastrin inhibitor	0.715	0.004
	Cholesterol antagonist	0.717	0.007
beta-Bisabolene 	Carminative	0.895	0.002
	Antieczematic	0.868	0.008
	Antineoplastic	0.856	0.006
	Mucomembranous protector	0.787	0.022
	Antiinflammatory	0.726	0.013
	Immunosuppressant	0.722	0.014
4-(4-Methylphenyl) pentanal 	Antieczematic	0.770	0.025
	Adenomatous polyposis treatment	0.718	0.006
	Carminative	0.711	0.006
	Fibrinolytic	0.708	0.02
	Mucomembranous protector	0.710	0.052
Isoamyl Acetate 	Phobic disorders treatment	0.947	0.003
	Anesthetic general	0.876	0.004
	Antiseborrheic	0.858	0.009
	Mucomembranous protector	0.837	0.011
	Fibrinolytic	0.733	0.013
	Antieczematic	0.709	0.043
Pentanoic Acid 	Mucomembranous protector	0.933	0.004
	Antieczematic	0.920	0.004
	Mucositis treatment	0.874	0.008
	Antiseborrheic	0.866	0.008
	Preneoplastic conditions	0.821	0.003
	Adenomatous polyposis treatment	0.819	0.002
	Antimutagenic	0.783	0.004
	Fibrinolytic	0.780	0.005
	Antiinflammatory intestinal	0.727	0.002
	Gastrin inhibitor	0.720	0.004
	Anesthetic general	0.706	0.006

Probable activity; Pi = Probable inactivity. Pa > 0.700 = probable activity greater than 70%. The PASS prediction results were interpreted and used as follows: (i) only activities with Pa > Pi are considered as possible for a particular compound; (ii) if Pa > 0.7, the chance to find the activity experimentally is high.

The toxic risk predictions performed (mutagenic, carcinogenic, cardiotoxic, hepatotoxic, skin sensitization and reproductive toxicity) for α-curcumene, β-bisabolene, 4-(4-methylphenyl) pentanal, pentanoic acid and isoamyl acetate were assessed by five online platforms and the results are present in Table 3. In general, the compounds displayed low toxicity probability. However, β-bisabolene and α-curcumene showed medium and high carcinogenic potential. For the α-curcumene, 4-(4-methylphenyl) pentanal and isoamyl acetate, the results showed a skin sensitization probability on two of the platforms employed. In the other evaluated parameters, isoamyl acetate and pentanoic acid demonstrated high risks to reproductive system. Moreover, theoretical toxicity of compounds in the others tools employed suggesting a low toxicity risk of the compounds.

Table 3. Toxicity prediction for the five major *Handroanthus chrisotrichus* compounds obtained via computer simulation.

Toxic risk by ACD/Labs[1]; admetSAR[2]; pkCSM[3]; PreADMET[4]; OSIRIS Property Explorer[5])						
Phytoconstituents	*Mutagenic*	*Carcinogenics*	*Cardiotoxic*	*Hepatotoxicity*	*Skin Sensitization*	*Reproductive system toxicity*
Alpha-Curcumene	ND[1] ND[2] ND[3] (-)[4] ND[5]	(++)[2] (+++)[4]	(+)[1] (+)[2] ND[3] (++)[4]	ND[2] ND[5]	(+)[3] (+++)[5]	ND[5]
Beta-Bisabolene	ND[1] ND[2] ND[3] (-)[4] ND[5]	(++)[2] (++)[4]	(+)[1] ND[2] ND[3] (++)[4]	ND[2] ND[5]	(+)[3] ND[5]	ND[5]
4-(4-Methylphenyl)-pentanal	ND[1] ND[2] ND[3] (-)[4] (+++)[5]	ND[2] ND[4]	ND[1] ND[3] ND[3] (++)[4]	ND[2] ND[5]	(+)[3] (+++)[5]	ND[5]
Isoamyl Acetate	ND[1] ND[2] ND[3] (-)[4] ND[5]	ND[2] (++)[4]	ND[1] ND[2] ND[3] (+)[4]	ND[2] ND[5]	(+)[3] (++)[5]	(+++)[5]
Pentanoic Acid	ND[1] ND[2] ND[3] (-)[4] ND[5]	ND[2] (++)[4]	ND[1] ND[3] ND[3] (+)[4]	ND[2] ND[5]	ND[3] ND[5]	(+++)[5]

The scale of toxicity risk ranges from low (+), medium (++), high (+++) and non-detected (ND).

HCBE antioxidant analyses

HCBE presented antioxidant potential in all performed tests (Table 4). The IC_{50} in TAC assay was 45.1 ± 1.55 µg. mL^{-1}. In $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ scavengers assay the IC_{50} was 543.15 ± 6.29 and 50.6 ± 3 µg. mL^{-1}, respectively. FRAP presented IC_{50} of 20.05 ± 0.2 µg. mL^{-1}. Finally, extract decreased the deoxyribose degradation induced by Fenton reaction at IC_{50} of 91.9 ± 5.56 µg. mL^{-1}.

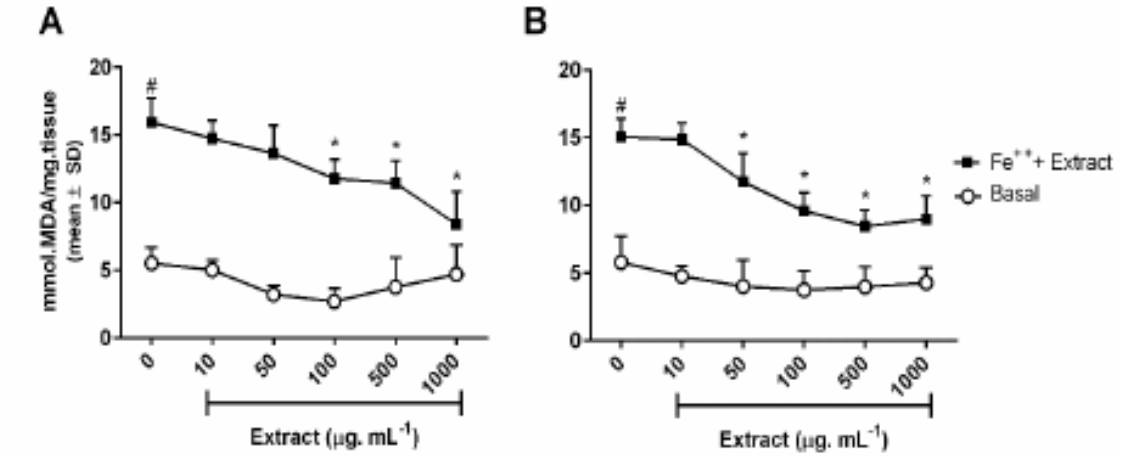
Table 4. IC_{50} values to different antioxidant assays of *Handroanthus chrysotrichus*.

Test	*H. chrysotrichus* IC_{50} (µg. mL^{-1})
TAC	45.1 ± 1.55
$DPPH^{\bullet}$ scavenger	543.15 ± 6.29
$ABTS^{\bullet+}$ scavenger	50.6 ± 3
FRAP	20.05 ± 0.2
Deoxyribose assay	91.9 ± 6.56

Values are expressed as mean ± SD (n=3).

Analyses with tissues of Swiss albino mice exposed to Fe^{++} showed the protective capacity of HCBE from 100 and 50 µg. mL^{-1} for brain and liver, respectively (Figure 3). In this context, the extract decreased TBA-RS levels for tissue reported.

Figure 3. Effects of *Handroanthus chrysotrichus* bark extract on TBA-RS levels from brain (A) and liver (B) tissues Fe^{+}-induced (#p ≤ 0.05 compared to baseline; * p ≤ 0.05 compared to concentration 0 of the same group).



HCBE LD50 and Toxicity assays

HCBE presents a LD_{50} of 276 µg. mL^{-1} (R^2 = 0.9912) to brine shrimp in *A. salina* bioassay. No toxicity of the HCBE was observed for the cell viability and comet assay (Table 5). Both results showed that HCBE did not present toxicity in PBMC in all tested concentrations compared to controls, including concentrations approaching double the LD_{50} found to *A. salina* (500 µg. mL^{-1}). Thus, there were not decrease in viable cells and significant increase in cells damage index ($p < 0.05$).

Table 5. Number of cells with comet, distribution of damage classes and damage index.

	CA	Viability (%)	Comet class (mean ± SD)					DI
			0	1	2	3	4	
Saline	100	93.7 ± 1.8	67 ± 4.2	30 ± 2.8	2.5 ± 2	0.5 ± 0.7	0	36.5 ± 4.9
H_2O_2 5 mM	100	84.9 ± 4.4*	32 ± 5.6	17.5 ± 3.5	18 ± 7	21 ± 8.4	11.5 ± 3	162.5 ± 6*
10 µg. mL^{-1}	100	94.3 ± 1.3	65.5 ± 19	26.5 ± 14.8	7.5 ± 4.9	0.5 ± 0.7	0	43 ± 22.5
50 µg. mL^{-1}	100	95.8 ± 0.3	66 ± 4.2	24 ± 8.4	5.5 ± 2.1	4 ± 1.4	0.5 ± 0.7	49 ± 2.8
100 µg. mL^{-1}	100	92 ± 1.9	62.5 ± 12	22 ± 5.6	9.5 ± 3.5	3 ± 1.4	3 ± 1	62 ± 22
500 µg. mL^{-1}	100	90.4 ± 0.1	52.5 ± 3.5	36	10 ± 1.4	1.5 ± 2.1	0	60.5 ± 9

CA: Total cells analyzed. DI: Damage index. * indicates significant difference compared to the negative control.

4. Discussion

This study aimed to evaluate the toxicological and antioxidant potential of HCBE *in silico* and *in vitro*. The extract showed five major compounds identified with pharmacological potential and low toxicity risks predictions *in silico*. In addition, HCBE presented antioxidant activity, protected brain and liver of mice against oxidative damage and no present cell toxicity *in vitro*. These data may justify its use in folk medicine, and it proves to be a promising extract for *in vivo* testing.

Initially, *A. salina* assays resulted medium toxicity. It indicate bioactive compounds presence, besides a positive correlation with cytotoxicity in cancer cell lines (McLaughlin, 1991). In agreement GC/MS analyses suggested the presence of substances as β-bisabolene which it was reported tumor-specific pro-apoptotic properties (Yeo et al., 2016). Although tests of α-curcumene in human colon cancer cell line did not inhibit cell proliferation (Tyagi et al., 2015).

Major compounds found α-curcumene and β-bisabolene are terpenes also presents in bark of *H. heptaphyllus* (Garcez et al., 2007) and act among other things against insects protecting the plant (Júnior, 2003). These insecticidal functions promoted the mortality of *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* larvae (AlShebly et al., 2017). *H. impetiginosus* bark also presented larvicidal activity in three mosquitos species and *H. serratifolius* steam had antileishmanial activity (Costa et al., 2017; Kim et al., 2013).

Other compounds are ester isoamyl acetate, well known in the food industry (Torres et al., 2010); and the short-chain fatty acid (SCFA) pentanoic acid, a superior class of cellulosic biofuels. This SCFA may be produced by intestinal bacteria (Topping, 1996) and induces thymic stromal lymphopoietin production (Mizuno et al., 2017). Thymic stromal lymphopoietin is an IL-7-like cytokine mainly derived from fibroblasts and epithelial cells and is related to allergic inflammation (Liu, 2006; Sims et al., 2000). Nevertheless, 4-(4-Methylphenyl) pentanal is not target of many studies.

Throughout chromatography analyzes β-lapachone and lapachol was expected in extract studied, since they are cited in literature as major compound in *H. chrysotricus* (Grazziotin et al., 1992), *H. impetiginosus* (Castellanos et al., 2009; Ferreira et al., 1989) and others Bignoniaceae plants. In addition, lapachol is soluble in ethanol (Kiage-mokua et al., 2012), being consistent with method here applied, but this compound was not detected by High Performance Liquid Chromatography (Supplementary material). Thus, it seems to be absent in the *H. chrysotricus* bark analyzed. These substantial differences between specimen may be due

to environmental conditions, because lapachol presence may vary in the same specie according rainfall, temperature and the soil content (Wright & Setzer, 2013).

The biological activity spectrum *in silico* of isolated compounds (Table 2) showed compatible action with popular practice. In this line, Salgueiro et al. (2018) suggest that *in silico* investigations can be used as a complementary route after the ethnopharmacological research. Indeed, *in silico* studies may be useful to point out several possibilities for conducting *in vitro* and *in vivo* studies (Salgueiro et al., 2018). Brazilian ethnopharmacological investigations indicated therapeutic use of genus *Handroanthus* as anti-inflammatory and for cancer treatment, back pain, stomachache, gastritis, ulcer and allergy (Bieski et al., 2015; Ribeiro et al., 2017). According to Bolson et al. (2015) *H. chrysotrichus* also may be used for cardiovascular system diseases, immunological and poisoning. Moreover, other *in silico* analyses were made, and the results showed low toxicity probability in most of the analyzed criteria (Table 3). Nevertheless, care should be taken with carcinogenic potential and topical use of the extract, due skin sensitization of some compounds.

The biological activity spectrum *in silico* no showed, but the HCBE had significant antioxidant activity (Table 4). These important effect may reduce oxidative damage induced by several diseases (Andréia Caroline Fernandes Salgueiro et al., 2016). The absence of antioxidant activities in *in silico* investigations can be explained by synergism among different molecules in a crude plant extract (Salgueiro et al., 2018). In the context of computational evaluations, the interaction between compounds cannot be evaluated (Salgueiro et al., 2018). The extract exhibited better activities in FRAP, TAC and $ABTS^{\bullet+}$ scavenger. Antioxidant analyses as ferric reducing antioxidant power assay, FRAP, propose the affinity of HCBE with Fe ions. We know that iron is an essential element for the body and may induce oxidative stress via Fenton reaction (Benzie & Strain, 1996; Halliwell & Gutteridge, 2007). In addition, HCBE decreased Fe^{+}-induced damage to brain and liver tissues of mice (Figure 3). TAC and $ABTS^{\bullet+}$ are based on the reduction of molybdenum and ABTS radicals, respectively. $ABTS^{\bullet+}$ assay is a simple method applicable to the extracts antioxidant study, but this radical is not found in biologicals systems (Magalhães et al., 2008; Prieto et al., 1999; Re et al., 1999). Therefore, these antioxidants properties may be linked to polyphenols found in the colorimetric phytochemical analyses. In fact, polyphenols have a high reactivity and ability to stabilize unpaired electron, including to chelate transition metal ions (Vertuani et al., 2004). On the other hand, antioxidant activity against $DPPH^{\bullet}$ showed high IC_{50}, compatible data with bark extract of *T. rosea* (Jimenez-Gonzalez et al., 2018), suggesting that bark extracts these trees have low efficiency in this test. The extract here analyzed not indicated reaction with nitric oxide ($NO^{\bullet}$)

(data not show), a pro-inflammatory mediator that plays an important role in the regulation of immune functions, neurotransmission, and vasodilation (Sharma, Al-Omran, & Parvathy, 2007).

Despite knowledge about vegetal extracts are necessary, because shows potential actions and may value the plant, we considered important to perform *in vitro* assays evaluating the cell responses (Fraga et al., 2020; Lima et al., 2020). There was not changes of viability and genotoxicity in cells exposed to HCBE (Table 5). These data are according to Boriollo et al. (2017), which suggested absence of genotoxicity of the *H. impetiginosa* bark. In addition, confirm the low toxicity found in *in silico* analyses.

Therefore, the species studied showed low toxic potential and exhibited a biological activity spectrum, including the antioxidant capacity and the protective action in liver and brain of mice. Literature suggested that its major compounds presents biological act. These data support point the HCBE as a promising extract for *in vivo* evaluations.

5. Conclusion

The present study observes different parameters of golden trumpet *Handroanthus chrysotrichus*. We identify five major volatile compounds from hydroalcoholic stem bark extract: α-curcumene, β-bisabolene, 4-(4-Methylphenyl) pentanal, pentanoic acid and isoamyl acetate. These compounds present pharmacological activities predicted which are compatible with popular practice. On other hand, β-bisabolene and α-curcumene show carcinogenic potential toxic risk predictions.

The results also verify high levels of polyphenols and show that *H. chrysotrichus* bark has antioxidant effect in different systems. Despite the extract had presented toxicity in *Artemia satureioides* bioassay, there was not cell toxicity. Finally, our tests indicate *H. chrysotrichus* as a potential agent for *in vivo* testing, especially for the treatment of diseases in which the bark is used and to ensure safety the medicinal use of this plant.

Referências

AlShebly, M. M., AlQahtani, F. S., Govindarajan, M., Gopinath, K., Vijayan, P., & Benelli, G. (2017). Toxicity of ar-curcumene and epi-β-bisabolol from *Hedychium larsenii* (Zingiberaceae) essential oil on malaria, chikungunya and Japanese encephalitis mosquito vectors. *Ecotoxicology and Environmental Safety*, *137*(2017), 149–157. https://doi.org/10.1016/j.ecoenv.2016.11.028

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry*, *239*(1), 70–76. https://doi.org/10.1006/abio.1996.0292

Bieski, I. G. C., Leonti, M., Arnason, J. T., Ferrier, J., Rapinski, M., Violante, I. M. P., Balogun, S. O., Pereira, J. F. C. A., Figueiredo, R. D. C. F., Lopes, C. R. A. S., Silva, D. R. da, Pacini, A., Albuquerque, U. P., & Oliveira, D. T. (2015). Ethnobotanical study of medicinal plants by population of Valley of Juruena Region , Legal Amazon , Mato Grosso , Brazil. *Journal of Ethnopharmacology*, *173*, 383–423. https://doi.org/10.1016/j.jep.2015.07.025

Bolson, M., Hefler, S. R., Dall'Oglio Chaves, E. I., Gasparotto Junior, A., & Cardozo Junior, E. L. (2015). Ethno-medicinal study of plants used for treatment of human ailments, with residents of the surrounding region of forest fragments of Paraná, Brazil. *Journal of Ethnopharmacology*, *161*, 1–10. https://doi.org/10.1016/j.jep.2014.11.045

Boriollo, M. F. G., Silva, T. A., Rodrigues-Netto, M. F., Silva, J. J., Marques, M. B., Dias, C. T. S., Höfling, J. F., Resck, M. C. C., Oliveira, N. M. S., Boriollo, M. F. G., Silva, T. A., Rodrigues-Netto, M. F., Silva, J. J., Marques, M. B., Dias, C. T. S., Höfling, J. F., Resck, M. C. C., & Oliveira, N. M. S. (2017). Reduction of doxorubicin-induced genotoxicity by *Handroanthus impetiginosus* in mouse bone marrow revealed by micronucleus assay. *Brazilian Journal of Biology*, *78*(1), 1–12. https://doi.org/10.1590/1519-6984.18515

Castellanos, J. R. G., Prieto, J. M., & Heinrich, M. (2009). Red Lapacho (*Tabebuia impetiginosa*) — A global ethnopharmacological commodity? *Journal of Ethnopharmacology*, *121*, 1–13. https://doi.org/10.1016/j.jep.2008.10.004

Cheng, F., Li, W., Zhou, Y., Shen, J., Wu, Z., Liu, G., Lee, P. W., & Tang, Y. (2012). admetSAR: A Comprehensive Source and Free Tool for Assessment of Chemical ADMET Properties. *Journal of Chemical Information and Modeling*, *52*(11), 3099–3105. https://doi.org/10.1021/ci300367a

Choi, C. W., Kim, S. C., Hwang, S. S., Choi, B. K., Ahn, H. J., Lee, M. Y., Park, S. H., & Kim, S. K. (2002). Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. *Plant Science*, *163*(6), 1161–1168. https://doi.org/10.1016/S0168-9452(02)00332-1

Costa, E. V. S., Brígido, H. P. C., Silva, J. V. da S. e, Coelho-Ferreira, M. R., Brandão, G. C., & Dolabela, M. F. (2017). Antileishmanial Activity of *Handroanthus serratifolius* (Vahl) S. Grose (Bignoniaceae). *Evidence-Based Complementary and Alternative Medicine*, *2017*, 1–6. https://doi.org/10.1155/2017/8074275

Drwal, M. N., & Griffith, R. (2013). Combination of ligand- and structure-based methods in virtual screening. *Drug Discovery Today. Technologies*, *10*(3), e395-401. https://doi.org/10.1016/j.ddtec.2013.02.002

Ferreira, D. T., Oliveira, A. B. de, & Castro, C. R. de. (1989). Constituintes químicos da *Tabebuia impetiginosa* (MART) Standl. Bignoniaceae. *Semina: Exact and Technological Sciences*, *10*(4), 272–273.

Fraga, L. N., Oliveira, A. K. de S., Aragão, B. P., Silva, A. M. de O. e, Wartha, E. R. S. de A., Bacci, L., Lobato, L. P., & Carvalho, I. M. M. de. (2020). Physico-chemical characterization of the pulp and peel of Brazilian Pitomba (*Talisia esculenta* (A. St.-Hill.) Radlk). *Research, Society and Development*, *9*(1), 1–14.

Garcez, F. R., Garcez, W. S., Mahmoud, T. S., Figueiredo, P. de O., & Resende, U. M. (2007). Novos constituintes químicos das cascas do caule de *Tabebuia heptaphylla*. *Quimica Nova*, *30*(8), 1887–1891. https://doi.org/10.1590/S0100-40422007000800017

Grazziotin, J. D., Schapoval, E. E. S., Chaves, C. G., Gleye, J., & Henriques, A. T. (1992). Phytochemical and analgesic investigation of *Tabebuia chrysotricha*. *Journal of Ethnopharmacology*, *36*(3), 249–251. https://doi.org/10.1016/0378-8741(92)90051-R

Halliwell, B., & Gutteridge, J. M. C. (2007). *Free radicals in biology and medicine* (4ª). Oxford University Press Inc.

Jardim Botânico do Rio de Janeiro. (2018). *Flora do Brasil 2020 em construção*. *Handroanthus Chrysotrichus*. http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB114078

Jimenez-Gonzalez, F. J., Vélez-Gómez, J. M., Melchor-Moncada, J. J., Veloza, L. A., & Sepúlveda-Arias, J. C. (2018). Antioxidant, anti-inflammatory, and antiproliferative activity of extracts obtained from *Tabebuia Rosea* (Bertol.) DC. *Pharmacognosy Magazine*, *14*(55), 25–31.

Júnior, C. V. (2003). Terpenos com atividade inseticida: uma alternativa para o controle químico de insetos. *Quimica Nova*, *26*(3), 390–400.

Kiage-mokua, B. N., Roos, N., & Schrezenmeir, J. (2012). Lapacho Tea (*Tabebuia impetiginosa*) Extract Inhibits Pancreatic Lipase and Delays Postprandial Triglyceride Increase in Rats †. *Phytotherapy Research*, *26*, 1878–1883.

Kim, M.-G., Jeon, J. H., & Lee, H.-S. (2013). Larvicidal activity of the active constituent isolated from *Tabebuia avellanedae* bark and structurally related derivatives against three mosquito species. *Journal of Agricultural and Food Chemistry*, *61*(45), 10741–10745. https://doi.org/10.1021/jf403679h

Lee, M. H., Choi, H. M., Hahm, D. H., Her, E., Yang, H. I., Yoo, M. C., & Kim, K. S. (2012). Analgesic and anti-inflammatory effects in animal models of an ethanolic extract of Taheebo, the inner bark of *Tabebuia avellanedae*. *Molecular Medicine Reports*, *6*(4), 791–796. https://doi.org/10.3892/mmr.2012.989

Lima, A. R. N., Macedo, R. G., Batista, G. G., Câmara, G. B., Lima, R. de F., & Oliveira, T. K. B. de. (2020). Antimicrobial and anti-inflammatory activity of *Anadenanthera colubrina* (Vell.) Brenan. *Research, Society and Development*, *9*(1), 1–12.

Liu, Y.-J. (2006). Thymic stromal lymphopoietin: master switch for allergic inflammation. *The Journal of Experimental Medicine*, *203*(2), 269–273. https://doi.org/10.1084/jem.20051745

Magalhães, L. M., Segundo, M. A., Reis, S., & Lima, J. L. F. C. (2008). Methodological aspects about in vitro evaluation of antioxidant properties. *Analytica Chimica Acta*, *613*(1), 1–19.

https://doi.org/10.1016/J.ACA.2008.02.047

McLaughlin, J. L. (1991). Crown gall tumors on potato discs and brine shrimp lethality: two simple bioassays for higher plant screening and fractions. In P.M.DEY & J. B. HARBONE (Eds.), *Methods in Plant Biochemistry* (1st ed., pp. 1–32). Academic Press.

Meyer, B., Ferrigni, N., Putnam, J., Jacobsen, L., Nichols, D., & McLaughlin, J. (1982). Brine Shrimp: A Convenient General Bioassay for Active Plant Constituents. *Planta Medica*, *45*(05), 31–34. https://doi.org/10.1055/s-2007-971236

Mizuno, N., Abe, K., Morishita, Y., Yamashita, S., Segawa, R., Dong, J., Moriya, T., Hiratsuka, M., & Hirasawa, N. (2017). Pentanoic acid induces thymic stromal lymphopoietin production through G q/11 and Rho-associated protein kinase signaling pathway in keratinocytes. *International Immunopharmacology*, *50*, 216–223. https://doi.org/10.1016/j.intimp.2017.06.024

Nurmi, K., Ossipov, V., Haukioja, E., & Pihlaja, K. (1996). Variation of total phenolic content and individual low-molecular-weight phenolics in foliage of mountain birch trees (Betula pubescens ssp.tortuosa). *Journal of Chemical Ecology*, *22*(11), 2023–2040. https://doi.org/10.1007/BF02040093

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, *95*(2), 351–358. https://doi.org/10.1016/0003-2697(79)90738-3

OSIRIS Properties Explorer. (n.d.). Retrieved September 16, 2018, from https://www.organic-chemistry.org/prog/peo

Park, B.-S., Lee, K.-G., Shibamoto, T., Lee, S.-E., & Takeoka, G. R. (2003). Antioxidant Activity and Characterization of Volatile Constituents of Taheebo (*Tabebuia impetiginosa* Martius ex DC). *Journal of Agricultural and Food Chemistry*, *51*, 295–300.

Park, B., Lee, H., Lee, S., Piao, X., Takeoka, G. R., Wong, R. Y., Ahn, Y., & Kim, J. (2006). Antibacterial activity of *Tabebuia impetiginosa* Martius ex DC (Taheebo) against *Helicobacter pylori*. *Journal of Ethnopharmacology*, *105*, 255–262. https://doi.org/10.1016/j.jep.2005.11.005

Perez, J. E., Isaza, G., Bueno, J. G., Arango, M. C., Hincapié, B. L., Nieto, A. M., & Londoño, D. P. (2004). Efecto de los extractos de *Phenax rugosus*, *Tabebuia chrysantha*, Althernantera williamsii y Solanum dolichosepalum sobre el leucograma y la producción de anticuerpos en ratas. *REVISTA MÉDICA DE RISARALDA*, *10*(2), 13–21.

Pires, D. E. V, Blundell, T. L., & Ascher, D. B. (2015). pkCSM: Predicting Small-Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures. *Journal of Medicinal Chemistry*, *58*(9), 4066–4072. https://doi.org/10.1021/acs.jmedchem.5b00104

PreADMET web-based, Online. (n.d.). Retrieved September 16, 2018, from https://preadmet.bmdrc.kr/

Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant

capacity through the formation of a phosphomolybdinum complex: specific application to the determination of Vitamin E. *Anal Biochem*, *269*, 337–341.

Puntel, R. L., Nogueira, C. W., & Rocha, J. B. T. (2005). Krebs cycle intermediates modulate thiobarbituric acid reactive species (TBARS) production in rat brain in vitro. *Neurochemical Research*, *30*(2), 225–235. https://doi.org/10.1007/s11064-004-2445-7

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant Activity Applying an Improved Abts Radical Cation Decolorization Assay. *Free Radical Biology and Medicine*, *26*(9), 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Reis, F. P., Senna Bonfa, I. M., Cavalcante, R. B., Okoba, D., De Souza Vasconcelos, S. B., Candeloro, L., De Oliveira Filiu, W. F., Duenhas Monreal, A. C., Da Silva, V. J., Santa Rita, P. H., Carollo, C. A., & Toffoli-Kadri, M. C. (2014). *Tabebuia aurea* decreases inflammatory, myotoxic and hemorrhagic activities induced by the venom of *Bothrops neuwiedi*. *Journal of Ethnopharmacology*, *158*(PART A), 352–357. https://doi.org/10.1016/j.jep.2014.10.045

Ribeiro, R. V., Bieski, I. G. C., Balogun, S. O., & Martins, D. T. de O. (2017). Ethnobotanical study of medicinal plants used by Ribeirinhos in the North Araguaia microregion, Mato Grosso, Brazil. *Journal of Ethnopharmacology*, *205*(April), 69–102. https://doi.org/10.1016/j.jep.2017.04.023

Salgueiro, A.C.F., Folmer, V., Bassante, F. E. M., Cardoso, M. H. S., da Rosa, H. S., & Puntel, G. O. (2018). Predictive antidiabetic activities of plants used by persons with Diabetes mellitus. *Complementary Therapies in Medicine*, *41*, 1–9. https://doi.org/10.1016/j.ctim.2018.08.009

Salgueiro, A. C. F., Folmer, V., da Silva, M. P., Mendez, A. S. L., Zemolin, A. P. P., Posser, T., Franco, J. L., Puntel, R. L., Puntel, G. O., Puntel, R. L., Puntel, G. O. (2016). Effects of *Bauhinia forficata* Tea on Oxidative Stress and Liver Damage in Diabetic Mice. *Oxidative Medicine and Cellular Longevity*, *2016*, 1–9. https://doi.org/10.1155/2016/8902954

Sharma, J. N., A Al-Omran, & Parvathy, S. S. (2007). Review Role of nitric oxide in inflammatory diseases. *Inflammopharmacology*, *15*, 252–259. https://doi.org/10.1007/s10787-007-0013-x

Sims, J. E., Williams, D. E., Morrissey, P. J., Garka, K., Foxworthe, D., Price, V., Friend, S. L., Farr, A., Bedell, M. A., Jenkins, N. A., Copeland, N. G., Grabstein, K., & Paxton, R. J. (2000). Molecular cloning and biological characterization of a novel murine lymphoid growth factor. *The Journal of Experimental Medicine*, *192*(5), 671–680. http://www.ncbi.nlm.nih.gov/pubmed/10974033

Singh, N. P., McCoy, M. T., Tice, R. R., & Schneider, E. L. (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. *Experimental Cell Research*, *175*(1), 184–191. https://doi.org/10.1016/0014-4827(88)90265-0

Soares, J. J., Rodrigues, D. T., Gonçalves, M. B., Lemos, M. C., Gallarreta, M. S., Bianchini, M. C., Gayer, M. C., Puntel, R. L., Roehrs, R., & Denardin, E. L. G. (2017). Paraquat exposure-induced Parkinson's disease-like symptoms and oxidative stress in *Drosophila melanogaster:* Neuroprotective effect of *Bougainvillea glabra* Choisy. *Biomedicine and Pharmacotherapy*,

95(July), 245–251. https://doi.org/10.1016/j.biopha.2017.08.073

Topping, D. L. (1996). Short-chain fatty acids produced by intestinal bacteria. *Asia Pacific Journal of Clinical Nutrition*, *5*, 15–19.

Torres, S., Pandey, A., & Castro, G. R. (2010). Banana flavor: Insights into isoamyl acetate production. *Cell*, *549*(1), 776–802.

Twardowschy, A., Freitas, C. S., Baggio, C. H., Mayer, B., dos Santos, A. C., Pizzolatti, M. G., Zacarias, A. A., dos Santos, E. P., Otuki, M. F., & Marques, M. C. A. (2008). Antiulcerogenic activity of bark extract of *Tabebuia avellanedae*, Lorentz ex Griseb. *Journal of Ethnopharmacology*, *118*(3), 455–459. https://doi.org/10.1016/j.jep.2008.05.013

Tyagi, A. K., Prasad, S., Yuan, W., Li, S., & Aggarwal, B. B. (2015). Identification of a novel compound (β-sesquiphellandrene) from turmeric (Curcuma longa) with anticancer potential: Comparison with curcumin. *Investigational New Drugs*, *33*(6), 1175–1186. https://doi.org/10.1007/s10637-015-0296-5

Vertuani, S., Angusti, A., & Manfredini, S. (2004). The Antioxidants and Pro-Antioxidants Network: An Overview. *Current Pharmaceutical Design*, *10*(14), 1677–1694. https://doi.org/10.2174/1381612043384655

Wright, C. R., & Setzer, W. N. (2013). Volatile components of organ pipe cactus , *Stenocereus thurberi* Engelm., growing in the Organ Pipe Cactus National Monument and the Arizona-Sonora Desert Museum. *American Journal of Essential Oils and Natural Products*, *1*(3), 19–22. https://doi.org/10.1086/497361

Percentage of contribution of each author in the manuscript

Márcio Tavares Costa – 30%

Aline da Silva Goulart – 14%

Jefferson de Jesus Soares – 12%

Andréia Caroline Fernandes Salgueiro – 6%

Hemerson Silva da Rosa – 6%

Elton Luis Gasparotto Denardin – 5%

Clésio Soldateli Paim – 12%

Robson Luiz Puntel – 5%

Vanderlei Folmer – 10%

Supplementary Material

1. Materials and Methods

1.1. HPLC analyses

1.1.1. Chemicals

Lapachol and β-lapachone reference standard were obtained by Sigma-Aldrich® (São Paulo, Brazil). Acetonitrile and ethanol were obtained by Hexis® (São Paulo, Brazil). Purified water was obtained by Milli-Q Plus® system from Millipore (Milford, MA, USA).

1.1.2. Apparatus and chromatographic conditions

A Shimadzu Prominence® liquid chromatograph (Kyoto, Japan) equipped with a model LC-20AD quaternary pump, SIL-20AC HT auto sampler, CTO-20AC column oven, SPD-M20A photodiode-array detector and LC Solution V. 1.24 SP1 manager system software was used. The chromatographic separation was performed in a Supelco Ascentis® column RP-18 (250 x 4.6 mm I.D., 5 μm, St. Louis, MO, United States) and the temperature was set at 30 °C in the column oven. The mobile phase comprised a mixture of water –acetonitrile ranging from 90:10 to 5:95, in a linear gradient from 0 to 55 min. at a flow-rate of 1.0 mL min^{-1}. The lapachol and β-lapachone substances were determined by UV detection at 254 nm using a diode array detector (DAD). The volume of solutions injected into the HPLC system was 20 μL.

1.1.3. Solutions preparation

Lapachol reference standard (10 mg. mL^{-1}) and β-lapachone reference standard (5 mg. mL^{-1}) solutions were diluted in ethanol. *Handroanthus chrysotrichus* bark extract were prepared in ethanol at 10 mg mL^{-1}.

2. Results

The results of the analytical method showed that the lapachol and β-lapachone substances are not present in the plant bark or the extraction process used was not suitable to extract its (Figure 1).

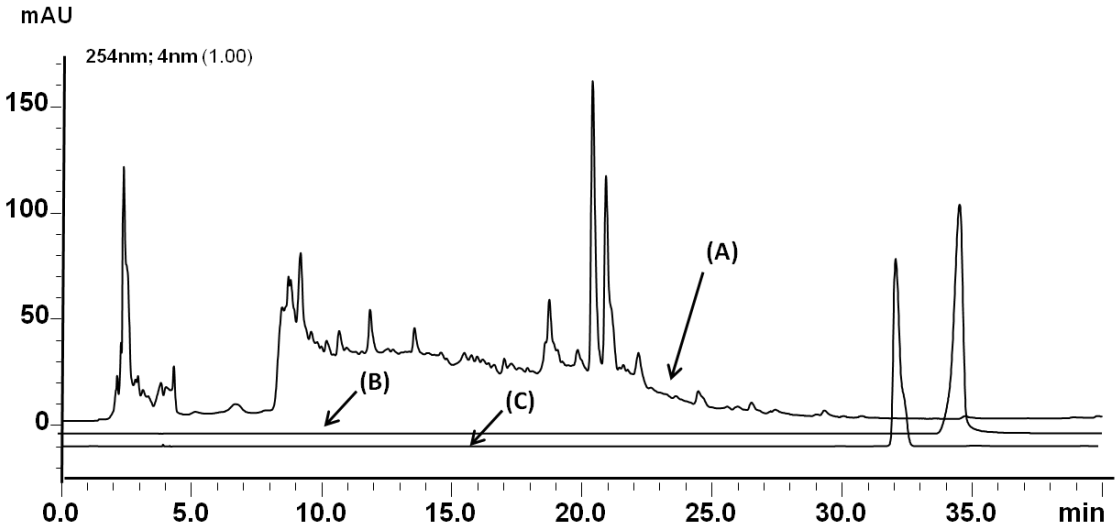


Figure 1. HPLC profile obtained from analysis of *Handroanthus chrysotrichus* bark extract at 10 mg. mL^{-1} (A). Lapachol reference standard (10 mg. mL^{-1}) (B), and β-lapachone reference standard (5 mg. mL^{-1}) (C). Chromatographic conditions: water –acetonitrile as mobile phase ranging from 90:10 to 5:95, in a linear gradient from 0 to 55 min, flow-rate of 1.0 mL min^{-1}, DAD detection at 254 nm.

6.2. Artigo científico 2

OXFORD Toxicology Research

Toxicology Research, 2021, 1–9

https://doi.org/10.1093/toxres/tfab076
Paper

PAPER

In vivo effects of exposure to Golden trumpet *Handroanthus chrysotrichus* in mice

Márcio Tavares Costa,[1] Aline da Silva Goulart,[1] Kellen Mariane Athaide Rocha,[1] Andréia Caroline Fernandes Salgueiro,[2] Morgana Duarte da Silva,[3] Robson Luiz Puntel[1] and Vanderlei Folmer[1,*]

[1]Biochemistry Graduate Program, Federal University of Pampa, Uruguaiana, RS 97501-970, Brazil, [2]Collaborating Professor of the Science Education, Life Chemistry and Health Graduate Program, Federal University of Pampa, Uruguaiana, RS 97501-970, Brazil and [3]Department of Physiological Sciences, Federal University of Santa Catarina, Florianópolis, SC 88040-900, Brazil

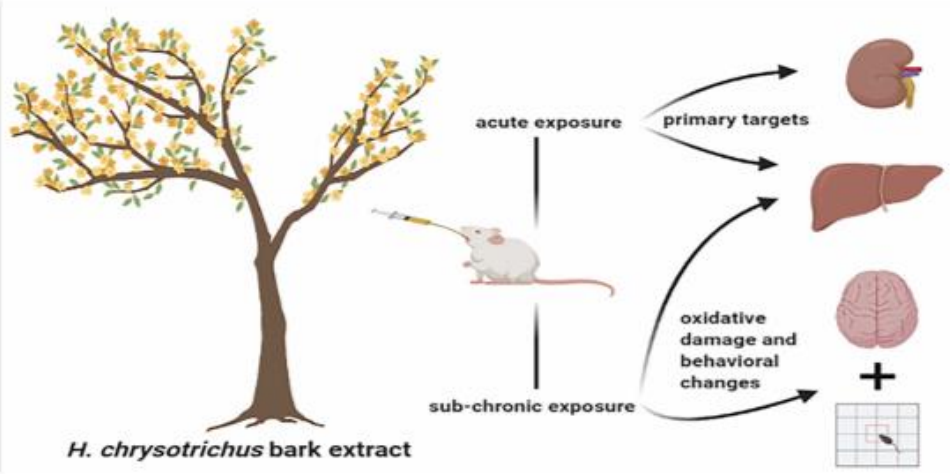
*Correspondence address. Federal University of Pampa, UNIPAMPA - Biochemistry Graduate Program - BR 472 Km 592, CEP 97500-970, Caixa Postal 118, Uruguaiana, RS, Brazil. Tel: +55-55-39110200; E-mail: vandfolmer@gmail.com

Abstract

The Golden trumpet *Handroanthus chrysotrichus* is a tree that presents beneficial health properties against various diseases. Thus, this study aims to verify the toxicity of *H. chrysotrichus* bark extract, observing the effects of exposure to this extract in mice. For this, mice were separated in groups: saline (sterile solution .9%); *H. chrysotrichus* crude extract (HCCE) 10; HCCE 50, and HCCE 100 mg. kg^{-1} (p.o.). We analyzed HCCE effects on acute (single exposure) and subchronic protocol (14 days exposure). After both exposures, acute, and subchronic, we collected samples from blood, brain, liver, and kidney tissues for biochemical evaluation. In addition, after subchronic exposure, we performed behavioral tests. Acute exposure caused an increase of lipid peroxidation in liver tissue. Moreover, we observed a significant carbonyl increase in liver and brain tissues from HCCE 50 mg. kg^{-1}. Kidneys presented carbonyl increase in mice treated with the highest concentration. Besides, creatinine increased in the group of the acute exposure at HCCE 100 mg. kg^{-1}. Total leukocyte count decreased in all concentrations tested. Sub-chronic exposure at HCCE 100 mg. kg^{-1} caused a decrease in the number of crossing and an increase in its self-grooming frequency in the open field test. In this exposure, the brain and liver had a significant increase in carbonyl levels in all concentrations. We concluded that *H. chrysotrichus* cause behavioral and biochemical alterations in mice. HCCE primary targets seem to be the liver, kidneys, and white cells.

Received: 16 March 2021; **Revised:** 9 July 2021; **Accepted:** 19 July 2021

Graphical Abstract



Key words: Tabebuia, pharmacology, oxidative stress, ethnology, toxicology

Introduction

Handroanthus chrysotrichus (Mart. ex DC.) Mattos, known as the Golden trumpet, is a tree from the Bignoniaceae family. *Handroanthus chrysotrichus* has been consumed due to its beneficial health properties in the treatment of cardiovascular and immunological system diseases, allergic process, and envenoming. Stem, flowers, and bark are the main product obtained from the plant by people in folk medicine to obtain its therapeutic virtues [1, 2].

This traditional knowledge has been employed as the starting point for many successful drug development projects. Besides, ethnobotany is a powerful tool to document and understand how different traditional people relate to natural resources [3, 4]. In this context, the scientific literature reports the analgesic and antiproliferative effects of Lapachol, found in *H. chrysotrichus* wood extract [5, 6]. On other hand, Costa *et al.* [7] showed five volatile compounds of *H. chrysotrichus* bark extract: α-curcumene, β-bisabolene, 4-(4-Methylphenyl)pentanal, pentanoic acid, and isoamyl acetate. According to the authors, these compounds presented other pharmacological properties *in silico*, as mucobembranous protector and antieczematic. In addition, the bark extract showed antioxidant activity. All these data are compatible with its use in folk medicine.

We highlight that many factors affect the plant extract characteristics and composition. These substantial differences between specimens may be due to environmental conditions and the conditions under which the plant was cultivated [8, 9]. Moreover, parts of the plant and the extraction method result in the acquisition of different secondary metabolites [10]. Important factors in the study of xenobiotics.

The use of *H. chrysotrichus*, as well as many plant species, may indicate the presence of active substances and serves as a clue to pharmacological and phytochemical explorations [11]. Thus, this is an initial study that aims to verify the putative toxicity of *H. chrysotrichus* bark extract, observing the behavioral and physiological effects of acute and subchronic exposure to this plant in mice.

Materials and Methods

Plant material

Handroanthus chrysotrichus bark was collected in 29°49′49.2″S 57°06′07.1″W geographical coordinates in Brazilian summer. After identification, a voucher specimen to be deposited at the herbarium of Federal University of Pampa (protocol number 142/2017).

To obtain an extract of the plant material, its bark was subjected to drying at 40°C for 5 days and reduced to powder. Subsequently, samples of the powdered material were used in the percolation technique. Percolation was carried out for 2 hours in a glass column and applied to bark using 70% hydroethanolic solution in a ratio of 1:10 (w/v). After that, the *H. chrysotrichus* crude extract (HCCE) was freeze-dried.

Animals

The Committee for Animal Research of the Federal University of Pampa approved this research (Protocol number 55/2019) and all guidelines instructed by the ethical research committee for laboratory animal care were followed thoroughly. Male Swiss mice (25–35 g) were kept in an appropriate animal cabinet with forced air ventilation, in a 12-hour light/dark cycle, at a controlled room temperature of 22°C, with food and water *ad libitum*.

Based in previous assays [7], mice were separated in four groups ($n = 5$ for each group): saline (sterile saline solution .9%); HCCE 10; HCCE 50; and HCCE 100 mg. kg^{-1}. Dry HCCE diluted in saline was given orally (p.o.). Then, we analyzed acute and subchronic exposure—with two lots of 20 animals each—at the same HCCE groups. In the first lot, mice were exposed once to

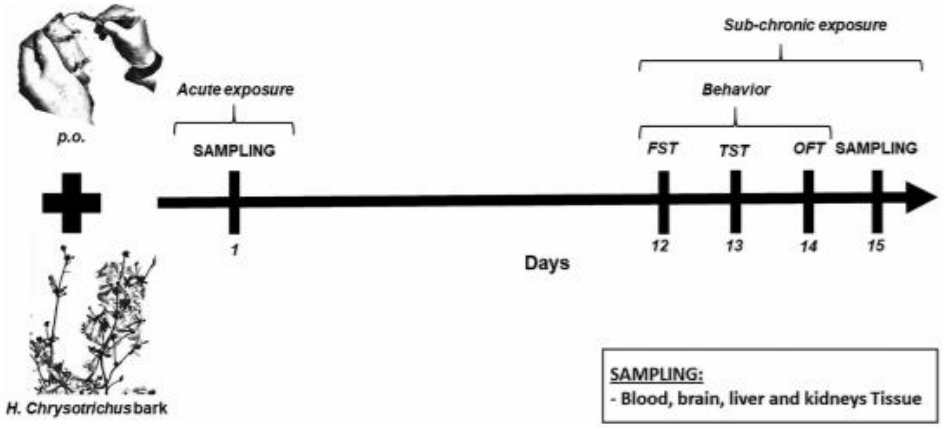


Figure 1: Study experimental design.

HCCE and the samples from brain, liver, and kidneys tissues and blood were collected for biochemical analyzes after 24 h. While in the second, there was an exposure daily for 14 days, after we performed the behavioral test and collected samples from brain, liver, and kidneys tissues and blood to perform biochemical analyses. Figure 1 shows the study experimental design.

Behavioral test

Open field test. Open field test (OFT) was performed according to Bond and Giusto [12], with modifications. Briefly, mice were treated for 14 days with saline or HCCE (10, 50, or 100 mg. kg^{-1}, p.o.), and on the 14th day, the test was performed 1 hour after gavage. They were enclosure a white quadrangular apparatus (100 × 100 cm diameter, with 12 equally spaced squares) in a silent room. Mice behavior response was recorded by a video camera during 6-min period. Finally, locomotor and anxiety conduct were assessed by (i) number of crossing with all paws; (ii) rearing, times standing on hind legs; (iii) self-grooming frequency; and (iv) defecation score [13].

Tissue toxicity evaluation

Organs/body weight ratio. Twenty-four hours after last HCCE administration, animals were euthanized by decapitation and their organs—liver, kidneys, spleen, heart, and brain—were weighed, and the results were expressed as a ratio of body weight in relation to body weight. The initial and final difference in animal weight *in vivo* in each group was also recorded. Data assessed toxicity about these tissues.

Oxidative stress assays. The following tests were used to obtain oxidative stress biomarkers in the brain, liver, and kidneys of treated mice. Soon after euthanasia, organs were quickly removed and carefully washed twice with cold saline and homogenized in cold NaCl (150 mM). Homogenates were centrifuged at 2000 g for 10 min at 4°C and the supernatants (S1) were collected for biochemical analyses.

Thiobarbituric acid reactive substances assay. Lipid peroxidation index was measured according to Ohkawa *et al.* [14] using S1 of tissues and corrected by protein content [15]. A standard curve of malondialdehyde (MDA) was constructed to determine thiobarbituric acid reactive substances (TBA-RS) content.

Protein carbonyl levels. Aliquots of S1 were derivatized using 2,4-dinitrophenylhydrazine (DNPH) and precipitated with an equal volume of 20% (w/v) trichloroacetic acid. Then, they were washed two times with an ethanol/ethyl acetate mixture (1:1). Precipitates were dissolved in 6 M guanidine HCl solution. Protein carbonyl levels were determined spectrophotometrically at 370 nm, against blanks [16]. Results were calculated using the DNPH molar extinction coefficient, corrected by protein content, and expressed as nanomoles of carbonyl per milligram of protein.

Nonprotein thiols levels. Nonprotein thiols levels (NPSH) levels were determined according to Ellman [17]. S1 samples were precipitated with 10% trichloroacetic acid (1:1) and centrifuged at 4000 g for 10 min at 4°C to obtain supernatants, which were added to a medium containing phosphate buffer (TFK .25 mM, pH 7.4) and Ellman reagent (DTNB 1 mM). The yellow pigment produced was measured spectrophotometrically at 420 nm. Results were calculated in relation to a standard curve constructed with glutathione (GSH) at known concentrations and corrected by protein content.

Blood biomarkers

Blood was collected in heparinized tubes by cardiac puncture after fasting for 12 hours. Analyzes performed were:

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity and creatine K levels. After centrifugation, ALT and AST activity and creatine K levels were determined in plasma using a commercial kit (Labtest, Minas Gerais/Brazil).

Total leukocyte count. After blood collection, total leukocyte count occurred according to Dornfest *et al.* [18] with modifications. Briefly, blood was collected and diluted (1:20) in Turkey dye and filled in the Neubauer Chamber. Total leukocyte count was made in the Optical Light Microscope and results being expressed as cell numbers. μL^{-1}.

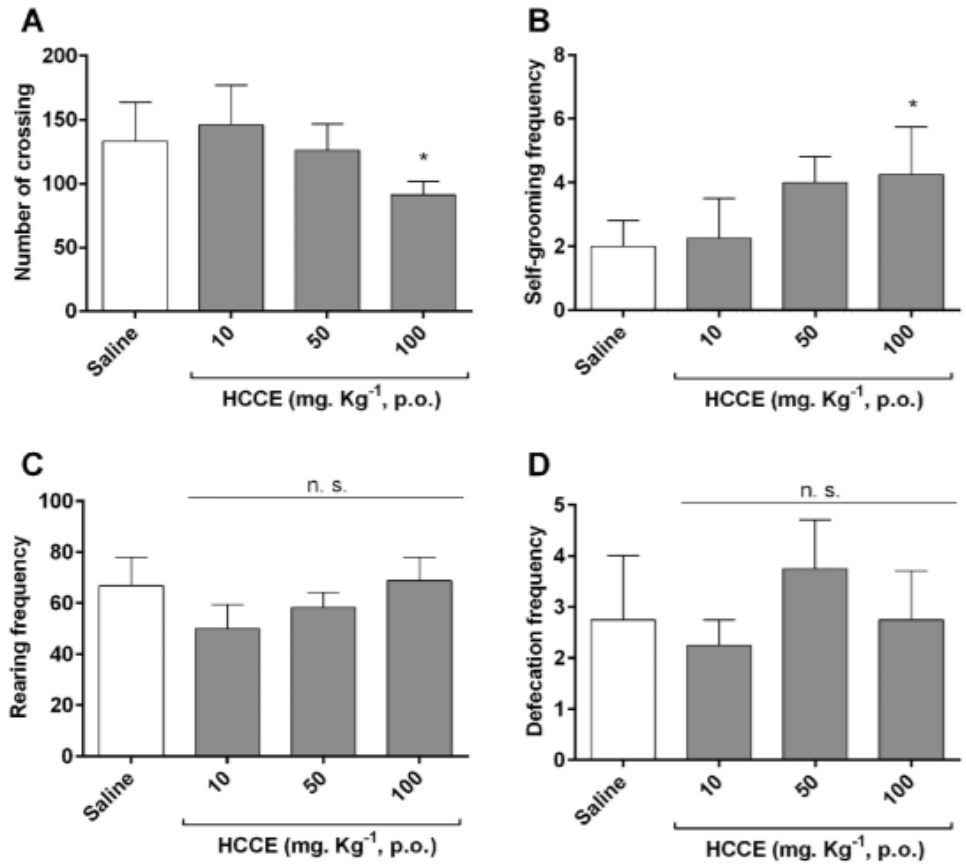


Figure 2: Open field test. A) Number of crossing; B) Self-grooming frequency; C) Rearing frequency and; D) defecation frequency. Data are expressed in mean ± SD and analyzed using one-way ANOVA followed by Bonferroni's test (* indicate significative difference compared to saline).

Statistical analyses

Results are presented as mean ± standard deviation (SD) for each group. Data obtained from behavioral tests and organs/-body weight ratio were analyzed using a one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparisons test. For data obtained from oxidative tests and creatinine K assay was employed two-way ANOVA followed by Tukey's multiple comparisons test. For leukocyte count was used one-way ANOVA followed by Bonferroni's test in the comparison between groups and two-way ANOVA followed by Tukey's test to compare exposure. P values less than .05 were considered indicative of significance.

Results

Behavioral test

The open field assay showed no significant difference in the rearing and defecation frequency between HCCE concentrations tested and the control group. Treatment with lower HCCE doses (10–50 mg. Kg^{-1}) tested in the subchronic model, no affected the spontaneous locomotor and emotional response (Fig. 2). However, mice exposed to HCCE at 100 mg. kg^{-1} concentration presented a significant decrease ($P < 0.05$) in the number of crossing (Fig. 2A) and an increase in its self-grooming frequency (Fig. 2B) compared with saline.

Other complementary behavioral tests were performed. Mice treated presents not significant changes in forced swimming test (FST) and tail suspension test (TST) (Supplementary material, Fig. 1).

Tissue toxicity evaluation

Organs/body weight ratio. There was no difference in the animal weight before and after treatment in both acute and subchronic exposures. The toxicity analyses by organs/body weight ratio (Fig. 3) showed that mice exposed acute and subchronically to HCCE did not show weight abnormalities in their main organs—heart, brain, liver, spleen, and kidneys—compared with saline control. Histological changes of liver exposed subchronically also were not observed, despite kidney glomeruli of the HCCE 50 and 100 groups have shown changes in their structure (Supplementary material, Fig. 2).

Oxidative stress. Oxidative stress was assessed in the brain, liver, and kidneys of mice acute and subchronically exposed to HCCE. Here, we quantified TBA-RS, NPSH, and carbonyl levels in each organ to characterize HCCE effects (Fig. 4 and 5).

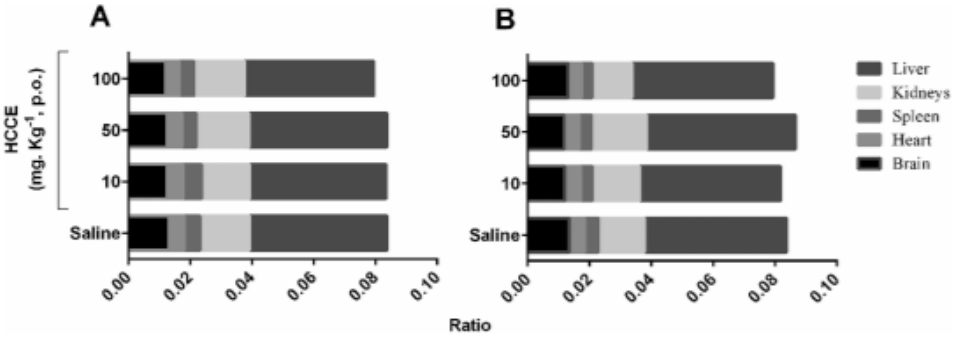


Figure 3: Ratio of organ/body weights in mice exposed to *Handroanthus chrysotrichus* A) acute and B) subchronically. Data are expressed in mean ± SD and analyzed using one-way ANOVA followed by Bonferroni's test.

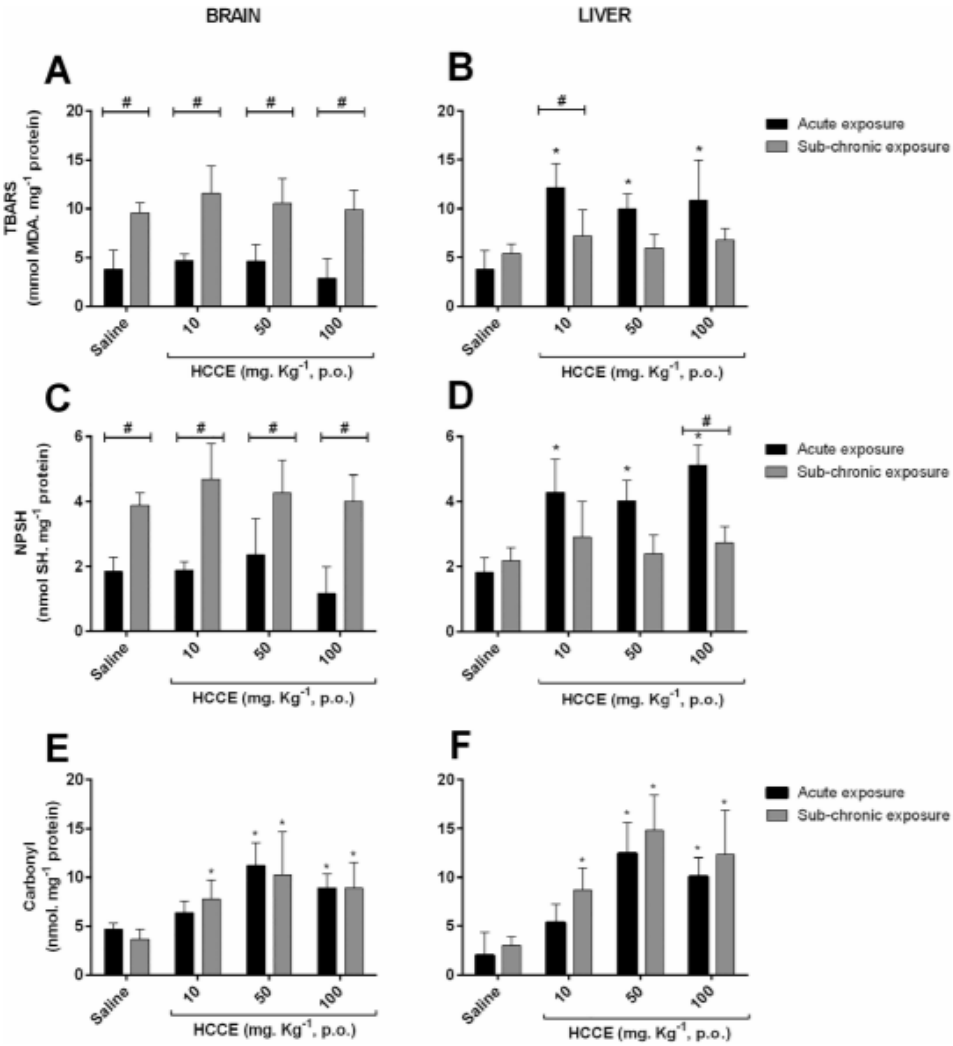


Figure 4: Evaluation of oxidative stress in the brain (A, C, and E) and liver (B, D, and F) of mice exposed acute and sub-chronically to *Handroanthus chrysotrihus* extract. Data are expressed in mean ± SD and analyzed using two-way ANOVA followed by Tukey's test (* indicate significative difference compared to saline; # indicate significative difference between exposure groups).

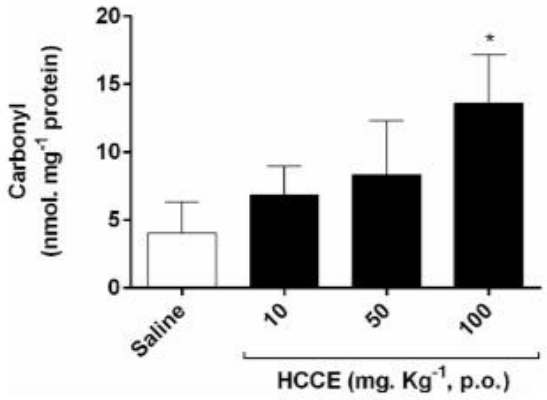


Figure 5: Evaluation of carbonyl levels in kidney of mice acutely exposed to Handroanthus chrysotrihus extract. Data are expressed in mean ± SD and analyzed using one-way ANOVA followed by Bonferroni's test (* indicate significative difference compared to saline).

Table 1: blood biomarkers analyzed

Parameter (Unit)	Exposure acute (mg. Kg^{-1})				Exposure sub-chronic (mg. Kg^{-1})			
	Saline	10	50	100	Saline	10	50	100
ALT (U/l)	71.1 ± 26.9	74.1 ± 16.4	117.4 ± 8,9	140.9 ± 41.3	99.2 ± 20.7	115.1 ± 4.7	115.9 ± 11.9	151.1 ± 41.9
AST (U/l)	201.8 ± 53.5	225.2 ± 42.5	194.9 ± 65.4	300.9 ± 39.1	169.5 ± 36.3	185.3 ± 15.7	165.6 ± 26.4	228.9 ± 99.9
Creatinine K (mg. dL^{-1})	0.24 ± .13	0.49 ± .08	0.23 ± .04	0.62 ± .19[a]	0.26 ± .1	0.36 ± .18	0.37 ± .14	0.35 ± .14
Leukocytes (10^3. μL^{-1})	7.65 ± .91	3.08 ± 1.14[a]	4.6 ± .93[a]	3.1 ± .54[a]	8.36 ± 2.2	13.31 ± 5.2[b]	6.41 ± 2[b]	10.03 ± 3.48[b]

Data represented by mean ± SD. Letter "a" indicates significative difference compared with saline using one-way ANOVA followed by Turkey's multiple comparisons test. Letter "b" indicates difference between groups of exposure with same concentration using two-way ANOVA followed by same post hoc.

Initially, the amount of TBA-RS and NPSH in the brain did not show significant alterations in both, acute, and subchronic exposure (Fig. 4A and C). In addition, animals of acute exposure showed a significant increase in carbonyl from 50 mg. Kg^{-1} compared with saline control (Fig. 4E). While animals of subchronic exposure presented a significant increase in carbonyl in all concentrations tested (10–100 mg. Kg^{-1}), compared with control (Fig. 4E). However, subchronic exposure had a significant difference from acute in brain TBA-RS and NPSH amount, including saline control (Fig. 4A, C).

Liver analysis indicated a TBA-RS increase in acute conditions for all groups tested when compared with saline control. There were no alterations for this parameter in subchronic evaluation (Fig. 4B). A similar situation was found for NPSH levels (Fig. 4D). Moreover, when the liver was exposed subchronically to HCCE presented a significant increase in carbonyl levels in all tested concentrations. While animals of acute exposure showed a significant increase from 50 mg. Kg^{-1} compared with control (Fig. 4F).

The only altered oxidative parameter in kidneys was carbonyl of mice treated with acute exposure to HCCE at its highest concentration (100 mg. Kg^{-1}) (Fig. 5). TBARS and NPSH did not change with treatment acute or subchronic (Data not show).

Blood biomarkers

All blood biomarkers analyzed are demonstrated in Table 1.

ALT, AST, and creatinine K. ALT and AST analysis were used as parameters for hepatic damage measure, which did not show significant differences between groups assessed. The biomarker used to indicate kidney damage was creatinine K. This biomarker had a significant increase in the mice group of the acute exposure at HCCE 100 mg. Kg^{-1}.

Total leukocyte count. Total leukocyte count showed that mice of the acute exposure group had a significant decrease of cell count in all HCCE concentrations tested (10–100 mg. Kg^{-1}). On other hand, no one of the animals subchronically exposed to HCCE showed a significant difference.

Discussion

Here, we verified alterations in mice behavior caused by subchronic exposure to *H. chrysotrichus* hydroalcoholic stem bark extract at the highest dose. The extract also caused an oxidative imbalance in liver tissue. Brain tissue had an increase in carbonyl levels. The animals of acute exposure showed in the kidney tissue a significant increase of carbonyl and creatinine K levels at the highest HCCE concentration. In addition, the acute exposure trigged a decrease of total leukocyte count in all concentrations tested.

General aspects were observed while the mice were exposed to the extract (death, contortions or tremors, vomit, diarrhea, lethargy, and irritation of the skin). These toxicity symptoms observed (Supplementary material, Table 1) indicated diarrhea and lethargy in animals exposed subchronically at 50- and 100-mg. Kg^{-1} concentrations. Although TST and FST behavioral tests were not significant (Supplementary material, Fig. 1), these observations are following OFT (Fig. 2A), where we see a decrease in the number of crossing at 100 mg. Kg^{-1}. This group presented also an increase in its self-grooming (Fig. 2B), a common behavior

in rodents that consist of stereotyped patterns known as a syntactic chain pattern [19]. These movements represent up to 30–50% of their waking time [20, 21]. The increase of this conduct compared with the mean of the control group may be considered as an anxiety signal. Thus, these changes in the central nervous system may have occurred in animals exposed subchronically to high concentrations of the extract. Although there are Bignoniaceae species that possesses anxiolytic and antidepressant-like activities [22].

In this context, dopamine is one of the main neurotransmitters present in the brain, and administration of its receptor agonist (D1R) in rodents may amplify behavioral stereotyped and production of self-grooming chains [23, 24]. The glutamate seems is also involved in the regulation of self-grooming [25], including there is the effect of neuronal glutamate transporter EAAC1 on D1R expression [26]. Another neurotransmitter is γ-aminobutyric acid (GABA), present more than 90% of striatal neurons in the brain [27]. Morland *et al.* [28] suggest that the inhibition of GABA transaminase causes its accumulation and increase of the extracellular concentration, which inhibits neuronal activity and causes lethargy. We highlight that our previous evaluation of HCCE [7] demonstrated five compounds of extract, among them the pentanoic acid, which present an effect GABA transaminase inhibitor *in silico* (Probable activity = 0.852; Probable inactivity = 0.002—Data not show). Although we have found no evidence that HCCE is neurotoxic, this datum may indicate a relationship between extract and the nervous system.

After the euthanasia of mice, we made other toxicological tests with their organs. There were no changes in the animal organs/body weight ratio (Fig. 3), even in histological analysis of the liver (Supplementary material, Fig. 2). In the brain, no differences were observed in TBA-RS and NPSH levels when compared with control. Moreover, the increase in carbonyl in the brain may be induced by stress in rodents, which is associated with the development of the depressive state, as we saw in OFT [29]. Even because, we did not verify any report of neurotoxicity in HCCE.

Still, in the brain analysis, mice of subchronic showed a significant difference when compared with acute exposure in TBA-RS and NPSH levels, including saline control (Fig. 4A and C). We believe that this fact does not have a relation with subchronic treatment. But with something that occurs in this period, such as animal manipulation, aging, or methodological interference. Moreover, according to Fisher–Wellman and Bloomer [30], exercises—like swimming incorporated in behavioral tests—possess the potential to result in increased reactive oxygen and nitrogen species production, and subsequent oxidative stress. These are some facts that may justify the difference found between acute and subchronic groups.

Altogether, the imbalance in the liver may have occurred due to its function as the main organ involved in the biotransformation of xenobiotics in the organism. However, serum enzymes that are considered sensitive markers of hepatic toxicity as ALT and AST [31] have not altered their activities (Table 1), suggesting that major liver damage occurred in its lipids during oxidative stress generated by acute exposure (Fig. 4B). Not all species in the Bignoniaceae family trigger this stress [32]. But, we highline that α-curcumene, the major constituent of the HCCE [7], exerts triglyceride-lowering activity on serum as well as liver triglycerides [33]. This set of transformations may influence oxidative stress. Moreover, carbonyl levels also were affected. We know that carbonylated proteins have a long half-life and take longer to suffer degradation when compared with normal proteins; therefore, they may be accumulating since acute exposure (Fig. 4F).

On the other hand, the kidney presented increased carbonyl and creatinine levels (Fig. 5 and Table 1, respectively) found only in the acute treatment at 100 mg. Kg^{-1} concentration. Nonetheless, the subchronically treatment at 100 mg. Kg^{-1} showed significant increase of glomeruli and capsular space area (Supplementary material, Fig. 2). The kidneys receive about 20–25% of the blood flow, and because of this, any chemical or drug that reaches the systemic circulation will reach the kidneys in high concentrations, possibly concentrating potential toxicants in the kidney fluid. Thus, renal transport, accumulation, and metabolism of xenobiotics contribute significantly to renal susceptibility to toxic damage [34].

We also found leukopenia in all concentrations tested of the acute treatment (Table 1). Leukocytes are the main blood cells involved in the immune response. Among the types of leukocytes, neutrophils are the most important in the pathogenesis of inflammation. They are predominant cells in the first 6–24 hours in acute cases of inflammation [35]. Despite HCCE presents some compounds anti-inflammatory act *in silico* [7], here seems to be occurring a leukocyte migration in the first 24 hours. Later, this process may have suffered a compensatory effect on subchronic exposure. Nevertheless, the leukocytes activated produce cytokines as inflammatory responses. These cytokines trigger changes behavioral such as reductions in activity, food intake, and social interaction, along with increased sleep and anhedonia [36, 37]. Similar to that seen in some treated groups of subchronic exposure (Fig. 2; Supplementary Material, Table 1).

Blood parameters are used to determine the extent of adverse effect of exogenous compounds as plant extracts [38]. Blood cells derive from the pluripotent hematopoietic stem cell, which capable of becoming leukocytes, erythrocytes, or thrombocytes. Changes in the hematopoietic system have a higher predictive value for human toxicity when data are translated from animal studies [39, 40]. In this context, treatments with others species of Bignoniaceae family also can change blood parameters, as red blood cells, hemoglobin, mean corpuscular volume, neutrophils, lymphocyte, and platelet, besides some serum electrolytes as calcium [32, 41, 42].

Thus, besides white cells, the HCCE primary targets seem to be the liver and kidneys, because they are the main sites for xenobiotics metabolism. On other hand, the brain is affected under subchronic exposure, causing long-term behavioral changes at the highest concentrations. Possibly, continuous stress produces brain damage and alterations in its neurotransmitters.

Conclusions

The present study showed that high concentrations of *H. chrysotrichus* cause behavioral and biochemical alterations in mice. HCCE trigger an oxidative imbalance in liver tissue in both exposures, acute, and subchronic. Besides, the carbonylated protein levels increased in brain tissue, which may be associated with a possible lethargy in mice exposed subchronically. We verified also that acute exposure induced leukopenia and caused kidney damage at the highest concentration.

The results obtained allow concluding that extract may cause significant damage, especially at high concentrations and in a long extract exposure. Our current study provided a comprehensive view for understanding the damage caused by HCCE. Moreover, a further detailed investigation is required for discovering the key metabolites and their roles in these damages and identifying how HCCE acts on white blood cells.

Supplementary Data

Supplementary data is available at *TOXRES Journal* online.

Abbreviations

(ALT), alanine aminotransferase; (ANOVA), analysis of variance; (AST), aspartate aminotransferase; (DNPH), 2,4-dinitrophenylhydrazine; (DTNB), Ellman reagent; (FST), forced swimming test; (HCCE), *Handroanthus chrysotrichus* crude extract; (GSH), glutathione; (MDA), malondialdehyde; (NPSH), nonprotein thiols levels; (OFT) open field test; (p.o.), given orally; (S1), supernatants; (SD), standard deviation; (TBA-RS), thiobarbituric acid reactive substances; (TFK), phosphate buffer; (TST), tail suspension test; (w/v), weight/volume.

Acknowledgements

The authors thank the Brazilian Government Agencies – Coordination for the Improvement of Higher Education Personnel (CAPES) and National Council for Scientific and Technological Development (CNPq)—for their financial support. We are grateful for the scientific initiation and post-doctoral fellowships promoted by the Research Support Foundation of the State of Rio Grande do Sul (FAPERGS). We also thank graduate Luciana de Mello Viana for helping to maintain the animals.

Conflict of Interest Statement

There are no conflicts of interest.

References

1. Bolson M, Hefler SR, Dall'Oglio Chaves EI *et al.* Ethnomedicinal study of plants used for treatment of human ailments, with residents of the surrounding region of forest fragments of Paraná, Brazil. *J Ethnopharmacol* 2015;**161**:1–10.
2. Ribeiro RV, Bieski IGC, Balogun SO, Martins DT D O. Ethnobotanical study of medicinal plants used by Ribeirinhos in the North Araguaia microregion, Mato Grosso, Brazil. *J Ethnopharmacol* 2017;**205**:69–102.
3. de Albuquerque UP, Hanazaki N. Five problems in current ethnobotanical research—and some suggestions for strengthening them. *Hum Ecol* 2009;**37**:653–61.
4. Heinrich M, Bremner P. Ethnobotany and ethnopharmacy - their role for anti-cancer drug development. *Curr Drug Targets* 2006;**7**:239–45.
5. Grazziotinv JD, Schapoval EES, Chaves CG *et al.* Phytochemical and analgesic investigation of *Tabebuia chrysotricha*. *J Ethnopharmacol* 1992;**36**:249–51.
6. Epifano F, Genovese S, Fiorito S *et al.* Lapachol and its congeners as anticancer agents: a review. *Phytochem Rev* 2014;**13**:37–49.
7. Costa MT, Goulart AS, Soares JJ *et al.* Antioxidant and toxicological potential of the Golden trumpet hydroalcoholic stem bark extract. *Res Soc Dev* 2020;**9**:1–23.
8. Wright CR, Setzer WN. Volatile components of organ pipe cactus, *Stenocereus thurberi* Engelm., growing in the Organ Pipe Cactus National Monument and the Arizona-Sonora Desert Museum. *Am J Essent Oils Nat Prod* 2013;**1**:19–22.
9. Cardozo EL, Ferrarese-Filho O, Filho LC *et al.* Methylxanthines and phenolic compounds in mate (*Ilex paraguariensis* St. Hil.) progenies grown in Brazil. *J Food Compos Anal* 2007;**20**:553–8.
10. Victório CP, Lage CLS, Kuster RM. Flavonoid extraction from *Alpinia zerumbet* (Pers.) Burtt et Smith leaves using different techniques and solvents. *Eclética Química* 2009;**34**:19–24.
11. Amorozo MCM. A abordagem etnobotânica na pesquisa de Plantas Medicinais. In: Di Statsi LC (ed). *Plantas medicinais: Arte e Ciência, um guia de estudo interdisciplinar*. São Paulo: EDUSP, 1996, 47–68.
12. Bond N, Di Giusto E. Open-field behavior as a function of age, sex, and repeated trials. *Psychol Rep* 1977;**41**:571–4.
13. Whimbey AE, Denenberg VH. Two independent behavioral dimensions in open-field performance. *J Comp Physiol Psychol* 1967;**63**:500–4.
14. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979;**95**:351–8.
15. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;**72**:248–54.
16. Levine RL, Garland D, Oliver CN *et al.* [49] Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol* 1990;**186**:464–78.
17. Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959;**82**:70–7.
18. Dornfest BS, Lapin DM, Naughton BA *et al.* Phenylhydrazine-induced leukocytosis in the rat. *J Leukoc Biol* 1986;**39**:37–48.
19. Kalueff AV, Aldridge JW, LaPorte JL *et al.* Analyzing grooming microstructure in neurobehavioral experiments. *Nat Protoc* 2007;**2**:2538–44.
20. Bolles RC. Grooming behavior in the rat. *J Comp Physiol Psychol* 1960;**53**:306–10.
21. Spruijt BM, van Hooff JA, Gispen WH. Ethology and neurobiology of grooming behavior. *Physiol Rev* 1992;**72**:825–52.
22. Murtala AA, Akindele AJ. Anxiolytic- and antidepressant-like activities of hydroethanol leaf extract of *Newbouldia laevis* (P.Beauv.) Seem. (Bignoniaceae) in mice. *J Ethnopharmacol* 2020;**249**:112420.
23. Berridge KC, Aldridge JW. Super-stereotypy II: enhancement of a complex movement sequence by intraventricular dopamine D1 agonists. *Synapse* 2000;**37**:205–15.
24. Berridge KC, Aldridge JW. Super-stereotypy I: enhancement of a complex movement sequence by systemic dopamine D1 agonists. *Synapse* 2000;**37**:194–204.
25. Goodman WK, Grice DE, Lapidus KAB, Coffey BJ. Obsessive-compulsive disorder. *Psychiatr Clin North Am* 2014;**37**:257–67.
26. Bellini S, Fleming KE, de M *et al.* Neuronal glutamate transporters control dopaminergic signaling and compulsive behaviors. *J Neurosci* 2018;**38**:937–61.
27. Gerfen CR. Synaptic organization of the striatum. *J Electron Microsc Tech* 1988;**10**:265–81.
28. Morland C, Frøland A-S, Pettersen MN *et al.* Propionate enters GABAergic neurons, inhibits GABA transaminase, causes GABA accumulation and lethargy in a model of propionic acidemia. *Biochem J* 2018;**475**:749–58.
29. Gorlova A, Pavlov D, Zubkov E *et al.* Alteration of oxidative stress markers and behavior of rats in a novel model of depression. *Acta Neurobiol Exp (Wars)* 2019;**79**:232–8.
30. Fisher-Wellman K, Bloomer RJ. Acute exercise and oxidative stress: a 30 year history. *Dyn Med* 2009;**8**:1.
31. Ramaiah SK. Preclinical safety assessment: current gaps, challenges, and approaches in identifying translatable

biomarkers of drug-induced liver injury. *Clin Lab Med* 2011;**31**:161–72.
32. Calil Brondani J, Reginato FZ, da Silva Brum E *et al.* Evaluation of acute and subacute toxicity of hydroethanolic extract of *Dolichandra unguis-cati* L. leaves in rats. *J Ethnopharmacol* 2017;**202**:147–53.
33. Yasni S, Imaizumi K, Sin K *et al.* Identification of an active principle in essential oils and hexane-soluble fractions of *Curcuma xanthorrhiza* Roxb. Showing triglyceride-lowering action in rats. *Food Chem Toxicol* 1994;**32**:273–8.
34. Davis BB, Mattammal MB, Zenser TV. Renal metabolism of drugs and xenobiotics. *Nephron* 1981;**27**:187–96.
35. Francischetti I, Moreno JB, Scholz M, Yoshida WB. Leukocytes and the inflammatory response in ischemia-reperfusion injury. *Rev Bras Cir Cardiovasc* 2010;**25**: 575–84.
36. Larson SJ, Dunn AJ. Behavioral effects of cytokines. *Brain Behav Immun* 2001;**15**:371–87.
37. Dantzer R, O'Connor JC, Freund GG *et al.* From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat Rev Neurosci* 2008;**9**:46–56.
38. Arora S, Pal M, Bordoloi M, Nandi SP. Effect of hexane extract of *Syzygium aromaticum* on haematological profile of rats. *J Environ Biol* 2018;**39**:347–52.
39. Guyton AC, Hall JE. *Textbook of Medical Physiology*, 11th edn. Mansfield: Elsevier, 2006.
40. Olson H, Betton G, Robinson D *et al.* Concordance of the Toxicity of pharmaceuticals in humans and in animals. *Regul Toxicol Pharmacol* 2000;**32**:56–67.
41. Gopalakrishnan V, Beulah U, Devaki K, Akila G. Effect of aqueous extract of *Passiflora edulis* on biochemical and hematological parameters of Wistar Albino rats. *Toxicol Int* 2012;**19**:63–7.
42. Ibrahim MB, Sowemimo AA, Sofidiya MO *et al.* Sub-acute and chronic toxicity profiles of *Markhamia tomentosa* ethanolic leaf extract in rats. *J Ethnopharmacol* 2016;**193**:68–75.

SUPPLEMENTARY MATERIAL

1. Materials and methods

During the study, all animals were observed and mice sub-chronically exposed to HCCE performed behavioral tests (tail suspension and forced swimming test).

1.1. General Observation

During the maintenance of animals, there were general observations about the health of the mice. These observations were performed every 12 hours for 10 minutes. Besides, toxicity symptoms observed were classified into lethality, pain, gastrointestinal and behavioral alterations, and sensitization skin. Symptoms were expressed flexibly: (+) low potential, reached one animal in the group; (++) medium risk, reached between 2 and 3 animals in the group; (+++) high risk, reached between 4 and 5 animals in the group; and non-observed risk (0).

1.2. Behavioral tests

1.2.1. Forced swimming test (FST)

Mice were individually forced to swim in a cylindrical container (diameter 10 cm, height 25 cm), containing 19 cm of water at 25 ± 1°C. The mouse was immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. The immobility time was measured by a blinded observer during a 6 min test. An increase in the duration of immobility was considered indicative of a depressant-like activity[1].

1.2.2. Tail suspension test (TST)

TST is based on the fact that animals subjected to inescapable stress will develop an immobile posture. The mice were individually suspended 50 cm above the floor using adhesive

tape placed approximately 1 cm from the tail and the total duration of immobility time was measured according to the method described by Steru et al.[2]. The immobility time was measured for 6 min by a blind observer. An increase in the immobility time was considered indicative of a depressant-like activity.

1.3. Histology

Three liver and kidneys samples were prepared to histological examination. Fresh portions of both tissues were cut out rapidly and fixed in neutral buffered formalin (10%). After, these samples were dehydrated with progressive percentages of ethanol (70, 80, 900, 95, and 100%) and clarified in xylene. Then, tissues were impregnated in paraffin, mounted in blocks, and cut (5 µm) slices by a microtome. The microscopic slides were stained with Hematoxylin and Eosin.

Twenty images of renal corpuscles were photographed per slide (Enlargement 400x), and using the Adobe Photoshop 2021 software, the glomerular and corpuscular area were measured in μm^2. The subtraction these two areas resulted in the capsular space.

1.4. Statistical analyses

Results are presented as mean ± standard deviation (SD) for each group. Data obtained were analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparisons test. P values less than 0.05 were considered indicative of significance.

2. Results

2.1. General observation

Our observations on animal health during treatment demonstrate the danger of exposure sub-chronic to HCCE, mainly at high concentrations (Table 1). Among the toxicity symptoms observed are diarrhea and lethargy.

Table 1. Observations of toxicity symptoms observed during the treatment of the mice.

Group		Toxicity symptoms observed					
		Mortality	**Pain**	**Gastrointestinal**		**Behavioral**	**Sensitization**
Exposure	**Dose (mg. Kg^{-1})**	**Death**	**Contortions or tremors**	**Vomit**	**Diarrhea**	**Lethargy**	**Skin**
Acute	Saline	0	0	0	0	0	0
	10	0	0	0	0	0	0
	50	0	0	0	0	0	0
	100	0	0	0	0	0	0
Sub-chronic	Saline	0	0	0	0	0	0
	10	0	0	0	0	0	0
	50	0	0	0	+	+	0
	100	0	0	0	++	+++	0

Low potential (+), medium risk (++), high risk (+++) and non-observed risk (0).

2.2. Behavioral tests

Results illustrated in Figure 1 represent depressive-like behavior expressed as the duration of immobility in TST (Figure 1A) and FST (Figure 1B). Group treated with HCCE (10 – 100 mg. kg^{-1}, p.o.) no presented a significant increase ($p < 0.05$) in the immobility time in both tests, TST and FST, compared with animals from the group treated with saline (10 ml. kg^{-1}, p.o.).

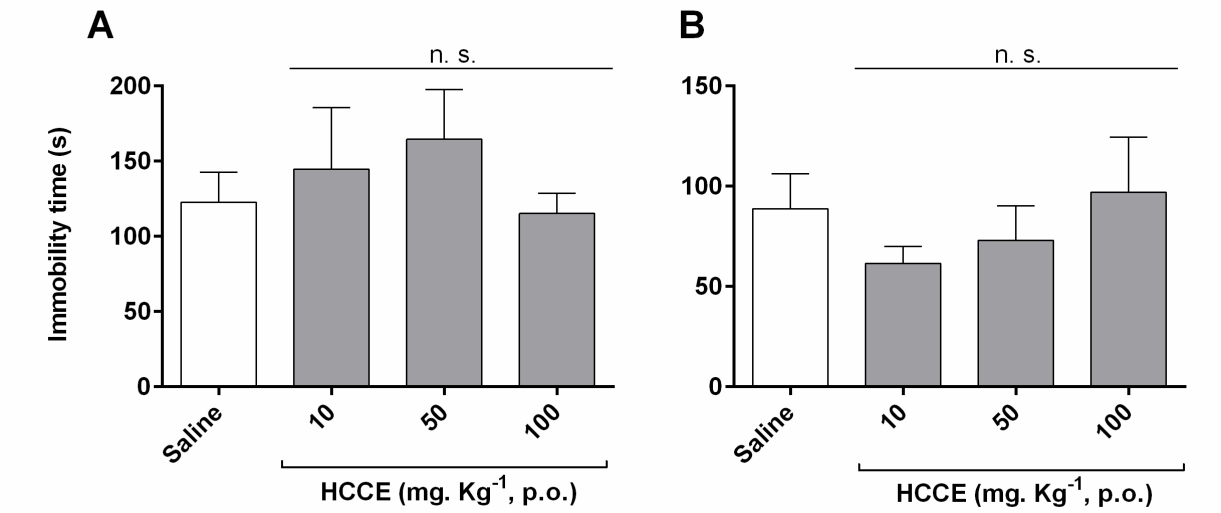


Figure 1. Immobility evaluation in A) tail suspension test (TST) and B) forced swimming test (FST) of mice sub-chronically exposed to Handroanthus chrysotrichus. Data are expressed in mean ± SD. There was non-significance (n. s.) between the groups evaluated.

No differences were found in the analyzed liver tissues (Figure 2). On the other hand, the group treated with HCCE 100 (100 mg. kg^{-1}, p.o.) presented a significant increase ($p < 0.05$) in the glomerular and capsular space area (Figure 2A, and C). The intermediate group HCCE 50 (50 mg. kg^{-1}, p.o.) presented an increase in its capsular space (Figure 2C).

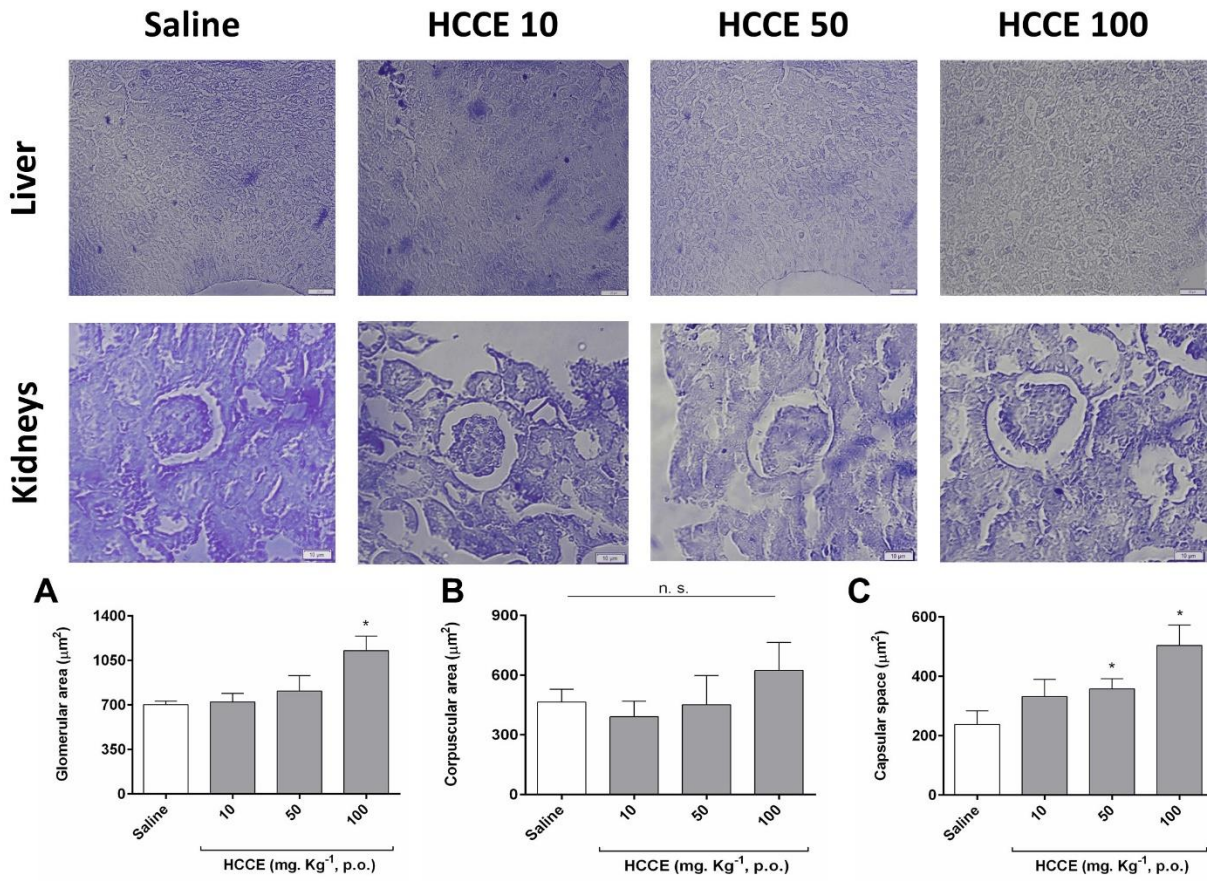


Figure 2. Effect of sub-chronically HCCE treatment on mice liver and kidneys histology. Kidney glomeruli were analyzed using A) the glomerular, B) corpuscular, and C) capsular space area. Data are expressed in mean ± SD (* indicate significative difference).

References

1 M. P. Kaster, V. M. Gadotti, J. B. Calixto, A. R. S. Santos and A. L. S. Rodrigues, *Neuropharmacology*, 2012, **62**, 419–426.

2 L. Steru, R. Chermat, B. Thierry and P. Simon, *Psychopharmacology (Berl).*, 1985, **85**, 367–370.

6.3. Manuscrito científico 1

Philodryas patagoniensis venom: status of the art and its potential use in toxicological and pharmacological fields

Márcio Tavares Costa[a]
ORCID: https://orcid.org/0000-0003-4940-3567
E-mail: marciocosta@unipampa.edu.br

Ana Paula Tavares Costa[b]
ORCID: https://orcid.org/0000-0003-1938-1299
E-mail: anapaulatavarescosta@gmail.com

Kellen Mariane Athaide Rocha[a]
ORCID: https://orcid.org/0000-0001-8038-5286
E-mail: kellen_a.rocha@hotmail.com

Aline da Silva Goulart[a]
ORCID: https://orcid.org/0000-0002-3222-5816
E-mail: alinefsgoulart@gmail.com

Robson Puntel[c]
ORCID: https://orcid.org/0000-0001-9047-2906
E-mail: robsonunipampa@gmail.com

Vanderlei Folmer[c]*
ORCID: https://orcid.org/0000-0001-6940-9080
E-mail: vanderleifolmer@unipampa.edu.br

[a]PhD student at the Graduate Program in Biochemistry at the Federal University of Pampa (UNIPAMPA), Uruguaiana, Rio Grande do Sul, Brazil;
[b]PhD student at the Graduate Program in Biology of Continental Aquatic Environments, Federal University of Rio Grande (FURG), Rio Grande, Rio Grande do Sul, Brazil;
[c]Professor PhD at the Graduate Program in Biochemistry at the Federal University of Pampa (UNIPAMPA), Uruguaiana, Rio Grande do Sul, Brazil;
*Corresponding author.

Abstract

The aim of this work was to present the state of the art of *Philodryas patagoniensis* venom (PpV) and to approach the possibility of its use in toxicological and pharmacological tests. We consulted Web of Science (WoS), Scopus, and PubMed databases to search for articles from 1969 until May 2020, with keyword combinations: "*Philodryas patagoniensis*" AND "venom". After filtering the number of articles was reduced from 133 to 37. Then, we assessed the similarity among databases and created categorizations to classify the lines of study: (I) Biochemistry; (II) Toxicology; and (III) Pharmacological works. Moreover, we extracted diverse information from articles. The results showed WoS and Scopus present a similarity of 60% in their papers. Biochemistry works are more abundant. *P. patagoniensis* is not considered a venomous snake, but its envenoming can trigger clinical manifestations. PpV has a complex mixture of compounds generated by Duvernoy's gland with the potential to generate protease and hemorrhagic activities greater than *Bothrops jararaca*. Most rear-fanged snake venoms have not been studied; therefore, many medical implications may be discovered. On the other hand, the interactive potential of toxins is a neglected area of research. Thus, based on collected data, we concluded that PpV is little explored in both research fields: Toxicological and Pharmacological. In addition, despite the difficulties, it seems plausible to use PpV as a Toxicological or Pharmacological assessment model due to the diverse reasons here mentioned.

Keywords: Poison. Dipsadidae. Colubridae. Review. Ophidism. Green-racer.

Abbreviations

Cysteine-rich secretory proteins (CRISPs); Ethylenediamine tetra-acetic acid solution (EDTA); Web of Science (WoS); Scopus (Sc); PubMed (PM); *Philodryas patagoniensis* venom (PpV); Snake venom metalloproteinase (SVMP).

Background

Philodryas patagoniensis is a snake known as Green racer and belongs to the Dipsadidae family, which is characterized by tree species. Nevertheless, *P. patagoniensis* is a species that occurs in open areas of the Pampa Biome, essentially terrestrial and including arboreal habits when foraging [1]. The Green racer is frequent in Rio Grande do Sul State and may be found until Rio Grande do Norte State. In addition, this species occurs in Argentina, Uruguay, and Bolivia. These snakes are diurnal species, with brownish color that allows them to camouflage, besides reaching up to 1.6m in length [2,3].

About 20 to 40% of snake accidents in Brazil occur due to species of the family Dipsadidae, the largest family of snakes in Brazil. Moreover, the main genera involved in these accidents are *Helicopters, Oxyrhopus, Thamnodynaster,* and *Philodryas* [4–8]. The number of accidents caused by venomous animals has been growing in recent years and the chance of death among snakebites is higher [9,10]. Brazil recorded 30,482 cases of exposure to snakes in 2019, with 147 deaths. The Northern region of the country was the most affected, with 34% of accidents. The bothropic group represented 68.5% of these cases and non-venomous or ignored species were 20% [11].

In Brazil, there are aglyphous, opisthoglyphous, and proteroglyphous snakes, but due to medical importance, venomous solenoglyphous snakes are more studied. Marcussi et al. [12,13] began genotoxic studies caused by these snakes in human leukocytes. On the other hand, the pharmacology field approaches the potentials of different compounds against their venoms. The use of ascorbic acid, vitamin E, and Vitamin B-complex are examples in cases of envenoming, in which the organisms were protected against damage induced by the venom of species of the genus *Bothrops* [14].

Especially, *P. patagoniensis* is an opisthoglyphous snake presents venom, which added to saliva is involved in food biology, acting as a complementary mechanism in the capture of

its prey [3,15]. However, accidents with this snake as well as other specimens of the Dipsadidae family may provide to their victim pain, edema, bruising, hemorrhage, muscle necrosis, and systemic effects, such as dizziness and vomiting [16–21].

In this context, despite *P. patagoniensis* is a frequent species, which presents cases of accidents with envenoming, it is a snake little used to obtain venom for toxicological and pharmacological tests. Thus, we aim to bring the state of the art of its venom and to approach the possibility of its use in these fields.

Methods

Carrying out this study we consulted Web of Science (WoS), Scopus (Sc), and PubMed (PM) databases to search articles. We used in each of these databases the following keyword combination: "*Philodryas patagoniensis*" AND "venom". Thus, there were searches based on the title, abstract, and keywords of articles published from 1969 until May 2020. This process resulted in a total of 133 papers, 50 found in Sc, followed by 48 in WoS, and 35 in PM.

After assessing the titles and abstracts (and the full text when needed), we excluded duplicate papers and those were not related to study *P. patagoniensis* venom (Figure 1). This

filtering reduced the number of articles from 133 to 37. For each paper, we obtained information such as the year of publication and the region which the survey was conducted.

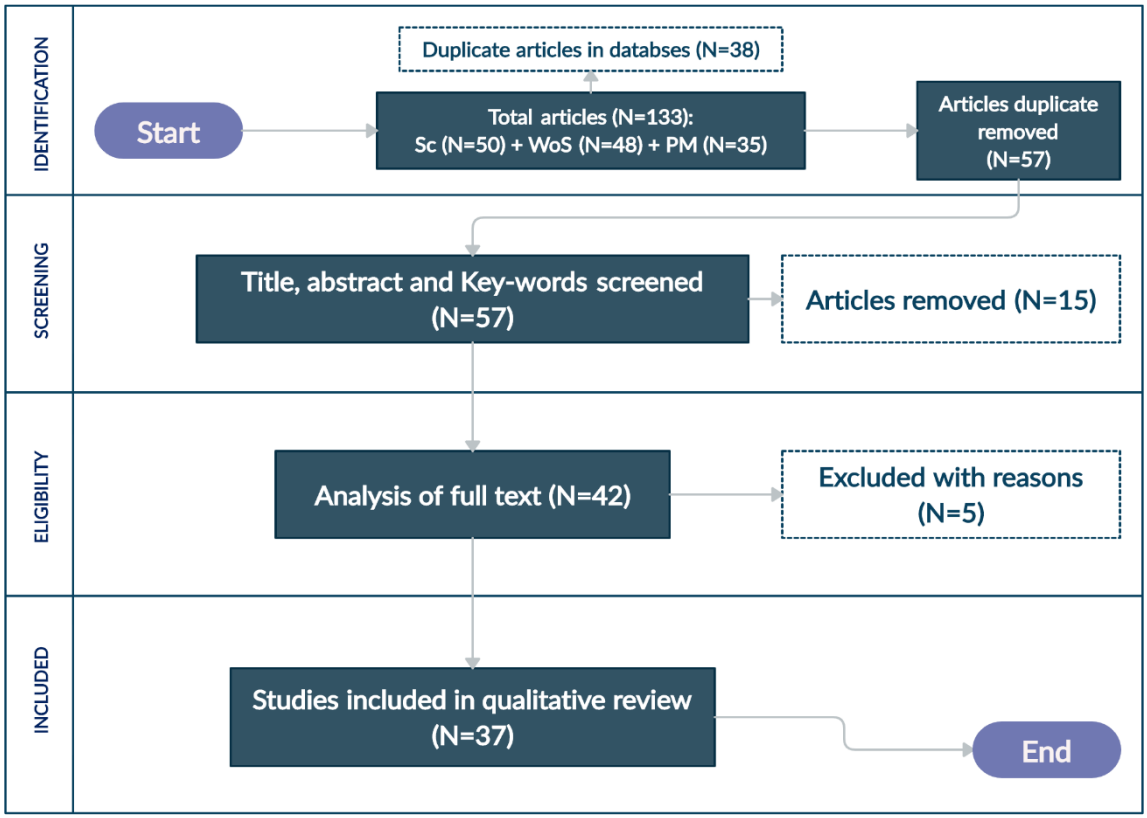


Figure 1. Bibliometric analysis step with the screening of articles.

The papers included in this work allowed to perform some steps:

1) The similarity among databases was analyzed by Jaccard similarity;
2) We developed the following categorizations to classify the lines of study for each paper according to their approaches: (I) Biochemistry – articles that characterize proteins and enzymatic activities of *P. patagoniensis* venom *in vitro*; (II) Toxicology – articles that aim to assess the consequences of envenoming or to assess a compound against venom in an animal model; and (III) Pharmacological – articles that determine possible drugs from the venom.

We clarified that item 1 was developed in the topic "*General information*". While item 2 was covered the remaining topics, according to the subject. In addition to the filtered works,

other articles were employed to contextualize and develop the themes related to general aspects and comparison among groups of snakes.

General information

When analyzing the three databases – WoS, Sc, and PM – there were 133 articles in all, which were abbreviated to 37. In this process, we noted a greater similarity between Sc and WoS. This similarity reached approximately 60%, which indicates the duplicated productions of these databases.

After choosing the works, we saw that the oldest paper was published in 1982, and the most recent in 2020. Along this timeline, there has been no publication regarding *P. patagoniensis* venom (PpV) for many years. The largest number of articles published was among 2010, 2011, and 2017, with four publications each year. The articles classification showed that 54.05% are defined as Biochemistry works, 40.54% as Toxicology, and 5% as Pharmacological studies.

Most studies were developed in Brazil (N=15) and Argentina (N=9). It was possible to verify a collaborative network between these countries (N=5), and collaborative studies between the United States and Argentina (N=2).

Thus, we see that the last decade has been remarkable in the production of the theme addressed. Most articles are related to Biochemistry and Brazil is the reference in the area.

Biochemistry of <u>Philodryas patagoniensis</u> venom

We know that *Bothrops* snakes are the main cause of accidents in Brazil, but coral snakes also present medical importance. These species are of the Viperidae and Elapidae families, respectively. In addition, both families Viperidae and Elapidae present front-fanged and their venoms are constituted essentially by snake venom metalloproteinase (SVMP), snake venom serine proteinase, L-amino acid oxidase, kunitz peptide, phospholipases A2, C-type

lectin, and cysteine-rich secretory proteins (CRISPs). Besides that, they also present minor venom components and different concentrations for each species. The main difference between elapid and viper venoms is the presence of three-finger toxin in elapid venoms and the absence in viper venoms [22–25].

In this context, Peichoto et al. [26] highlighted that PpV and other snakes of their groups possess many venom proteins in common with the venoms of front-fanged snakes, as the proteins found in PpV and also in Russian viper venoms [27]. These similarities between different groups occur because the venom glands of caenophidian snakes are homologous. In Brazil, the Caenophidia clade includes Colubridae, Dipsadidae, Elapidae, and Viperidae families. Thus, venoms produced among the rear-fanged snakes present likeness with the venoms of the front-fanged snakes, including peptides, which are a remarkable example of functional convergence [28–30]. However, considering that their venoms are adapted to facilitate feeding, they vary in composition with several important factors as phylogeny [28], geographic location, seasonal variation, diet, age, and sex of the animal [31].

The responsible for forming the venom in rear-fanged species like *Philodryas* is Duvernoy's gland. Duvernoy’s nomenclature is disuse due to the issues raised above, such as homology. Anyway, this gland is characterized by seromucous cells, which show neutral mucosubstance and protein radicals. Furthermore, there is an acinar area around the gland that is formed by mucous and mucoserous cells [30,32]. The Duvernoy’s gland provides to *P. patagoniensis* a venom formed mainly of proteins that have a molecular mass between 25-80 KDa [33]. Basically SVMP, metal-dependent proteinases that could have their toxic activities blocked by Ethylenediamine tetra-acetic acid solution (EDTA), for example [34].

The patagonfibrase is a SVMP present in the PpV with approximately 53KDa. Activities of this protein increase in the presence of calcium ions, and it can degrade fibrinogen and azocasein. Likewise, it participates in the genesis edema, leukocyte recruitment, bleeding,

myotoxic effects, and inhibits platelet aggregation [35–37]. Moreover, patagonfibrase increased tissue factor and reduced protein disulfide isomerase locally in the rat model and triggered local inflammatory reactions that characterize snake bites [37,38]. The venom features also the patagonin, a CRISPs that exhibited a molecular mass of 24.8KDa and myotoxic activity [39]. Patagonin belongs to snake venom CRISPs, which are proteins found in the majority of crude venoms but at low levels, and can exhibit diverse activities [40]. Literature also reported minor venom components presence as caseinolytic activity, acetylcholinesterase, carbohydrate, besides phospholipase A2 in this venom [26,41].

We highlight in this topic that there is a homology in the venom of rear-fanged and front-fanged snakes. Nevertheless, diverse aspects influence the venom composition, which contributes to the synthesis of different compounds. Large protein groups such as SVMP, CRISPs, and phospholipase A2 are present in PpV, as well as in other families of snakes. Until now, two compounds of PpV have been properly characterized. Thus, we noted a limitation in research about this venom, which can provide access to unknown compounds with toxicological and pharmacological potential.

Toxicological potential of the Philodryas patagoniensis venom

Envenomation represents a serious public health problem. We know that most accidents with *P. patagoniensis* occur in spring and summer. Although this species is considered non-venomous, there are accidents with clinical manifestations such as pain, brief bleeding, erythema, and edema. Rare are cases with systemic symptoms [18]. Araújo and Santos [16] reported an accident caused by *P. patagoniensis*:

> The bite itself did not cause immediate problems. However, the patient complained about a constant itching, that could have been caused by an allergic reaction due to the poison of the snake. After a few minutes, a local swelling rapidly develops and spreads to the hand and fingers, expanding later to the forearm and arm, and reaching the axilla, 72 hours later. This edema remained for 15 days. During that period, arm and hand were hampered and painful, having lost part of their movements. Despite the local signs, the patient did not present systemic symptoms.

In addition, another case of envenoming by *P. patagoniensis* was reported. A 5-year-old boy bitten also showed signs of local envenoming characterized by swelling and warmth on the bitten limb [42]. These are not the only envenoming cases, but they are the ones documented in the literature. All these symptoms occur because venoms have a complex mixture of protein and non-protein components. Despite SVMP seems to be primarily responsible for these effects, as well as the venom of others snake species [41,43], Lopes et al. [44] suggest that SVMP is not related to pain induced by PpV.

Some symptoms of exposure to *P. patagoniensis* as bleeding are already well known [45]. This symptom is caused by SVMP, which degrades proteins in the basement membrane of blood vessels, resulting in a loss of capillary integrity and leading to local bleeding. Implications are worsened by fibrinogenolytic enzymes, which reduce plasma fibrinogen by hydrolysis and prevent coagulation. Including a stronger fibrinogen degradation activity than other rear-fanged snakes such as *Rhabdophis tigrinus* and *R. lateralis* [46–48].

Histological analysis indicated that the damages caused by PpV are dependent on its reach. When administrated intravenously, PpV can generate damage to the cerebellum, cerebrum, lung, kidney, and liver. The subcutaneous route also can affect the same tissues, except for the cerebellar region. Finally, intramuscular administration causes impairment in the lung, kidney, and liver tissue of rats. Moreover, there is a deficient muscular regeneration observed in *P. patagoniensis* envenomation, both experimentally and clinically. These facts, consequently, cause behavioral changes in the model adopted [49,50].

In addition, mice were used for initial tests to elucidate nociception – physiological components of pain – and edema pathway of PpV. The study showed that both symptoms present cyclooxygenase eicosanoids as the main mediators. Besides the angiotensin-converting enzyme inhibitors as captopril can increase nociception, indicating that kinins participate in the pathophysiology [44].

Other side effects of the PpV in animal models are neuromuscular blockade, dermonecrotic activity, and myotoxic potential reaching necrosis, together with increase of creatine kinase in serum as a marker for muscle damage. Moreover, PpV indicates a protease activity greater than the *Bothrops jararaca* [33,34,51]. The peak of the hemorrhagic activity differed between venoms, being 24h for *B. jararaca* and 4h for PpV [34]. Later, Rocha and Furtado [45] performed other comparisons between PpV and bothropic venom. According to the authors, PpV presents biological activities like the venom of species of *Bothrops* genus, with a faster development time of the effects. Including a lethal dose of 50% of 58.85 µg. mouse^{-1}, while *B. jararacussu, B. alternatus,* and *B. jararaca* present a lethal dose of 58.8, 67.5, and 24.7 µg. mouse $^{-1}$, respectively [52].

Possibly due to this similarity, PpV is reactive to bothropic antivenom [33]. As PpV is immunogenic, hyperimmune sera produced by injection of this venom present cross-reactive with sera produced by *B. jararaca* venom. Thus, *Bothrops* antivenom produced may be useful in human serum therapy in cases of envenomation by *P. patagoniensis*, with concentration adjustments [34]. This is important data since ophidism caused by *Philodryas* is neglected in some countries, despite the fact that antivenom therapy is not very effective locally [38,53].

We highlighted that these biological effects are common among other snakes of the *Philodryas* genus, as *P. nattereri* and *P. olfersii* [54]. Especially *P. olfersii,* which can also increase the levels of insulin-like growth factor I (IGF-1) in the serum and trigger a cellular response involving leukopenia and lymphopenia. It is, probably, mediated by metalloproteinases, serine proteinases, CRISPs, and C-type lectins present in the venom [55]. These triggered hazards by envenoming include the exposure to *P. baroni* snake, an exotic species traded as a pet [56].

Therefore, the activities described so far for PpV stem from its biology. All these effects are important for the success in the *P. patagoniensis* predation, especially in juveniles. Because

the juveniles still do not perform the constriction. Moreover, the potential of *P. patagoniensis* is demonstrated by comparing its venom with the *B. jararaca* venom. Furthermore, the literature suggests that SVMP is not solely responsible for the effects of envenoming, as was once believed. This fact opens new possibilities in the toxicological field.

Pharmacological potential of the Philodryas patagoniensis venom potential

Animal toxins are in the spotlight of biomedical research due to their potential pharmacological relevance. Some examples of this potential are 1) Captopril, the first inhibitor of angiotensin-converting enzyme developed from peptides studied and isolated from *Bothrops jararaca* venom [57]; 2) Fibrin sealant from *Crotalus durissus terrificus* venom, a biological and biodegradable product without adverse reactions and that does not transmit of infectious diseases [58]; and 3) Mambalgins, peptides isolated from black mamba venom which have a potent analgesic effect that can be as strong as morphine [59]. Additionally, we find several snake venom-based aesthetic products for sale.

Anticancer properties are another line of research on snake venom. Interestingly, all anti-cancer evaluations on tumorigenic properties – such as proliferation, angiogenesis, invasion, and metastasis of malignant cells – have been performed with isolated toxins from front-fanged snake species, especially from Viperidae species. However, the potential therapeutic applications of toxins described from rear-fanged snake species remain unexplored [60].

Although there has been an increase in the amount of data in the pharmacological field, there is still little data on PpV. This venom showed low activity for the growth of the *Leishmania major* parasite [61]. Nevertheless, CRISPs isolated as patagonin present promising activity against Trypanosomes and *Leishmania* [62]. Three databases currently acquire experimental data, annotate them, and try to provide a global view of the knowledge about these

compounds [63]. To date, we have found no further information in the literature about PpV. Which suggests a wide range of possibilities be explored.

Challenges when it comes to <u>Philodryas patagoniensis</u> venom

Despite the high potential, a major obstacle in exploring snake venom is the low amount of venom usually obtained during the process of milking (extracting venom from the venom glands of a live snake). It is difficult to extract venom from many of the rear-fanged snakes [64]. Not only that, but it is also difficult to keep rear-fanged snakes as *P. patagoniensis* for venom production. The first three months in captivity are the most critical [65]. Because of these, venom research has focused mainly on those snakes with medical importance [64].

In the same sense, the vast most of snake venoms with rear-fanged have not been studied. Therefore, there are opportunities to discover novel biological molecules with possible medical implications [26]. In addition, discoveries in snake venom composition have revealed an unexpectedly high degree of variation of snake venoms of the same species linked to variables such as diets, geographical distribution, and ontogeny. These factors increase the potential pool that snake venom represents for pharmacological prospecting [64]. Besides, it allows understanding the composition and evolution of venoms from rear-fanged snakes [26].

It should be noted that Modahl and Mackessy [66] alert to the reductionist approaches usually applied in the study of purified toxins. Authors point out that few studies have attempted to evaluate interactions between toxins and suggest that the interactive or synergistic potential of toxins is a neglected area of research. Consequently, venoms from rear-fanged snakes have the potential to make an important contribution to our understanding of many phenomena still outstanding in Toxinology.

Ultimately, even if the works occur, another challenge is the gap existing between basic research and clinical trials. Brazil has a wide biodiversity of venomous animals, with the

potential for drug discovery and development. But most research occurs isolated in experimental laboratories. Thus, basic research findings are published but rarely advanced [67]. Remaining a gap in translational Toxinology research.

Conclusions

In conclusion, it was noticed that Biochemistry works are highlighted between papers about *Philodryas patagoniensis* venom, followed by Toxicology. Pharmacological studies are scarce. Moreover, Brazil is the country with the highest number of papers on the subject and some works with a collaborative network with Argentina.

Here, we also showed that, despite this species being considered as non-venomous, accidents with clinical manifestations due to a complex mixture of venom produced by Duvernoy's gland. Venom is homologous to the venoms of the front-fanged snakes; therefore, it can cause similar or greater biological activity than *Bothrops*. Even so, accidents with *Philodryas* are being neglected.

Based on collected data, there is much to understand of *P. patagoniensis* Toxicology and many possibilities to test in Pharmacological fields. Furthermore, despite the difficulties, it seems plausible to use *P. patagoniensis* venom in tests, as a model of myotoxicity, edematogenic, nociception, and hyperalgesia. Mainly in distant labs, due 1) to the species richness in South America, and easy to find; 2) there are cases of envenoming by *P. patagoniensis;* 3) being an opisthoglyphous species, offering minimal risk of serious accidents in labs; and 4) the fact that its venom presents be similar or greater biological activity than *Bothrops.* Furthermore, our research group also knows its genotoxic potential in mice (data not yet published). Thus, this promotion would aid to emphasize the importance of the conservation of snake biodiversity and to offer new possibilities for study.

Acknowledgements

The authors thank Andréia Salgueiro, PhD, for critical reading of the manuscript and editorial assistance.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interest.

Authors' contributions

All authors wrote the review, read, and approved the final manuscript.

Ethics approval

Not applicable.

Consent for publication

Not applicable.

References

1. Lema T de. Os répteis do Rio Grande do Sul: atuais e fósseis, biogeografia, ofidismo. 1st ed. Porto Alegre: EDIPUCRS; 2002. 264 p.

2. Hartmann PA, Marques OAV. Diet and habitat use of two sympatric species of *Philodryas* (Colubridae), in south Brazil. Amphibia-Reptilia. 2005;26:25–31.

3. Loebmann D, Quintela FM. Guia ilustrado: os répteis da região costeira do extremo sul do Brasil. 1st ed. Pelotas: USEB; 2009. 82 p.

4. Carvalho MA de, Nogueira F. Serpentes da área urbana de Cuiabá, Mato Grosso: aspectos ecológicos e acidentes ofídicos associados. Cad Saude Publica.

1998;14(4):753–63.

5. Puorto G, França FOS. Serpentes não peçonhentas e aspectos clínicos dos acidentes. In: Cardoso JLC, França FOS, Wen FH, Málaque CMS., Haddad V, editors. Animais peçonhentos no Brasil: biologia, clínica e terapêutica dos acidentes. São Paulo: FAPESP; 2003. p. 468.

6. Salomão MG, Albolea ABP, Almeida-Santos SM. Colubrid snakebite: a public health problem in Brazil. Herpetol Rev. 2003;34(3):307–12.

7. Santos-Costa MC, Outeiral AB, D'Agosttini F, Cappelari L. Frequência de acidentes ofídicos na região da grande Porto Alegre e cidades próximas, RS, Brasil. Comun do Mus Ciências e Tecnol da PUCRS, Série Zool. 2001;14(1):89–93.

8. Silveira PVP, Nishioka S de A. Non-venomous snake bite and snake bite without envenoming in a brazilian teaching hospital: analysis of 91 cases. Rev Inst Med Trop Sao Paulo. 1992;34(6):499–503.

9. Tavares AV, Araújo KAM de, Marques MR de V, Leite R. Epidemiology of the injury with venomous animals in the state of Rio Grande do Norte, Northeast of Brazil. Cien Saude Colet. 2020;25(5):1967–78.

10. Gonçalves JE, Medeiros SM de FR dos S, Cavalcanti IDL, Mendes RCMG, Bezerra INM, Nóbrega MM, et al. Acidentes por animais peçonhentos: uma análise do perfil epidemiológico na região Nordeste do Brasil no período de 2010 a 2019. Res Soc Dev. 2020;9(10):e4679108843.

11. Sinan. ACIDENTE POR ANIMAIS PEÇONHENTOS - Notificações registradas no Sistema de Informação de Agravos de Notificação - Sinan Net [Internet]. Ministério da Saúde/SVS - Sistema de Informação de Agravos de Notificação - Sinan Net. 2020.

12. Marcussi S, Santos PRS, Menaldo DL, Silveira LB, Santos-Filho NA, Mazzi M V., et al. Evaluation of the genotoxicity of *Crotalus durissus terrificus* snake venom and its isolated toxins on human lymphocytes. Mutat Res - Genet Toxicol Environ Mutagen. 2011;724(1–2):59–63.

13. Marcussi S, Stábeli RG, Santos-Filho NA, Menaldo DL, Silva Pereira LL, Zuliani JP, et al. Genotoxic effect of *Bothrops* snake venoms and isolated toxins on human lymphocyte DNA. Toxicon. 2013;65:9–14.

14. Oliveira CHDM, Simão AA, Marcussi S. Inhibitory effects of ascorbic acid, Vitamin E, and Vitamin B-complex on the biological activities induced by *Bothrops* venom. Pharm Biol. 2016;54(5):845–52.

15. Abegg AD, Neto OME. Serpentes do Rio Grande do Sul. 1st ed. Tapera: LEW; 2012. 148 p.

16. Araújo ME de, Santos ACMCA dos. Cases of human envenoming caused by *Philodryas olfersii* and *Philodryas patagoniensis* (serpentes: Colubridae). Rev Soc Bras Med Trop. 1997;30(6):517–9.

17. Assakura MT, Salomão MG, Puorto G, Mandelbaum FR. Hemorrhagic, fibrinogenolytic and edema-forming activities of the venom of the colubrid snake *Philodryas olfersii* (green snake). Toxicon Off J Int Soc Toxinology. 1992;30(4):427–38.

18. de Medeiros CR, Hess PL, Nicoleti AF, Sueiro LR, Duarte MR, de Almeida-Santos SM, et al. Bites by the colubrid snake *Philodryas patagoniensis*: A clinical and epidemiological study of 297 cases. Toxicon. 2010;56(6):1018–24.

19. Prado-Franceschi J, Hyslop S, Cogo JC, Andrade AL, Assakura M, Cruz-Höfling MA, et al. The effects of Duvernoy's gland secretion from the xenodontine colubrid *Philodryas olfersii* on striated muscle and the neuromuscular junction: partial characterization of a neuromuscular fraction. Toxicon Off J Int Soc Toxinology. 1996;34(4):459–66.

20. Prado-Franceschi J, Hyslop S, Cogo JC, Andrade AL, Assakura MT, Reichl AP, et al. Characterization of a myotoxin from the Duvernoy's gland secretion of the xenodontine colubrid *Philodryas olfersii* (green snake): effects on striated muscle and the neuromuscular junction. Toxicon Off J Int Soc Toxinology. 1998;36(10):1407–21.

21. Ribeiro LA, Puorto G, Jorge MT. Bites by the colubrid snake *Philodryas olfersii*: a clinical and epidemiological study of 43 cases. Toxicon Off J Int Soc Toxinology. 1999;37(6):943–8.

22. Terra RMS, Pinto AFM, Guimarães JA, Fox JW. Proteomic profiling of snake venom metalloproteinases (SVMPs): Insights into venom induced pathology. Toxicon. 2009;54(6):836–44.

23. Gren ECK, Kitano ES, Andrade-Silva D, Iwai LK, Reis MS, Menezes MC, et al. Comparative analysis of the high molecular mass subproteomes of eight *Bothrops* snake venoms. Comp Biochem Physiol Part D Genomics Proteomics. 2019;30:113–21.

24. Tasoulis T, Isbister G. A review and database of snake venom proteomes. toxins. 2017;9(9):290.

25. Corrêa-Netto C, Junqueira-de-Azevedo I de LM, Silva DA, Ho PL, Leitão-de-Araújo M, Alves MLM, et al. Snake venomics and venom gland transcriptomic analysis of Brazilian coral snakes, *Micrurus altirostris* and *M. corallinus*. J Proteomics. 2011;74(9):1795–809.

26. Peichoto ME, Tavares FL, Santoro ML, Mackessy SP. Venom proteomes of South and North American opisthoglyphous (Colubridae and Dipsadidae) snake species: A preliminary approach to understanding their biological roles. Comp Biochem Physiol Part D Genomics Proteomics. 2012;7(4):361–9.

27. Kovalchuk S, Ziganshin R, Starkov V, Tsetlin V, Utkin Y. Quantitative proteomic analysis of venoms from Russian Vipers of *Pelias* Group: Phospholipases A2 are the main venom components. Toxins. 2016;8(4):105.

28. Junqueira-de-Azevedo I, Campos P, Ching A, Mackessy S. Colubrid venom composition: An -Omics perspective. toxins. 2016;8(8):230.

29. Brust A, Sunagar K, Undheim EAB, Vetter I, Yang DC, Casewell NR, et al. Differential evolution and neofunctionalization of snake venom metalloprotease domains. Mol Cell Proteomics. 2013;12(3):651–63.

30. Jackson TNW, Young B, Underwood G, McCarthy CJ, Kochva E, Vidal N, et al. Endless forms most beautiful: the evolution of ophidian oral glands, including the venom system,

and the use of appropriate terminology for homologous structures. Zoomorphology. 2017 Mar 15;136(1):107–30.

31. Chippaux JP, Williams V, White J. Snake venom variability: methods of study, results and interpretation. Toxicon. 1991;29(11):1279–303.

32. Lopes RA, Contrera MG, da Costa JR, Petenusci SO, Lima-Verde JS. [The salivary glands of *Philodryas patagoniensis* Girard, 1857 (Serpentes, Colubridae). A morphological, morphometric and histological study]. Arch Anat Microsc Morphol Exp. 1982;71(3):175–82.

33. Costa RSC da, Prudêncio L, Ferrari EF, Souza GHMF, de Mello SM, Prianti Júnior ACG, et al. Neuromuscular action of venom from the South American colubrid snake *Philodryas patagoniensis*. Comp Biochem Physiol Part C Toxicol Pharmacol. 2008;148(1):31–8.

34. Rocha MMT, Paixão-Cavalcante D, Tambourgi D V., Furtado MDFD. Duvernoy's gland secretion of *Philodryas olfersii* and *Philodryas patagoniensis* (Colubridae): Neutralization of local and systemic effects by commercial bothropic antivenom (*Bothrops* genus). Toxicon. 2006;47(1):95–103.

35. Peichoto ME, Leme AFP, Pauletti BA, Batista IC, Mackessy SP, Acosta O, et al. Autolysis at the disintegrin domain of patagonfibrase, a metalloproteinase from *Philodryas patagoniensis* (Patagonia Green Racer; Dipsadidae) venom. Biochim Biophys Acta - Proteins Proteomics. 2010;1804(9):1937–42.

36. Peichoto ME, Teibler P, Mackessy SP, Leiva L, Acosta O, Gonçalves LRC, et al. Purification and characterization of patagonfibrase, a metalloproteinase showing α-fibrinogenolytic and hemorrhagic activities, from *Philodryas patagoniensis* snake venom. Biochim Biophys Acta - Gen Subj. 2007;1770(5):810–9.

37. Peichoto ME, Zychar BC, Tavares FL, de Camargo Gonçalves LR, Acosta O, Santoro ML. Inflammatory effects of patagonfibrase, a metalloproteinase from *Philodryas patagoniensis* (Patagonia Green Racer; Dipsadidae) venom. Exp Biol Med. 2011;236(10):1166–72.

38. Peichoto ME, Santoro ML. Patagonfibrase modifies protein expression of tissue factor and protein disulfide isomerase in rat skin. Toxicon. 2016;119:330–5.

39. Peichoto ME, Mackessy SP, Teibler P, Tavares FL, Burckhardt PL, Breno MC, et al. Purification and characterization of a cysteine-rich secretory protein from *Philodryas patagoniensis* snake venom. Comp Biochem Physiol Part C Toxicol Pharmacol. 2009;150(1):79–84.

40. Tadokoro T, M. Modahl C, Maenaka K, Aoki-Shioi N. Cysteine-Rich Secretory Proteins (CRISPs) from Venomous Snakes: An Overview of the Functional Diversity in a Large and Underappreciated Superfamily. Toxins. 2020;12(3):175.

41. Zelanis A, Teixeira da Rocha MM, de Fátima Domingues Furtado M. Preliminary biochemical characterization of the venoms of five Colubridae species from Brazil. Toxicon. 2010;55(2–3):666–9.

42. Nishioka S de A, Silveira PVP. *Philodryas patagoniensis* bite and local envenoming.

Rev Inst Med Trop Sao Paulo. 1994;36(3):279–81.

43. Girón ME, Estrella A, Sánchez EE, Galán J, Tao WA, Guerrero B, et al. Purification and characterization of a metalloproteinase, Porthidin-1, from the venom of Lansberg's hog-nosed pitvipers (*Porthidium lansbergii hutmanni*). Toxicon. 2011;57(4):608–18.

44. Lopes PH, Rocha MMT, Kuniyoshi AK, Portaro FCV, Gonçalves LRC. Edema and nociception induced by *Philodryas patagoniensis* venom in mice: A pharmacological evaluation with implications for the accident treatment. J Pharmacol Exp Ther. 2017;361(3):349–54.

45. Rocha MMT, Furtado MDFD. Análise das atividades biológicas dos venenos de *Philodryas olfersii* (Lichtenstein) e *P. patagoniensis* (Girard) (Serpentes, Colubridae). Rev Bras Zool. 2007;24(2):410–8.

46. Acosta O, Leiva LC, Peichoto ME, Maruñak S, Teibler P, Rey L. Hemorrhagic activity of the Duvernoy's gland secretion of the xenodontine colubrid *Philodryas patagoniensis* from the north-east region of Argentina. Toxicon. 2003;41(8):1007–12.

47. Komori Y, Hifumi T, Yamamoto A, Sakai A, Ato M, Sawabe K, et al. Comparative study of biological activities of venom from colubrid snakes *Rhabdophis tigrinus* (Yamakagashi) and *Rhabdophis lateralis*. Toxins. 2017;9(11):373.

48. Peichoto ME, Leiva LC, Guaimás Moya LE, Rey L, Acosta O. Duvernoy's gland secretion of *Philodryas patagoniensis* from the northeast of Argentina: Its effects on blood coagulation. Toxicon. 2005;45(4):527–34.

49. Peichoto ME, Teibler P, Ruíz R, Leiva L, Acosta O. Systemic pathological alterations caused by *Philodryas patagoniensis* colubrid snake venom in rats. Toxicon. 2006;48(5):520–8.

50. Peichoto ME, Sánchez MN, Teibler GP, Maruñak SL, Acosta OC. Comparative study of muscular regeneration after necrosis induced by *Philodryas patagoniensis* and *Bothrops alternatus* snake venoms. In: 2014 ACT Annual Meeting Poster Presentations. International Journal of Toxicology; 2015. p. 77–111.

51. Peichoto ME, Acosta O, Leiva L, Teibler P, Maruñak S, Ruíz R. Muscle and skin necrotizing and edema-forming activities of Duvernoy's gland secretion of the xenodontine colubrid snake *Philodryas patagoniensis* from the north-east of Argentina. Toxicon. 2004;44(6):589–96.

52. Furtado MFD, Colleto GMDD, Silva WD DA. Controle de qualidade dos venenos animais e dos correspondentes antivenenos. I Padronização dos métodos de ensaio das atividades bioquímicas e farmacológicas dos venenos de algumas espécies do gênero *Bothrops* e *Crotalus* usando amostras secas a temperatura. Mem Inst Butantan. 1991;53(2):149–59.

53. Urra FA, Miranda-calle AB, Araya-maturana R. *Philodryas* (Serpentes: Dipsadidae) envenomation, a neglected issue in Chile. Toxins. 2019;11(697):1–14.

54. Vasconcelos-Filho FSL, Rocha-e-Silva RC da, Vasconcelos RH, Oliveira Neto J de, Silveira JA de M, Morais GB de, et al. Natural history and biological aspects of dipsadidae snakes: *P. olfersii* , *P. patagoniensis* and *P. nattereri*. Rev Bras Hig e

Sanidade Anim. 2015;9(3).

55. Oliveira JS, Sant'Anna LB, Oliveira Junior MC, Souza PRM, Andrade Souza AS, Ribeiro W, et al. Local and hematological alterations induced by *Philodryas olfersii* snake venom in mice. Toxicon. 2017;132:9–17.

56. Sánchez M, Timoniuk A, Maruñak S, Teibler P, Acosta O, Peichoto M. Biochemical and biological analysis of *Philodryas baroni* (Baron's Green Racer; Dipsadidae) venom. Hum Exp Toxicol. 2014;33(1):22–31.

57. Maxwell RA, Eckhardt SB. Captopril. In: Drug Discovery. Totowa, NJ: Humana Press; 1990. p. 19–34.

58. Barros LC, Ferreira RS, Barraviera SRCS, Stolf HO, Thomazini-Santos IA, Mendes-Giannini MJS, et al. A new fibrin sealant from *Crotalus durissus terrificus* venom: Applications in medicine. J Toxicol Environ Heal Part B. 2009;12(8):553–71.

59. Diochot S, Baron A, Salinas M, Douguet D, Scarzello S, Dabert-Gay A-S, et al. Black mamba venom peptides target acid-sensing ion channels to abolish pain. Nature. 2012;490(7421):552–5.

60. Urra F, Araya-Maturana R. Targeting metastasis with snake toxins: Molecular mechanisms. Toxins. 2017;9(12):390.

61. Peichoto ME, Tavares FL, DeKrey G, Mackessy SP. A comparative study of the effects of venoms from five rear-fanged snake species on the growth of *Leishmania major*: Identification of a protein with inhibitory activity against the parasite. Toxicon. 2011;58(1):28–34.

62. Adade CM, Carvalho ALO, Tomaz MA, Costa TFR, Godinho JL, Melo PA, et al. Crovirin, a snake venom Cysteine-Rich Secretory Protein (CRISP) with promising activity against Trypanosomes and *Leishmania*. PLoS Negl Trop Dis. 2014;8(10):e3252.

63. Jungo F, Estreicher A, Bairoch A, Bougueleret L, Xenarios I. Animal Toxins: How is complexity represented in databases? Toxins. 2010;2(2):262–82.

64. Vonk FJ, Jackson K, Doley R, Madaras F, Mirtschin PJ, Vidal N. Snake venom: From fieldwork to the clinic. BioEssays. 2011;33(4):269–79.

65. Braz H, Rocha M, Furtado M. Maintaining rear-fanged snakes for venom production: an evaluation of mortality and survival rates for *Philodryas olfersii* and *P. patagoniensis* in captivity. J Venom Anim Toxins Incl Trop Dis. 2012;18(2):164–72.

66. Modahl CM, Mackessy SP. Venoms of rear-fanged snakes: New Proteins and Novel Activities. Front Ecol Evol. 2019;7.

67. Barraviera B. How to overcome the gap between basic research and clinical trials? J Venom Anim Toxins Incl Trop Dis. 2011;17(4):361.

6.4. Manuscrito científico 2

Analgesic and antioxidant effects of the Golden trumpet on snake-envenomed mice

Márcio Tavares Costa[a]; Aline da Silva Goulart[b]; Kellen Mariane Athaide Rocha[a]; Morgana Duarte da Silva[c]; Andréia Caroline Fernandes Salgueiro[d]; Robson Puntel[e]; Vanderlei Folmer[e]*

[a] PhD student at the Graduate Program in Biochemistry at the Federal University of Pampa (UNIPAMPA), Uruguaiana, Rio Grande do Sul, Brazil;
[b] PhD student at the Graduate Program in Biochemistry at the Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, Rio Grande do Sul, Brazil;
[c] Department of Physiological Sciences, Federal University of Santa Catarina, Florianópolis, SC;
[d] Collaborating professor of the Science Education, Life Chemistry and Health Graduate Program, Federal University of Pampa, Uruguaiana, RS, Brazil;
[e] Professor PhD at the Graduate Program in Biochemistry at the Federal University of Pampa (UNIPAMPA), Uruguaiana, Rio Grande do Sul, Brazil;
*Corresponding author: vanderleifolmer@unipampa.edu.br

Abbreviations:

(COX), cyclooxygenase; (DNPH), 2,4-dinitrophenylhydrazine; (DTNB), Ellman reagent; (GSH), glutathione equivalents; (HCCE), *Handroanthus chrysotrichus* crude extract; (MDA), malondialdehyde; (NPSH), nonprotein thiols levels; (PBMC), Peripheral blood mononuclear cells; (PLA2s), phospholipases A2; (PGs), prostaglandins; (p.o.), oral administration; (PpV), *Philodryas patagoniensis* venom; (S1) supernatants; (TBA-RS), thiobarbituric acid reactive substances; (TFK), phosphate buffer; (w/v), weight/volume.

ABSTRACT

Handroanthus chrysotrichus is a tree used in the traditional medicine against snake bites. Thus, this study aims to investigate whether the hydroethanolic extract of *H. chrysotrichus* bark attenuates the envenoming symptoms induced by *P. patagoniensis* venom (PpV) in mice. For this, we captured and milked *P. patagoniensis* to obtain the venom. The damages were induced in the right hind paw with a single intraplantar (i.pl.) injection of vehicle or PpV (1.5 µg). Pretreatment (1 hour before i.pl.) and treatment (daily for 3 days) were administered orally (p.o.). Mice were separated in groups: saline (vehicle, p.o. + i.pl.); *H. chrysotrichus* crude extract (HCCE) at 100 mg. kg^{-1} (HCCE, p.o. + vehicle, i.pl.); venom (vehicle, p.o. + PpV, i.pl.); and HCCE + venom (HCCE, p.o. + PpV, i.pl.). We performed behavioral tests (nociception and hyperalgesia) and evaluated edema, and temperature of the paw for three days. After euthanasia, the protective potential of the extract on systemic damages were observed though the organs/body weight ratio, creatinine levels in the blood, and oxidative stress trigged in leukocytes and main organs. HCCE decreased significatively nociception, hyperalgesia, edema, and temperature *in situ*. The liver/body weight ratio increased in the venom group, and HCCE could not protect. Blood analysis indicated that extract minimized creatinine levels. HCCE decreased oxidative damages in leukocytes and main organs. In conclusion, hydroethanolic bark extract of *H. chrysotrichus* has a protective action against *P. patagoniensis* envenoming, especially against local inflammation. In addition, HCCE minimizes the systemic oxidative stress triggered by envenoming.

Keywords: Poison. Dipsadidae. Colubridae. Review. Ophidism. Green-racer.

1. Introduction

Handroanthus chrysotrichus (Mart. ex DC.) Mattos, also known as Golden trumpet, is a tree that belongs to Bignoniaceae family with a wide distribution in Brazil. This species presents a consolidated diversity throughout Brazilian Biomes such as Cerrado, Atlantic Forest, and Pampa, occurring from the Northeast region to the South (Jardim Botânico do Rio de Janeiro, 2020). In the traditional medicine, extracts of Golden trumpet may be used to treat cardiovascular and immunological system diseases, allergic process, and envenoming by insect and snake bites (Bolson *et al.*, 2015; Ribeiro *et al.*, 2017).

Extracts of species from the Bignoniaceae family as *Tabebuia rosea* and *T. aurea*, were effective to treat snake bites (Núñez *et al.*, 2004; Reis *et al.*, 2014). However, limited medical resources for snakebite envenoming treatment is an important concern in health centers in several countries and leads to the importance of further studies and validation of plant extracts used in traditional treatments. It is fundamental to emphasize that distant rural communities from specialized medicals centers suffer from the lack of adequate immediate treatment. Indeed, approximately 60% of snake bite victims in rural communities are initially treated by local healers and treated with plants as antidotes (Otero *et al.*, 2000).

We highlighted that snake bites represent a public health problem. Accordingly, snakebite envenoming occur worldwide and affects between 1.8 and 2.7 million people per year, reaching mortality estimates ranging from 81.410 to 137.880 deaths (Chippaux, 1998; Kasturiratne *et al.*, 2008). Brazil recorded 28.961 cases of exposed to snakes in 2018, with 105 deaths. North region of the country was the most affected, with 33% of accidents. The bothropic group represented 69.3% these cases. However, nonvenomous and ignored species were 19.1% (Croda *et al.*, 2020).

Thus, previous studies of Costa et al. (2020) demonstrated that *H. chrysotrichus* has an antioxidant effect, besides presenting compounds with pharmacological activities *in silico*

compatible with the popular use. Grazziotin et al. (1992) also indicated the analgesic potential of this plant species. These effects are important in snake bite cases because according to Peichoto and Santoro (2016), antivenom therapy is not very effective locally. Consequently, the extract of *H. chrysotrichus* could help in the treatment of these victims.

Philodryas spp. belong to the group of nonvenomous and ignored species. Nevertheless, its envenoming is associated with evolving ecchymoses, systemic bleeding, coagulopathy and acute kidney injury. Moreover, the *Philodryas patagoniensis* envenoming, species used in this study, can cause pain, local brief bleeding, erythema, and edema (Medeiros *et al.*, 2010; Gutiérrez *et al.*, 2017). In this context, our study aimed to investigate whether the hydroethanolic extract of *H. chrysotrichus* bark acts as a mitigation of the main symptoms of snake envenoming caused by *P. patagoniensis* in mice.

2. Materials and methods

2.1. Plant material

H. chrysotrichus was identified by biologist and a branch with flowers and fruits was collected in Uruguaiana (29°49'49.2"S, 57°06'07.1"W). Then a voucher was deposited at the herbarium of Federal University of Pampa (number 142/2017).

Bark samples were used to obtain hydroethanolic extract. These barks were collected in Brazilian summer, dried at 40°C for five days, reduced to powder, and used in the percolation technique for two hours using 70% hydroethanolic solution at a ratio of 1:10 (w/v). Finally, the *H. chrysotrichus* crude extract (HCCE) was freeze-dried and kept frozen (-6ºC). At the time treatment, HCCE was diluted in sterile saline solution (0.9%) in order to obtain a stock solution, from which we calculated the desired concentration based on weight of mice for their oral administration.

2.2. Animals and venom

The Institutional Animal Care and Use Committee at Federal University of Pampa (Protocol number 48/2018) approved all the experimental procedures, according to "Principles of Laboratory Animal Care" (NIH Publication Vol 25, No. 28 revised 1996). The Ministry of Environment of Brazil, through the Chico Mendes Institute (protocol number 45691), approved acquisition and use of the *Philodryas patagoniensis* venom.

2.2.1. *Philodryas patagoniensis* venom*:*

Ten specimens of *Philodryas patagoniensis* were captured in Rio Grande do Sul State, Brazil (-29º 46' 53" S, -57º 2' 16" W). After identified and measured (between 79 and 103 cm in length, rostrum-cloaca) these animals were milked according to Ferlan *et al.* (1983) with modifications. We collected venom with the aid of capillary tubes, but without the use of medication.

Venom solutions were immediately cooled and their protein content was evaluated by Bradford (1976) method using bovine serum albumin as standard. Then, *P. patagoniensis* venom (PpV) was diluted in sterile saline solution (0.9%) for use.

2.2.2. Mice

We use twenty male Swiss mice weighing 25-35g. Animals were housed in an appropriate cabinet with forced air ventilation, controlled humidity (60-80%) and temperature (22±2°C). They were kept on a 12-hour light-dark cycle (07:00 am - 07:00 pm) and the experiments were carried out in the light phase of the cycle. The animals received free food and drink.

After acclimatization, animals were separated in four groups (n = 5 for each group), and each group had damage induced in the right hind paw with subcutaneous injection of 50 µl of

solutions containing vehicle (saline solution) or 1.5 µg of PpV. Pretreatments (HCCE or vehicle) were administered orally (p.o.) one hour before injection:

I. Saline group: mice were pretreated with saline solution (10 mL. kg^{-1}; p.o.) one hour before receiving a single intraplantar (i.pl.) injection of vehicle (50µl of sterile saline 0.9%). Afterward, these animals were treated daily for 3 days with saline solution (p.o.) always one hour before tests;

II. HCCE group: mice were pretreated with HCCE (100 mg. kg^{-1}, p.o.) one hour before receiving an i.pl. of vehicle (50µl of sterile saline 0.9%). After, these animals were treated daily for 3 days with HCCE (100 mg. kg^{-1}, p.o.) one hour before tests;

III. Venom group: mice were pretreated with saline solution (10 mL. kg^{-1}, p.o.) one hour before receiving an i.pl. of 1.5 µg of PpV diluted in 50µl of sterile saline 0.9%. Subsequently, these animals were treated daily for 3 days with saline solution (p.o.) one hour before tests;

IV. HCCE + Venom group: mice were pretreated with HCCE (100 mg. kg^{-1}, p.o.) one hour before receiving an i.pl. of 1.5 µg of PpV diluted in 50µl of sterile saline 0.9%. Later, mice were treated daily for 3 days with HCCE (100 mg. kg^{-1}, p.o.) one hour before tests.

HCCE dose was established in pilot studies (Costa *et al.*, 2020, 2021).

2.3. Nociceptive test

We used an intraplantar injection of PpV, based on formalin tests described by Santos *et al.* (1998), to verify the venom nociceptive potential. In addition, we use this tool to verify the potential of treatment with HCCE. Mice had a pretreatment with HCCE or vehicle, one hour later occurred the injection (PpV or vehicle) in the right paw, and immediately placed in an acrylic chamber. Time spent licking and biting the injected paw was recorded 30 minutes (reaction time in seconds). The values were considered as nociception response.

2.4. Mechanical hyperalgesia

Hyperalgesia was evaluated using the von Frey filament test, according to Chaplan *et al.* (1994) with modifications. After spontaneous reaction analysis, mice were placed individually in acrylic compartments (7 × 9 × 11 cm) that allowed access to the ventral surface of the hind legs and acclimated for 30 minutes. There were performed stimuli directed perpendicular to the plantar surface of these animals, and the responses (paw withdrawal or not) analyzed in relation to the tension applied by the different von Frey filaments, calibrated to produce forces (0.02, 0.04, 0.07, 0.16, 0.4, 1.0, 2.0, 4.0 g) which produce different levels of mechanical stimuli. Evaluations were performed using the method known as "up-and-down" (Dixon, 1980). The results obtained were expressed in 50% of the withdrawal threshold (g), and intense reduction in the paw withdrawal threshold characterized the presence of mechanical hyperalgesia.

Basal response of the animals was evaluated one hour before any intervention. Then, there were treatments (p.o.) and after one hour we applied the injection of saline or venom in mice. Mechanical hyperalgesia assays evaluated with von Frey filaments were tested at 1, 3, 6, and 12 hours, as well 24, 48, and 72 hours after injection. Analyses were also repeated 1 hour before and 1 hour after daily treatment (p.o.) in HCCE + Venom group, in order to verify the extract effect.

2.5. Paw edema measurement

Paw edema progression was verified by an electronic caliper (Starrett, model 797B-8, with LCD display), and was expressed in millimeters (mm) as the difference between the tested injected paw and contralateral paw (left paw). The paw measurements were assessed daily for three days, always one hours after saline or HCCE administration.

2.6. Paw temperature measurement

Paw temperature was verified by an infrared digital thermometer (Incoterm, model ST-600, with LCD display), and was expressed in degrees Celsius (ºC) as the difference between the tested injected paw and contralateral paw (left paw). The paw measurements were assessed daily for three days, always one hours after saline or HCCE treatment.

2.7. Organ toxicity evaluation

Animals were euthanized by decapitation 24 hours after last HCCE administration.

2.7.1. Creatinine K levels

Serum creatinine levels were evaluated in order to verify kidney damage. In this way, the blood of the mice was collected in heparinized tubes by cardiac puncture after fasting for 12 hours. After centrifugation, creatinine K levels were determined in plasma using a commercial kit (Labtest, Minas Gerais/Brazil).

2.7.2. Organs/body weight ratio

The organs of the mice – liver, kidneys, spleen, heart, and brain – were weighed and the results were expressed as a ratio of organ weight in relation to body weight. The initial and final difference in animal weight *in vivo* in each group was also recorded. The data assessed toxicity about these tissues.

2.8. Oxidative stress assays

2.8.1. Viability and genotoxicity in peripheral blood mononuclear cells

A fraction of collected blood was used for cell viability and genotoxicity determination. Thus, mononuclear leukocytes were isolated using Histopaque® 1077 (1:1), centrifuged (500 rpm, at 4º C, for 35 min). The interface band containing peripheral blood mononuclear cells (PBMC) was collected and buffered with phosphate-buffer saline in order to test viability and genotoxicity.

a) Cell viability was tested with Trypan blue (0.2%) dye exclusion test. Cells were counted in a Neubauer chamber. Stained cells were considered dead. The ratio between dead and alive cells was expressed as a percentage;

b) Genotoxicity was evaluated through comet assay from Singh *et al.* (1988). After mounting slides and performing electrophoresis, the lengths of tails were measured under fluorescence microscopy after adding ethidium bromide on the slides. One hundred cells from each of the three replicate slides were analyzed. Cells were scored visually according to tail length and received scores from 0 (no migration) to 4 (maximal migration). Analysis was based on the reading scores, which represented the sum of cells identified in each class multiplied by the class values.

2.8.2. Oxidative stress in the main organs

The following tests were used in order to obtain oxidative stress biomarkers in brain, liver and kidneys of treated mice. Soon after euthanasia, organs were quickly removed and carefully washed twice with cold saline and homogenized in cold NaCl (150 mM). Homogenates were centrifuged at 2000 g for 10 min at 4°C and the supernatants (S1) were collected for biochemical analyses.

a) Thiobarbituric acid reactive substances (TBA-RS) assay was used as lipid peroxidation index and measured according to Ohkawa et al. (1979). S1 of tissues were used for this assay and corrected by protein content (Bradford, 1976). A standard curve of malondialdehyde (MDA) was constructed to determine TBA-RS content.

b) Protein carbonyl levels. Aliquots of S1 were derivatized using 2,4-dinitrophenylhydrazine (DNPH) and precipitated with an equal volume of 20% (w/v) trichloroacetic acid. Then they were washed twice with an ethanol/ethyl acetate mixture (1:1). Precipitates were dissolved in 6M guanidine HCl solution. Protein carbonyl levels were determined spectrophotometrically at 370 nm, against blanks (Levine *et al.*, 1990). Results were

calculated using the DNPH molar extinction coefficient, corrected by protein content, and expressed as nanomoles of carbonyl per milligram of protein.

c) Nonprotein thiols levels (NPSH) were determined according to Ellman (1959). S1 samples were precipitated with 10% trichloroacetic acid (1:1) and centrifuged at 4000 g for 10min at 4◦C to obtain supernatants, which were added to a medium containing phosphate buffer (TFK 0.25mM, pH 7.4) and Ellman reagent (DTNB 1mM). The yellow pigment produced was measured spectrophotometrically at 420 nm. Results were calculated in relation to a standard curve constructed with glutathione equivalents (GSH) at known concentrations and corrected by protein content.

2.9. Statistical analysis

The experimental design was performed with four groups with five animals each. The data obtained in nociceptive test, mechanical hyperalgesia, paw edema, and paw temperature were expressed as mean ± SEM. In addition, nociceptive test was analyzed by one-way ANOVA followed by Tukey's multiple comparisons test, while mechanical hyperalgesia tests, edema, and temperature were analyzed by two-way ANOVA followed by Bonferroni's test.

The organs toxicity evaluation and oxidative stress assays were expressed as mean ± SD. Creatinine levels and organs/body weight ratio were analyzed by one-way and two-way ANOVA, respectively, followed by Tukey's test. All oxidative stress assays were analyzed by ordinary one-way ANOVA followed by Bonferroni's test. P-values ≤ 0.05 were considered significant.

3. Results

3.1. Nociceptive test

Venom group showed reaction time significantly higher than Saline, an approximate increase of 3837%. The HCCE + Venom group also had a significant increase in time spent compared to Saline (1365%), but 63% lower when compared to the Venom group. HCCE *per se* did not indicate a difference compared to Saline (Figure1A).

We recorded here that the time spent by the groups licking and biting the injected paw did not show a significant difference in the first 5 min of the test (data not shown).

3.2. Mechanical hyperalgesia

This assay allowed verifying the hiperalgesia triggered by PpV since the first hours (Figure 1B), in which during 12 hour the Venom group had a significant decrease in its withdrawal threshold for all time tested, compared to Saline. The Venom group also showed a difference in relation to the HCCE + Venom group until the sixth hour. Up to the first 6 hours after the venom injection, the HCCE + Venom group increased withdrawal threshold between 2322 and 10853% compared to the Venom group. However, in the 12th hour, the HCCE + Venom group began to show a significant difference compared to Saline, but no longer in relation to Venom. The HCCE group showed no difference related to the Saline group.

In order to check the act of the extract, we performed the von Frey assay one hour before and after gavage in the HCCE + Venom group (Figure 1C). The comparation between tests suggests that HCCE presents analgesic effect in the mice tested. Moreover, apparently the extract does not induce tolerance because it was efficient throughout the days of treatment.

When compared over the days (Figure 1D), we noted a significant mechanical sensitivity to touch in the Venom group compared to Saline. Animals of this group also presented difference in relation to the HCCE + Venom group every day. The HCCE + Venom group showed no difference compared to Saline. In addition, the HCCE group remained similar

to the Saline group on days 1 and 2, but on day 3 there was a significant decrease in its paw withdrawal threshold. Thus, HCCE appears to have an effect *per se*.

3.3. Paw edema and temperature measurements

Paw edema and temperature measurement showed a similar result. The Venom group of both, edema and temperature, had a significant increase in all the days tested when compared to Saline. The HCCE + Venom group remained without a significant difference in relation to Saline. However, in the third day, HCCE + Venom also was statically equivalent to the Venom group in these assays. The HCCE group did not present a difference when compared to Saline on any day (Figure 2A and B).

3.4. Organ toxicity evaluation

Here, we evaluated serum creatinine levels and the ratio of organ weight in relation to body weight. None of the groups showed a significant difference in body weight during *in vivo* monitoring.

Creatinine levels increased significantly in the Venom group compared to the Saline group. The HCCE and HCCE + Venom groups maintained their baseline levels (Figure 3A).

When analyzing the ratio of organ weight in relation to body weight, Figure 3B indicates that the Venom and HCCE + Venom groups had significantly higher ratio liver than the Saline and HCCE groups. Brain, heart, kidneys, and spleen showed no statistical changes.

3.5. Oxidative stress

PBMC were used to verify the cell viability and DNA damage induced by PpV in mice and the potential of HCCE to protect animals from these damages. There is no evidence of a significant reduction in cell viability among the groups tested (Figure 4A). Nevertheless, a

significant increase of DNA index damage was verified in the Venom group compared to Saline. The HCCE *per se* and HCCE + Venom groups maintained their baseline damage levels and did not show a significant difference when compared with the Saline group (Figure 4B).

Main organs were also used to verify oxidative stress caused by PpV and the protective potential of Golden trumpet against this damage. The TBA-RS measure did not indicate changes in the brain (Figure 5A), but the liver and kidneys had a significant increase of TBA-RS in the Venom group. In both cases, liver and kidneys, the HCCE and HCCE + Venom groups did not show a significant difference in relation to Saline group (Figure 5B, C).

NPSH levels measured in the brain tissue showed a significant increase in the Venom and HCCE + Venom groups, compared to Saline (Figure 5D). Liver NPSH showed an increase in the HCCE, Venom, and HCCE + Venom groups (Figure 5E). Kidney NPSH levels did not show a difference in any tested groups (Figure 5F).

Brain protein carbonyl levels presented a significant increase in the Venom group compared to Saline. Carbonyl levels in the HCCE and HCCE + Venom groups were not statistically different from the Saline in the brain (Figure 5G). In the liver, protein carbonyl had a significant increase in the Venom and HCCE + Venom groups compared to Saline. The HCCE group showed no difference between Saline and Venom (Figure 5H). Finally, kidney tissue showed an increase of carbonyl in the Venom group compared to Saline. The HCCE + Venom group had not a statistical difference in relation to Saline and Venom. HCCE showed no difference compared to the Saline group (Figure 5I).

4. Discussion

H. chrysotrichus extract has been used to treat injuries for several decades, including snake bites. Here, we tested the effectiveness of *H. chrysotrichus* bark extract against local and systemic damages induced by PpV in mice. Results suggest that pretreatment with HCCE

minimizes damages such as nociception, hyperalgesia, edema, and temperature at the site of the bite. Moreover, HCCE can act systemically protecting cells and organs from oxidative stress triggered by the envenoming.

De Medeiros *et al.* (2010) reported that pain, hemorrhage, erythema, and edema are visible signs of *P. patagoniensis* snake bites. Here, the nociception test suggests that HCCE pretreatment can reduce licking and biting behaviors in approximately 63% (Figure 1A). We did not see a significant difference in the first 5 min of the test, and when we compare these data with the original formalin test, this fact indicates no changes in the neurogenic phase, not affecting directly nociceptors of types C afferent and Aδ fibers (Hunskaar and Hole, 1987). Thus, PpV triggered a nociception response based in inflammatory process, therefore it is related to the release of several pro-inflammatory mediators, such prostaglandin and bradykinin, as occurs with others snake envenoming cases, including those caused by *Bothrops* genus (Chacur *et al.*, 2002; Barbosa *et al.*, 2003; Teixeira *et al.*, 2009).

Although the acting of histamine and serotonin were discarded from inflammatory process and pain induced by PpV, kinins as bradykinin appears to be present in these processes (Lopes *et al.*, 2017). Indeed, serine proteinases are present in the PpV, can act as kallikrein-like and support the involvement of bradykinin in the pain generated by envenoming (Peichoto *et al.*, 2012; Serrano, 2013). Moreover, the nociception induced by PpV may be inhibited by Indomethacin (cyclooxygenase inhibitor) in mice (Lopes *et al.*, 2017).

In this sense, several extracts of the Bignoniaceae family are able to interfere in prostaglandins (PGs) synthesis or in the mediators liberation as cytokines (Twardowschy *et al.*, 2008; Reis *et al.*, 2014; Maria-Ferreira *et al.*, 2020). Therefore, it is plausible to think that HCCE presents anti-inflammatory and antinociceptive activities through one of these routes. Costa *et al.* (2020) identified the five most representative volatile compounds: α-curcumene, β-bisabolene, 4-(4-Methylphenyl) pentanal, pentanoic acid, and isoamyl acetate. *In silico* anti-

inflammatory properties were pointed to β-bisabolene and pentanoic acid. Moreover, α-curcumene is the majority compound in the bark of *H. chrysotrichus* and occurs in many extracts with anti-inflammatory and analgesic properties too (Lisa *et al.*, 2020).

Mechanical hyperalgesia evaluation suggests an analgesic effect of the extract (Figure 1B, C, and D). These data are in agreement with Grazziotin *et al.* (1992), mentioned above. If HCCE really interferes in the PGs or cytokines cascade, this analgesic effect is expected. Because the peripheral pain is signaled by primary afferent sensory nerves, which detect chemical substances (PGs and bradykinin, for example) and gradually undergo a peripheral sensibilization to nearby mechanical changes (Mantyh *et al.*, 2002). This fact also explains the increase in algesia caused by HCCE on 3th day (Figure 1D), because we know that long-term or high-dose exposure to this extract can be harmful (Costa *et al.*, 2021).

Here, the used concentration of the PpV was edematous over the days of experiment. Its peak was within the first 24 hours (day 1), as illustrated in Figure 2A. As well as the mice paw edema, the increase of local temperature was also a symptom caused by PpV injected in the paw (Figure 2B). Both, edema and temperature, decrease with pretreatment followed by daily treatments. The snake venom metalloproteinases are the main group related to the edematogenic effect in this venom. Especially the patagonfibrase, a metalloproteinase capable of inducing cell recruitment and contributing to edema, hemorrhage, and local inflammation. The edema presents a different modulation than nociception. In addition, the cyclooxygenase (COX) 1 cascade metabolites appear to participate in edema modulation (Peichoto *et al.*, 2011; Lopes *et al.*, 2017). These data indicate a possible interference of the extract in PGs production, especially along COX-1, which seems to modulate the edematous process.

It should be noted that phospholipases A2 (PLA2s) are a protein group related to COX and widely distributed in snake venoms, which can trigger diverse effects in victims, among them neurotoxicity, myotoxicity, anticoagulant effects, cytotoxicity, cardiotoxicity, and edema

(Xiao *et al.*, 2017). However, PpV appears to present low or lack PLA2s activity, thus this enzyme does not participate in the edema-forming effect produced by PpV (Peichoto *et al.*, 2004, 2012; Zelanis *et al.*, 2010). Therefore, the beginning of the COX process is probably due to endogenous PLA2, in which plant extract could be interfering.

Pretreatment with the extract also decreased creatinine to baseline levels in the group tested (HCCE + Venom), although this reduction was not significant compared to the Venom group (Figure 3A). In addition, when analyzing the ratio of organ weight in relation to body weight (Figure 3B), there are evidences that mice liver was significatively damaged by PpV. In this case, HCCE cannot protect the organ. These records are in accordance with Peichoto *et al.* (2006) who showed that PpV can induce a systemic effect. Authors also indicated that a subcutaneous administration, as applied here, can cause damage in the cerebrum, lung, besides severe peritubular capillary congestion in kidney and hydropic degeneration in liver. However, pretreatment with HCCE, accompanied by daily administration of the extract for 3 days, presented a partial protector effect in these cases.

On the other hand, the genotoxicity verified in PBMC was inhibited by HCCE (Figure 4B) possibly due to antioxidant potential of the extract (Costa *et al.*, 2020). The oxidative stress also reached organs of the mice such as brain, liver, and kidneys (Figure 5). These harmful effects of the venom on these organs are in agreement with damages mentioned above by Peichoto *et al.* (2006). Measured lipid peroxidation affected the liver and kidneys, while the carbonylation attained all the tested organs. These tests showed that oxidative stress may persist for days after venom exposure, as in *Bothrops* envenomation (Strapazzon *et al.*, 2015). In addition, the antioxidant potential of HCCE decreases the lipoperoxidation verified in the liver and kidneys. Besides, the extract reduced carbonylation in the brain. In this context, *H. chrysotrichus* bark aided in the treatment of envenomed animals.

5. Conclusion

This is the first work to analyze the therapeutic potential of the Golden trumpet *Handroanthus chrysotrichus* against snake envenomation. In conclusion, results obtained after pretreatment with hydroethanolic bark extract of *H. chrysotrichus* suggest a protective action against snake envenoming, especially against cardinal signs of inflammation such as pain, edema, and heat. A hypothesis would be the interference of the extract in the synthesis or degradation of prostaglandins and kinins. Moreover, *H. chrysotrichus* seems to minimize the organs damage and to treat systemic oxidative stress triggered by envenoming.

Finally, we highlighted the importance of new researches in order to find agents that can assist snake envenoming therapy. *H. chrysotrichus* bark extract constitutes a rich source of natural compounds with the potential to neutralize the toxic activities of snake venoms. A better understanding of its mechanisms and compounds may elucidate the scientific bases of its use in folk medicine and provide alternatives for clinical management of snakes envenoming.

Acknowledgments

The authors thank the Brazilian Government Agencies – Coordination for the Improvement of Higher Education Personnel (CAPES) and National Council for Scientific and Technological Development (CNPq) – for their financial support.

Declaration of Competing Interest

There are no conflicts of interest.

References

Barbosa AM, do Amaral RO, Teixeira CF., Hyslop S, and Cogo JC (2003) Pharmacological characterization of mouse hind paw oedema induced by *Bothrops insularis* (jararaca ilhoa) snake venom. *Toxicon* **42**:515–523.

Bolson M, Hefler SR, Dall'Oglio Chaves EI, Gasparotto Junior A, and Cardozo Junior EL (2015) Ethno-medicinal study of plants used for treatment of human ailments, with residents of the surrounding region of forest fragments of Paraná, Brazil. *J Ethnopharmacol* **161**:1–10.

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**:248–254.

Chacur M, Picolo G, Teixeira CF., and Cury Y (2002) Bradykinin is involved in hyperalgesia induced by *Bothrops jararaca* venom. *Toxicon* **40**:1047–1051.

Chaplan SR, Bach FW, Pogrel JW, Chung JM, and Yaksh TL (1994) Quantitative assessment of tactile allodynia in the rat paw. *J Neurosci Methods* **53**:55–63.

Chippaux JP (1998) Snake-bites: appraisal of the global situation. *Bull World Health Organ* **76**:515–24.

Costa MT, Goulart A da S, Rocha KMA, Salgueiro ACF, Silva MD da, Puntel RL, and Folmer V (2021) *In vivo* effects of exposure to Golden trumpet *Handroanthus chrysotrichus* in mice. *Toxicol Res (Camb)* 1–9.

Costa MT, Goulart ADS, Soares JDJ, Salgueiro ACF, Da Rosa HS, Denardin ELG, Paim CS, Puntel RL, and Folmer V (2020) Antioxidant and toxicological potential of the Golden trumpet hydroalcoholic stem bark extract. *Res Soc Dev* **9**:122942936.

Croda JR, Haraki CAC, Ciarlini ACL, Dourado FS, Pereira LRM, Castro APB de, Júnior FEF de L, and Wada MY (2020) Acidentes ofídicos no Brasil, 2018, in *Boletim Epidemiológico nº 09*. Ministério da Saúde. Secretaria de Vigilância em Saúde, Brasília, DF.

de Medeiros CR, Hess PL, Nicoleti AF, Sueiro LR, Duarte MR, de Almeida-Santos SM, and França FOS (2010) Bites by the colubrid snake *Philodryas patagoniensis*: A clinical and epidemiological study of 297 cases. *Toxicon* **56**:1018–1024.

Dixon WJ (1980) Efficient analysis of experimental observations. *Ann Rev Pharmacol Toxicol* **20**:441–462.

Ellman GL (1959) Tissue sulfhydryl groups. *Arch Biochem Biophys* **82**:70–77.

Ferlan I, Ferlan A, King T, and Russell FE (1983) Preliminary studies on the venom of the colubrid snake *Rhabdophis subminatus* (red-necked keelback). *Toxicon* **21**:570–574.

Grazziotin JD, Schapoval EES, Chaves CG, Gleye J, and Henriques AT (1992) Phytochemical and analgesic investigation of *Tabebuia chrysotricha*. *J Ethnopharmacol* **36**:249–251.

Gutiérrez JM, Calvete JJ, Habib AG, Harrison RA, Williams DJ, and Warrell DA (2017) Snakebite envenoming. *Nat Rev Dis Prim* **3**:17063.

Hunskaar S, and Hole K (1987) The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. *Pain* **30**:103–114.

Jardim Botânico do Rio de Janeiro (2020) Flora do Brasil 2020.

Kasturiratne A, Wickremasinghe AR, de Silva N, Gunawardena NK, Pathmeswaran A, Premaratna R, Savioli L, Lalloo DG, and de Silva HJ (2008) The Global Burden of Snakebite: A literature analysis and modelling based on regional estimates of envenoming

and deaths. *PLoS Med* **5**:e218.

Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz A-G, Ahn B-W, Shaltiel S, and Stadtman ER (1990) [49] Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol* **186**:464–478, Academic Press.

Lisa SR, Islam MK, and Qais N (2020) Plants and Plant Constituents with Analgesic and Anti-inflammatory Activities: A Systematic Review. *Dhaka Univ J Pharm Sci* **19**:207–224.

Lopes PH, Rocha MMT, Kuniyoshi AK, Portaro FCV, and Gonçalves LRC (2017) Edema and nociception induced by *Philodryas patagoniensis* venom in mice: A pharmacological evaluation with implications for the accident treatment . *J Pharmacol Exp Ther* **361**:349–354.

Mantyh PW, Clohisy DR, Koltzenburg M, and Hunt SP (2002) Molecular mechanisms of cancer pain. *Nat Rev Cancer* **2**:201–209.

Maria-Ferreira D, Carlotto J, Dallazen JL, da Luz BB, de Souza LM, de Paula Werner MF, and Cipriani TR (2020) A polysaccharide fraction from *Handroanthus albus* (yellow ipê) leaves with antinociceptive and anti-inflammatory activities. *Int J Biol Macromol* **159**:1004–1012.

Núñez V, Otero R, Barona J, Fonnegra R, Jiménez S, Osorio RG, Quintana JC, and Díaz A (2004) Inhibition of the Toxic Effects of *Lachesis muta*, *Crotalus durissus cumanensis* and *Micrurus mipartitus* snake venoms by plant extracts. *Pharm Biol* **42**:49–54.

Ohkawa H, Ohishi N, and Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* **95**:351–358.

Otero R, Fonnegra R, Jiménez SL, Núñez V, Evans N, Alzate SP, García ME, Saldarriaga M, Del Valle G, Osorio RG, Díaz A, Valderrama R, Duque A, and Vélez HN (2000) Snakebites and ethnobotany in the northwest region of Colombia: Part I: Traditional use of plants. *J Ethnopharmacol* **71**:493–504, Elsevier.

Peichoto ME, Acosta O, Leiva L, Teibler P, Maruñak S, and Ruíz R (2004) Muscle and skin necrotizing and edema-forming activities of Duvernoy's gland secretion of the xenodontine colubrid snake *Philodryas patagoniensis* from the north-east of Argentina. *Toxicon* **44**:589–596.

Peichoto ME, and Santoro ML (2016) Patagonfibrase modifies protein expression of tissue factor and protein disulfide isomerase in rat skin. *Toxicon* **119**:330–335.

Peichoto ME, Tavares FL, Santoro ML, and Mackessy SP (2012) Venom proteomes of South and North American opisthoglyphous (Colubridae and Dipsadidae) snake species: A preliminary approach to understanding their biological roles. *Comp Biochem Physiol Part D Genomics Proteomics* **7**:361–369.

Peichoto ME, Teibler P, Ruíz R, Leiva L, and Acosta O (2006) Systemic pathological alterations caused by *Philodryas patagoniensis* colubrid snake venom in rats. *Toxicon* **48**:520–528.

Peichoto ME, Zychar BC, Tavares FL, de Camargo Gonçalves LR, Acosta O, and Santoro ML (2011) Inflammatory effects of patagonfibrase, a metalloproteinase from *Philodryas patagoniensis* (Patagonia Green Racer; Dipsadidae) venom. *Exp Biol Med* **236**:1166–

1172.

Reis FP, Senna Bonfa IM, Cavalcante RB, Okoba D, De Souza Vasconcelos SB, Candeloro L, De Oliveira Filiu WF, Duenhas Monreal AC, Da Silva VJ, Santa Rita PH, Carollo CA, and Toffoli-Kadri MC (2014) *Tabebuia aurea* decreases inflammatory, myotoxic and hemorrhagic activities induced by the venom of *Bothrops neuwiedi*. *J Ethnopharmacol* **158**:352–357, Elsevier.

Ribeiro RV, Bieski IGC, Balogun SO, and Martins DT de O (2017) Ethnobotanical study of medicinal plants used by Ribeirinhos in the North Araguaia microregion, Mato Grosso, Brazil. *J Ethnopharmacol* **205**:69–102, Elsevier Ireland Ltd.

Santos ARS, Vedana EMA, and De Freitas GAG (1998) Antinociceptive effect of meloxicam, in neurogenic and inflammatory nociceptive models in mice. *Inflamm Res* **47**:302–307.

Serrano SMT (2013) The long road of research on snake venom serine proteinases. *Toxicon* **62**:19–26.

Singh NP, McCoy MT, Tice RR, and Schneider EL (1988) A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp Cell Res* **175**:184–191.

Strapazzon J de O, Parisotto EB, Moratelli AM, Garlet TR, Bastos J, Zimermann IR, Zanin M, Fagundez R, Lino MR de O, Fröde TS, and Wilhelm Filho D (2015) Systemic oxidative stress in victims of *Bothrops* snakebites. *J Appl Biomed* **13**:161–167, Korea Institute of Oriental Medicine.

Teixeira C, Cury Y, Moreira V, Picolo G, and Chaves F (2009) Inflammation induced by *Bothrops asper* venom. *Toxicon* **54**:988–997.

Twardowschy A, Freitas CS, Baggio CH, Mayer B, dos Santos AC, Pizzolatti MG, Zacarias AA, dos Santos EP, Otuki MF, and Marques MCA (2008) Antiulcerogenic activity of bark extract of *Tabebuia avellanedae*, Lorentz ex Griseb. *J Ethnopharmacol* **118**:455–459.

Xiao H, Pan H, Liao K, Yang M, and Huang C (2017) Snake venom PLA 2 , a promising target for broad-spectrum antivenom drug development. *Biomed Res Int* **2017**:1–10.

Zelanis A, Teixeira da Rocha MM, and de Fátima Domingues Furtado M (2010) Preliminary biochemical characterization of the venoms of five Colubridae species from Brazil. *Toxicon* **55**:666–669.

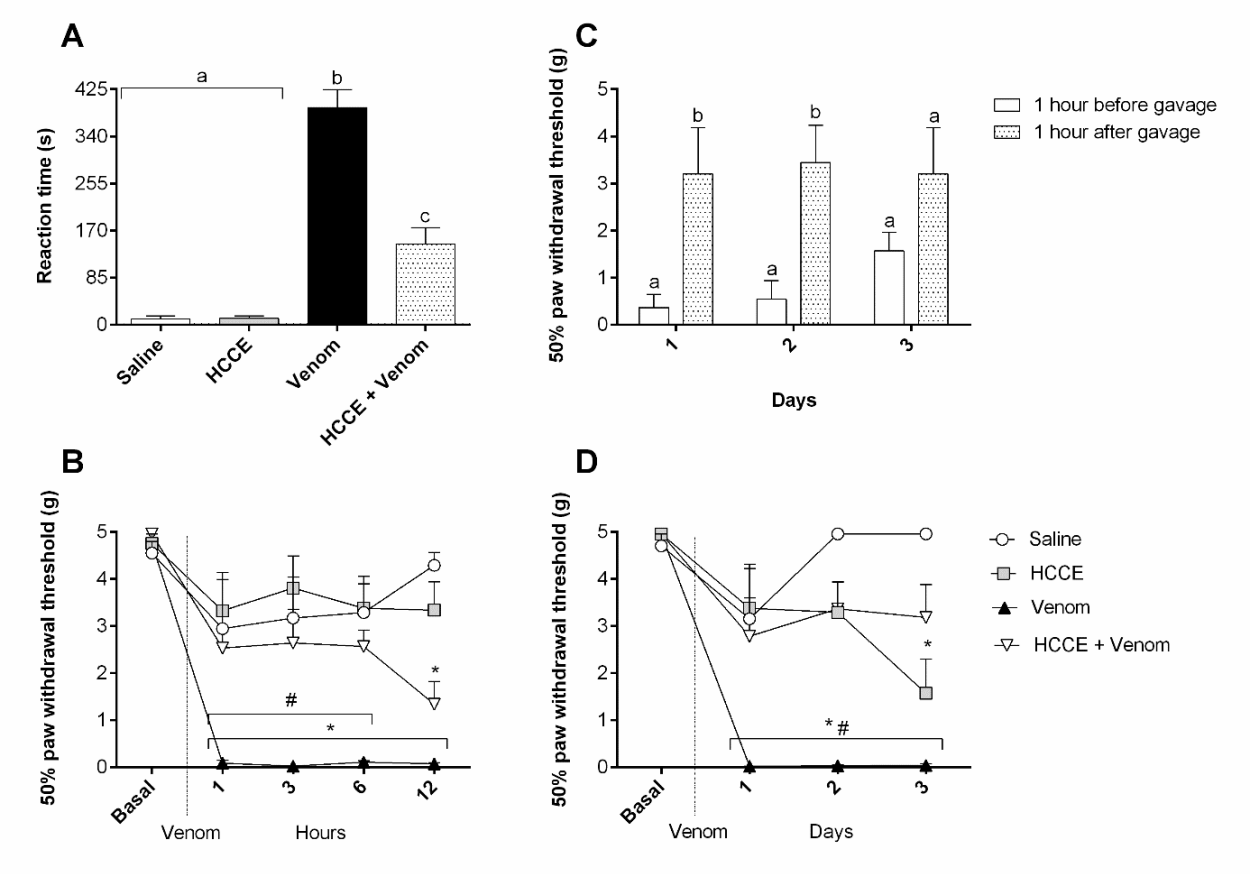


Figure 1. Nociception test (A) and mechanical hyperalgesia (B, C, and D) evaluated in mice. Data are expressed in mean ± SEM. Nociceptive test was analyzed using one-way ANOVA followed by Tukey's multiple comparisons test, and mechanical hyperalgesia tests analyzed by two-way ANOVA followed by Bonferroni's test (letters different indicate significative difference between bars; * indicate significative difference compared to Saline; and # indicate significative difference compared to HCCE + Venom group).

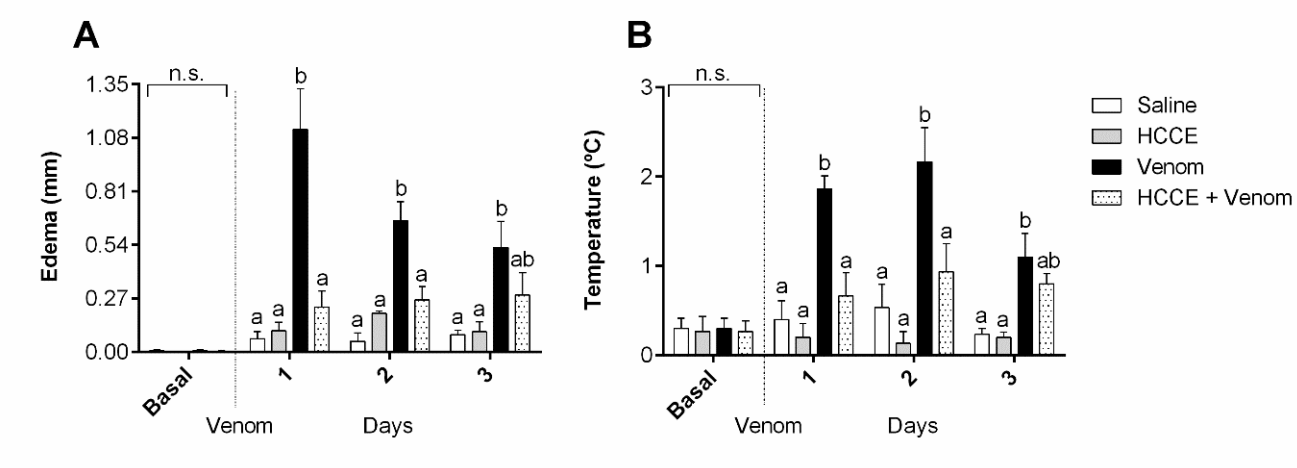


Figure 2. Paw edema (A) and temperature (B) measurements over the treatment days. Data are expressed in mean ± SEM and analyzed using two-way ANOVA followed by Bonferroni's test (letters different indicate significative difference between bars).

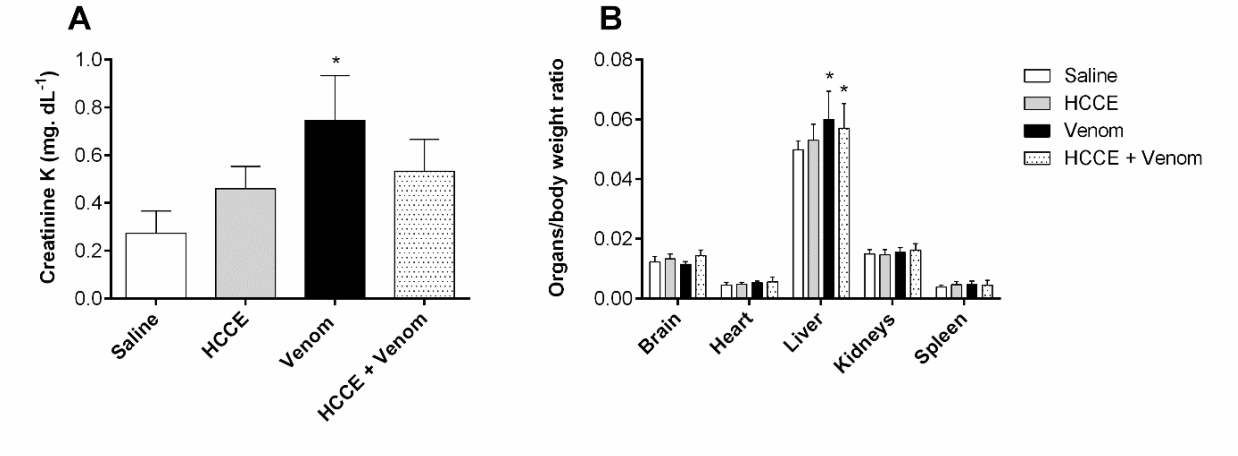


Figure 3. Organ toxicity evaluation. Creatinine K levels (A) and ratio of organ/body weights (B) in mice treated. Data are expressed in mean ± SD and analyzed using by one and two-way ANOVA, respectively, followed by Tukey's test (* indicate significative difference compared to Saline).

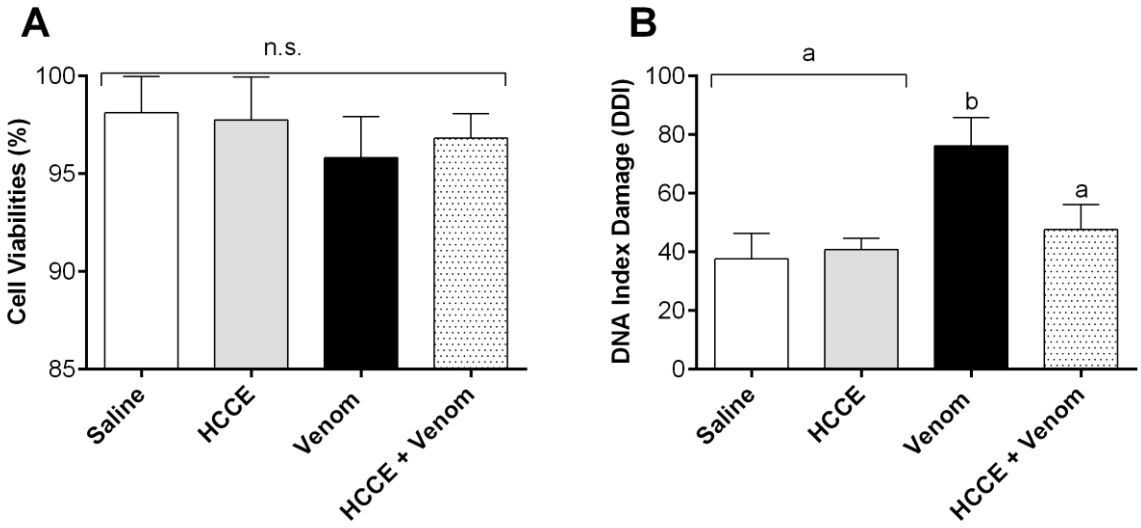


Figure 4. Viability (A) and genotoxicity (B) evaluated peripheral blood mononuclear cells. Data were expressed as mean ± SD and analyzed by one-way ANOVA followed by Bonferroni's test (letters different indicate significative difference between bars).

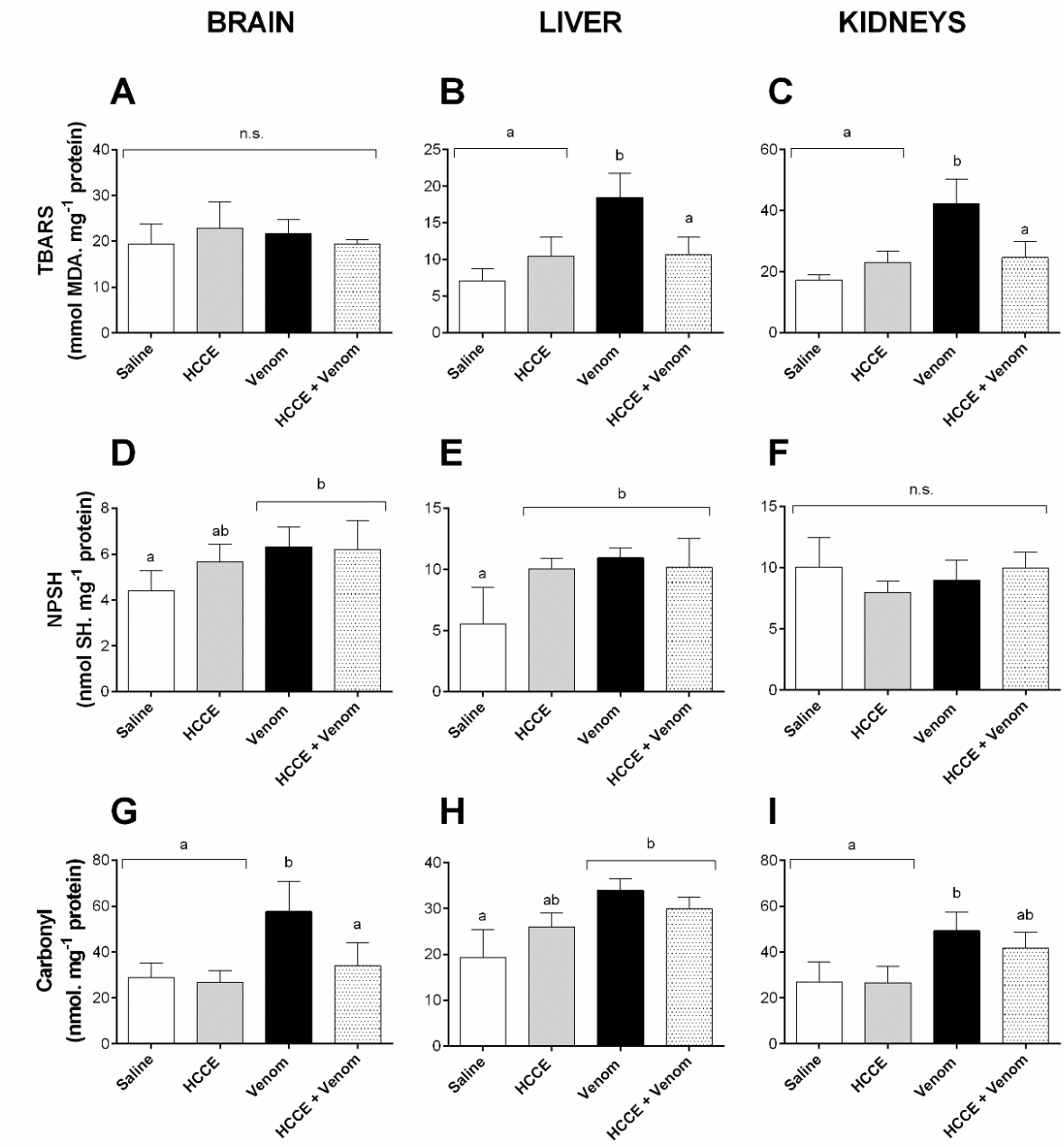


Figure 5. Evaluation of oxidative stress in brain (A, D, and G), liver (B, E and H), and kidneys (C, F, and I) of mice treated. Data are expressed in mean ± SD and analyzed by one-way ANOVA followed by Bonferroni's test (letters different indicate significative difference between bars).

7. DISCUSSÃO GERAL

O propósito deste estudo foi investigar o perfil fitoquímico do extrato hidroetanólico da casca de ipê-amarelo *Handroanthus chrysotrichus*, além do seu potencial toxicológico e farmacológico *in silico* e *in vitro* **(Artigo 1)** e avaliar, *in vivo*, os riscos associados ao uso medicinal de forma aguda e subcrônica **(Artigo 2)**. Após estas análises, verificou-se a ação protetora do extrato em casos de envenenamentos pela serpente *Philodryas patagoniensis* **(Manuscrito 2)**.

A fim de alcançar estes objetivos, utilizou-se metodologias e modelos diversos. Inicialmente, após a obtenção do extrato, buscou-se algum indicativo da presença de compostos bioativos no mesmo. O ensaio utilizando o modelo *Artemia salina* indicou esta possibilidade (MCLAUGHLIN, 1991). Identificados os principais compostos do extrato, o próximo passo foi a avaliação farmacológica e toxicológica *in silico*. Tal abordagem é caracterizada por realizar uma triagem eficiente de protótipos de fármacos e análogos, por meio da modelagem molecular, sem que ocorra experimentação desnecessária (ARROIO; HONÓRIO; SILVA, 2010). Análises *in vitro* complementaram estas avaliações e demonstraram o potencial do extrato para os testes *in vivo*. Destaca-se que os resultados obtidos *in vitro* nem sempre condizem com experimentos *in vivo*. Assim, a utilização de animais (vertebrados) na pesquisa biomédica é fundamental, pois representam a fisiologia como um todo (MENDES; SOUZA, 2017). Ao seguir estes passos ao longo do estudo, o conhecimento acerca do ipê-amarelo *H. chrysotrichus* se enriqueceu e respeitou-se os aspectos éticos que resguardam o bem-estar animal.

Desta forma, os resultados iniciais sugerem altos níveis de polifenóis no extrato estudado e a ocorrência de cinco compostos majoritários: α-curcumeno, β-bisaboleno, 4-(4-metilfenil) pentanal, ácido pentanóico e acetato de isoamila **(Artigo 1)**. Salienta-se que substâncias consideradas como princípios ativos vegetais advém, na sua maioria, do metabolismo secundário das plantas. Esses componentes do extrato possuem prioritariamente funções ecológicas e biológicas, como defesa (contra insetos, microorganismos), gustativas, odoríferas e visuais (coloração) (MAZID; KHAN; MOHAMMAD, 2011; VERPOORTE; MEMELINK, 2002). Desta forma, por exemplo, o composto majoritário α-curcumeno age biologicamente protegendo a planta contra insetos (JÚNIOR, 2003), e com potencial propriedades anti-inflamatória e analgésica (LISA; ISLAM; QAIS, 2020).

Simulações computacionais apontaram atividades biológicas que estão de acordo com o uso tradicional do ipê-amarelo e, em geral, os compostos encontrados apresentaram baixa probabilidade de toxicidade nestas previsões, exceto pelo efeito carcinogênico do α-curcumeno

e do β-bisaboleno **(Artigo 1)**. As predições realizadas com o uso de bioinformática são abordagens iniciais úteis na avaliação de compostos encontrados, pois os extratos vegetais são misturas complexas de diferentes metabólitos. Após uma elucidação estrutural do extrato, essas avaliações prévias podem predizer possíveis ações terapêuticas ou tóxicas destes fitoquímicos de maneira independente (ARVIDSON et al., 2008). No entanto, o sinergismo entre estes compostos deve ser considerado na determinação das atividades atribuídas às plantas na medicina tradicional, pois a avaliação *in silico* pode mascarar a real atividade medicinal ou toxicológica da planta.

In vitro, o extrato exibiu atividade antioxidante, sem demonstrar citotoxicidade ou genotoxicidade **(Artigo 1)**. Em geral, extratos vegetais com níveis de compostos fenólicos proeminentes, como os encontrados aqui, revelam-se como eficazes neutralizadores de radicais livres e inibidores de peroxidação lipídica (efeito danoso a membranas celulares) (RICE-EVANS; MILLER; PAGANGA, 1997). Para Halliwell e Gutteridge (2007), um antioxidante é uma substância que, quando presente em baixas concentrações, comparadas a de um substrato oxidável, retarda ou inibe significativamente a oxidação deste substrato. E, desta forma, é capaz de reduzir os danos oxidativos induzidos por várias doenças (SALGUEIRO et al., 2016b), bem como os gerados em casos de envenenamento da *P. patagoniensis* **(Manuscrito 2)**.

Contudo, testes *in vivo* demonstraram alguns efeitos adversos que merecem atenção, tanto nos casos de exposição aguda quanto subcrônica **(Artigo 2)**. Análises do extrato de *H. chrysotrichus* indicam alterações comportamentais e bioquímicas em camundongos causadas pela mesma. E caracterizam o fígado, os rins e os glóbulos brancos como alvos primários do extrato. Portanto, ao considerar esta pesquisa como translacional, deve haver preocupação com o uso correto e seguro desta planta medicinal. Os efeitos adversos ocasionados pelo consumo de plantas é um tema frequentemente ignorado. Em média, apenas 5% dos usuários conhecem os efeitos adversos provenientes do uso de plantas medicinais (SOUZA et al., 2013). Logo, é uma questão importante a ser trabalhado por pesquisadores, profissionais de educação e da saúde.

Ainda em nosso estudo, os testes realizados contra os efeitos lesivos ocasionados pela peçonha da *P. patagoniensis,* agente tóxico utilizado nos testes **(Manuscrito 1)**, indicam que o ipê-amarelo *H. chrysotrichus* é capaz de minimizar os sinais cardinais de inflamação: dor, edema e calor. Além de proteger os camundongos contra danos oxidativos desencadeados pela peçonha, como mencionado anteriormente **(Manuscrito 2)**. Estes resultados alicerçam o uso da casca do ipê-amarelo pela medicina tradicional e estão de acordo com pesquisadores que apontam extratos vegetais como uma rica fonte de compostos naturais, os quais podem

neutralizar as atividades tóxicas das peçonhas de cobra (NÚÑEZ et al., 2004). Ainda que, a administração contínua do extrato tenha causado efeito *per se* nos testes de hiperalgesia mecânica, em linha com testes anteriores **(Artigo 2)**. Dados que reforçam a necessidade de cautela na recomendação desse composto para consumo contínuo.

Em suma, com base nos resultados aqui apresentados, pode-se conferir ao extrato da casca do ipê-amarelo efeitos antioxidantes e anti-inflamatórios, considerando o alívio de sinais cardinais da inflamação – dor, edema e calor (Figura 6). Efeitos relevantes nos casos de exposição a serpentes, pois a administração do soro antiofídico neutraliza a toxicidade sistêmica induzida pela peçonha, mas não oferece proteção contra o estresse oxidativo induzido pela peçonha. E, sem a adoção de uma terapia alternativa, os danos oxidativos e a infiltração de mediadores inflamatórios continuarão mesmo após a administração do soro (SEBASTIN SANTHOSH et al., 2013).

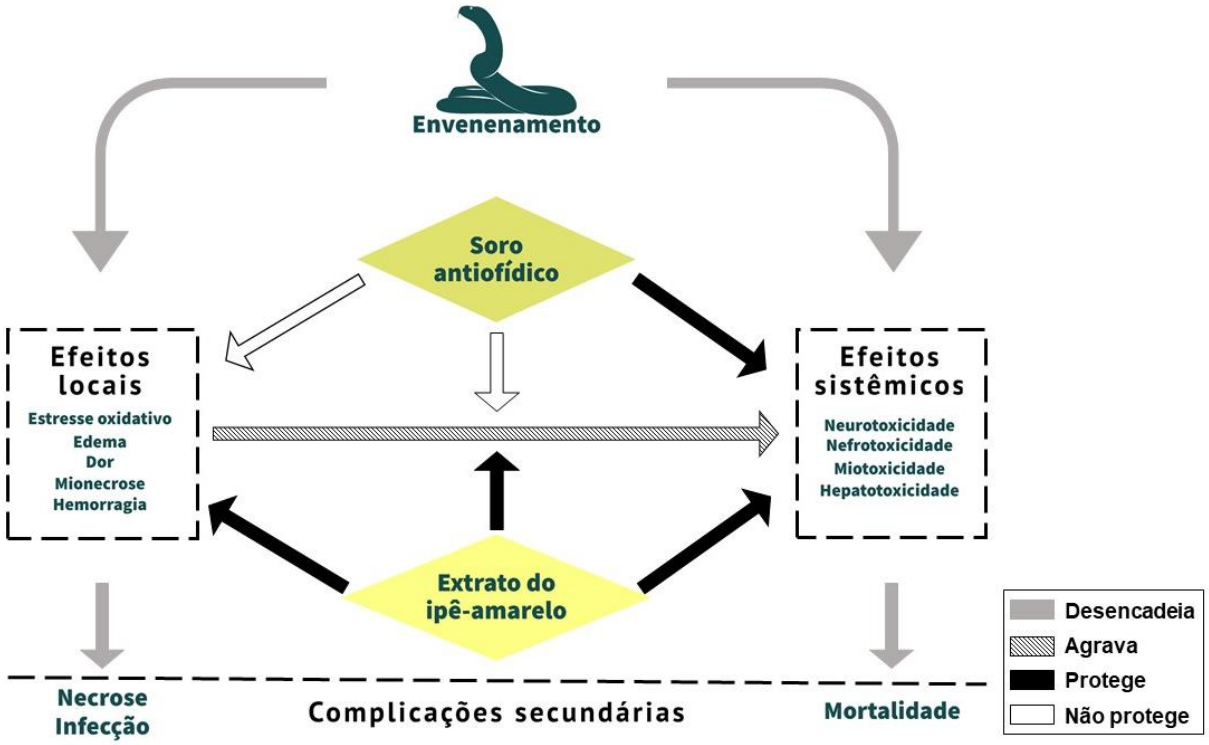


Figura 6. Representação esquemática das interações do extrato hidroetanólico da casca do ipê-amarelo *Handroanthus chrysotrichus* com as complicações causadas pela peçonha do gênero *Philodryas.* Fonte: autor.

8. CONCLUSÕES

Em suma, os resultados aqui apresentados culminam na tese de que o extrato hidroetanólico da casca do ipê-amarelo *Handroanthus chrysotrichus* apresenta atividades, *in silico* e *in vitro,* condizentes com seu uso medicinal. O extrato também exibe, nas formas testadas, ação protetora contra envenenamento por serpente, especialmente contra os sinais cardinais de inflamação como dor, edema e calor. Fato que fundamenta, nestes casos de envenenamentos, o tratamento com o ipê-amarelo adotado por populares.

Por outro lado, ensaios *in silico* e *in vivo,* sugerem que o extrato pode desencadear efeitos adversos quando administrado em altas concentrações ou em casos de exposições de longa duração. Assim, seguindo às devidas precauções, esta planta pode vir a ser um potencial agente terapêutico para o tratamento complementar do estresse oxidativo e da inflamação relacionados aos envenenamentos por serpentes.

Ainda, abrem-se novas perspectivas para avaliação do extrato da casca do ipê-amarelo *H. chrysotrichus,* como a composição e efeitos desta planta em outras formas de extração, visto que alguns componentes podem não ser extraídos ou ainda serem perdidos no extrato hidroetanólico. Além disso, uma investigação mais detalhada poderia descobrir metabólitos-chave e seus papéis na atuação do ipê-amarelo, elucidando as bases científicas de seu uso na medicina popular. Outros meios de administração do extrato também poderiam ser testados, como cataplasma *in situ*.

Por fim, com base nos dados experimentais e ensaios biológicos, dá-se uma nova dimensão às ações do ipê-amarelo nos níveis orgânico, celular e bioquímico. E destaca-se a importância de pesquisas para encontrar, em uma extensa diversidade vegetal, agentes que possam auxiliar nas diversas terapias, em especial no envenenamento por cobras.

9. REFERÊNCIAS BIBLIOGRÁFICAS

ALAM, M. I.; GOMES, A. Viper venom-induced inflammation and inhibition of free radical formation by pure compound (2-hydroxy-4-methoxy benzoic acid) isolated and purified from anantamul (*Hemidesmus indicus* R.Br) root extract. **Toxicon**, v. 36, n. 1, p. 207–215, jan. 1998.

ARAÚJO, M. E. DE; SANTOS, A. C. M. C. A. DOS. Cases of human envenoming caused by *Philodryas olfersii* and *Philodryas patagoniensis* (serpentes: Colubridae). **Revista da Sociedade Brasileira de Medicina Tropical**, v. 30, n. 6, p. 517–519, dez. 1997.

ARAÚJO, S. D. et al. Effect of dexamethasone associated with serum therapy on treatment of *Bothrops jararaca* venom-induced paw edema in mice. **Inflammation Research**, v. 56, n. 10, p. 409–413, 2007.

ARROIO, A.; HONÓRIO, K. M.; SILVA, A. B. F. DA. Propriedades químico-quânticas empregadas em estudos das relações estrutura-atividade. **Química Nova**, v. 33, n. 3, p. 694–699, 2010.

ARVIDSON, K. B. et al. *In silico* toxicological screening of natural products. **Toxicology Mechanisms and Methods**, v. 18, n. 2–3, p. 229–242, 9 jan. 2008.

ASSAKURA, M. T. et al. Hemorrhagic, fibrinogenolytic and edema-forming activities of the venom of the colubrid snake *Philodryas olfersii* (green snake). **Toxicon: official journal of the International Society on Toxinology**, v. 30, n. 4, p. 427–38, abr. 1992.

B. MORS, W. et al. Plant natural products active against snake bite — the molecular approach. **Phytochemistry**, v. 55, n. 6, p. 627–642, nov. 2000.

BARBOSA, K. B. F. et al. Estresse oxidativo: conceito, implicações e fatores modulatórios. **Revista de Nutrição**, v. 23, n. 4, p. 629–643, ago. 2010.

BARREIROS, A. L. B. S.; DAVID, J. M.; DAVID, J. P. Estresse oxidativo: Relação entre geração de espécies reativas e defesa do organismo. **Quimica Nova**, v. 29, n. 1, p. 113–123, 2006.

BEECH, E. et al. GlobalTreeSearch: The first complete global database of tree species and country distributions. **Journal of Sustainable Forestry**, v. 36, n. 5, p. 454–489, 4 jul. 2017.

BENÍTEZ, N. P.; VALOIS, H. Ethnobotany of four black communities of the municipality of Quibdo, Choco - Colombia. **Lyonia**, v. 7, n. 2, p. 61–69, 2004.

BIANCHI, M. DE L. P.; ANTUNES, L. M. G. Radicais livres e os principais antioxidantes da dieta. **Revista de Nutrição**, v. 12, n. 2, p. 123–130, ago. 1999.

BITTENCOURT, J. A. H. M. et al. Antiophidian activity of *Brosimum guianense* (AUBL) HUBER. **American Journal of Pharmacology and Toxicology**, v. 9, n. 2, p. 148–156, 1 fev. 2014.

BOLSON, M. et al. Ethno-medicinal study of plants used for treatment of human ailments, with residents of the surrounding region of forest fragments of Paraná, Brazil. **Journal of Ethnopharmacology**, v. 161, p. 1–10, 2015.

BOOM, B. M. Ethnobotany of the Chácobo Indians, Beni, Bolivia: Second Edition. **Advances in Economic Botany**, v. 4, p. 1–74, 1996.

BRACESCO, N. et al. Analysis of radioprotection and antimutagenic effects of *Ilex paraguariensis* infusion and its component rutin. **Brazilian Journal of Medical and Biological Research**, v. 51, n. 9, 2018.

BRANDELLI, C. L. C. Plantas medicinais: histórico e conceitos. In: MONTEIRO, S. DA C.; BRANDELLI, C. L. C. (Eds.). . **Farmacobotânica: aspectos teóricos e aplicação [recurso eletrônico]**. Porto Alegre: Artmed, 2017a. p. 1–12.

BRANDELLI, C. L. C. Introdução à fitoterapia: conceitos e definições. In: MONTEIRO, S. DA C.; BRANDELLI, C. L. C. (Eds.). . **Farmacobotânica: aspectos teóricos e aplicação [recurso eletrônico]**. Porto Alegre: Artmed, 2017b. p. 95–107.

BRASIL. **Política nacional de plantas medicinais e fitoterápicos / Ministério da Saúde, Secretaria de Ciência, Tecnologia e Insumos Estratégicos, Departamento de Assistência Farmacêutica**. Brasília: Ministério da Saúde, 2006a.

BRASIL. **A fitoterapia no SUS e o Programa de Pesquisa de Plantas Medicinais da Central de Medicamentos / Ministério da Saúde, Secretaria de Ciência, Tecnologia e Insumos Estratégicos, Departamento de Assistência Farmacêutica**. Brasília, DF: Ministério da Saúde, Secretaria de Ciência, Tecnologia e Insumos Estratégicos, Departamento de Assistência Farmacêutica, 2006b.

BRASIL. **Programa Nacional de Plantas Medicinais e Fitoterápicos /Ministério da Saúde, Secretaria de Ciência, Tecnologia e Insumos Estratégicos, Departamento de Assistência Farmacêutica e Insumos Estratégicos**. Brasília: Ministério da Saúde, 2009.

BRASIL, M. DA S. **Acidentes por animais peçonhentos: o que fazer e como evitar**. Disponível em: <https://antigo.saude.gov.br/saude-de-a-z/acidentes-por-animais-peconhentos>. Acesso em: 11 set. 2021.

BUCARETCHI, F. et al. Acidentes por *Philodryas olfersii*: Relato de dois casos. In: **Resumos do III Congresso Latino Americano de Herpetologia**. Campinas: Universidade Estadual de Campinas, 1993. p. 195.

CAMPBELL, N. A. et al. **Biologia**. 8ª Ed. ed. Porto Alegre: Artmed, 2010.

CARDOSO, J. L. C. A fitoterapia antiveneno na medicina brasileira. In: CARDOSO, J. L. C. et al. (Eds.). **Animais peçonhentos no Brasil: biologia, clínica e terapêutica dos acidentes**. 2. ed. São Paulo: Sarvier, 2009. p. 481–485.

CARVALHO, M. A. DE; NOGUEIRA, F. Serpentes da área urbana de Cuiabá, Mato Grosso: aspectos ecológicos e acidentes ofídicos associados. **Cadernos de Saúde Pública**, v. 14, n. 4, p. 753–763, out. 1998.

CARVALHO, J. C. T. et al. Effects of *Bothrops alternatus* venom in zebrafish: a histopathological study. **Inflammopharmacology**, v. 26, n. 1, p. 273–284, 2018.

CASTOLDI, L. et al. Evaluation of *in vitro* cytotoxic effects of Especifico Pessoa phytotherapic tincture on Ehrlich tumor cells and mice spleen cells. **Fronteiras: Journal of Social, Technological and Environmental Science**, v. 9, n. 1, p. 458–472, 5 mar. 2020.

CHUANG, H. et al. Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P2-mediated inhibition. **Nature**, v. 411, n. 6840, p. 957–962, jun. 2001.

CITELI, N. et al. **Lista dos Polos de Soro para atendimento de acidentes ofídicos no Brasil**. Manguinhos: SINITOX, 2018.

CLISSA, P. B. et al. The effect of jararhagin, a metalloproteinase from *Bothrops jararaca* venom, on pro-inflammatory cytokines released by murine peritoneal adherent cells. **Toxicon**, v. 39, n. 10, p. 1567–1573, out. 2001.

COLPO, A. C. et al. Yerba mate (*Ilex paraguariensis* St. Hill.)-based beverages: How successive extraction influences the extract composition and its capacity to chelate iron and scavenge free radicals. **Food Chemistry**, v. 209, p. 185–195, 2016.

COSTA, R. S. C. DA et al. Neuromuscular action of venom from the South American colubrid snake *Philodryas patagoniensis*. **Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology**, v. 148, n. 1, p. 31–38, jul. 2008.

CRODA, J. R. et al. Acidentes ofídicos no Brasil, 2018. In: OLIVEIRA, W. K. DE et al. (Eds.). . **Boletim Epidemiológico nº 09**. Brasília, DF: Ministério da Saúde. Secretaria de Vigilância em Saúde, 2020. v. 51.

DE MEDEIROS, C. R. et al. Bites by the colubrid snake *Philodryas patagoniensis*: A clinical and epidemiological study of 297 cases. **Toxicon**, v. 56, n. 6, p. 1018–1024, nov. 2010.

DE MOURA, V. M. et al. Plants used to treat snakebites in Santarém, western Pará, Brazil: An assessment of their effectiveness in inhibiting hemorrhagic activity induced by *Bothrops jararaca* venom. **Journal of Ethnopharmacology**, v. 161, p. 224–232, 23 fev. 2015.

FAN, H. W.; CARDOSO, J. L. C. Clinical toxicology of snakebites in South America. In: MEIER, J.; WHITE, J. (Eds.). . **Handbook of clinical toxicology of animal venoms and poisons**. Boca Raton: CRC Press, 1995. p. 667–688.

FARSKY, S. H. P. et al. *Bothrops asper* snake venom and its metalloproteinase BaP–1 activate the complement system. Role in leucocyte recruitment. **Mediators of Inflammation**, v. 9, n. 5, p. 213–221, 2000.

FERREIRA, A. L. A.; MATSUBARA, L. S. Radicais livres: conceitos, doenças relacionadas, sistema de defesa e estresse oxidativo. **Revista da Associação Médica Brasileira**, v. 43, n. 1, mar. 1997.

FRANCISCHETTI, I. et al. Os leucócitos e a resposta inflamatória na lesão de isquemia-reperfusão. **Revista Brasileira de Cirurgia Cardiovascular**, v. 25, n. 4, p. 575–584, dez. 2010.

FUNASA. **Manual de Diagnóstico e Tratamento de Acidentes por Animais Peçonhentos**. 2ª ed. Brasília: Fundação Nacional de Saúde, 2001.

GAY, C. C. et al. Proteolytic, edematogenic and myotoxic activities of a hemorrhagic metalloproteinase isolated from *Bothrops alternatus* venom. **Toxicon**, v. 46, n. 5, p. 546–554, 2005.

GONÇALVES, J. E. et al. Acidentes por animais peçonhentos: uma análise do perfil epidemiológico na região Nordeste do Brasil no período de 2010 a 2019. **Research, Society and Development**, v. 9, n. 10, p. e4679108843, 4 out. 2020.

GRAZZIOTIN, J. D. et al. Phytochemical and analgesic investigation of *Tabebuia chrysotricha*. **Journal of Ethnopharmacology**, v. 36, n. 3, p. 249–251, 1992.

GROSE, S. O.; OLMSTEAD, R. G. Taxonomic revisions in the polyphyletic genus *Tabebuia* (Bignoniaceae). **Systematic Botany**, v. 32, n. 3, p. 660–670, 2007.

HALLIWELL, B.; GUTTERIDGE, J. M. C. **Free radicals in biology and medicine**. 4ª ed. New York: Oxford University Press Inc, 2007.

HALLIWELL, B.; WHITEMAN, M. Measuring reactive species and oxidative damage *in vivo* and in cell culture: how should you do it and what do the results mean? **British Journal of Pharmacology**, v. 142, n. 2, p. 231–255, maio 2004.

HARRIS, M. L. et al. Free radicals and other reactive oxygen metabolites in inflammatory bowel disease: cause, consequence or epiphenomenon? **Pharmacology & Therapeutics**, v. 53, n. 3, p. 375–408, jan. 1992.

HARTMANN, P. A.; MARQUES, O. A. V. Diet and habitat use of two sympatric species of *Philodryas* (Colubridae) in south Brazil. **Amphibia-Reptilia**, v. 26, p. 25–31, 2005.

HOUGHTON, P. J.; OSIBOGUN, I. M. Flowering plants used against snakebite. **Journal of Ethnopharmacology**, v. 39, n. 1, p. 1–29, 1 maio 1993.

HUERRE, M. R.; GOUNON, P. Inflammation: Patterns and new concepts. **Research in Immunology**, v. 147, n. 7, p. 417–434, set. 1996.

JÚNIOR, C. V. Terpenos com atividade inseticida: uma alternativa para o controle químico de insetos. **Quimica Nova**, v. 26, n. 3, p. 390–400, 2003.

LISA, S. R.; ISLAM, M. K.; QAIS, N. Plants and plant constituents with analgesic and anti-inflammatory activities: A systematic review. **Dhaka University Journal of Pharmaceutical Sciences**, v. 19, n. 2, p. 207–224, 10 dez. 2020.

LOEBMANN, D.; QUINTELA, F. M. **Guia ilustrado: os répteis da região costeira do extremo sul do Brasil**. 1. ed. Pelotas: USEB, 2009.

LOPES, P. H. et al. Edema and nociception induced by *Philodryas patagoniensis* venom in mice: A pharmacological evaluation with implications for the accident treatment . **Journal of Pharmacology and Experimental Therapeutics**, v. 361, n. 3, p. 349–354, 2017.

LORENZI, H. **Brazilian trees : a guide to the identification and cultivation of brazilian native trees**. 4. ed. Nova Odessa: Instituto Plantarum de Estudos da Flora, 2002.

LORENZI, H.; MATOS, F. J. A. **Plantas medicinais no Brasil: nativas e exóticas**. Nova Odessa: Instituto Plantarum, 2002.

MACKESSY, S. P. Biochemistry and pharmacology of colubrid snake venoms. **Journal of Toxicology: Toxin Reviews**, v. 21, n. 1–2, p. 43–83, 2002.

MALANGE, K. F. et al. *Tabebuia aurea* decreases hyperalgesia and neuronal injury induced by snake venom. **Journal of Ethnopharmacology**, v. 233, p. 131–140, 2019.

MARTINS, E. N. et al. Protective effect of *Melissa officinalis* aqueous extract against Mn-induced oxidative stress in chronically exposed mice. **Brain Research Bulletin**, v. 87, n. 1, p. 74–79, 4 jan. 2012.

MAZID, M.; KHAN, T. A.; MOHAMMAD, F. Role of secondary metabolites in defense mechanisms of plants. **Biology and Medicine**, v. 3, n. 2, p. 232–249, 2011.

MCLAUGHLIN, J. L. Crown gall tumors on potato discs and brine shrimp lethality: two simple bioassays for higher plant screening and fractions. In: P.M.DEY; HARBONE, J. B. (Eds.). . **Methods in Plant Biochemistry**. 1. ed. New York: Academic Press, 1991. p. 1–32.

MENDES, M. M. P. G.; SOUZA, C. S. A. DE J. Aplicação de modelos animais na pesquisa biomédica experimental. **Revista de Saúde - RSF**, v. 4, n. 2, 2017.

MISE, Y. F.; LIRA-DA-SILVA, R. M.; CARVALHO, F. M. Envenomation by *Bothrops* in the State of Bahia: epidemiological and clinical aspects. **Rev Soc Bras Med Trop**, v. 40, n. 5, p. 569–573, 2007.

MONEIM, A. et al. Protective effects of melatonin against oxidative damage induced by Egyptian cobra (*Naja haje*) crude venom in rats. **Acta Tropica**, v. 143, p. 58–65, 2015.

NISHIOKA, S. DE A.; SILVEIRA, P. V. P. *Philodryas patagoniensis* bite and local envenoming. **Revista do Instituto de Medicina Tropical de São Paulo**, v. 36, n. 3, p. 279–281, jun. 1994.

NÚÑEZ, V. et al. Inhibition of the toxic effects of *Lachesis muta*, *Crotalus durissus cumanensis* and *Micrurus mipartitus* snake venoms by plant extracts. **Pharmaceutical Biology**, v. 42, n. 1, p. 49–54, 3 jan. 2004.

OGA, S.; SEKINO, T. Toxicidade e actividade antiinflamatoria de *Tabebuia avellanedae* Lorentz e Griesebach (ipê-roxo). **Revista da Faculdade de Farmacia e Bioquimica de São Paulo**, v. 7, n. 1, p. 47–53, 1969.

OLIVEIRA, C. H. D. M.; SIMÃO, A. A.; MARCUSSI, S. Inhibitory effects of ascorbic acid, Vitamin E, and Vitamin B-complex on the biological activities induced by *Bothrops* venom. **Pharmaceutical Biology**, v. 54, n. 5, p. 845–852, 2016.

OLIVEIRA, C. Z. et al. Anticoagulant and antifibrinogenolytic properties of the aqueous extract from *Bauhinia forficata* against snake venoms. **Journal of Ethnopharmacology**, v. 98, n. 1–2, p. 213–216, abr. 2005.

OSPINA, L. A. F. et al. Antiinflammatory , antioxidant and antibacterial activity of two species of *Tabebuia genus*. **Revista Cubana de Plantas Medicinales**, v. 18, n. 1, p. 34–46, 2013.

OTERO, R., R. J.; FONNEGRA E JIMÉNEZ, S. L. **Plantas utilizadas contra mordeduras de serpientes em Antioquia y Chocó, Colombia**. Medellín: Universidad de Antioquia, 2000.

OTERO, R. et al. Snakebites and ethnobotany in the northwest region of Colombia: Part I: Traditional use of plants. **Journal of Ethnopharmacology**, v. 71, n. 3, p. 493–504, 1 ago. 2000a.

OTERO, R. et al. Snakebites and ethnobotany in the northwest region of Colombia. Part II: Neutralization of lethal and enzymatic effects of Bothrops atrox venom. **Journal of Ethnopharmacology**, v. 71, n. 3, p. 505–511, 1 ago. 2000b.

OTERO, R. et al. Snakebites and ethnobotany in the northwest region of Colombia: Part III: Neutralization of the haemorrhagic effect of *Bothrops atrox* venom. **Journal of Ethnopharmacology**, v. 73, n. 1–2, p. 233–241, 1 nov. 2000c.

PARK, B.-S. et al. Antioxidant activity and characterization of volatile constituents of taheebo (*Tabebuia impetiginosa* Martius ex DC). **Journal of Agricultural and Food Chemistry**, v.

51, p. 295–300, 2003.

PATRÃO-NETO, F. C. et al. Dexamethasone antagonizes the *in vivo* myotoxic and inflammatory effects of *Bothrops* venoms. **Toxicon**, v. 69, p. 55–64, 2013.

PEICHOTO, M. E. et al. Purification and characterization of patagonfibrase, a metalloproteinase showing α-fibrinogenolytic and hemorrhagic activities, from *Philodryas patagoniensis* snake venom. **Biochimica et Biophysica Acta (BBA) - General Subjects**, v. 1770, n. 5, p. 810–819, maio 2007.

PEICHOTO, M. E. et al. Purification and characterization of a cysteine-rich secretory protein from *Philodryas patagoniensis* snake venom. **Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology**, v. 150, n. 1, p. 79–84, jul. 2009.

PEICHOTO, M. E. et al. Inflammatory effects of patagonfibrase, a metalloproteinase from *Philodryas patagoniensis* (Patagonia green racer; Dipsadidae) venom. **Experimental Biology and Medicine**, v. 236, n. 10, p. 1166–1172, out. 2011.

PEREIRA, N. A.; JACCOUD, R. J. S.; MORS, W. B. Triaga brasilica: Renewed interest in a seventeenth-century panacea. **Toxicon**, v. 34, n. 5, p. 511–516, maio 1996.

PRADO-FRANCESCHI, J. et al. The effects of Duvernoy's gland secretion from the xenodontine colubrid *Philodryas olfersii* on striated muscle and the neuromuscular junction: partial characterization of a neuromuscular fraction. **Toxicon: official journal of the International Society on Toxinology**, v. 34, n. 4, p. 459–66, abr. 1996.

PRADO-FRANCESCHI, J. et al. Characterization of a myotoxin from the Duvernoy's gland secretion of the xenodontine colubrid *Philodryas olfersii* (green snake): effects on striated muscle and the neuromuscular junction. **Toxicon: official journal of the International Society on Toxinology**, v. 36, n. 10, p. 1407–21, out. 1998.

PRADO-FRANCESCHI, J.; HYSLOP, S. SOUTH AMERICAN COLUBRID ENVENOMATIONS. **Journal of Toxicology: Toxin Reviews**, v. 21, n. 1–2, p. 117–158, 6 jan. 2002.

RAI, M. K. et al. **Medicinal Plants: Biodiversity and Drugs**. 1st. ed. Boca Raton: CRC Press, 2012.

RANG, H. P.; DALE, M. M. **Farmacologia**. 6ª ed. Rio de Janeiro: Elsevier, 2007.

REIS, F. P. et al. *Tabebuia aurea* decreases inflammatory, myotoxic and hemorrhagic activities induced by the venom of *Bothrops neuwiedi*. **Journal of Ethnopharmacology**, v. 158, n. PART A, p. 352–357, 2014.

RIBEIRO, L. A.; PUORTO, G.; JORGE, M. T. Bites by the colubrid snake *Philodryas olfersii*: a clinical and epidemiological study of 43 cases. **Toxicon: official journal of the International Society on Toxinology**, v. 37, n. 6, p. 943–8, jun. 1999.

RIBEIRO, R. V. et al. Ethnobotanical study of medicinal plants used by Ribeirinhos in the North Araguaia microregion, Mato Grosso, Brazil. **Journal of Ethnopharmacology**, v. 205, n. April, p. 69–102, 2017.

RICE-EVANS, C.; MILLER, N.; PAGANGA, G. Antioxidant properties of phenolic compounds. **Trends in Plant Science**, v. 2, n. 4, p. 152–159, abr. 1997.

ROCHA, M. M. T. et al. Duvernoy's gland secretion of *Philodryas olfersii* and *Philodryas patagoniensis* (Colubridae): Neutralization of local and systemic effects by commercial bothropic antivenom (*Bothrops* genus). **Toxicon**, v. 47, n. 1, p. 95–103, 2006.

ROCHA, M. M. T.; FURTADO, M. D. F. D. Análise das atividades biológicas dos venenos de *Philodryas olfersii* (Lichtenstein) e *P. patagoniensis* (Girard) (Serpentes, Colubridae). **Revista Brasileira de Zoologia**, v. 24, n. 2, p. 410–418, 2007.

ROSENFELD, G. Simptomatology, pathology, and theatment of snake bites in South America. In: BUCHERL, W.; BUCKLEY, E. E.; DEULOFEU, V. (Eds.). **Venoms animals and their venoms**. 2. ed. New York: Academic Press, 1971. p. 687.

S. GIRISH, K.; KEMPARAJU, K. Overlooked issues of snakebite management: Time for strategic approach. **Current Topics in Medicinal Chemistry**, v. 11, n. 20, p. 2494–2508, 1 out. 2011.

SACHETT, J. DE A. G. et al. *Bothrops atrox* snakebite: How a bad decision may lead to a chronic disability: A case report. **Wilderness & Environmental Medicine**, v. 31, n. 3, p. 317–323, set. 2020.

SALGUEIRO, A. C. F. et al. *In vitro* and *in silico* antioxidant and toxicological activities of *Achyrocline satureioides*. **Journal of Ethnopharmacology**, v. 194, p. 6–14, 2016a.

SALGUEIRO, A. C. F. et al. Effects of *Bauhinia forficata* tea on oxidative stress and liver damage in diabetic mice. **Oxidative Medicine and Cellular Longevity**, v. 2016, p. 1–9, 2016b.

SALOMÃO, M. G.; ALBOLEA, A. B. P.; ALMEIDA-SANTOS, S. M. Colubrid snakebite: a public health problem in Brazil. **Herpetological Review**, v. 34, n. 3, p. 307–312, 2003.

SANTOS-COSTA, M. C. et al. Frequência de acidentes ofídicos na região da grande Porto Alegre e cidades próximas, RS, Brasil. **Comunicação do Museu de Ciências e Tecnologia da PUCRS, Série Zoologia**, v. 14, n. 1, p. 89–93, 2001.

SANTOS, R. A. F.; PRUDENCIO, G. D. A. Avaliação da potencialidade antioxidante e citotóxica do "Específico pessoa" encontrado em feira livre de Ji-Paraná (RO). **Multi-Science Journal**, v. 2, n. 2, p. 48, 18 out. 2019.

SCHMID-SCHÖNBEIN, G. W. Analysis of inflammation. **Annual Review of Biomedical Engineering**, v. 8, n. 1, p. 93–151, ago. 2006.

SEBASTIN SANTHOSH, M. et al. *Vipera russelli* venom-induced oxidative stress and hematological alterations: Amelioration by crocin a dietary colorant. **Cell Biochemistry and Function**, v. 31, n. 1, p. 41–50, jan. 2013.

SERAPICOS, E. DE O.; MERUSSE, J. L. B. Morfologia e histoquímica das glândulas de Duvernoy e supralabial de seis espécies de colubrídeos opistoglifodontes (serpentes, Colubridae). **Papéis Avulsos de Zoologia (São Paulo)**, v. 46, n. 15, p. 187–195, 2006.

SILVA, M. C. G. DA; WESLLING, L.; GABRIEL, V. A. Plantas medicinais: rompendo fronteiras étnicas. **Desenvolvimento Regional: Processos, Políticas e Transformações Territoriais**, p. 1–21, 2019.

SILVEIRA, P. V. P.; NISHIOKA, S. DE A. Non-venomous snake bite and snake bite without envenoming in a brazilian teaching hospital: analysis of 91 cases. **Revista do Instituto de**

Medicina Tropical de São Paulo, v. 34, n. 6, p. 499–503, dez. 1992.

SINAN. **Acidente por animais peçonhentos - Notificações registradas no Sistema de Informação de Agravos de Notificação - Sinan Net**. Disponível em: <http://tabnet.datasus.gov.br/cgi/tabcgi.exe?sinannet/animaisp/bases/animaisbrnet>. Acesso em: 8 dez. 2020.

SOGA, F. et al. Serotonin activates human monocytes and prevents apoptosis. **Journal of Investigative Dermatology**, v. 127, n. 8, p. 1947–1955, ago. 2007.

SOUZA, C. M. . et al. Utilização de plantas medicinais com atividade antimicrobiana por usuários do serviço público de saúde em Campina Grande - Paraíba. **Revista Brasileira de Plantas Medicinais**, v. 15, n. 2, p. 188–193, 2013.

SOUZA, V. C.; LORENZI, H. **Botânica sistemática: guia ilustrado para identificação das famílias de Angiospermas da flora brasileira**. Nova Odessa: Instituto Plantarum de Estudos da Flora, 2005.

STRAPAZZON, J. DE O. et al. Systemic oxidative stress in victims of *Bothrops* snakebites. **Journal of Applied Biomedicine**, v. 13, n. 2, p. 161–167, 2015.

SUNITHA, K. et al. Inflammation and oxidative stress in viper bite: An insight within and beyond. **Toxicon**, v. 98, p. 89–97, maio 2015.

TAVARES, A. V. et al. Epidemiology of the injury with venomous animals in the state of Rio Grande do Norte, Northeast of Brazil. **Ciência & Saúde Coletiva**, v. 25, n. 5, p. 1967–1978, maio 2020.

THEAKSTON, R. D. .; WARRELL, D. .; GRIFFITHS, E. Report of a WHO workshop on the standardization and control of antivenoms. **Toxicon**, v. 41, n. 5, p. 541–557, 1 abr. 2003.

USHANANDINI, S. et al. The anti-snake venom properties of *Tamarindus indica* (leguminosae) seed extract. **Phytotherapy Research**, v. 20, n. 10, p. 851–858, out. 2006.

VEIGA JUNIOR, V. F. DA. Estudo do consumo de plantas medicinais na Região Centro-Norte do Estado do Rio de Janeiro: aceitação pelos profissionais de saúde e modo de uso pela população. **Revista Brasileira de Farmacognosia**, v. 18, n. 2, p. 308–313, jun. 2008.

VERPOORTE, R.; MEMELINK, J. Engineering secondary metabolite production in plants. **Current Opinion in Biotechnology**, v. 13, n. 2, p. 181–187, abr. 2002.

VIVIER, E.; MALISSEN, B. Innate and adaptive immunity: specificities and signaling hierarchies revisited. **Nature Immunology**, v. 6, n. 1, p. 17–21, 1 jan. 2005.

WHO. **WHO Guidelines for the Production, Control and Regulation of Snake Antivenom Immunoglobulins**. Geneva: WHO Technical Report Series, 2008.

WRIGHT, A. Recent concepts in the neurophysiology of pain. **Manual Therapy**, v. 4, n. 4, p. 196–202, nov. 1999.

ZAMUNER, S. R. et al. *Bothrops asper* and *Bothrops jararaca* snake venoms trigger microbicidal functions of peritoneal leukocytes in vivo. **Toxicon**, v. 39, n. 10, p. 1505–1513, out. 2001.

ZELANIS, A.; TEIXEIRA DA ROCHA, M. M.; DE FÁTIMA DOMINGUES FURTADO, M.

Preliminary biochemical characterization of the venoms of five Colubridae species from Brazil. **Toxicon**, v. 55, n. 2–3, p. 666–669, 2010.

10. ANEXO 1 - Certificado de Aprovação de protocolo para uso de animais em pesquisa (Artigo 1).

MINISTÉRIO DA EDUCAÇÃO
FUNDAÇÃO UNIVERSIDADE FEDERAL DO PAMPA
(Lei nº 11.640, de 11 de janeiro de 2008)

Pró-Reitoria de Pesquisa

COMISSÃO DE ÉTICA NO USO DE ANIMAIS - CEUA

Fone: (55) 3413 4321, E-mail: ceua@unipampa.edu.br

CERTIFICADO DE APROVAÇÃO DE PROTOCOLO PARA USO DE ANIMAIS EM PESQUISA

Número de protocolo da CEUA: **009/2016**

Título: **Avaliação do potencial antinociceptivo e anti-inflamatório de extratos de *Sida tuberculata* em camundongos**

Data da aprovação: **06.05.2016**

Período de vigência do projeto: 20.05.2017

Pesquisador: Vanderlei Folmer

Campus: **Uruguaiana**

Telefone: 55 39110200

E-mail: vanderleifolmer@unipampa.edu.br

Prof. Dr. Vanusa Manfredini
Coordenadora CEUA/UNIPAMPA

11. ANEXO 2 – Certificado de Aprovação de protocolo para uso de animais em pesquisa (Artigo 2).

MINISTÉRIO DA EDUCAÇÃO
FUNDAÇÃO UNIVERSIDADE FEDERAL DO PAMPA
(Lei nº 11.640, de 11 de janeiro de 2008)

Pró-Reitoria de Pesquisa, Pós-Graduação e Inovação (PROPII)

CEUA
Comissão de Ética em Uso de Animais

COMISSÃO DE ÉTICA NO USO DE ANIMAIS - CEUA

Fone: (55)3911-0200, E-mail: ceua@unipampa.edu.br

CERTIFICADO DE APROVAÇÃO DE PROTOCOLO PARA USO DE ANIMAIS EM PESQUISA/ENSINO

Número de protocolo da CEUA: 055/2019

Título: Avaliação dos efeitos toxicológicos da exposição aguda e crônica do extrato hidroetanólico da casca do ipê-amarelo *Tabebuia chrysotricha* sobre camundongos

Data da aprovação: 16/03/2020

Período de vigência do projeto: 31/12/2020

Pesquisadores(a): Márcio Tavares Costa

Campus: Uruguaiana

Telefone: (55) 3911-0200 Ramal: 9513

E-mail: marciocosta@unipampa.edu.br

CEUA

Finalidade	**( ) Ensino (X) Pesquisa**
Espécie/Linhagem/Raça	Camundongos Swiss
Nº de animais	40
Peso/Idade	30-40 g / 4 semanas
Sexo	Machos
Origem	Biotério de instituição federal

Comissão de Ética em Uso de Animais

Cátia A. Veiverberg

Profª. Drª. Cátia Aline Veiverberg
Coordenadora CEUA/UNIPAMPA

12. ANEXO 3 – Certificado de Aprovação de protocolo para uso de animais em pesquisa (Manuscrito 2).

MINISTÉRIO DA EDUCAÇÃO
FUNDAÇÃO UNIVERSIDADE FEDERAL DO PAMPA
(Lei nº 11.640, de 11 de janeiro de 2008)

Pró-Reitoria de Pesquisa, Pós-Graduação e Inovação (PROPII)

CEUA
Comissão de Ética em Uso de Animais

COMISSÃO DE ÉTICA NO USO DE ANIMAIS - CEUA

Fone: (55)3911-0200, E-mail: ceua@unipampa.edu.br

CERTIFICADO DE APROVAÇÃO DE PROTOCOLO PARA USO DE ANIMAIS EM PESQUISA

Número de protocolo da CEUA: 048/2018

Titulo: Avaliação fitoquímica do ipê-amarelo *Tabebuia chrysotricha* e seu efeito antiofídico frente ao veneno de *Philodryas patagoniensis*

Data da aprovação: 31/01/2019

Período de vigência do projeto: 31/01//2021

Pesquisadores(a): Márcio Tavares Costa

Campus: Uruguaiana

Telefone: (55) 3911-0200 ramal 9513

E-mail: marciocosta@unipampa.edu.br

CEUA

Finalidade	**( ) Ensino (X) Pesquisa**	
Espécie/Linhagem/Raça	Camundongos Swiss	*Philodryas patagoniensis* (cobra papa-pinto)
Nº de animais	20	10
Peso/Idade	30-40 gramas/40-50 dias	indiferente
Sexo	Machos	Machos e Fêmeas
Origem	Biotério	Captura na natureza (protocolo SISBIO nº 45691)

Comissão de Ética em Uso de Animais

Cátia A. Veiverberg

Profª. Drª. Cátia Aline Veiverberg
Coordenadora Substituta CEUA/UNIPAMPA