UNIVERSIDADE FEDERAL DE MINAS GERAIS

ESCOLA DE VETERINÁRIA

TOXICOLOGIA DO FERRO APLICADA A *LEPORINUS FRIDERICI* E DE EXTRATOS VEGETAIS EM *MACROBRACHIUM AMAZONICUM*

TAINÁRA CUNHA GEMAQUE

Belo Horizonte

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TOXICOLOGIA DO FERRO APLICADA A *LEPORINUS FRIDERICI* E DE EXTRATOS VEGETAIS EM *MACROBRACHIUM AMAZONICUM*

Dissertação apresentada ao Programa de Pós-Graduação em Zootecnia da Universidade Federal de Minas Gerais para a obtenção do título de Mestre em Zootecnia.

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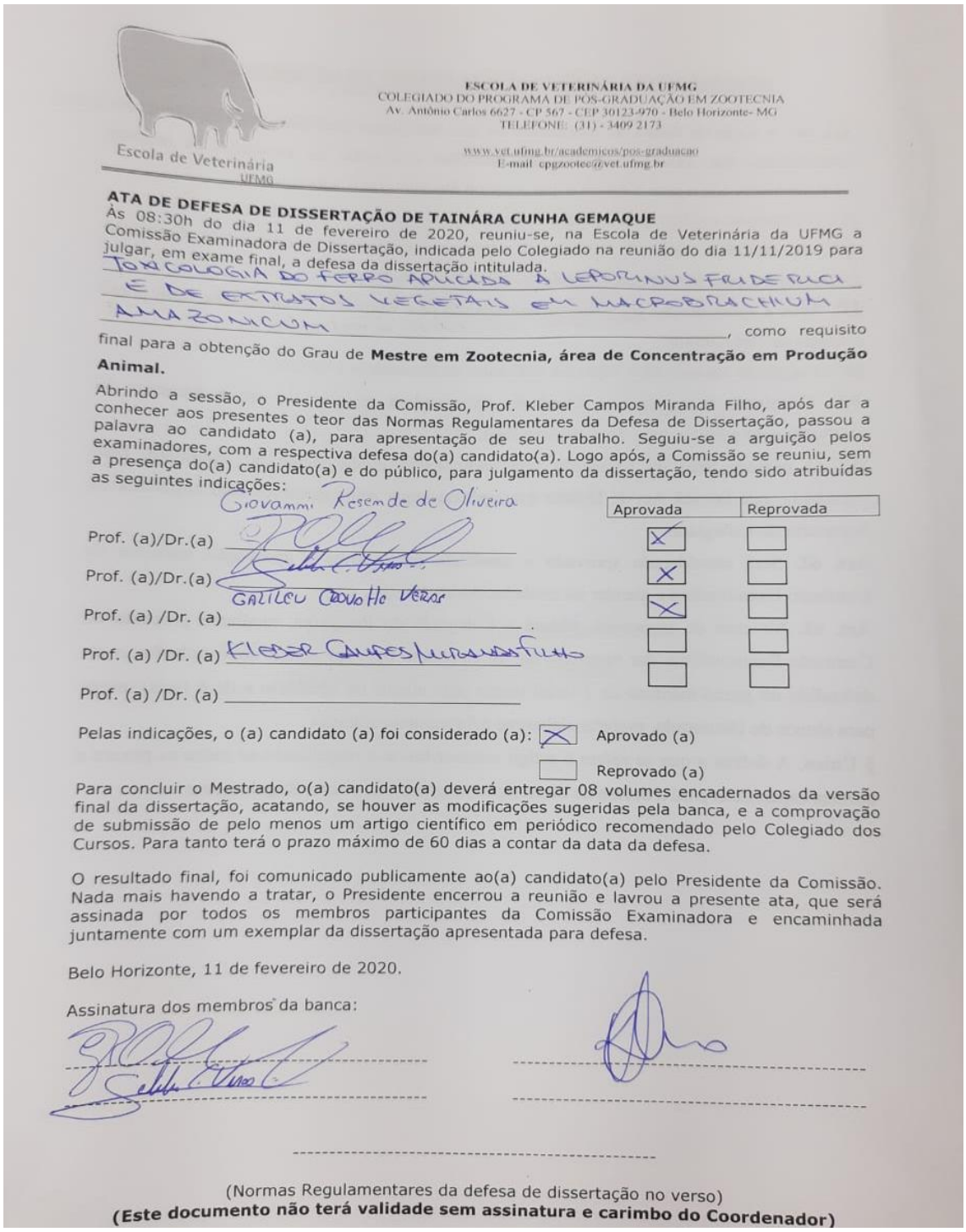

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Às 08:30h do dia 11 de fevereiro de 2020, reuniu-se, na Escola de Veterinária da UFMG a Comissão Examinadora de Dissertação, indicada pelo Colegiado na reunião do dia 11/11/2019 para julgar, em exame final, a defesa da dissertação intitulada.

TOXICOLOGIA DO FERRO APLICADA A LEPORINUS FRIDERICI E DE EXTRATOS VEGETAIS EM MACROBRACHIUM AMAZONICUM

, como requisito final para a obtenção do Grau de **Mestre em Zootecnia, área de Concentração em Produção Animal.**

Abrindo a sessão, o Presidente da Comissão, Prof. Kleber Campos Miranda Filho, após dar a conhecer aos presentes o teor das Normas Regulamentares da Defesa de Dissertação, passou a palavra ao candidato (a), para apresentação de seu trabalho. Seguiu-se a arguição pelos examinadores, com a respectiva defesa do(a) candidato(a). Logo após, a Comissão se reuniu, sem a presença do(a) candidato(a) e do público, para julgamento da dissertação, tendo sido atribuídas as seguintes indicações:

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Belo Horizonte, 11 de fevereiro de 2020.

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Dedico

Em primeiro lugar, a Deus, pela força e coragem durante toda esta longa caminhada.

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Sumário

LIST OF TABLES

LIST OF FIGURES

LISTA DE ABREVIATURAS

ABNT	Associação Brasileira de Normas Técnicas
BO	Batimentos operculares
CL_{50}	Concentração letal mediana
DL	Dose letal
DMSO	Dimetilsulfóxido
DNA	Ácido desoxirribonucleico
FeS_2	Pirita
Fe^{2+}	Íon Férrico
Fe^{3+}	Íon Ferroso
Fe_2O_3	Hematita
$Fe_2O_3H_2O$	Limonita
Fe_3O_4	Magnetita
$FeCO_3$	Siderita
GSH	Glutationa
IEPA	Instituto de Pesquisas Cientificas e Tecnológicas do Estado do Amapá
MeHb	Metahemoglobina

TOXICOLOGIA DO FERRO APLICADA A *LEPORINUS FRIDERICI* E DE EXTRATOS VEGETAIS EM *MACROBRACHIUM AMAZONICUM*

Resumo

Esse trabalho teve como objetivo testar a tolerância de duas espécies aquícolas amazônicas frente ao desafio toxicológico agudo com substâncias oriundas da região Norte do país. Os artigos se pautaram em: Avaliação da toxidade do ferro no peixe tropical *Leporinus friderici*, Toxicidade de extratos de plantas medicinais amazônicas frente ao *Macrobrachium amazonicum*. O presente estudo teve como objetivo determinar a toxicidade de íons de ferro (Fe^{2+} e Fe^{3+}) em juvenis de piau (*Leporinus friderici*), por meio de testes de toxicidade aguda, observação do batimento opercular e hematologia. Foram utilizados 88 espécimes de *L. friderici* divididos em 11 tratamentos: controle; 1 mg / L; 3 mg / L; 7,5 mg / l; 15 mg / L e 30 mg / L de íons Fe^{2+} e Fe^{3+}, obtidos a partir de soluções tamponadas. Parâmetros biométricos e fisiológicos de peixes (0-96 h após intoxicação), batimento opercular por um minuto na primeira hora de exposição e parâmetros sanguíneos foram avaliados. Os resultados indicam uma variabilidade dos efeitos tóxicos, os dois íons de ferro eram tóxicos para o piau, pois ambos na concentração de 30 mg / L eram 100% letais para os organismos expostos. O Fe^{3+} causou letalidade total também nas concentrações de 15 mg / L e 7,5 mg / L. Na concentração de 1 mg / L e 3 mg / L Fe^{3+}, os peixes foram tolerantes e não ocorreu mortalidade. Em concentrações de 1 mg / L; 3 mg / L; 7,5 mg / L e 15 mg / L, Fe^{2+} também não mostraram mortalidade, diminuição dos níveis de glutationa (GSH) e aumento dos níveis de hemoglobina e metahemoglobina (MeHb) nos dois grupos, com maiores alterações nos grupos Fe^{3+} em relação à hemoglobina e MeHb. Alterações observáveis nos níveis de GSH ocorreram em animais expostos ao Fe^{2+}. Os dois íons de ferro (Fe^{2+} e Fe^{3+}) eram muito tóxicos para os juvenis de “piau”. As plantas medicinais da Amazônia são comercializadas há décadas, mas poucos estudos científicos comprovam sua eficácia e segurança no uso em atividades de aquicultura. O objetivo do presente estudo foi utilizar o camarão amazônico *Macrobrachium amazonicum* para prever a toxicidade dos extratos naturais de nove plantas medicinais: pariri *Arrabidaea chica*, sacaca *Croton cajucara*, muirapuama *Ptychopetalum olacoides*, anauerá *Licania macrophylla*, barbatimão *Ouratea hexasperma*, faveira *Vatairea guianensis*, jacareúba *Calophyllum brasilliense*, pau d'arco *Tabebuia sp*. e verônica *Dalbergia subcymosa*, nas concentrações de 1, 10, 100, 500 e

1000 µg / mL. O meio foi preparado em dimetilsulfóxido (DMSO) 0,5%, diluído com água. Foram adicionadas 10 pós-larvas (0,5 ± 0,1 g) a cada triplicado e, após 24 h, as mortalidades foram avaliadas, com os resultados expressos em CL_{50} utilizando o método estatístico Probit. Os resultados dos testes de toxicidade agudam indicam variabilidade nos efeitos tóxicos de plantas medicinais, com o *Tabebuia* sp. (CL_{50} = 758,31 µg / mL) e as *C. cajucara* e *V. guianensis* mais tóxicas (CL_{50} = 72,16 e 75,23 µg / mL), respectivamente. Os extratos demonstraram letalidade contra *M. amazonicum*, que prevê toxicidade e alerta para seu uso como fitoterápicos.

Palavras Chaves: Amazônia, Aquicultura, Plantas medicinais, Ferro, Toxicidade.

ABSTRACT

This work aimed to test the tolerance of two Amazonian aquaculture species in face of the acute toxicological challenge with substances from the northern region of the country. The articles were based on: Evaluation of iron toxicity in tropical fish *Leporinus friderici*, Toxicity of extracts from Amazonian medicinal plants against *Macrobrachium amazonicum*. The present study aimed to determine the toxicity of iron ions (Fe^{2+} and Fe^{3+}) in juvenile piau (*Leporinus friderici*), by means of acute toxicity tests, observation of the opercular beat and hematology. 88 specimens of *L. friderici* were used, divided into 11 treatments: control; 1 mg / L; 3 mg / L; 7.5 mg / L; 15 mg / L and 30 mg / L of Fe^{2+} and Fe^{3+} ions, obtained from buffered solutions. Biometric and physiological parameters of fish (0-96 h after intoxication), opercular beat for one minute in the first hour of exposure and blood parameters were evaluated. The results indicate a variability of the toxic effects, the two iron ions were toxic to piau, since both at the concentration of 30 mg / L were 100% lethal for the exposed organisms. Fe^{3+} also caused total lethality at concentrations of 15 mg / L and 7.5 mg / L. At the concentration of 1 mg / L and 3 mg / L Fe^{3+}, the fish were tolerant and there was no mortality. In concentrations of 1 mg / L; 3 mg / L; 7.5 mg / L and 15 mg / L, Fe^{2+} also showed no mortality, decreased levels of glutathione (GSH) and increased levels of hemoglobin and methaemoglobin (MeHb) in both groups, with greater changes in the Fe^{3+} groups in relation to hemoglobin and MeHb. Observable changes in GSH levels occurred in animals exposed to Fe^{2+}. The two iron ions (Fe^{2+} and $Fe3^{+}$) were very toxic for juveniles of "piau". Medicinal plants from the Amazon have been commercialized for decades, but few scientific studies prove their effectiveness and safety in use in aquaculture activities. The objective of the present study was to use the Amazonian shrimp *Macrobrachium amazonicum* to predict the toxicity of the natural extracts of nine medicinal plants: pariri *Arrabidaea chica*, sacaca *Croton cajucara*, muirapuama *Ptychopetalum olacoides*, anauerá *Licania macrophylla*, barbatimão *Ouratea hexasperma*, faveira *Vatairea guianensis*, jacareúba *Calophyllum brasa Tabebuia* sp. and verônica *Dalbergia subcymosa*, in concentrations of 1, 10, 100, 500 and 1000 µg / mL. The medium was prepared in 0.5% dimethyl sulfoxide (DMSO) diluted with water. 10 post-larvae (0.5 ± 0.1 g) were added to each triplicate and, after 24 h, mortality was evaluated, with the results expressed in LC_{50} using the Probit statistical method. The results of acute toxicity indicate variability in the toxic effects of medicinal plants, with *Tabebuia* sp. (LC_{50} = 758.31 µg / mL) and the most toxic *C. cajucara* and *V.*

guianensis (LC_{50} = 72.16 and 75.23 µg / mL), respectively. The extracts demonstrated lethality against *M. amazonicum*, which predicts toxicity and warns of its use as herbal medicines.

Keywords: Amazon, Aquaculture, Medicinal plants, Iron, Toxicity.

TOXICOLOGIA DO FERRO APLICADA A *LEPORINUS FRIDERICI* E DE EXTRATOS VEGETAIS EM *MACROBRACHIUM AMAZONICUM*

1. INTRODUÇÃO

A região amazônica é considerada um dos ambientes mais ricos em diversidade biológica do planeta devido à sua extensa área geográfica e ao mosaico diversificado de habitats, que inclui: rios, igarapés, igapós, terras firmes, várzeas, savanas, manguezais, entre outros (PIMENTEL, 2003). Dentro dessa diversidade, os crustáceos e peixes ocupam quase todos os hábitats aquáticos desde lagoas, rios, igarapés, lagoas salinas, fontes térmicas e lagos profundos (VIEIRA, 2003).

A aquicultura é a atividade agropecuária que mais cresce no Brasil, produzindo importantes fontes de proteínas para consumo humano. Dentre as atividades desenvolvidas pela aquicultura, destaca-se a carcinicultura (produção de crustáceos) com grande crescimento mundial e representatividade na aquicultura brasileira, tendo o *gênero Macrobrachium* como o mais cultivado em água doce no Brasil (KUBITZA, 2015). Ainda dentro da atividade aquícola, a piscicultura é praticada há muito tempo, existindo registros de que os chineses já a praticavam há vários séculos antes de nossa era. De forma semelhante, os egípcios já criavam a tilápia-do-Nilo há 4000 anos. No Brasil, a maior parte das atividades relacionadas à piscicultura ocorre em propriedades rurais, em tanques escavados, tanque-rede na grande maioria, em fazendas dotadas de açudes ou represas (ZACARDI et al., 2017).

A espécie *Macrobrachium amazonicum* apresenta uma ampla distribuição geográfica na América do Sul, estendendo pela bacia do Rio Orinoco, bacia do rio Amazonas e bacia do rio Paraguai (HOLTHUIS, 1952). É chamado popularmente de camarão regional, camarão canela, camarão sossego, camarão de água doce, e camarão-da-Amazônia (VALENTI, 1996). Apresentam importância comercial na região Norte, sendo explorado comercialmente nos Estados do Amazonas, Pará e Amapá em especial pela pesca artesanal e populações tradicionais (LIMA et al., 2014). Segundo Moraes-Valenti e Valenti (2010), essa espécie apresenta significativo valor de mercado, atendendo às necessidades alimentícias e econômicas da população.

Leporinus friderici, é uma espécie da família Anostomidae. O gênero *Leporinus* é considerado um dos mais ricos dentre os Characiformes, com grande potencial na piscicultura (GARAVELLO e BRITSKI, 2003). É uma espécie com ampla distribuição na América do sul, principalmente na região amazônica com boa aceitação de mercado e com elavado consumo na região Norte do Brasil (SILVA-SANTOS et al., 2018).

A toxicologia é a ciência que estuda os efeitos nocivos decorrentes das interações de substâncias químicas com o organismo (LOMBARDI et al., 2004). Entende-se por Concentração Letal Mediana (CL_{50}), a concentração de uma determinada substância que causa mortalidade em 50% dos animais em teste de toxicidade aguda (LI et al., 2014). Os testes toxicológicos são empregados para se descobrir os efeitos deletérios de poluentes em meios aquáticos e usados também para se testar a toxicidade de compostos presentes nos meios onde os organismos são cultivados.

Entre os metais mais abundantes no planeta encontra-se o ferro que desempenha um papel importante para muitos organismos vivos, desde que em baixas concentrações. Este elemento é vital para a sobrevivência dos seres humanos, mamíferos e peixes, pois é essencial para os múltiplos processos metabólicos como transporte de oxigênio, síntese de DNA, transporte de elétrons e cofator para muitas proteínas (BURY, 2003).

Em 2013, ocorreu um desmoronamento no porto de Santana - Amapá, e toneladas de minérios de ferro foram despejadas acidentalmente no leito do rio Amazonas, causando contaminação na água, bem como distúrbios ambientais aos organismos aquáticos que habitam esta região, que abriga uma diversificada ictiofauna, relativamente pouco conhecida, dada a grande extensão de sua costa e a enorme diversidade de espécies (SILVA, 2014). Esse fato comprometeu a pesca e a aquicultura da região, com efeito sobre as comunidades que vivem dessas atividades.

Muitas espécies capturadas na área do desmoronamento de minério de ferro são utilizadas para o consumo familiar e comercialização no próprio porto onde ocorre a pesca (SILVA, 2014). Devido ao consumo de peixes retirados dessa água contaminada por metais, é necessário averiguar os possíveis danos provocados nesses peixes que são consumidos por seres humanos. Pois os metais pesados tais como o ferro tem efeito cumulativo e podem ser encontrados nos peixes de captura vendidos em mercados (FRASER et al., 2013).

O uso de extratos de plantas vem sendo estudados na aquicultura, dentre as pesquisas realizadas destacam-se os fitoterápicos ou fitomedicamentos no tratamento de doenças e estudos sobre a toxicologia dos extratos (ALBUQUERQUE e HANAZAKI, 2006).

Um dos principais problemas relacionados à ação das plantas medicinais no Brasil é a ideia do que vem da natureza não faz mal, excluindo a possibilidade de uma planta causar uma reação adversa ou efeito tóxico. Toda planta apresenta alguma toxicidade em determinada concentração, porém a denominação de plantas tóxicas se conceitua a todos os vegetais que, através do contato, inalação ou ingestão, acarretam danos à saúde, tanto para o homem como para animais, podendo inclusive levá-los a óbito (MONSENY et al., 2015). Muitas plantas tóxicas são tidas como ornamentais, logo estando presente em diversos ambientes ao nosso redor, por conseguinte facilitando o risco de intoxicação para o homem. Para tanto, os estudos da toxicidade pré-clínica, além da avaliação de segurança e eficácia dos fitoterápicos se torna de extrema importância (HOLLENBACH, 2008), e no caso da aquicultura, para futura aplicação nos organismos aquáticos cultivados.

2. REVISÃO DE LITERATURA

2.1 Toxicidade

Toxicidade é a capacidade inerente e potencial do agente tóxico de provocar efeitos nocivos em organismos vivos. O efeito tóxico é geralmente proporcional à concentração do agente tóxico no sítio de ação (LIMA et al., 2013). A toxicidade aguda é caracterizada pela administração ou exposição da substância química numa dose única (ou múltipla), sendo analisados os efeitos adversos ocorridos nesse intervalo de tempo. Decorre de um único contato (dose única) ou múltiplos contatos (efeitos cumulativos), os efeitos surgem de imediato ou no decorrer de algumas horas ou dias (BORGES, 2018).

O teste de toxicidade aguda estima a concentração letal mediana (CL_{50}) e classifica os agentes tóxicos quanto à periculosidade e possibilitam estabelecer limites permissíveis para várias substâncias químicas, além de avaliar o impacto de misturas de poluentes sobre os organismos aquáticos em testes que simulam as condições naturais em laboratório (ALBINATI et al., 2017).

Com a realização de testes toxicológicos é possível avaliar a segurança ambiental, pois a presença de agentes químicos nos ecossistemas aquáticos representa sempre um

risco aos seres vivos, o risco que um agente químico impõe aos organismos aquáticos é avaliado por meio do julgamento científico da probabilidade de danos que suas concentrações ambientais, conhecidas ou estimadas, podem causar dentro da aquicultura (GUIMARÃES et al., 2014).

2.2 O Ferro

O ferro é um dos metais mais abundantes na Terra, sendo importante para muitos organismos vivos, desde que em baixas concentrações. Este elemento é vital para a sobrevivência dos seres humanos, outros mamíferos e peixes, pois é essencial para os múltiplos processos metabólicos como transporte de oxigênio, síntese de DNA, transporte de elétrons e cofator para muitas proteínas (BURY, 2003). Nos peixes, o ferro presente na água pode ser absorvido pelas brânquias, pele ou por meio da alimentação (SILVA et al., 2018). Quando ultrapassa a quantidade mínima tolerável e necessária para o organismo, este metal torna-se tóxico e até mesmo letal em um curto período (COTTET et al., 2015).

O ferro participa de processos hepáticos e está associado ao transporte de oxigênio através da hemoglobina, sendo considerado um dos elementos mais importantes para homeostase dos peixes. O ferro é mais conhecido na forma de íons de ferro ferroso (Fe^{2+}) e íons de ferro férrico (Fe^{3+}). Nos casos de intoxicação aguda por ferro pode haver a necrose do tecido branquial (SLANINOVA et al., 2014).

Apesar de ser o quarto elemento mais abundante na crosta terrestre, o ferro não se encontra isolado na natureza, mas somente em minérios, sendo que os principais são: hematita (Fe_2O_3), magnetita (Fe_3O_4), siderita ($FeCO_3$), limonita ($Fe_2O_3H_2O$) e pirita (FeS_2) (DE DOMENICO et al., 2013).

Os peixes utilizados em estudos toxicológicos, reagem imediatamente frente a qualquer alteração no ecossistema aquático, através de alterações fisiológicas como mudanças no batimento opercular, natação incomum ao comportamento da espécie, alterações branquiais e mudanças ou dificuldades na alimentação. Tais reações são facilmente notáveis perante intoxicação por substâncias químicas (HUNDLEY et al., 2018).

O uso indiscriminado e a liberação de resíduos de minérios em ambientes aquáticos podem levar a perturbação ambiental que poderia ser considerada como

potenciais fontes de estresse para a biota. Muitos poluentes ambientais, como o ferro, são capazes de induzir o estresse oxidativo em animais aquáticos, incluindo peixes (FRASER, 2013).

Em estudo realizado por Benedito Cecilio et al. (2005), foram avaliadas as variações fisiológicas de *L. friderici* em decorrência de alterações ambientais e contaminação por mercúrio, afetando o crescimento, natação, atividade respiratória e alimentação.

2.3 Contaminação de peixes por ferro

O excesso de ferro dissolvido na água pode causar o acúmulo desse metal nas brânquias de muitos organismos aquáticos, resultando na obstrução branquial e acarretando perturbações respiratórias (ZAHEDI et al., 2014).

Os peixes que consomem dietas com níveis elevados de ferro podem apresentar crescimento reduzido, pior conversão alimentar, rejeição da dieta, mortalidade e danos histopatológicos nas células do fígado (BURY, 2003).

Nos peixes, o local inicial de bioacumulação de metais absorvidos da água é o tecido branquial (ZAHEDI et al., 2014; QIAN et al., 2015). De acordo com o “National Research Council” (NRC, 2011), um dos efeitos da toxicidade do Fe nos peixes é a perda de crescimento e em alguns casos a mortalidade.

Os fatores mais importantes que podem influenciar na absorção do ferro são as proporções do mineral na forma orgânica e inorgânica na ração e na água, a quantidade ingerida e as condições no trato digestório (WATANABE et al., 1997). Considera-se que os efeitos toxicológicos do ferro podem estar relacionados à sua acumulação nos tecidos (CHEN et al., 2012).

2.4 *Leporinus friderici*

Leporinus friderici é um peixe Characiforme pertencente à família Anostomidae, popularmente conhecido no Brasil como "piava" ou "piau-três-pintas" (SILVA-SANTOS et al., 2018). É amplamente distribuído nas bacias hidrográficas do Amazonas e Paraguai, nos países da América do Sul (Suriname, Brasil, Paraguai, Argentina e Uruguai) (GRAÇA e PAVANELLI 2007). Sua migração é sazonal e de curta distância (AGOSTINHO et al. 2003).

O *L. friderici* necessita da corrente fluvial para ter acesso a um local de reprodução e de outro local para alimentação, crescimento e engorda (NOMURA, 1970). Essa espécie apresenta fecundação externa, com período reprodutivo de novembro a fevereiro (GARAVELLO, 1979). Possui aceitação para pesca esportiva e apresenta elevada qualidade de carne (ANDRADE e VIDAL, 1991), características importantes para a pesca comercial e de subsistência (VAZ et al., 2000). O comprimento médio de primeira maturação varia entre 11,5 a 32,0 cm em fêmeas e 11,5 a 22,5 cm em machos (NOMURA, 1970; LOPES et al., 2000; RÊGO, 2008).

2.5 Os extratos vegetais

O uso de compostos bioativos oriundos de extratos vegetais na aquicultura está se tornando cada vez mais comum, sendo utilizados como uma alternativa ao tratamento com antibióticos como é o caso da planta amendoeira (*Terminalia catappa*) (FUJIMOTO, 2012). Haja vista que produz um menor impacto ambiental, reduz a quantidade de resíduos químicos nos animais, por isso a necessidade de se estudar a toxicidade de extratos aos organismos cultivados antes de iniciar seu uso na aquicultura, pois possuem diversas propriedades biológicas capazes de impedir o crescimento e a disseminação de patógenos quando utilizados na concentração errada (SANTOS, 2013).

2.6 *Macrobrachium amazonicum*

O camarão-da-Amazônia é uma espécie de ampla distribuição geográfica, ocorrendo desde o Equador até a Argentina, passando pela Venezuela e com distribuição em vários estados brasileiros (e.g. Amapá, Amazonas, Pará, Maranhão, Piauí, Ceará, Rio Grande do Norte, Paraíba, Pernambuco, Mato Grosso, Paraná, Acre, Goiás e Mato Grosso do Sul) (MAGALHÃES et al., 2003).

A espécie é caracterizada morfologicamente pela presença de um rostro longo com espinhos muito curtos. Quando vivos, apresentam-se transparentes, quase incolores. Os machos adultos são geralmente maiores que as fêmeas, embora não haja um consenso na literatura, e apresentam o cefalotórax e o segundo par de quelípodes proporcionalmente mais desenvolvidos. No segundo par de pleópodes observa-se uma estrutura alongada, chamada de petasma (apêndice masculino), que é adjacente ao apêndice interno e é usado durante a cópula. As fêmeas adultas apresentam o segundo par de quelípodes proporcionalmente menor e com poucos espinhos, como relatado por Magalhães e Walker

(1988). As pleuras dos segmentos abdominais das fêmeas são mais compridas e arcadas para fora, formando a câmara incubadora (GOMES CORRÊA, 1977).

Algumas espécies do gênero *Macrobrachium*, dentre as quais *M. amazonicum*, reproduzem-se durante todo o ano, o que torna a espécie vantajosa para produção comercial (VALENTI et al., 1986).

Atualmente *M. amazonicum* é uma espécie com alta produtividade pela pesca e recria na região norte do Brasil (MARQUES et al., 2012), sendo estudada para sua introdução na aquicultura devido às suas características favoráveis, sua aceitação de mercado e introdução em outras regiões fora da sua região de origem, ou seja, a bacia Amazônica (KUTTY et al., 2000; NEW 2005; MACIEL e VALENTI, 2009; SILVA et al., 2017).

2.7 Região Amazônica

A região amazônica abriga diversos organismos aquáticos, como peixes e camarões. Devido à sua extensa área (com diversos habitats) pode favorecer o desenvolvimento de espécies aquícolas, apresentando grande potencial para expansão da aquicultura, sobretudo pela elevada demanda regional por pescado (REIS et al., 2016).

2.8 Considerações

A toxicidade é um fator limitante para o desenvolvimento e sobrevivência de peixes e camarões, os resíduos de ferro presentes no meio aquático são consequências de atividades humanas como a atividade mineradora.

Os compostos bioativos de extratos de plantas necessitam ser avaliados quanto a sua toxicidade para posterior uso na aquicultura, sendo assim é importante conhecer a quantidade tolerável das substâncias testadas para estes animais.

3. OBJETIVOS

3.1 Objetivo geral

Avaliar a tolerância de espécies aquícolas amazônicas frente ao desafio toxicológico agudo com substâncias presentes na região Norte do país.

3.2 Objetivos específicos

Testar a tolerância do piau (*Leporinus friderici*) frente à exposição ao ferro.

Testar a tolerância do camarão da amazônia (*Macrobrachium amazonicum*) à exposição a nove extratos de plantas medicinais.

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5. ARTICLE 1

Evaluation of Iron Toxicity in the Tropical Fish *Leporinus friderici*

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641 **Evaluation of Iron Toxicity in the Tropical Fish *Leporinus friderici***

642

643 **Abstract**

644 The present study aimed to determine the toxicity of iron ions (Fe^{2+} and Fe^{3+}) in juveniles
645 of "piau" (*Leporinus friderici*), by means of acute toxicity tests, observation of the
646 opercular beat and hematology. We used 88 specimens of *L. friderici* divided into 11
647 treatments: control; 1 mg/L; 3 mg/L; 7.5 mg/L; 15 mg/L and 30 mg/L of Fe^{2+} and Fe^{3+}
648 ions, obtained from buffered solutions. Biometric and physiological parameters of fish
649 (0-96 h after intoxication), opercular beating for one minute in the first hour of exposure
650 and blood parameters were evaluated. The results indicate a variability of the toxic effects,
651 the two iron ions were toxic to "piau", since both in the concentration of 30 mg/L, were
652 100% lethal to the exposed organisms. Fe^{3+} caused total lethality also at concentrations
653 of 15 mg/L and 7.5 mg/L. At the concentration of 1 mg/L and 3 mg/L Fe^{3+}, the fish were
654 tolerant and no mortality occurred. At concentrations of 1 mg/L; 3 mg/L; 7.5 mg/L and
655 15 mg/L, Fe^{2+} also showed no mortality, decreased glutathione levels (GSH), and
656 increased levels of hemoglobin and methaemoglobin in both groups, with higher changes
657 in Fe^{3+} groups in relation to hemoglobina and and methaemoglobin .Obervable chnages
658 in GSH levels ocurred in animais exposed to Fe^{2+}. The two ion (Fe^{2+} and Fe^{3+}) were toxic
659 to the ''piau'' juveniles.

660

661 Keywords: Toxic Effects; "piau"; Lethality

662

Introduction

Toxicity is the inherent and potential capacity of the toxic agent to cause harmful effects on living organisms Borges [1]. The toxic effect is generally proportional to the concentration of the toxic agent at the site of action Klüver et al. [2]. Acute toxicity is characterized by the administration or exposure of the chemical in a single or multiple dose in a short time (hours), analyzing the adverse effects occurring within that time interval Albinati et al. [3]. The effects are due to a single contact (single dose) or multiple contacts (cumulative effects), arising immediately or over the course of a few hours or days Oga [4]. The acute toxicity test estimates the median lethal concentration (LC_{50}) and classifies hazardous substances intoxicants and allows the establishment of permissible limits for various chemical substances, as well as assessing the impact of mixtures of pollutants on aquatic organisms of water bodies by tests simulating the natural conditions in the laboratory Qu et al. [5]. Metals from mining may be responsible for contamination of aquatic environments. Some metals, such as iron, zinc, magnesium, cobalt, are useful in small amounts as they form some cell structures. But if the limiting amount of these metals is exceeded, they will become toxic, causing problems to organisms exposed to these metals Teixeira & Bessa [6]. The element iron is considered a heavy metal being found in high concentrations in aquatic environments near the miners that exploit this ore. In Brazil, the dam located in the municipality of Mariana - MG was built to serve as a deposit of the wastes generated during the mining process of iron, and ruptured, causing an unprecedented environmental disaster in Brazil's history Barba [7]. Iron is also regarded as an important chemical element for many living organisms (as a biometal) and is vital for survival at low concentrations as it is essential for multiple metabolic processes such as oxygen transport, DNA synthesis, electron transport, and cofactor for many proteins Bury [8]. Iron present in water can be absorbed by the fish via gills, skin or food Cottet et al. [9].

Excess iron dissolved in the water can cause the formation of flakes of this metal in the gills of the fish resulting in its obstruction, causing respiratory disorders (Zahedi 2014). Animals that consume diets with high levels of iron may have reduced growth, worse feed conversion, diet rejection, mortality, and histopathological damage in liver cells, where excess of iron in the body is stored Bury [8]. Fish can be used as environmental indicators, reacting immediately to any changes in the aquatic ecosystem, through physiological changes such as changes in opercular beating, unusual swimming,

696 gill changes and feeding changes or difficulties Hundley et al. [10]. Such reactions are
697 easily noticeable due to chemical intoxication Niencheski et al. [11]. The observable
698 effects in the face of poisoning are various. Opercular beats are responses to changes in
699 the aquatic environment, such as contamination by substances with toxic potential Gibson
700 & Mathis [12]. Blood also has elements that respond to exposure to pollutants. The same
701 is responsible for the transport of substances in vertebrates, this tissue being directly
702 linked to physiological dynamics and the body's immune responses. The study of its
703 cellular components, especially the study of glutathione (GSH), hemoglobin (Hb) and
704 methaemoglobin (MeHb), can provide important information about stress and innate
705 immunity of fish Maciel et al. [13]. Some metal ions (especially copper, zinc and iron)
706 can bind to proteins involved in neurodegeneration. It is known that iron is responsible
707 for several neurodegenerative pathologies in humans such as Alzheimer's and
708 Parkinson's disease Koslowski et al. [14,15]. These diseases are triggered by genetic
709 predisposition, aging and by environmental factors such as exposure to heavy metals Tan
710 et al. [16]. Fish are important sources of dietary iron for the human organism, contributing
711 to the formation of hemoglobin; however, over accumulation of iron ions in the human
712 body by ingestion of foods with excess of this element may promote neurodegeneration
713 Wojtunik-Kulesza et al. [17]. In this way, the aquaculture activity carried out by riverine
714 communities in water bodies affected by iron pollution can be alarming. The "piau"
715 Leporinus friderici (Characiformes, Anostomidae) is one of the most widely distributed
716 species in the Neotropical region. In addition, it is considered to be one of the most
717 abundant species in the various water systems of the Amazon region with relevant
718 economic importance Olivatti et al. [18]. The species of the genus Leporinus present great
719 potential for fish farming because they have good commercial acceptance Baldisserotto
720 & Gomes et al. [19]. Sexual maturation occurs when the individual reaches approximately
721 20 cm in total length, concentrating his reproductive period in the months of November
722 to February. The "piau" performs seasonal reproductive migration (piracema). There is
723 demand for *L. friderici* fingerlings for aquaculture and repopulation programs in rivers
724 and reservoirs in much of the national territory Suplicy [20]. It is important to consider
725 the risk of contamination of fish such as the "piau" with iron ions and the possibility of
726 bioaccumulation and transfer to the human body through feeding. The presente study
727 aimed to determine the toxicity of iron ions (Fe^{2+} and Fe^{3+}) in "piau" (*L. friderici*)
728 juveniles by means of acute toxicity tests, opercular beating and blood parameters.

Material and Methods

The experiments were carried out in the ichthyology laboratory of the Institute of Scientific and Technological Research of the State of Amapá - IEPA. Eighty eight specimens of "piau" (*L. friderici*) with an average weight of 1.70 ± 0.68 g were used. All animals were measured with digital caliper QS-50 (Jakemy, China), weighed in precision digital scale 0.01 g BL series (Shimadzu do Brasil Comércio Limitada, Barueri, SP, Brazil). The fish were purchased at a fisherman's shop, acclimated for a week and fed daily (08:00 and 17:00 h). Water with an average temperature of 27°C ± 1.0 and measured using the mercury thermometer (Accumed Produtos Médico Hospitalares Ltda., Duque de Caxias, RJ, Brazil). The pH presented an average value of 7.25 ± 0.75, using a HI 98129 digital parameter (Hanna Instruments Inc, Woonsocket Rhode Island, USA). Dissolved oxygen concentrations were always close to saturation. Feeding was suspended 24 h before the start of the experiment, according to the recommendations of the Brazilian Association of Technical Standards - ABNT ABNT [21] for acute fish toxicity tests. The water employed in the experimental media was treated with AquaSafe (water chlorine remover) and parasite controller Labcon Ictio (Alcon) to avoid parasites in the animals. The treatments were carried out in 2 L beakers (one fish per beaker) with aeration (24 h) with the use of compressor pumps for aquariums (model U-2800, Boyu). The three "piau" specimens were divided into 11 groups and added iron in the water for the test with buffer solutions of ferrous sulphate and ferric sulphate and maintained for up to 96 h. The concentrations tested were: control (without addition of iron); 1 mg/L Fe2+ and Fe^{3+}; 3 mg/L Fe^{2+} and Fe^{3+}; 7.5 mg/L Fe^{2+} and Fe^{3+}; 15 mg/L Fe^{2+} and Fe^{3+}; 30 mg/L Fe^{2+} and Fe^{3+}, the same tests were performed for each concentration, each fish being one replicate (n = 8). For the analysis of the effects caused by exposure to Fe, the opercular beats (OB) were observed. At this time, the specimens were kept individually in a beaker for one minute before and after submission to the treatments cited for the period of one hour where OB counts were performed per minute. Mortality was also assessed throughout the experiment. For the collection of blood, the animals were collected still dying, avoiding blood clotting. To determine the GSH, 50 microliters of whole blood were collected from caudal venous puncture with the use of heparinized capillaries, diluted 60 times in 3 mL cuvette, for further reading in a spectrophotometer at 412 nm. The determination of hemoglobin was performed according to the methodology described by Clark et al. [22]. Blood samples were collected from the dying fish and analyzed immediately in a

spectrophotometer at 540 nm. Methemoglobin levels (MeHb) were determined according to Benesch et al. [23], also with the use of capillaries with heparin, with subsequent reading made at 630 nm in a spectrophotometer. The data were submitted to non-parametric ANOVA by the Kruskal-Wallis test by the INFOSTAT version 2017 program Casanoves et al. [24].

Results and Discussion

The results of the iron ion toxicity in juveniles of *L. friderici* indicated variability of the toxic effects. Fe^{3+} showed toxicity at lower concentrations and at shorter exposure times in comparison with Fe^{2+}. Total lethality was observed after 3 h of exposure at concentrations of 7.5 mg/L; 15 mg/L and 30 mg/L Fe^{3+}. Fe^{2+} ions showed lower toxicity, but the total lethality was observed in 24 h of exposure at 30 mg/L Fe^{2+} concentration. At concentrations of 1 mg/L; 3 mg/L; 7.5 mg/L and 15 mg/L Fe^{2+}, no mortalities were observed after 96 h of exposure (Table 1). It was observed that for 30 mg/L Fe^{2+} (1h = 112.25 ± 1.96 OB/min, 24h = total lethality); 15 mg/L Fe^{2+} (1h = 97.88 ± 2.17 OB/min); 7.5 mg/L de íons Fe^{2+} (1h = 87.38 ± 3,62 OB/min; 96h = no lethality), 3 mg/L Fe^{2+} (1h = 83.63 ± 13.3 OB/min; 96h = no lethality.

Table 1: Mortality in "piau" juveniles after acute exposure to iron ions (Fe^{2+} and Fe^{3+}) in water for 96 hours.

Total nominal concentration of iron ions (mg/L)	n	Mortality (%)
Fe^{2+}		
0 (control)	8	0 ± 0.0
1	8	0 ± 0.0
3	8	0 ± 0.0
7.5	8	0 ± 0.0
15	8	0 ± 0.0
30	0	100 ± 0.0
Fe^{3+}		
0 (control)	8	0 ± 0.0
1	8	0 ± 0.0
3	8	0 ± 0.0
7.5	0	100 ± 0.0
15	0	100 ± 0.0
30	0	100 ± 0.0

Tests that presented the highest lethality were those performed with Fe $^{3+}$. It was observed that at 30 mg/L Fe^{3+} (1h = 128.25 ± 3.41 OB/min; 3h = total lethality); 15 mg/L Fe^{3+} (1h = 98.50 ± 3.63 OB/ min; 3h = total mortality); 7.5 mg/L Fe^{3+} (1h = 94.38 ± 5.73 OB/ min; 3h = total lethality); 3 mg/L Fe^{3+} (1h = 84.50 ± 2.88 BO/min; 96h = no lethality); 1 mg/L Fe^{3+} (1h = 79.88 ± 2.03 OB/min; 96h = no lethality). Iron is an element considered

786 to be of low toxicity at concentrations below 3 mg/L and can cause harmful effects to fish
787 in their soluble form (Fe^{2+} or Fe^{3+}), as demonstrated by Geertz Hansen & Rasmussen [25].
788 According to these authors, iron soluble at concentrations higher than 0.5 mg/L caused a
789 significant decrease in survival in trout larvae (*Salmo trutta*).

790 In the present study, lethality was lower when fish was exposed to Fe^{2+}, 30 mg/L
791 (24h = total lethality); 15 mg/L (24h = no lethality); 7.5 mg/L (96h = no lethality); 3 mg/L
792 (96h = no lethality). Studies show that other species of fish are tolerant to these
793 concentrations of iron ions, such as zebra fish Danio rerio. According to Chua et al. [26]
794 D. rerio has accumulated iron in the liver when exposed to 50 mg/L, suggesting that this
795 metal could be accumulated in this organ. Significant increases were observed when
796 compared to the control group (92.00 ± 05.95 BO/min), to the groups intoxicated with 30
797 mg/L of Fe^{3+} and Fe^{2+} ($p<0.05$) in the first hour (while all the specimens were still alive)
798 (Table 2).

799 Table 2: Opercular beating in "piau" juveniles after acute exposure to iron ions (Fe^{2+} and
800 Fe^{3+}) in the water during the first hour of testing. Total nominal concentration of iron ions
801 (mg/L).

Total nominal concentration of iron ions (mg/L)	Opercular beats / minute
Fe^{2+}	
0 (control)	92.00 ± 05.95^{bc}
1	78.50 ± 1.60^{a}
3	83.63 ± 13.30^{ab}
7.5	87.38 ± 3.62^{ab}
15	97.88 ± 2.17^{cd}
30	112.25 ± 1.96^{d}
Fe^{3+}	
0 (control)	92.00 ± 5.95^{bc}
1	79.88 ± 2.03^{a}
3	84.50 ± 2.88^{ab}
7.5	94.38 ± 5.73^{bc}
15	98.50 ± 3.63^{cd}
30	128.25 ± 3.41^{d}

802

803 Different letters in the same column showed significant differences $p<0.05$ by the Kruskal-Wallis test.

804 The increase in opercular beats was higher in the groups contaminated with Fe^{3+}
805 ions, since this was the most toxic ion. With the increase of the concentrations to 7.5
806 mg/L, 15 mg/L and 30 mg/L Fe^{3+}, the beats changed, because, with the increase of this
807 ion in water, dissolved oxygen (DO) decreased, causing the animals to keep their

808 swimming altered in search of oxygen, provoking a growing stress in the organism since
809 the search for oxygen increases along with BO. The liver, gills and skin are the major
810 sites of iron deposition and storage under conditions of overload, the liver metabolizes
811 excess iron in the plasma and stores it in the form of ferritin and hemosiderin Chua et al.
812 [26]. The variation in the number of opercular beats is related to the concentrations of
813 Fe^{2+} and Fe^{3+} from which *L. friderici* was submitted. At low concentrations 3 mg/L and 1
814 mg/L of the two ions, there was little oscillation in the beats, because they are
815 concentrations in which the organism can survive and make sufficient withdrawal of DO
816 from the water to supply its needs Watanabe et al. [27]. Bury et al. [28] concluded that
817 the ferrous form (Fe^{3+}) is more toxic than the ferric form (Fe^{2+}) for European plaice
818 (Platichtys flesus) and iron uptake occurred mostly in the final part of the intestine. The
819 most important factors that may influence iron uptake are the proportions of the mineral
820 in organic and inorganic form (in feed and water), the amount ingested and the
821 physiological conditions of the digestive tract Watanabe et al. [27]. In fish, the initial site
822 of accumulation of metals absorbed from water is the gill tissue Zahedi et al. According
823 to NRC [29], one of the effects of Fe toxicity on fish is loss of growth. Glutathione levels
824 decreased in the contaminated groups when compared to the control group (Figure 1).
825 The greatest decrease in GSH occurred in the group contaminated with Fe^{2+}, the time of
826 exposure in these groups was higher, because it was where the greatest survival occurred
827 during the 96 h of experiment, occurring only in the group of 30 mg/L Fe^{2+} in 24 h. In the
828 groups contaminated with Fe^{3+}, there was a lower decrease in GSH levels, since the
829 exposure time was lower, that is, in less than 24 h there were total lethality in the groups
830 of 30 to 3 mg/L of Fe^{3+}.

831

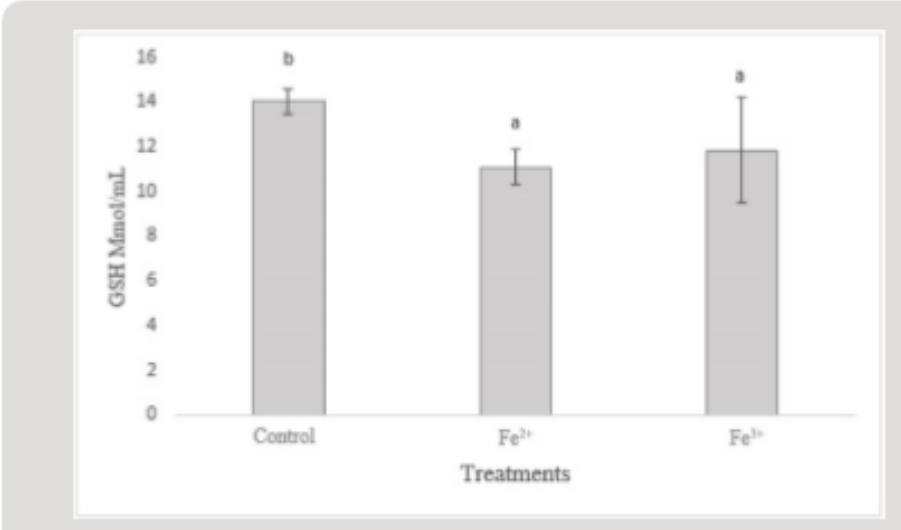

Figure 1: Levels of glutathione (GSH) in *L. friderici* exposed to iron ions (Fe^{2+} and Fe^{3+}).

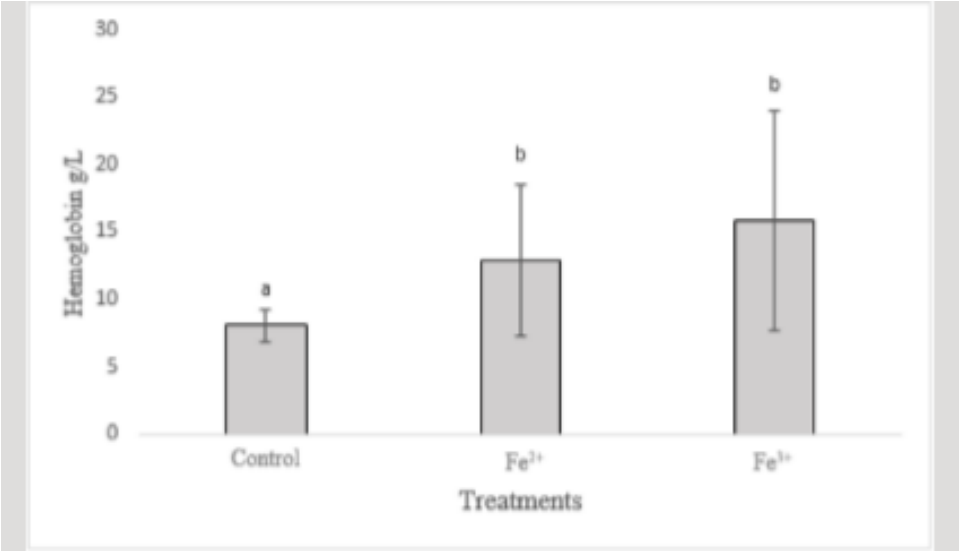

Figure 2: Levels of hemoglobin in *L. friderici* exposed to iron ions (Fe^{2+} and Fe^{3+}).

GSH can be considered one of the most important agents in the defense of cells against oxidative stress and plays a central role in the biotransformation and elimination of pollutants. This molecule has a reducing role in many reactions of peroxides and free radicals Huber et al. [30]. According to Van Der Oost et al. [31], the decrease in GSH concentration would indicate acute stress, affecting the immune system, the nervous system, and the gastrointestinal system. There was an increase in hemoglobin levels in the contaminated groups in relation to the control group (Figure 2). It is noted that increased hemoglobin may be associated with increased energy/oxygen demand by the body, which usually occurs in acute stress situations. In the groups contaminated with Fe^{3+}, the hemoglobin increase was more relevant, due to the toxicity of this contaminant

being greater and causing damage to the organism in a shorter time. With the Fe^{2+} contaminated groups, there was also an increase, but smaller when compared to the Fe^{3+} treatments. Fish exposed to Fe ions have demonstrated a functional increase in blood hemoglobin levels, as Hb is converted to MeHb, causing intoxication in animals Aggergaard et al. [32]. Iron is involved in hepatic processes, and is also associated with oxygen transport through Hb, being considered one of the most important elements for fish homeostasis (Neves 2016). However, excess iron dissolved in the water can cause iron flakes to form in the gills of the fish resulting in their obstruction, causing respiratory disorders BURY et al. [8] and the increase of OB to overcome this provoked deficiency. In acute cases of iron poisoning there may be necrosis of the gill tissue and loss of ammonia excretion capacity capacity that is concentrated in the blood of the fish Slaninova et al. [33].

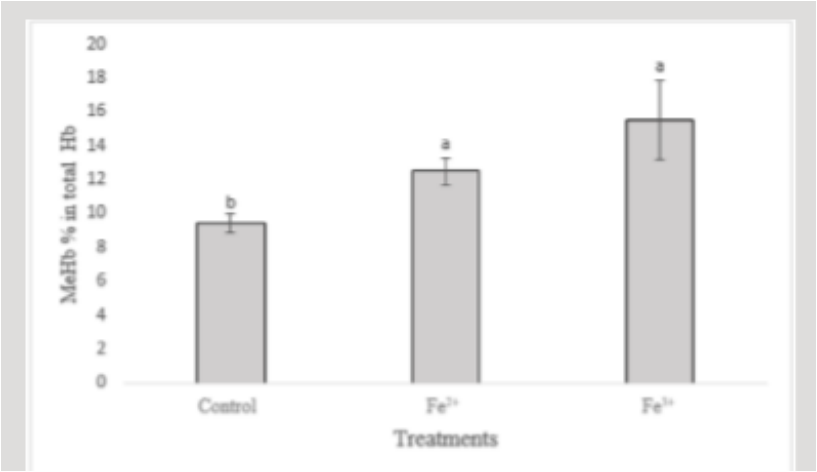

Figure 3: Methemoglobin levels in *L. friderici* exposed to iron ions (Fe^{2+} and Fe^{3+}).

Methemoglobin levels increased in groups contaminated with iron ions (Figure 3). The increase of MeHb in the organisms exposed to Fe^{3+} was higher in relation to the control group and the fish exposed to Fe^{2+}, the latter being also larger than the control. It has already been shown that Fe^{3+} are more lethal than Fe^{2+}, thus toxicological effects are more conspicuous in the groups contaminated with this pollutant. The most important effect of iron on fish refers to the ability of this compound to oxidize Hb in the blood, converting it to MeHb Jensen [34], making it impossible to transport oxygen to tissues and possibly causing the death of the animal through asphyxia Avilez et al. [35]. In humans, the malfunctioning in the iron metabolism or its excess in the organism, generated oxidative stress and the production of oxygen radicals, being able to be related

to Parkinson's and Alzheimer's disease Kozlowski et al. [15]. However, it is not known whether this accumulation is the cause or consequence of this disease Bury et al. [8]. Inflammatory problems of the central nervous system have been reported due to excess iron causing neuronal degradation Andersen et al. [36]. Therefore, it is advisable to avoid the consumption of fish contaminated with iron ions [37,38].

Conclusion

The two iron ions (Fe^{2+} and Fe^{3+}) are toxic to the "piau", and Fe^{2+} was toxic only at the concentration of 30 mg/L. Fe^{3+} causes total lethality at concentrations of 30 mg/L, 15 mg/L, 7.5 mg/L. At concentrations below 3 mg/L Fe^{3+}, the organism tolerates acute exposure with no mortality. Therefore, Fe^{2+} is less toxic when compared to Fe^{3+}, being tolerated by *L. friderici* at concentrations below 30 mg/L for 96 h of exposure. When compared to the control group, the opercular beats (OB) were higher with the concentrations of 30 mg/L with both iron ions. Considering the above, it is possible to observe that the decrease of GSH occurs in both groups, being higher in the group contaminated with Fe^{2+} ions, in these groups the survival time was higher, thus increasing the exposure to the pollutant. It is observed that the increase of hemoglobin was higher in the groups contaminated with Fe^{3+}, since this one is more toxic. In the groups contaminated with Fe^{2+} also increased, but smaller when compared to the Fe^{3+} groups. Methaemoglobin increased in the Fe^{3+} groups, being higher in relation to the control group and the Fe^{2+} groups, and the latter being also larger than the control group.

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6. ARTICLE 2

TOXICITY OF BRAZILIAN MEDICINAL PLANT EXTRACTS ON *Macrobrachium amazonicum*

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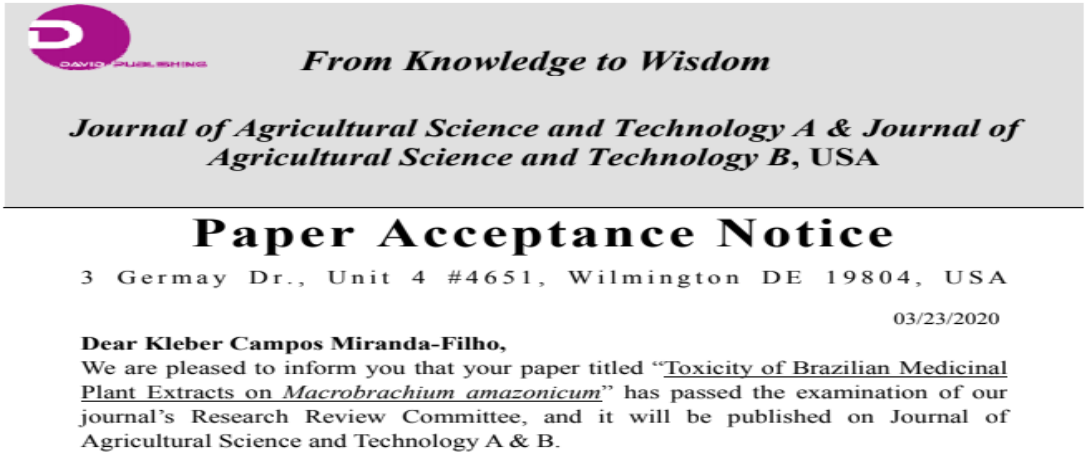


From Knowledge to Wisdom

***Journal of Agricultural Science and Technology A & Journal of Agricultural Science and Technology B*, USA**

Paper Acceptance Notice

3 Germay Dr., Unit 4 #4651, Wilmington DE 19804, USA

03/23/2020

Dear Kleber Campos Miranda-Filho,

We are pleased to inform you that your paper titled "Toxicity of Brazilian Medicinal Plant Extracts on *Macrobrachium amazonicum*" has passed the examination of our journal's Research Review Committee, and it will be published on Journal of Agricultural Science and Technology A & B.

ABSTRACT

Medicinal plants from Amazon have been commercialized for decades, but few scientific studies prove their effectiveness and safety in use in aquaculture activities. The objective of the present study was to use the Amazonian prawn *Macrobrachium amazonicum* to predict the toxicity of the natural extracts of nine medicinal plants viz pariri *Arrabidaea chica*, muirapuama *Ptychopetalum olacoides*, anauerá *Licania macrophylla*, barbatimão *Ouratea hexasperma*, faveira *Vatairea guianensis*, sacaca *Croton cajucara*, jacareúba *Calophyllum brasilliense*, pau d'arco *Tabebuia sp.* and verônica *Dalbergia subcymosa*, in concentrations of 1, 10, 100, 500 and 1000 µg / mL. The media was prepared in 0.5% dimethyl sulfoxide (DMSO) diluted with water. 10 post-larvae (0.5 ± 0.1 g) were added to each triplicate and, after 24 h, the mortalities were evaluated, with the results expressed in LC_{50} using the Probit statistical method. In order to obtain the concentrations of a common bioactive compound of plant extracts, the concentrations of flavonoids were analyzed using a methodology based on the formation of chromophores. The results of acute toxicity indicate variability in the toxic effects of medicinal plants, taking into account the concentration of total flavonoids, with the least toxic *Tabebuia* sp. (LC_{50} = 758.31 µg / mL) and the most toxic *C. cajucara* and *V. guianensis* (LC_{50} = 72.16 and 75.23 µg / mL), respectively. The extracts demonstrated lethality against *M. amazonicum*, which predicts toxicity and warns of its use them as herbal medicines. More studies must be carried out to determine other bioactive compounds in the plant extracts used since there is an unparalleled availability of chemical diversity.

Keywords: Amazon, aquaculture, lethality, medicinal plants, post-larvae.

1. INTRODUCTION

The use of medicinal plants is an accessible and traditional form of treatment for human use and with great potential for use in aquaculture. In Brazil, because of its rich biodiversity, many researches have been focused on natural products, from new renewable sources of energy, from biomass (biofuels), or other industrial uses, to those directed to drugs. Currently, the pharmaceutical industry handles high numbers worldwide, selling derivatives of medicinal plants [1]. Among the medicinal plants studied for this purpose, the following stand out:

1.1 *Arrabidaea chica* (Pariri)

A scandent shrub traditionally indicated to treat symptoms of inflammation. Its ethanolic extracts are chemically investigated and tested against yeasts and dermatophyte fungi [2].

1.2 *Ptychopetalum olacoides* (Muirapuama)

The indigenous tribes of Brazil use the bark and roots in infusion for many purposes as a treatment for neuromuscular disorders, cardiac and gastrointestinal asthenia acting as a muscle tonic [3].

1.3 *Licania macrophylla* (Anauerá)

It is popularly known as “anauerá” or “anuera”, being found mainly on the lowland margins of the lower Amazon regions. Amazonian communities use the stem bark of this plant as an antibiotic in the treatment of amoebic parasites and dysentery disorders. The *Licania* genus is rich in bioactive compounds such as flavonoids, terpenoids, steroids, among others [4].

1.4 *Ouratea hexasperma* (Barbatimão)

The *Ouratea* genus comprises 300 tropical species that occur mainly in South America and has been reported to be used in folk medicine for the treatment of inflammation [5].

1.5 *Vatairea guianensis* (Faveira)

Native plant to the Amazon, known as “fava bolacha”, “fava-de impingem” or “faveira”. Its fruits, stem bark, roots, leaves and juices are commonly used for treatment as a dermatological antifungal [6].

1.6 *Tabebuia* sp. (Pau d’arco)

It is a species from the North and Northeast regions of the country, known as “ipê-roxo” and used in traditional medicine to contain inflammation [7].

1.7 *Croton cajucara* (Sacaca)

Popularly known as Sacaca, it is a shrub plant with odorous leaves, which is very common in the Amazon. In northern Brazil, the leaves and bark of the trunk of this plant are used in the preparation of teas, tinctures or tablets as a hepatic-protective and antibiotic substance [8].

1.8 *Calophyllum brasiliense* (Jacareúba)

It is of great interest in popular medicine and is used in the treatment of disorders in the glycemic index such as diabetes, as a healing and anti-viral [9].

1.9 *Veronica officinalis* (Verônica)

Used popularly to treat hemorrhage, and it has antioxidant, healing and antifungal, antimicrobial, anthelmintic and anti-inflammatory properties [10].

Toxicological studies assess the harmful effects (acute and chronic) of chemical substances on living organisms. It operates on the principle that it is based on exploring the risk of animal and human exposure to various products in order to be able to establish safe conditions of exposure to these agents [11].

Lethality is the inherent and potential ability of the toxic agent to cause harmful effects and death in living organisms. The toxic effect is generally proportional to the concentration of the toxic agent against the site of action [12].

The use of plant extracts has increased in aquaculture as an alternative for prophylactic control. The extracts have some advantages over synthetic products for the cultivation of aquatic organisms such as less toxicity because they are less concentrated; they have multiple modes of action, resulting in less likelihood of causing resistance; in addition to reducing the environmental impact, as these are biodegradable, helping in the quality of cultivation, and reducing production costs [13].

There are several medicinal plants that have great potential to help aquaculture in this regard. For use with prophylactic purposes, it is essential to know the concentrations that may cause toxicity (chronic and lethal) to the organisms. Toxicity tests with herbal products aim the safety use of plant extracts without causing harmful effects to organisms kept in captivity [14, 15].

The bioactive compounds of plant extracts are substances that the plant synthesizes and stores during its growth. The active ingredients are not evenly distributed in the vegetable. They concentrate preferentially on flowers, leaves and roots, and sometimes on seeds, fruits and bark. These compounds are responsible for helping plants adapt to environments they are in, being sources of biologically active substances [16]. In the plant extracts studied, flavonoids (phenolic compounds with activities such as antioxidant, anticancer, antibacteria, cardioprotective agents, anti-inflammation, immune system promoting, and so on) were analyzed, as they form a very large group, due to the number of their natural constituents and wide distribution in the plant kingdom [17, 18, 19, 20, 21]. Flavonoids can be found in the most diverse structural forms, but their fundamental nucleus has 15 carbon atoms that form a tricyclic compound [22]. We

1114 believe in the relationship between the toxicity of plant extracts and their constitution in
1115 terms of flavonoids.

1116 In order to test the different plant extracts from North of Brazil, we choose an
1117 important species of crustacean, the *Macrobrachium amazonicum*. This prawn is a native
1118 species in Brazil and can be found in the Amazon Basin, São Francisco Basin, Paraguay
1119 Basin, North and Northeast Coastal Basins, as well as countries like Guyana, French
1120 Guiana, Venezuela, Ecuador, Peru and Bolivia [23, 24]. It is also a species with great
1121 potential for aquaculture activities [25, 26].

1122 Social aspects are also relevant when it comes to *M. amazonicum*, because there
1123 are riverside populations that use species fishing as a means of survival in certain
1124 locations, such as Pará state [27], where the activity generates jobs and income for
1125 families [28].

1126 Thus, the objective of the present work was to use *M. amazonicum*, as a model to
1127 predict the toxicity of natural products (taking in consideration the flavonoids as bioactive
1128 compounds), for later use in aquaculture as aids in the treatment of animal diseases and
1129 organ disorders.

1130

1131 **2. MATERIAL AND METHODS**

1132 Herbal extracts containing tinctures from pariri, mairipuama, anauerá,
1133 barbatimão, faveira, sacaca, pau d'arco, jacareúba and verônica were obtained from the
1134 Scientific and Technological Research Institute of Amapá (IEPA).

1135 Briefly, the content of total flavonoids was determined according to the
1136 methodology described by [30], using aluminum chloride that reacts with the sample
1137 flavonoids generating a chromophore, which represents the characteristic molecule by
1138 identifying the characteristic yellow color of the flavonoids, which is evaluated at 420

nm. A calibration curve was plotted with rutin. The result was expressed in mg of rutin equivalents / g of extract. The validated parameters were linearity, specificity, precision, accuracy and robustness, as well as the determination of impurities and identification of the extract.

10 post-larvae weighing 0.5 ± 0.1 g were used per treatment, maintained in Becker of 500 mL with aeration, containing the extracts under analysis, concentrations of 1, 10, 100, 500 and 1000 µg / mL were tested. The concentrations were prepared in dimethyl sulfoxide (DMSO) and diluted in water, not exceeding 0.5% DMSO in the final solution [31].

After 24 hours, the dead prawns were removed. The tests with the nine extracts were performed in triplicates. Safety levels were defined based on the most estimated values for pollutants in relation to the lethal concentration [32].

For the statistical tests, the Probit program was used to estimate the LC_{50}, and the Kruskal-Wallis test was used to determine the different toxicities between the extracts at the 5% level of significance using the Infostat version 2019 program.

For better sampling organization, the data collected were organized in the form of descriptive analysis, which according to [33], is a type of analysis used to describe and summarize the data. The data collected regarding the quantification of total flavonoids were obtained by means of the relative standard deviation (DPR%) in Excel® software.

3. RESULTS

Regarding the study of total flavonoids for the different plant extracts, the results obtained are shown in table 1.

Extract	Total flavonoids (mg/g ± SD)
Licania macrophylla	9.65± 0.25
Calophyllum brasiliense	8.05 ± 0.15
Veronica officinalis	7.18 ± 0.13
Vatairea guianensis	6.33 ± 0.80
Arrabidaea chica	6.20 ± 0.07
Croton cajucara	6.00 ± 0.83
Ouratea hexasperma	2.59 ± 0.14
***Tabebuia* sp.**	N.D
Ptychopetalum olacoides	N.D

1164

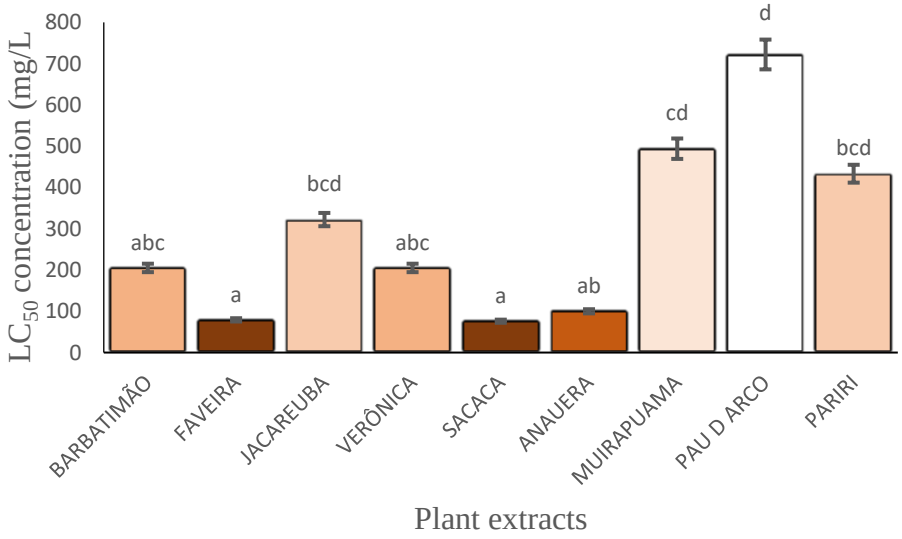
1165 Table 1. Total concentration of flavonoids for the different plant extracts.

1166

1167 The LC_{50}-24h of plant extracts for *M. amazonicum* post-larvae with the respective upper

1168 and lower limit values are shown in figure 1.

1169



1170

1171 Figure 1. Median lethal concentration (LC_{50}) of plant extracts to Amazon freshwater

1172 prawn *Macrobrachium amazonicum*. Different letters show significant differences (P

1173 <0.05) by the Kruskal Wallis test. Toxicity a> b> c> d.

Of all evaluated extracts, the bag was the one with the highest toxicity for *M. amazonicum* post-larvae, with the LC_{50}-24h of 75.96 µg / mL. The second most toxic extract was faveira, which had an LC_{50}-24h of 79.19 µg / mL, followed by anauera, whose LC_{50}-24h was 99.94 µg / mL.

Barbatimão and verônica showed the same toxicity for *M. amazonicum* post-larvae at the end of the acute toxicity test (Tab. 2). The jacareúba showed LC_{50}-24h of 322.2 µg / mL, followed by pariri and muirapuama plants with the respective LC_{50}-24h of 433.3 and 493.9 µg / mL.

Based on the results obtained, the least toxic medicinal plant for amazonian prawn was pau d'arco, with the LC_{50}-24h of 722.2 µg / mL. Table 2 shows the safety levels of the tested extracts.

Plant Extract	Safety Level* (µg/mL) ±SD
***Tabebuia* sp.** **(Pau d'arco)**	36.11 ± 1.81
Ptychopetalum olacoides **(Muirapuama**)	24.70 ± 1.24
Arrabidaea chica **(Pariri)**	21.67 ± 1.09
Calophyllum brasiliense **(Jacareúba)**	16.11 ± 0.80
Veronica officinalis **(Verônica)**	10.25 ± 0.52
Ouratea hexasperma **(Barbatimão)**	10.25 ± 0.52
Licania macrophylla **(Anauerá)**	5.00 ± 0.25
Vatairea guianensis **(Faveira)**	3.96 ± 0.20
Croton cajucara **(Sacaca)**	3.69 ± 0.19

*5% LC_{50} 24h.

Table 2. Safety levels for the use of Amazon plant extracts for freshwater prawn *Macrobrachium amazonicum*.

4. DISCUSSION

Regarding the study of flavonoids, some identifications for *Licania* were observed in a publication referring to the traditional, phytochemical and pharmacological use of the genus [34]. These authors carried out the identification of the chemical composition of several species of *Licania*, finding potential for flavonoids in some of them, such as

Licania pettiere and *L. carii*. Additionally, [35], in studies conducted with *Licania* sp. and *Parinari* sp., showed more than six positive results for flavonoids. [36] also discovered a new flavonol, licanol, in *L. macrophylla*. In the present study, a very favorable potential for flavonoids was identified in the species *L. macrophylla*, 9.76 ± 0.25 mg / g.

In a phytochemical approach performed on leaves of *A. chica*, [37], reported the presence of different chemical classes, among them flavonoids. Our quantification of total flavonoids performed in this work identified 6.22 ± 0.07 mg / g in *A. chica*.

Calophyllum brasiliense is a species of great medicinal interest in the treatment of diabetes, however in the reviewed literature there were no phytochemical studies related to the identification and quantification of flavonoids. In the present work, the compound was identified and quantified by spectrophotometry in the samples of extracts, with a concentration of 8.07 ± 0.15 mg / g.

Ptychopetalum olacoides did not show potential for flavonoids in the quantification analyzes performed in the present work. In the literature, no studies were found regarding the quantification of flavonoids performed with the species.

In a phytochemical study carried out on the bark of the *C. cajucara* tree, carried out by [38], demonstrated the presence of several diterpene clerodanes such as trans-dehydrocrotonina, trans-crotonina, cis-cajucarina and sacacarina. Pharmacological analysis, the activity of the largest component of the bark extract, has been extensively studied, as well as its anti-inflammatory, antimicrobial, analgesic, antiulcerogenic or antilipemic properties. The bark essential oil has also been reported to have gastroprotective activity. In research by [39], a new flavonoid was found in *C. cajucara*, which represents a very rare group of flavonols with specific cyclization. In the present

study, the quantification of flavonoids for this species was performed, presenting 6.00 ± 0.83 mg / g.

There is no quantification of flavonoids for *Ouratea* in this study, nor in the other literature reviewed. In the present study, the potential for flavonoids in the species showed a low result, with 2.61 ± 0.14 mg / g.

Veronica officinalis is a common species in the Amazon. [40] carried out studies regarding the identification of flavones in 29 species of *Veronica* and, in fact, found eight flavone aglycones. There are no comparative data regarding the quantification of flavonoids in *V. officinalis* in the reviewed literature. In the present study, one of the highest quantified concentrations (7.20 ± 0.13 mg / g) was identified in this species.

Vatairea guianensis presented few scientific references that mention biological activities for the species, as well as no data regarding the quantification of flavonoids. Although [41] portrayed in his research for the first time in the species, substances such as four isoflavones and five triterpenes identified in a lupeol mixture. The quantification of total flavonoids performed in this work, identified 6.38 ± 0.80 mg / g for *V. guianensis*.

[42] in studies carried out regarding the antioxidant activity and phytochemical screening endophytes, noted that *Tabebuia* sp. proved to be a rich source of many organic compounds (e.g. tannins, flavonoids, steroids, alkaloids, etc), especially phenolic and polyphenol. Such substances have been classified as cytotoxic, antimicrobial and antifungal due to the presence of anthraquinone compounds. In our study, we did not identify total flavonoids in *Tabebuia* sp.

The study of flavonoids contained in plant extracts aims to quantify one of the main bioactive compounds that can cause intoxication in animals when used incorrectly. Although flavonoids are commonly found in plants for medicinal purposes, other bioactive compounds must be quantified in the extracts of the nine plants studied in the

present study, in view of the additive effect of them on the biota treated with plant extracts.

Some crustaceans are widely used in ecotoxicological tests. Once the safety levels of plant extracts are defined to different animals, they can be used to treat them in closed cultivation systems without compromising their normal development. For this, it is also necessary to know their effectiveness in deal with specific pathogens.

Several toxicological studies are carried out with in small crustaceans observing some aspects such as mobility *Daphnia similis*, reproduction in *Ceriodaphnia dubia* or lethality in *M. amazonicum* post-larvae [43].

Plant extracts may have antibacterial activity against pathogens that affect humans [44] and animals [45], and the replacement of current antimicrobials with herbal products in aquaculture is not utopia, as several medicinal plants have shown activity to control and prevent pathogenic bacteria in fish, such as *Aeromonas hydrophila* [46, 47], *Streptococcus iniae* [48, 49], *Streptococcus agalactiae* [50], *Flavobacterium columnare* [51], *Pseudomonas fluorescens* and *Edwardsiella tarda* [46].

These plant extracts can be of low cost to the producer and some are considered immunostimulants that increase the resistance of animals and stimulate non-specific responses of the immune system [52].

In an acute toxicological study carried out with *Artemia salina* exposed to three species of medicinal plants of the genus *Phyllanthus*, in Northeastern Brazil, the LC_{50}-24h were similar to those found in this work for the species muirapuama, pau d'arco and pariri. The authors estimated the LC_{50}-24h from 404.43 ± 49.64 µg / mL to 770.84 ± 51.78 for *Phyllanthus niruri*, 837.65 ± 61.45 µg / mL to 1,075.89 ± 70.72 µg / mL for *Phyllanthus amarus* and 534.60 ± 46.83 µg / mL at 1,003.62 ± 65.15 µg / mL for *Phyllanthus tenellus* [53].

The medicinal plant *A. chica* (pariri) is used as an anti-inflammatory, astringent, and against intestinal colic, diarrhea, anemia and skin diseases [54]. It has low toxicity to *M. amazonicum* post-larvae, corroborating the study of Amaral et al. (2012) [55]. The authors observed that this medicinal plant had practically no toxic effect on *M. amazonicum* post-larvae, demonstrating the low toxicity of the ethanolic extract present in the leaves.

In addition, pariri also presented low toxicity to some mammals, as demonstrated in a study carried out with rats, whose median lethal dose (LD_{50}) = 2g / Kg [56]. Barbatimão, which is also used as an herbal medicine, should be administered with caution due to the phytotoxicity presented to animals such as Winstar rats [57] and *A. salina* [58].

Tests with *A. salina* exposed to ethanolic extract of Anauerá bark showed low toxicity to this small crustacean [59]. In the same way, Faveira has low toxicity for Winstar Rats, which can be used to treat integumentary tissue [60]. The use of trans-dehydrocrotonin from Sacaca in rats demonstrates a gastro-protective effect; however, at doses of 100 mg / kg, they caused liver damage [61]. The use of Jacareúba extract in rats above 1,000 mg / kg demonstrated deleterious effects and intoxication signals such as agitation and depression [62]. Studies with verônica extract in pregnant rats did not caused embryo-toxicity and the authors indicate its use as an anti-inflammatory [63].

In general, *M. amazonicum* post-larvae are also important for monitoring the natural environments where the species occurs, as described in the literature [64, 65, 66, 67]. Therefore, toxicity tests with prawn post-larvae are used in several research areas, such as aquaculture, medicinal chemistry, pharmacology, agriculture and ecotoxicity, the latter being of great importance in the evaluation of the toxic potential of extracts and isolated substances [68]. Some authors have reported the effect of bioactive compounds

from Brazilian plant extracts against bacteria (by antimicrobial activity) [69], favoring the prawn *M. amazonicum* [70, 71].

In the present study, it was possible to verify the toxicity of plant extracts and their safety levels for use. However, further work is needed to test its phytotherapic action (bioactive compounds) against pathogens, such as anti-inflammatories and other uses in the cultivation of *M. amazonicum*. The less toxic extracts (pau d'arco, pariri, muirapuama, jacareúba) with anti-inflammatory and protective function of the organism can be used as aids in the treatment of various disorders [2, 3, 7, 9]. The other extracts (veronica, sacaca, barbatimão, anauerá, faveira) must be used with greater care due to their toxic potential and has application as fungicides and bactericides in the systems and as aids in treatments for infection of these pathogens [4, 5, 6, 8, 10].

5. CONCLUSIONS

The method carried out to flavonoid determination showed high specificity at 420 nm for the extracts of the species provided by the Institute of Scientific and Technological Research of Amapá (IEPA), which gives reliability in the quantification of flavonoids.

Sacaca and faveira extracts were the most toxic to post-larvae of *M. amazonicum*, with the LC_{50} = 72.16 and 75.23 µg / mL respectively, and the least toxic medicinal plant was pau d'arco, with the LC_{50}- 24h at 722.2 µg / mL.

The post-larvae of *M. amazonicum* showed sensitivity to the nine extracts evaluated, which predicts care in view of the toxic effects in the use of products derived from these plants if they are used in the commercial cultivation of this crustacean.

The chemical diversity of the natural extracts encourages the study of other bioactive compounds (in addition to flavonoids) contained in the plant extracts tested.

The research carried out with medicinal plants from the Amazon region was of paramount importance, in view of the plant richness of that region. Thus, the advancement in this line of knowledge becomes of fundamental importance to assist in the use of plant extracts within aquaculture in a safe manner.

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7. CONSIDERAÇÕES FINAIS

Saber a toxicidade de ferro para peixes e de extratos de plantas para camarões é fundamental dentro da aquicultura, o ferro está presente na atividade mineradora na região amazônica, local onde habita o *Leporinus friderici*, conhecer sobre a tolerância e contaminação deste animal pelo contaminante em estudo faz-se necessário não somente para fins de cultivo, assim como para consumo humano, pois na região amazônica o consumo de pescados é muito elevado.

Extratos de plantas são comumente utilizados na região amazônica para fins medicinais, desta forma são necessários estudos sobre a toxicidade desses extratos para averiguar a possibilidade do uso de fitoterápicos de *Arrabidaea chica, Ptychopetalum, Licania macrophylla, Ouratea hexasperma, Vatairea guianensis, Tabebuia* sp., *Croton cajucara, Calophyllum brasilliense* e *Dalbergia subcymosa* para o *Macrobrachium amazonicum*.