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EFEITO DO CONSUMO DA POLPA DE MACAÍBA (*Acrocomia intumescens* Drude) SOBRE PARÂMETROS BIOQUÍMICOS, ESTRESSE OXIDATIVO E COMPORTAMENTO DE RATOS EXERCITADOS

JOÃO PESSOA - PB

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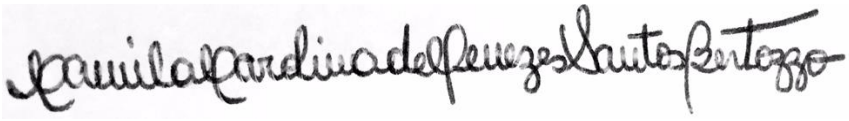

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A Deus, meu marido, meus pais, irmãos e familiares,

Dedico

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RESUMO

A polpa de macaíba (*Acrocomia intumescens* Drude) contém ácidos graxos insaturados, além de antioxidantes, o que a torna uma opção de consumo para indivíduos praticantes de exercício físico. Desta forma, objetivou-se com esta pesquisa verificar os efeitos do consumo da polpa de macaíba sobre os parâmetros físicos, bioquímicos, estresse oxidativo e comportamental em ratos exercitados. Para tanto, foram utilizados 44 ratos, sendo estes divididos em quatro grupos, com um número de 11 animais cada, sendo: controle sedentário (CT), macaíba sedentário (MC), controle exercitado (CT-EX) e macaíba exercitado (MC-EX). Os grupos CT e CT-EX receberam, por gavagem, água destilada, enquanto MC e MC-EX, receberam, por gavagem, polpa de macaíba na concentração de 1000 mg/kg de peso do animal. Todos os animais receberam ração comercial *ad labitum*. Os animais dos grupos CT-EX e MC-EX, foram submetidos ao protocolo de exercício, que consistia em um período de natação (nado livre), por um período de oito semanas. Na primeira semana de treinamento, fase de adaptação, em que o tempo do exercício seguiu ordem crescente, iniciando com 10 min e diariamente acrescentando 10 min. Ao final do tratamento, todos os animais foram submetidos a testes comportamentais, sendo usados os aparatos do campo aberto (CA), labirinto em cruz elevado (LCE) e caixa claro-escuro (CCE), para verificação de parâmetros de ansiedade. Quarenta e oito horas após os testes comportamentais os animais foram eutanasiados e realizada aferição dos parâmetros físicos e com o sangue coletado foram quantificados parâmetros bioquímicos. Com os valores das lipoproteínas verificou-se o risco cardiovascular. O fígado foi retirado e avaliou-se a concentração de malonaldeído (MDA), gordura total, colesterol total (TC), triglicerídios (TG) e histologia. O tecido do coração foi retirado e quantificado o MDA. Foi considerado estatisticamente diferente quando $p < 0,05$. A partir dos dados obtidos, verificou-se que os animais que consumiram polpa de macaíba, sedentários e exercitados, apresentaram redução de ansiedade, com maior número de ambulação e de *rearing* e menor tempo de *grooming* no CA, eles também apresentaram maior número de entradas e tempo de permanência nos braços abertos, maior número de mergulhos de cabeça e menor tempo nos braços fechados no LCE. MC-EX ainda apresentou maior número de entradas e permanência na parte claro do CCE. Quanto aos parâmetros físicos avaliados, verificamos que tanto MC quanto MC-EX apresentaram redução de peso, sendo esta redução mais expressiva para MC-EX. MC e MC-EX apresentaram redução de gordura corporal (mesentérica, retroperitoneal e epididimal), de lipoproteínas de baixa e muito baixa densidade, TC, aspartato e alanina aminotransferase, colesterol, MDA no coração, além de redução riscos coronários e cardiovasculares. Ainda verificamos aumento de HDL em MC e MC-EX. Concluindo desta forma que o consumo da polpa de macaíba induziu efeito ansiolítico, cardioprotetor, modulou parâmetros bioquímicos, além de contribuir na redução de gorduras viscerais.

Palavras-chave: Natação, malonaldeído, ansiedade, palmeira brasileira, efeito cardioprotetor.

ABSTRACT

Macaíba pulp (Acrocomia intumescens Drude) contains unsaturated fatty acids, in addition to antioxidants, which makes it a consumption option for individuals who practice physical exercise. Thus, the objective of this research was to verify the effects of the consumption of Macaíba pulp on physical, biochemical, oxidative and behavioral stress parameters in exercised rats. For this purpose, 44 rats were used, divided into four groups, with a number of 11 animals each, as follows: sedentary control (CT), sedentary macaw (MC), exercised control (CT-EX) and exercised macaw (MC- EX). The CT and CT-EX groups received, by gavage, distilled water, while MC and MC-EX received, by gavage, macaiba pulp at a concentration of 1000 mg/kg of animal weight. All animals received commercial feed ad labitum. The animals in the CT-EX and MC-EX groups were submitted to the exercise protocol, which consisted of a swimming period (free swimming), for a period of eight weeks. In the first week of training, adaptation phase, in which the exercise time followed ascending order, starting with 10 min and daily adding 10 min. At the end of the treatment, all animals were submitted to behavioral tests, using the open field (AC), elevated plus maze (LCE) and light-dark box (CCE) apparatus to verify anxiety parameters. Forty-eight hours after the behavioral tests, the animals were euthanized and the physical parameters were checked, and with the blood collected, biochemical parameters were quantified. With the values of lipoproteins, the cardiovascular risk was verified. The liver was removed and the concentration of malonaldehyde (MDA), total fat, total cholesterol (TC), triglycerides (TG) and histology were evaluated. Heart tissue was removed and MDA quantified. It was considered statistically different when $p < 0.05$. From the data obtained, it was found that sedentary and exercised animals that consumed Macaíba pulp presented reduced anxiety, with a greater number of ambulation and rearing and shorter grooming time in the CA, they also had a greater number of entries and time spent in open arms, greater number of head dives and less time in closed arms in the CSF. MC-EX still presented a greater number of entries and permanence in the clear part of the CCE. As for the physical parameters evaluated, we found that both MC and MC-EX showed a reduction in weight, with this reduction being more expressive for MC-EX. MC and MC-EX showed a reduction in body fat (mesenteric, retroperitoneal and epididymal), low and very low density lipoproteins, CT, aspartate and alanine aminotransferase, cholesterol, MDA in the heart, in addition to reduced coronary and cardiovascular risks. We still found an increase in HDL in MC and MC-EX. Thus, concluding that the consumption of Macaíba pulp induced anxiolytic, cardioprotective effect, modulated biochemical parameters, in addition to contributing to the reduction of visceral fat.

Keywords: Swimming, malonaldehyde, anxiety, Brazilian palm, cardioprotective effect.

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1 INTRODUÇÃO

A presença de componentes como fibras, minerais, vitaminas e compostos fenólicos em diversas frutas, além de ácidos graxos insaturados, têm sido correlacionadas com benefício na diminuição do estresse oxidativo em estudos *in vitro*, com animais e seres humanos (CILLA et al., 2018; KUMARA et al., 2018; LEVERS et al., 2015; LÓPES et al., 2017). O consumo dessas frutas está associado a diminuição de processos inflamatórios e melhoria de desempenho em esportistas (AFFOURTIT et al., 2015; NADERI et al., 2018).

Estudos com diferentes frutas foram realizados e nelas detectado capacidades antioxidantes (AMMAR et al., 2016; LEVERS et al., 2015; TOSCANO et al., 2015). Os frutos de palmeiras de origem brasileira também têm demonstrado benefícios. É o caso da carnaúba (*Copernicia prunifera*), cuja cera foi associada a efeito antioxidante e antifúngico *in vitro* (ANDRDADE et al., 2018), do óleo de amêndoa de bocaiuva (*Acrocomia aculeata* (Jacq.) Lodd. Ex. Martius), que apresentou atividade fotoprotetora em produtos cosméticos (DARIO et al., 2018) e do açaí (*Euterpe oleraceae*), que contribuiu na melhora da resposta inume *in vitro* (CANTU-JUNGLES et al., 2017).

Outra palmeira brasileira com potencial ação benéfica *in vivo* é a espécie *Acrocomia intumescens* Drude, conhecida popularmente como macaíba, macaúba ou palmeira barriguda. Produz frutos comestíveis, os quais apresentam uma polpa com teores significativos de ácidos graxos insaturados, cerca de 89,81%, com predominância de ácido oleico e linoleico. Além de aminoácidos (isoleucina, leucina, fenilalanina e treonina), fibras, também apresenta carotenoides, vitamina C e minerais como o K, Fe, P e Ca (BORA; ROCHA, 2004; COMBRA; JORGE, 2011; LESCANO et al., 2015; SOUZA, 2016). A presença de ácidos graxos livres e compostos antioxidantes na amêndoa da macaíba já foram relacionados a efeito ansiolítico em ratos dislipidêmicos (SILVA et al., 2021). Além disso, a polpa da macaíba demonstrou benefícios sobre parâmetros bioquímicos *in vivo,* contribuindo para melhor perfil lipídico de ratos dislipidêmicos (SOUZA, 2017).

A *Acrocomia intumescens* é uma palmeira com potencial para investimento tanto pela indústria alimentícia como pela farmacêutica. No entanto, ainda são escassos dados na literatura científica que demonstrem os efeitos da polpa da macaíba sobre processos metabólicos e comportamentais.

Dentre os transtornos comportamentais bastante pesquisados atualmente, está a ansiedade e depressão que apresentam como uma possível causa do seu aparecimento o estresse oxidativo e o aumento de processos inflamatórios. O desequilíbrio oxidante e o aumento do processo pró-inflamatório promovem danos celulares que inibem o processo de sobrevivência celular, acarretando danos estruturas e prejudicando a neurogênese e neuroplasticidade. Esses danos podem levar a comportamentos de ansiedade e depressão, além de patologias como a Alzheimer, Parkinson e Huntington (MOYLAN et al., 2013; PEÑA-BAUTISTA et al., 2019).

A alimentação balanceada juntamente com a prática regular de exercício podem contribuir na redução do estresse oxidativo, o que, por sua vez, favorecerá no tratamento não só de transtornos comportamentais, mas também da obesidade e doenças associadas (BELGUITH-HADRICHE et al., 2013; CASEY et al., 2013; HOUDEBINE et al., 2017; NADERI et al., 2018).

Desta forma, este trabalho justifica-se pela escassez de estudos utilizando a polpa de macaíba associada a exercício físico e influência sobre parâmetros bioquímicos e comportamentais. Este estudo se apresenta como uma proposta inovadora e que pode contribuir com a expansão no consumo e cultivo de frutos nativos brasileiros, podendo contribuir para a agroindústria especializada nestes produtos, fortalecendo a economia local e gerando renda para comunidade.

Considerando que componentes antioxidantes podem contribuir para reduzir danos oxidativos celulares e auxiliar na redução de comportamento ansiogênico, objetivou-se avaliar os efeitos da polpa de macaíba sobre os parâmetros físicos, bioquímicos, estresse oxidativo e comportamental em ratos exercitados.

2 REFERENCIAL TEÓRICO

2.1 PALMEIRA MACAIBEIRA (*Acrocomia intumescens* Drude**)**

No Brasil há uma diversidade de palmeiras, sendo em torno de 39 gêneros e 119 espécies, que estão espalhadas principalmente entre as regiões Centro, Norte e Nordeste (LEITMAN et al., 2015; NASCIMENTO et al., 2016).

Dentre as espécies de palmeiras, temos a *Acrocomia intumescens* Drude, popularmente conhecida como macaíba, macaúba ou palmeira barriguda. Pode ser encontrada na região Nordeste, nos Estados de Alagoas, Bahia, Ceará, Paraíba e Pernambuco, e na região Sudeste, nos Estados do Rio de Janeiro e São Paulo. Devido as condições de clima semiárido na região Nordeste, esta é a espécie de palmeira com maior abundância (LEITMAN et al., 2015; NASCIMENTO et al., 2016).

A *A. intumescens* (Figura 1), possui uma estrutura composta por caule, sendo este constituído de espinhos quando esta se encontra na fase jovem e caule liso em sua fase adulta (LORENZI et al., 2004; LEITMAN et al., 2015; NASCIMENTO et al., 2016). Possui frutos compostos por casca dura (epicarpo), e em seu interior podem ser vistos a polpa (mesocarpo) e a amêndoa (endocarpo) (ANDRADE, 2013; LORENZI et al., 2010; SILVA et al., 2020) (Figura 2).

Figura 1. Palmeira *Acrocomia intumescens* Drude (Macaíba).

Fonte: Acervo fotográfico do autor.

Figura 2. Fruto da palmeira *Acrocomia intumescens* Drude (Macaíba). A – Fruto completo (epicarpo); B – Polpa (mesocarpo); C – Amêndoa (endocarpo).

Fonte: Acervo fotográfico do autor.

Alguns estudos vêm sendo desenvolvidos voltados a caracterização da composição nutricional de seus frutos e dos óleos extraídos de sua polpa e amêndoa (NASCIMENTO et al., 2016; SILVA et al., 2020; SOUZA, 2016).

A polpa de macaíba é constituída predominantemente por ácidos graxos insaturados, especialmente os ácidos oleico e linoleico, além de apresentar carotenoides totais, fenólicos como a catequina, flavonoides, vitamina C, e minerais como P, Ca, Zn, Cu, Fe e Mn, sendo encontrado em maior quantidade o K e o Mg. Na amêndoa pode-se verificar a presença de ácidos graxos como o ácido oleico, ácido láurico, mirístico e palmítico, além de ácidos graxos estão presentes compostos fenólicos e flavonoides, e minerais como Ca, Zn, Cu, Fe e Mn, estando mais abundante o P, K e Mg (SOUZA, 2016; SILVA et al., 2020).

No estudo desenvolvido por Nascimento et al. (2016) com óleos extraídos da polpa e amêndoa de macaíba, empregando-se formas distintas de extração, verificou-se a presença de graxos livres como o ácido palmítico (C 16:0), ácido esteárico (C 18:0), ácido oleico (C 18:1), ácido linolênico (C 18:3), ácido linolênico (C 18: 3) no óleo extraído da polpa de macaíba. Já no óleo extraído da amêndoa de macaíba foi encontrado o ácido caprílico (C 8:0), ácido láurico (C 12:0), ácido láurico (C 12:0), ácido mirístico (C 14:0), ácido palmítico (C 16:0), ácido esteárico (C 18:0), ácido oleico (C 18:1), ácido linoleico (C 18:2), ácido linolênico (C 18:3).

As folhas da macaíba são utilizadas para alimentação animal e a polpa e amêndoa para consumo humano. Na medicina popular o óleo obtido da polpa e amêndoa de macaíba é utilizado como tônico, ao qual é atribuído atividades anti-inflamatória e antioxidante (MOTOIKE et al. 2013; NASCIMENTO et al., 2016).

Alguns estudos sobre o gênero *Acrocomia*, relaciona-se a espécie *Acrocomia aculeata* (SOUZA, 2016). A espécie A. *aculeata*, conhecida comumente por bocaiuva ou macaúba, possui frutos compostos por aproximadamente 20% de casca, 40% de polpa, 33% de endocarpo e 7% de amêndoa. A polpa deste fruto é consumida tanto na forma fresca quanto cozida, além de ser empregada na elaboração de sorvetes e produtos de panificação como bolos e biscoitos (COIMBRA; JORGE, 2011; CICONINI, 2013).

A raiz da A. *aculeata* tem sido usada popularmente na obtenção de óleo, ao qual tem sido atribuída ação diurética. Seu consumo popular também tem sido associado para tratar de doenças cardiovasculares, hipertensão, diabetes e inflamação (COIMBRA; JORGE, 2011; LESCANO et al., 2015). No entanto, só há, até o presente momento, comprovação científica de sua ação diurética e anti-inflamatória, quando administrado óleo microencapsulado de macaúba em estudos desenvolvidos com animais (LESCANO et al., 2015).

Silva et al. (2021) quando avaliou o efeito da amêndoa de macaíba sobre o comportamento de ansiedade de ratos previamente dislipidêmicos e verificou-se que a amêndoa de macaíba induziu comportamento ansiolítico e diminuiu a peroxidação lipídica no cérebro dos ratos dislipidêmicos. Souza (2017) verificou que a polpa de macaíba quando administrada a ratos dislipidêmicos foi capaz de reverter o quadro de dislipidemia e melhorar parâmetros bioquímicos *in vivo*. Em ambos estudos, os benefícios encontrados foram atribuídos a presença de componentes lipídicos, ácidos graxos insaturados, antioxidantes, como flavonoides e carotenoides, e a presença de fibra na polpa e amêndoa do fruto de macaíba.

2.2 ESTRESSE OXIDATIVO E ANTIOXIDANTES ALIMENTARES

A produção de radicais livres é um processo que ocorre naturalmente no corpo. Em condições normais, os níveis intracelulares de espécies reativas de oxigênio (EROs) são encontrados em baixas concentrações, isso ocorre devido a ação de

sistemas enzimáticos que participam do processo *in vivo*, homeostase redox. Protegendo o organismo de danos causados pelos radicais livres e contribuindo prevenindo ou retardando patologias cardiovasculares e neurológicas. Mas quando há um desequilíbrio entre pró-oxidantes e antioxidantes no corpo, temos o chamado estresse oxidativo (ALAM; BRISTI; RAFIQUZZAMAN, 2013; HALLIWELL; GUTTERIDGE, 2010; HASSANAIN; KHOURI, 2012; POLJSAK; DAHMANE, 2012; TINKEL; RAHAL et al, 2014).

Os antioxidantes endógenos, composto pelo sistema enzimático, não protegem por completo os componentes celulares. Desta forma a ingestão alimentar de fontes antioxidantes torna-se fundamental para preservação celular. Estes compostos alimentares com propriedades antioxidantes fazem parte do sistema antioxidante não enzimático e dentre os componentes com estas propriedades podemos apontar os polifenóis, flavonoides e carotenoides (ANILA, VIJAYALAKSHMI, 2003; BARREIROS, DAVID, DAVID, 2006; RAHAL et al, 2014).

Os carotenoides apresentam atividades biológicas, incluindo a atividade provitamina A. Esta vitamina age sobre o sistema imune, promovendo regulação do ciclo de apoptose, modulação de fatores de crescimento e diferenciação celular (FIEDOR; BURDA, 2014; RIBEIRO et al., 2018) e agindo sobre os radicais livres, aumentando a atividade de enzimas como a catalase, superóxido dismutase, glutationa redutase e glutationa peroxidase, que compõem o sistema antioxidante enzimático (RIBEIRO et al., 2018).

Os flavonoides por sua vez, tem sido apontado como hepatoprotetor e neuroprotetor. Segundo Xiang et al. (2018) os flavonoides apresentaram ação antioxidante, citoprotetor e hepatoprotetor. Estando estes benefícios associados com a diminuição de concentrações de alanina transaminase (ALT), de aspartato transaminase (AST) e dos níveis de malonaldeído (MDA) no tecido hepático *in vivo.*

Os polifenóis encontrados abundantemente em alimentos como frutas, ervas e vegetais, estão sendo cada vez mais estudados devido sua relação com a prevenção de doenças cardiovasculares e neurodegenerativas, através da inibição do estresse oxidativo verificados em culturas de células e em modelos animais (ALBARRACIN et al., 2012).

No estudo desenvolvido por Cilla et al. (2018), com o objetivo de verificar o efeito citoprotetor de polpa de laranja sobre o estresse oxidativo, foi verificado que as células tratadas com as polpas de laranja, apresentaram redução da peroxidação

lipídica, de espécies reativas de oxigênio e de alterações no potencial de membrana mitocondrial, demonstrando ação protetora contra o estresse oxidativo.

Jayesh et al. (2017), estudando o efeito do extrato aquoso de frutas de *Terminalia bellirica* (Gaertn Roxb) contra o estresse oxidativo induzido por CCL4 e de danos hepáticos em modelo animal, observaram que o extrato foi capaz de reverte o dano hepático através de sua atividade antioxidante.

A *Terminalia chebula* é outra planta da qual seu fruto tem sido estudado devido sua associação no tratamento de epilepsia e distúrbios do sistema nervoso central. Kumara et al. (2018) com o objetivo de avaliar os efeitos do extrato do fruto de *Terminalia chebula* sobre a convulsão, comprometimento cognitivo induzido por convulsão e estresse oxidativo em ratos, verificaram que a dose de 1000 mg/kg, apresentou proteção contra convulsões, além de atenuar o estresse oxidativo e comportamento cognitivo gerados pelas convulsões.

Os frutos e sementes da palma *Bactris guineensis*, também tem demonstrado ação citoprotetora contra o estresse oxidativo em astrócitos e células neuronais, diminuindo a produção de radicais superóxido, sendo este efeito atribuído a presença de componentes fenóis em sua constituição (LÓPEZ et al., 2017).

Frutos de palmeiras também tem demonstrado capacidade antioxidante. No estudo de Leow et al. (2013), avaliando a ação dos fenólicos obtidos do dendê em modelo animal, os autores verificaram que o extrato de fenólicos de dendê atenuou a inflamação por meio da modulação do eixo Th1/Th2, e aumentou a atividade antioxidante sérica.

Segundo Yeap et al. (2015) o óleo de coco virgem quando administrado *in vivo*, na dose de 10 ml/kg de peso de animal, foi capaz elevar antioxidantes cerebrais e reduzir níveis séricos de colesterol, triglicérides, glicose e corticosterona, apresentando assim, efeito antiestresse.

2.3 EXERCÍCIO FÍSICO E ESTRESSE OXIDATIVO

O exercício aeróbico é definido como sendo qualquer forma de atividade física capaz de promover aumento da frequência cardíaca e do volume respiratório em decorrência da demanda do organismo por maior quantidade de oxigênio pelo muscular ativo (WANG; XU, 2017).

A demanda aumentada de oxigênio muscular, gera por sua vez um aumento de espécies reativas de oxigênio (ERO). Essas alterações fisiológicas também são responsáveis por induzir adaptações biológicas ao treinamento, no entanto, o excesso de EROs pode gerar um impacto deletério nas células e tecidos, com desenvolvimento acentuado de peroxidação lipídica e proteica (MANKOWSKI et al., 2015).

Durante o exercício, existem várias fontes potenciais para a produção de espécies reativas de oxigênio, como ânions superóxido, peróxido de hidrogênio e radicais hidroxila. O desequilíbrio entre a produção de substâncias oxidantes e as defesas antioxidantes do organismo é conhecido por estresse oxidativo. Esse estresse oxidativo levar a um aumento na peroxidação lipídica e causar danos ao DNA celular e carbonilação de proteínas, promovendo alterações das funções celulares e danos teciduais (DEATON; MARLIN, 2003; GOTO; RADÁK, 2007). Estas alterações ocorrem devido aos radicais livres modificarem a permeabilidade, fluidez e integridade das membranas celulares e assim modificar sua funcionalidade (MAHATTANATAWEE et al., 2006).

O aumento do estresse oxidativo no cérebro pode apresentar como causa a absorção de oxigênio excessiva e geração elevada de espécies reativas de oxigênio (ROS), sendo estas reações catalisadas por enzimas, tais como oxidase de xantina e desidrogenase NADPH (FULK et al., 2004; VOLLERT et al., 2011). A elevação do estado oxidante, bem como o aumento do processo pró-inflamatório danificam a estrutura celular normal e desta forma inibem processos normais de sobrevivência celular, neurogênese e neuroplasticidade o que desencadeia alterações no comportamento de ansiedade (MOYLAN et al., 2013).

As doenças neurodegenerativas têm grande impacto social, tendo como possíveis causas o processo de peroxidação e podem estar relacionadas ao surgimento de patologias como Alzheimer, Parkinson e Huntington (PEÑA-BAUTISTA et al., 2019).

Doenças neurológicas como a esclerose múltipla, a qual se caracteriza por perda cíclica e reparação de bainhas de mielina associadas a inflamação crônica e perda de neuronal. Pode ter sua origem associada a níveis alterados de oxiesterois, derivados oxidativos do colesterol, implicados no metabolismo do colesterol. O acúmulo desses derivados pode estar relacionado com o início e desenvolvimento desta patologia e de sua implicação na inflamação, estresse oxidativo, desmienilização e neurodegeneração. O exercício físico moderado tem sido

associado como estratégia terapêutica, por contribuir no aumento da plasticidade neuronal, diminuição da inflamação e o estresse oxidativo (HOUDEBINE et al., 2017).

A realização de exercício físico moderado com regularidade contribui para a melhora na qualidade de vida, perda de peso corporal e melhora as defesas antioxidantes do organismo. No entanto, quando realizado de forma muito intensa pode levar a um aumento na atividade metabólica e favorecer a ocorrência de lesões oxidativas em biomoléculas, prejudicando as funções celulares (MARIN et al., 2013).

Durante a prática de exercício físico há um aumento da resposta inflamatória de fase aguda, caracterizada pelo processo de infiltração fagocitária no músculo, produção espécies reativas de oxigênio e elevação de citocinas inflamatórias, podendo levar a lesões musculares, além de aumentar a produção de radicais livres (ISANEJAD et al., 2015; KIRSCHVINK; MOFFARTS; LEKEUX, 2008).

Desta forma, o tipo de exercício assim como o tempo e intensidade devem ser levados em consideração quando se busca uma alternativa para controlar o estresse oxidativo e processos inflamatórios nos tecidos a fim de evitar maiores danos celulares. Visando a redução do estresse oxidativo o consumo de alimentos que possuem componentes antioxidantes e que possam contribuir para maior adaptação do corpo aos processos metabólicos gerados durante exercício torna-se uma estratégia (RAHAL et al., 2014).

2.4 ANTIOXIDANTES ALIMENTARES E EXERCÍCIO

O consumo de alimentos que possuem componentes antioxidantes é uma forma de contribuir para maior adaptação do corpo aos processos metabólicos gerados durante exercício e assim reduzir o estresse oxidativo e possíveis danos decorrentes (RAHAL et al., 2014).

Alguns estudos têm demonstrado que a associação entre dieta e exercício podem contribuir para uma recuperação mais rápida e melhor desempenho esportivo. Robinson et al., (2019) avaliando a associação da ingestão do óleo de coco ao exercício, em humanos, com 34 jovens adultos realizando o exercício de ciclismo de intensidade moderada, observou-se que ocorreu uma melhora a função endotelial vascular no grupo que consumiu o óleo de coco.

Manio, Matsumura e Inoue (2018) investigando as adaptações metabólicas, bioquímicas e genéticas em decorrência de exercício de esteira rolante *in vivo* e associando ao consumo de óleo de soja e de óleo de coco. Verificaram que o óleo de coco assim como o treinamento melhoram a taxa de troca respiratória (RER). Ainda, que o treinamento aumentou as atividades das enzimas mitocondriais provavelmente relacionadas ao aumento da expressão do receptor relacionado ao estrogênio (ERR) α e β. E que o óleo de coco promoveu a utilização de glicogênio no músculo treinado durante o exercício, favorecendo ao aumento da resistência.

De acordo com Yeap et al. (2015), a dose de 10 ml/kg de peso corporal, do óleo de coco virgem (VCO) administrada a camundongos com lesão induzida por estresse promoveu aumento dos níveis de antioxidantes cerebrais, níveis mais baixos de 5-hidroxitriptamina cerebral e peso reduzido das glândulas suprarrenais. Consequentemente, os níveis séricos de colesterol, triglicerídeos, glicose e corticosterona também foram menores nos camundongos tratados com VCO.

Segundo Esquius et al. (2019) o consumo de azeite está associado a um risco diminuído de doenças cardiovasculares e mortalidade. Quando avaliado o efeito da suplementação com azeite extravirgem na resposta cardiorrespiratória (CRC) e no desempenho esportivo, em comparação ao óleo de palma, verificou-se que a suplementação com azeite de oliva extravirgem aumentou a CRC durante um teste de caminhada progressiva em intensidade moderada, mas não alterou o desempenho e outros marcadores fisiológicos.

Capó et al. (2016), ao analisar os efeitos de uma bebida composta por amêndoa e azeite e enriquecida com α-tocoferol e docosaexaenoico, verificaram melhora do processo inflamatório em resposta ao exercício em jovens e em idosos atletas, embora esta resposta antinflamatória tenha sido menos expressiva grupo sênior.

2.5 TRANSTORNO DE ANSIEDADE E DEPRESSÃO

Os transtornos de ansiedade são problemas de saúde pública prevalentes, debilitantes e dispendiosos e afetam duas vezes mais mulheres do que homens (WHO, 2017). Embora a ansiedade e a depressão apresentem características sintomatológicas semelhantes e que suas ocorrências sejam em grande parte

simultâneas, ainda não está bem elucidado seus processos neurobiológicos (MAGGIONI et al., 2019).

As respostas fisiológicas e comportamentais decorrentes de eventos estressores diários são mediados pelo sistema nervoso através de hormônios circulantes (McEWEN, 2003). O estresse tende a gerar respostas imediatas e adaptativas, sendo estas respostas desenvolvidas pelo eixo hipotálamo-hipófise-adrenal (HPA), pelo sistema imunológico e pelo sistema nervoso autônomo (SNA). Todavia, quando há alterações ou falha na resposta dos mediadores após o estresse, verifica-se um desgaste a longo prazo do corpo. Levando a alterações neurais que podem ser observados nos sistemas cardiovascular, metabólico e imunológico, com comprometimento da imunidade, desenvolvimento de aterosclerose, obesidade, além de favorecer um processo de desmineralização óssea e atrofia das células nervosas no cérebro. Sendo percebida através do aparecimento da doença depressiva e também de transtornos de ansiedade crônica (McEWEN, 2003; VAN LEEUWEN et al., 2018).

Os mediadores desses sistemas de estresse, como o cortisol e noradrenalina são importantes na etiologia dos distúrbios afetivos, assim como a desregulação do sistema de estresse contribuem para o início da doença. O desenvolvimento e a recuperação de um transtorno afetivo podem prejudicar a funcionalidade dos sistemas de estresse. O que pode gerar independente do estresse um quadro de transtorno de humor ou ansiedade devido a falhas de resposta do sistema biológico ao estresse, perdurando por um longo período. Processos inflamatórios mediados por interleucinas em particular a (IL-6), além do estresse, têm sido relacionados ao aparecimento de quadros de ansiedade e depressão (HERMANS et al., 2014; VAN LEEUWEN et al., 2018; VINKERS et al., 2021).

Em contrapartida, o consumo de compostos antioxidantes provenientes de frutas, sementes e vegetais tem sido associado a um papel na redução do estresse oxidativo e com isso atuando para um efeito ansiolítico (KOMAKI et al., 2016).

Com base em tudo que fora anteriormente descrito, nosso trabalho se mostra relevante para conhecimento sobre o consumo do fruto da macaibeira e sobre seus efeitos fisiológicos sobre o organismo. Além de contribuir para valorização de frutos nativos, por vezes pouco explorados.

3 MATERIAL E MÉTODOS

3.1 TIPO DE ESTUDO

O presente estudo trata-se de uma pesquisa de caráter experimental, ensaio pré-clínico, com condições controladas (GIL, 2008; THOMAS; NELSON; SILVERMAN, 2012). Realizada a partir da administração de polpa de macaíba em modelo animal com protocolo de exercício de natação.

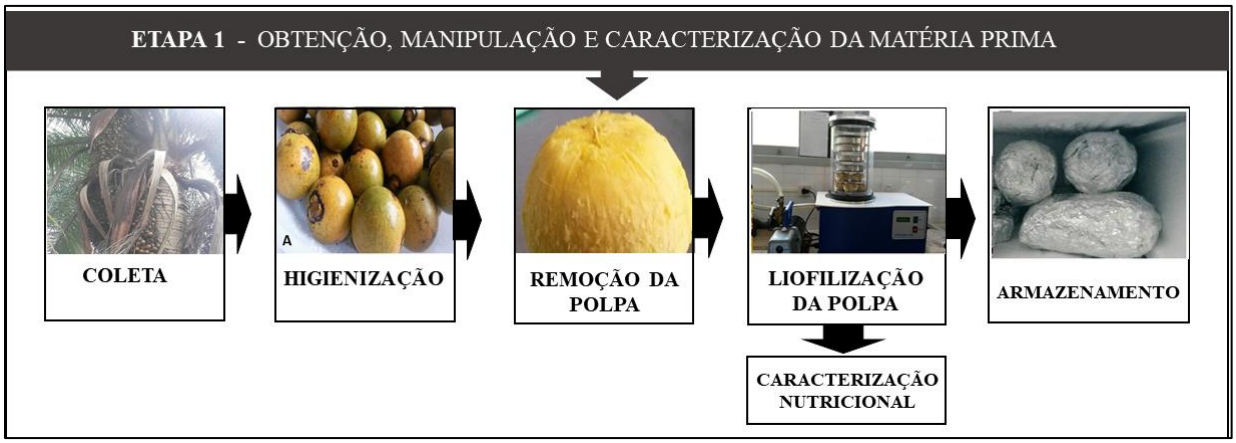
Esta pesquisa teve a contribuição para execução de pesquisa e análises da Universidade Federal de Campina Grande – campus Cuité, Universidade Federal da Paraíba, campus central localizado na cidade de João Pessoa e no campus de Areia, além da Universidade Federal do Rio Grande do Norte.

3.2 AQUISIÇÃO E PROCESSAMENTO DA MATÉRIA-PRIMA

Os frutos da Macaibeira (*Acrocomia intumescens* Drude) foram obtidos na cidade de Areia, local -6,963845 (latitude) e -35,749738 (longitude), estado da Paraíba, região Nordeste do Brasil. Registro no sistema nacional de gestão do patrimônio genético e conhecimentos tradicionais associados (SISGEN): ADD854A. Os frutos foram selecionados no início da fase de maturação, sem lesões, coletados entre 6 e 7 horas da manhã, transportados em caixas isotérmicas e armazenados sob refrigeração a 4 ± 1 ºC até o processamento.

Os frutos da macaíba foram limpos e descascados. Após serem despolpados manualmente, foram congelados em freezer a -20 ºC e posteriormente submetidos ao processo de liofilização em Liofilizador L101 (Liotop®, 212Vca), a -50 ºC, por 48 horas. Após a obtenção do liofilizado da polpa, este transferido para embalagem fechada a vácuo, e acondicionada em freezer a temperatura de -20 ºC (Figura 3).

Figura 3. Obtenção e preparo de matéria-prima.



3.3 ANÁLISE DE MACRONUTRIENTES E PERFIL DE ÁCIDOS GRAXOS DA POLPA

A polpa de macaíba liofilizada foi utilizada para determinação de proteína pelo método de micro Kjedhal e carboidrato, segundo metodologia descrita em Association of Official Agricultural Chemists (AOAC, 2000), lipídios pela metodologia descrita por Folch, Less e Stanley (1957) e perfil lipídico por Metodologia de Hartman e Lago (1973).

3.3 ANÁLISE DE COMPOSTOS BIOATIVOS DA POLPA DE MACAÍBA

3.3.1 Extração

Os constituintes da polpa da macaíba liofilizada foram extraídos com etanol 80% (v / v) e avaliados quanto à capacidade de eliminação do radical $ABTS^{\bullet+}$, atividade redutora do ferro (FRAP), compostos fenólicos totais e flavonoides totais. Para a determinação do teor de fenólicos totais 1 g da polpa de macaíba foi suspendida em 10 ml de metanol a 80% em um tubo de ensaio , o qual foi submetido à temperatura ambiente, na ausência de luz por 60 minutos e após filtração, o volume foi completado para 10 mL com o solvente de extração e armazenado em freezer (-18 °C) até as análises. Todas as extrações foram realizadas em triplicatas.

3.3. 2 Determinação de compostos fenólicos totais

Para determinar o conteúdo de compostos fenólicos totais, foi utilizada a metodologia descrita por Liu et al. (2002), com modificações. Foram misturados 250 µL de extrato com 1250 µL de um reagente Folin-Ciocalteau diluído 1:10. As soluções foram misturadas e deixadas à temperatura ambiente (27 °C) durante 6 minutos. Após a incubação, 1000 mL de solução de carbonato de sódio a 7,5% (Na_2CO_3) foram adicionados e levados em banho-maria a 50 °C por 5 minutos. A absorbância das misturas de reação foi medida em 765 nm usando um espectrofotômetro (BEL Photonics, Piracicaba, São Paulo, Brasil). A absorbância do extrato foi comparada com uma curva padrão de ácido gálico para estimar a concentração de CFT na amostra. Os CFT foram expressos em mg de equivalentes de ácido gálico (EAG) por cem gramas de polpa de macaíba com base no peso seco (mg EAG/100 g).

3.4.3 Determinação de flavonoides totais

O conteúdo total de flavonoides foi medido por meio do ensaio colorimétrico desenvolvido por Zhishen, Mengcheng e Jianming (1999). Alícota de 0,5 mL do extrato foi adicionado a um tubo de ensaio e, 150 µL de NaNO2 a 5% foram adicionados. Após 5 min, foram adicionados 150 µL de AlCl3 a 10% e, após 6 min, 1 mL de NaOH 1 M, seguido pela adição de 1,2 mL de água destilada. A absorbância da amostra foi lida em 510 nm usando um espectrofotômetro (BEL Photonics, Piracicaba, São Paulo, Brasil). A absorbância do extrato foi comparada com uma curva padrão de catequina para estimar a concentração do conteúdo de flavonoides na amostra. O conteúdo de flavonoides foi expresso em mg de equivalentes de catequina (EC) por cem gramas de polpa de macaíba com base no peso seco (mg EC/100 g).

3.4.4 Determinação de carotenoides totais

A determinação de carotenoides totais foi medida de acordo com a metodologia descrita por Higby (1962). Onde, os extratos foram preparados utilizando-se 1 g de polpa em 10 mL de hexano PA e carbonato de cálcio os quais permaneceram em ambiente com ausência de luz durante 12 horas sob refrigeração. Logo após, as amostras foram centrifugadas a 8.000 rpm por 10 minutos, em seguida foi realizada a leitura em espectrofotômetro a 450 nm. Para quantificação dos carotenoides totais foi utilizado a fórmula: Carotenoides totais =42 (A450 x 100)/(250 x L x W), em que, A450

corresponde a absorbância; L = largura da cubeta em cm; e W = quociente entre a massa da amostra em gramas e o volume final da diluição em mL, resultado expresso em mg.100 g^{-1}.

3.4.5 Determinação de flavonoides amarelos

A determinação de carotenoides totais seguiu a metodologia descrita por Francis (1982). Os extratos foram preparados a partir da maceração 1 g de polpa em 10 mL de solução extratora (etanol PA: HCl 1,5 M - 85:15), estes permaneceram protegidos da luz dpor período de 12 horas sob refrigeração. Logo após, foram centrifugados a 9.000 rpm por 15 min, e em seguida foi realizada a leitura em espectrofotômetro a 374 nm. Com os resultados obtidos, utilizou-se a fórmula: fator de diluição x absorbância/76,6, para obtenção do teor de flavonoides amarelos, sendo estes expressos em mg.100 g^{-1}.

3.4.6 Atividade antioxidante - método FRAP

O método FRAP foi realizado de acordo com Benzie e Strain (1996), com modificações propostas por Pulido, Bravo e Saura-Calixto (2000). Neste ensaio, 3,6 mL de reagente FRAP (0,3 M, tampão acetato de pH 3,6, TPTZ 10 mM e cloreto férrico 20 mM) foram misturados com 200 µL de extrato diluído em água destilada, sendo incubados por 30 min em banho-maria a 37 ºC. A solução FRAP foi usada como reagente de referência, e a absorbância foi lida a 593 nm em espectrofotômetro (BEL Photonics). Os resultados foram expressos em µmol de equivalentes de trolox por grama de polpa de macaíba com base no peso seco ($\mu mol\ TE\ /\ g^{-1}$).

3.4.7 Atividade antioxidante - método $ABTS^{\bullet+}$

O método ABTS foi realizado de acordo com a metodologia descrita por Surveswaran et al. (2007), com modificações. O radical $ABTS^{\bullet+}$ foi formado a partir da reação de persulfato de potássio 140 mM com solução estoque 7 mM de $ABTS^{\bullet+}$, mantido no escuro e em temperatura ambiente por 16 h. Para a análise, o radical $ABTS^{\bullet+}$ foi diluído em água destilada até a obtenção de uma solução com absorbância de 700 nm ± 0,02 nm a 734 nm. Uma alíquota de 100 µL de cada extrato foi então

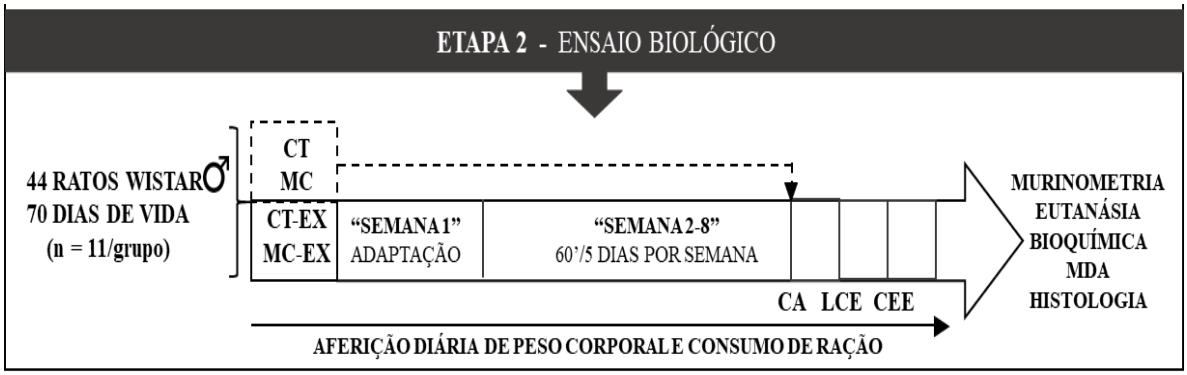
homogeneizada com 500 µL do radical $ABTS^{\bullet+}$. A absorbância das amostras foi lida a 734 nm após 6 min de reação. Os resultados foram expressos em µmol de equivalente trolox por grama de polpa de macaíba com base no peso seco (µmol TE / g^{-1}).

3.6 ENSAIOS BIOLÓGICOS

3.6.1 Animais e dietas

Foram utilizados 44 ratos com 70 dias de idade, pesando 250-300 g. Os animais foram mantidos em condições padrão, com ciclo claro/escuro de 12 horas (início da fase clara às 6:00 h), umidade relativa de 60 ± 5% e temperatura controlada de 22 ± 3 ºC. os animais receberam ração padrão para roedores (ração padrão (Presence Purina®, São Paulo, Brasil) e água *ad libitum*. Foram divididos aleatoriamente em quatro grupos experimentais, de acordo com a suplementação e programa de exercícios: grupo controle sedentário (CT - dieta padrão e água destilada) (n = 11), grupo controle exercitado (CT-EX - dieta padrão e água destilada) (n = 11), grupo macaíba sedentário (MC - dieta padrão e polpa de macaíba) (n = 11), grupo polpa de macaíba exercitado (MC-EX - dieta padrão e polpa de macaíba) (n = 11). Foi administrado polpa de macaíba numa dose de 1000 mg/kg de peso corporal do animal, diariamente por gavagem, 30 minutos antes da realização do exercício de natação, durante as oito semanas, para os grupos tratados. A pesquisa seguiu um protocolo experimental de acordo com as recomendações éticas do Instituto Nacional de Saúde (Bethesda, EUA), e foi aprovada pelo Comitê de Ética em Pesquisa da Universidade Federal de Campina Grande, nº 010/2019 (Anexo C) (Figura 4).

Figura 4. Delineamento experimental.



3.6.2 Treinamento físico de natação

Aos 70 dias de vida, foi iniciado o treinamento de natação, segundo metodologia adaptada de Nakao (2000), em que os ratos nadavam 1 hora por dia, 5 dias por semana, durante 8 semanas, sem uso de sobrecarga. A primeira semana de treinamento correspondeu ao período de adaptação, no qual os grupos treinados foram inicialmente aclimatados ao meio aquático, iniciando com 10 minutos e adicionando 10 minutos diários. A partir da segunda até a oitava semanas os animais nadaram 60 minutos/dia, 5 dias/semana, em um tanque de 85 cm de comprimento, 50 cm de largura e 40 cm de profundidade, com temperatura da água de 32 ºC (± 1 ° C). Os grupos não exercitados permaneceram durante o mesmo período em gaiolas com lâmina d'água (4 cm), com temperatura de 32 ºC (± 1 ° C), mas sem realizar esforço físico de natação. Este procedimento teve como objetivo proporcionar o mesmo estresse aquático (Tabela 1).

Tabela 1. Protocolo de treinamento.

Período de Treinamento	Tempo
1ª semana de treinamento (adaptação)	10 a 50 min
1º dia de adaptação	10 min
2º dia de adaptação	20 min
3º dia de adaptação	30 min
4º dia de adaptação	40 min
5º dia de adaptação	50 min
2ª a 8ª de semana de treinamento	60 min

Fonte: adaptada de Nakao (2000).

3.6 Testes comportamentais

3.6.1 Campo Aberto (CA)

O aparelho de campo aberto para realização do teste consistiu em caixa quadrada preta uniformemente iluminada, medindo 60 x 60 x 60 cm, sendo

subdivididas em 9 quadrantes medindo 20 x 20 cm (Figura 5). O campo aberto é um teste utilizado para avaliar o comportamento de ansiedade e atividade exploratória em ratos (PELLOW et al., 1985).

Figura 5. Aparato do Campo Aberto.

Fonte: Laboratório de Nutrição Experimental.

Após o período de 24 horas de repouso, cada animal foi colocado no centro do campo aberto, onde permaneceu durante 10 minutos para livre exploração. Foram avaliados os parâmetros de ambulação (número de cruzamentos dos segmentos pelo animal com as quatro patas), número do comportamento de levantar (*rearing*) e tempo do comportamento de autolimpeza *(grooming).* A locomoção/ambulação, assim como o ato de levantar-se são observados através da exploração forçada uma vez que o animal não pode escapar da área de teste, podendo assim avaliar o comportamento de ansiedade. O comportamento de *grooming* indica aumento de ansiedade no animal (RACHETTI et al., 2013).

Antes de iniciar os testes foi adotado o protocolo de higienização do aparelho com álcool a 70% e papel toalha, sendo que a após cada troca de animal a arena foi higienizada com álcool a 10% e papel toalha. A manipulação dos animais foi realizada sempre pelo mesmo pesquisador. Todas as sessões foram registradas com uma câmera filmadora fixada no teto do aparelho para posteriormente os vídeos serem analisados.

3.6.2 Labirinto em Cruz Elevado (LCE)

O LCE (Figura 6) é frequentemente utilizado como um modelo experimental para avaliar o comportamento de ansiedade em roedores (PELLOW; FILE, 1986). O teste consiste em colocar o animal em um LCE feito de madeira em forma de cruz, elevado do solo, formado por dois braços fechados por paredes e dois abertos (perpendiculares aos primeiros), objetivando analisar a frequência de entradas e o tempo gasto pelo animal em cada tipo de braço, além do tempo de permanência na área central. Quanto a esse tipo de teste, observa-se que o animal tende a explorar os dois tipos de braços, entrando e permanecendo por mais tempo nos braços fechados, uma vez que os roedores evitam espaços abertos. Segundo Handley e Mithani (1984) e Pellow e File (1986), a preferência pelos braços abertos ou fechados é considerada um indicador confiável de ansiedade, no qual, quanto maior o nível de ansiedade, menor é a preferência pelos braços abertos bem como o tempo de permanência nos mesmos, e vice-versa.

O referido teste ocorreu com todos os grupos experimentais, onde: o animal foi colocado no centro do aparelho, voltado para um dos braços fechados, onde foi permitida a livre exploração durante 5 (cinco) minutos. As sessões foram registradas com uma câmera de vídeo instalada no teto em ambiente de pouca luz. Posteriormente, foram analisados os seguintes parâmetros: número de entradas nos braços abertos e fechados, tempo gasto em cada um dos braços, tempo gasto na área central, número de mergulho de cabeça.

Figura 6. Aparato do Labirinto em Cruz Elevado.

Fonte: Laboratório de Nutrição Experimental.

3.6.3 Caixa claro-escuro (CCE)

O teste de transição claro-escuro é amplamente utilizado como modelo de ansiedade em roedores (BOURIN et al., 2007). Este método consiste em colocar o animal em um ambiente contendo dois compartimentos: um claro e um escuro, e baseia-se na aversão inata de roedores a ambientes iluminados e no comportamento exploratório espontâneo destes animais. A transição entre os compartimentos é definida como a entrada do animal com todas as quatro patas.

Substâncias com efeito ansiolítico promovem o aumento nas transições, e no número de entradas e tempo gasto no compartimento claro.

A caixa de transição claro-escuro é feita de madeira, com dimensões totais de: 27 cm (A) x 45 cm (L) x 27 cm (C), e consiste em dois compartimentos, sendo o compartimento claro com dimensão maior (27 x 27 x 27), e com o piso dividido em 9 quadrados (9 cm x 9 cm), e o compartimento escuro com menor dimensão (27 x 18 x 27). Na divisória entre os dois compartimentos, há uma abertura central medindo 7 cm x 7 cm. Esta caixa possui ainda uma tampa superior, que é pintada de preto na

extensão que cobre o compartimento escuro (Figura 7). Além disso, deve ser utilizada uma lâmpada (luminária) para iluminar o compartimento claro.

Figura 7. Caixa Claro-escuro.

Fonte: Laboratório de Nutrição Experimental.

O animal foi colocado no centro do compartimento claro com o focinho voltado para a entrada central, onde foram avaliados os seguintes parâmetros:

- ✓ Número de transições entre os compartimentos claro e escuro – sendo considerado quando o animal atravessa para o compartimento com as quatro patas;
- ✓ Tempo de permanência no compartimento claro (em segundos) – tempo gasto no compartimento claro;
- ✓ Tempo total de observação: 5 minutos

3.7 Parametros murinométrica e bioquímica

3.7.1 Avaliação murinométrica

Os animais, 24 horas após os testes comportamentais e após jejum de 8 a 10 horas foram eutanasiados, sendo posteriormente aferidos: peso corporal,

comprimento naso-anal, circunferência abdominal e torácica e Índice de Massa Corporal (NOVELLI et al., 2007).

3.7.2 Análises bioquímicas

O sangue coletado após a eutanásia foi centrifugado em uma centrífuga Novatecnica® NT 810 (22.438 x*g*, durante 10 minutos). Após centrifugação, foram medidos glicose (λ = 505 nm), colesterol total (TC) (λ = 500 nm), triglicerídeos (tag) (λ = 505 nm), e HDL-colesterol (λ = 500 nm), por meio de método enzimático - Trinder (Diagnóstico de Labtest, St Louis, EUA), usando um espectrofotômetro BEL Photonics®. A fração de lipoproteína de baixa densidade (LDL-c) foi estimada utilizando a equação (FRIEDWALD, LEVY, FREDRICKSON, 1972)

3.7.3 Determinação de riscos cardiovasculares

A partir dos dados bioquímicos foram calculados: o índice aterogênico (IA), coeficiente aterogênico (CA), risco cardíaco (RC) e risco caronariano (RC2) (AHMADVAND ET AL. 2016; EREJUWA ET AL. 2016; IKEWUCHI; IKEWUCHI, 2009).

3.7.4 Determinação do Conteúdo de Malonaldeído (MDA)

A determinação do conteúdo de MDA foi realizada pelo método descrito por Esterbauer e Cheeseman (1990) no fígado, coração e cérebro. Os resultados foram expressos em nmol/g de tecido.

3.7.5 Histologia do músculo solear e do fígado

Fragmentos de tecido do fígado e músculo solear foram preservados em solução de formaldeído tamponado a 10% até o processamento histológico. Os tecidos foram submetidos a procedimento histológico e incluídos em parafina

histológica e seccionados com espessura de 4 µm. Os tecidos seccionados foram corados com hematoxilina-eosina (HE) para análise microscópica (Motic BA 200).

3.8 ANÁLISES ESTATÍSTICAS

Os dados comportamentais foram analisados usando o software ANY-maze Video Tracking System sob a licença número 5REM-G7WG-W4Z5-ACZB. Em seguida, os resultados foram submetidos à análise de normalidade (D'Agostino-Pearson e Shapiro-Wilk), Análise de Variância de duas vias (ANOVA – two way) seguido por um teste de Tukey quando apropriado. Considerando o nível de significância de 5% ($p < 0,05$). Todos os dados foram analisados no GraphPad Prism® versão 5.01 (GraphPad Software Inc., San Diego, CA, EUA).

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4 RESULTADOS

Os resultados obtidos nesta tese estão apresentados em forma de Artigos Científicos submetidos em periódicos de impacto na área de Ciência e Tecnologia de Alimentos e de Nutrição, que se encontram entre os quatro estratos superiores (A1-A4).

Artigo 1: **BRAZILIAN PALM SEED PULP (***Acrocomia intumescens* **Drude) INDUCES ANXIOLYTIC BEHAVIOR IN EXERCISED RATS**

Artigo 2: **CONSUMPTION OF BRAZILIAN PALM FRUIT (***Acrocomia intumescens* **Drude) IMPROVES BIOCHEMICAL PARAMETERS AND REDUCES CARDIOVASCULAR RISK IN EXERCISED RATS**

Artigo 1 - BRAZILIAN PALM SEED PULP (*Acrocomia intumescens* Drude) INDUCES ANXIOLYTIC BEHAVIOR IN EXERCISED RATS

(Periódico: Food Bioscience. Fator de Impacto: 4.240)

Brazilian Palm seed pulp (*Acrocomia intumescens* Drude) induces anxiolytic behavior in exercised rats

Abstract

Introduction: *Acrocomia intumescens* is a native Brazilian palm that presents antioxidant components, in addition to unsaturated fatty acids, but it is still little explored.

Objective: To analyze the association of macaibeira (*Acrocomia intumescens* Drude) fruit pulp with physical exercise on behavioral parameters in rats.

Methodology: 44 rats were used, divided into 4 groups (n = 11 each): sedentary control (CT), exercised control (CT-EX), sedentary macaíba (MC) and exercised macaíba (MC-EX). MC and MC-EX received 1000 mg/kg/day of macaíba pulp by gavage. CT and CT-EX received distilled water. The exercised animals were exposed to one week of adaptation. After adaptation until the eighth week of training, the animals swam five days a week, for 60 minutes. Anxiety parameters were evaluated using the open field (OF), elevated plus maze (EPM), and light-dark box (LDB). The animals' brains were removed to measure malondialdehyde.

Results: MC-EX showed a higher rate of exploration, more time in the open arms in EMP, more time in the light space in LDB, and lower level of malondialdehyde. An anxiolytic -like behavior was observed in MC. MC entered and spent more time in the open arms. MC and MC-Ex spent more time in the central area in EPM and realized more rearing in OF.

Conclusion: Data showed that the consumption of macaíba pulp induces anxiolytic-like behavior and reduces lipid peroxidation in the brain of animals. In addition, this effect was enhanced when the animals were submitted to swimming exercise.

Keywords: Swimming. Malondialdehyde. Anxiety. Lipid peroxidation. Macaíba.

1. Introduction

Anxiety disorder affects a large part of the world population and its treatment occurs using drugs that despite proving effective has significant side effects as sedation, insomnia, and nausea, as well as being costly (Bartley, Hay, & Bloch, 2013). Because of this, other treatments have been sought to help improve the symptoms of these pathologies, such as changing eating habits and the regular practice of physical exercises to reduce stress damage and improve symptoms of anxiety and depression. Bearing in mind that oxidative stress has been associated with the emergence of neurological pathologies, such as anxiety and depression (W. Liu, Xue, Xia, Liu, & Qi, 2018; Z. Liu et al., 2017).

Physical exercise, as well as diet, are associated with decreased fatigue, tension and muscle pain, and feelings of anxiety and depression (Bartley et al., 2013), in addition to favoring an increase in neuronal plasticity, decreasing inflammation and oxidative stress by reducing free radicals responsible for cell damage (Abad, Miladi-Gorji, & Bigdeli, 2016; Houdebine, Gallelli, Rastelli, Sampathkumar, & Grenier, 2017). The oxidative stress reduction by physical exercise occurs by stimulating an enzymatic antioxidant system that acts by removing superoxide anion, organic hydroperoxides, and hydrogen peroxide (W. Liu et al., 2018; Z. Liu et al., 2017). While diet plays an important role in the non-enzymatic defense system, which includes vitamins, minerals and phenolic compounds (Neha, Haider, Pathak, & Yar, 2019). These compounds can be observed in some palm fruits which have already been proven to have antioxidant action (da Silva Andrade et al., 2018; Lescano, Iwamoto, Sanjinez-Argandoña, &

Kassuya, 2015; Turola Barbi et al., 2019). Palm trees are already widely used for medicinal purposes by local populations. However, more scientific studies that prove their effects *in vivo* are necessary (Agostini-Costa, 2018).

Acrocomia intumescens Drude, known as macaibeira, is a native Brazilian species, from the *Arecaceae* family, occurring in Brazil Northeast region, where is located the tropical and subtropical Atlantic Forest, consumed by the local population. Due to its nutritional value, this species of palm is promising for fresh consumption and in the preparation of food products (for example, oil). The fruit, known as macaíba, consists of two edible portions, pulp and kernel. The pulp, consisting of predominantly monounsaturated and polyunsaturated fatty acids, in addition to vitamin C, carotenoids, flavonoids and minerals (Bora & Rocha, 2004; Coimbra & Jorge, 2011). In spite of the macaíba presenting compounds with antioxidant potential and that can bring health benefits, until now, data in the literature investigating the association of this fruit with physical exercise on anxiety parameters have not been found. In this way, this study becomes a pioneer in the investigation of this relationship and contributes to the realization of future clinical studies.

Therefore, we hypothesized that supplementation with macaíba pulp associated with physical training may induce anxiolytic effects and decrease lipid peroxidation in the brain of rats. Thus, with the present study aimed to evaluate the effects of supplementation with macaíba pulp on anxiety parameters and lipid peroxidation in the brain tissue of rats submitted to physical exercise.

2. Methodology

2.1 Raw material and execution place

Macaibeira fruits were obtained from Areia city, -6.963845 (latitude) and -35.749738 (longitude) location, Paraíba state, Brazil Northeast region. Registration in the national system for the management of genetic heritage and associated traditional knowledge (SISGEN): ADD854A.

2.2 Macaíba pulp lyophilization

Macaibeira fruits were cleaned and pulped. After being pulped, they were frozen and subsequently subjected to the lyophilization process in Lyophilizer L101 (Liotop®), 212Vca, -50 ºC for 48 hours. After obtaining the lyophilizate, the pulp was transferred to plastic packaging, vacuum-sealed, -20 ºC.

2.3 Macronutrients characterization and fatty acid profile of the pulp

Lyophilized macaíba pulp was used for protein and carbohydrate determination by the methodology of Kjedhal, and carbohydrate described by AOAC (2000), lipids by the methodology described by Folch, Less and Stanley (1957) and lipid profile by Hartman and Lago's methodology (1973).

2.4 Analysis of bioactive compounds from macaíba pulp

2.4.1 Extraction

Macaíba pulp constituents were extracted with both ethanol 80% (v/v) and evaluated for ABTS• scavenging capacity, ferric reducing activity (FRAP) and total flavonoids. For the total phenolic content, methanol was used for extractions, 1 g of macaíba pulp was weighed in a test tube and immediately afterwards, 10 ml of solvent were added. The test tube was subjected to room temperature for 60 minutes and after

filtration, the volume was completed to 10 mL with the extraction solvent and stored in a freezer (-18 °C) until analysis. All the extractions were performed in triplicate.

2.4.2 Determination of total phenolic compounds (TPC)

To determine the content of total phenolic compounds, the methodology described by Liu et al. (2002) was used with minor modifications. Briefly, 250 µL of extract was mixed with 1250 µL of a 1:10 diluted Folin–Ciocalteau reagent. The solutions were mixed and left at room temperature (27°C) for 6 minutes. After incubation, 1000 ml of 7.5% sodium carbonate solution (Na_2CO_3) was added and stained in a 50 °C water bath for 5 minutes. The absorbance of the reaction mixtures was measured at 765 nm using a spectrophotometer (BEL Photonics®, Piracicaba, São Paulo, Brazil). The absorbance of the extract was compared with a gallic acid standard curve for estimating concentration of TPC in the sample. The TPC was expressed as mg of gallic acid equivalents (GAE) per hundred grams of macaíba pulp on the basis of dry weight (DW).

2.4.3 Determination of total flavonoids

The total flavonoid content was measured using the colorimetric assay developed by Zhishen, Mengcheng and Jianming (Zhishen, Mengcheng, & Jianming, 1999). A known volume (0.5 mL) of the extract was added to a test tube and at zero time, 150 µL of 5% $NaNO_2$ was added. After 5 min, 150 µL of 10% $AlCl_3$ was added, and, after 6min, 1 mL of 1 M NaOH, followed by the addition of 1.2 mL distilled water. Sample absorbance was read at 510 nm using a spectrophotometer (BEL Photonics®, Piracicaba, São Paulo, Brazil). The absorbance of the extract was compared with a catechin standard curve for estimating concentration of flavonoids contents in the

sample. The flavonoids contents were expressed as mg of catechin equivalents (QE) per hundred grams of macaíba pulp on the basis of dry weight (DW).

2.4.4 Antioxidant activity – FRAP method

The FRAP method was performed according to Benzie & Strain (1996), with modifications proposed by Pulido, Bravo & Saura-Calixto (2000). In this assay, 3.6 mL of FRAP reagent (0.3 M, pH 3.6 acetate buffer, 10 mM TPTZ and 20 mM ferric chloride) were mixed with 200 µL of extract diluted in distilled water, being incubated for 30 min at 37 ºC. The FRAP solution was used as reference reagent, and absorbance was read at 593 nm. The results were expressed in µmol of trolox equivalents per gram of macaíba pulp on dry weight (DW) basis (µmol TE/g^{-1}).

2.4.5 Antioxidant activity – ABTS method $^{+}$

The ABTS method was carried out according to the methodology described by Surveswaran et al. (2007), with modifications. The ABTS radical was formed from the reaction of 140 mM potassium persulfate with 7 mM ABTS stock solution, kept in the dark and at room temperature for 16 h. For the analysis, ABTS radical was diluted in distilled water until a solution with absorbance of 700 nm ± 0.02 nm at 734 nm was obtained. A 100 µL aliquot of each extract was then homogenized with 500 uL of the ABTS radical. Absorbance of the samples was read at 734 nm after 6 min of reaction. The results were expressed in µmol of trolox equivalent per gram macaíba pulp on dry weight (DW) basis (µmol TE/g^{-1}). Table 1 shows the nutritional composition and lipid profile of the macaíba pulp.

TABLE 1

2.5 Biological tests

2.5.1 Animals and diet

Forty-eight Wistar rats (n = 44) with 70 days old, weighing 250-300 g, were used. The animals were maintained in standard conditions, with 12 hours light/dark cycle, a relative humidity of 80 ± 5% and controlled temperature 22ºC ± 3ºC. Fed standard rodent diet and water *ad libitum*. They were randomly assigned to four groups, according to supplementation and exercise program: sedentary control group (CT - standard diet and distilled water) (n = 11), exercised control group (CT-EX - standard diet and distilled water) (n = 11), sedentary macaíba group (MC - standard diet and macaíba pulp) (n = 11), exercised macaíba pulp group (MC-EX - standard diet and macaíba pulp) (n = 11). The pulp of macaíba was administered, 1000 mg / kg of body weight of the animal (dose established based on previous trial), daily by gavage, before swimming exercise, during the eight weeks, for the treated groups. At the end of the experiment, the animals were euthanized using a guillotine. The entire experimental protocol was carried out upon approval by the Ethics Committee on the Use of Animals - CEUA / CSTR, nº 010/2019, in accordance with Animal Research: Reporting of In Vivo Experiments - The ARRIVE Guidelines (Kilkenny, Browne, Cuthill, Emerson, & Altman, 2010).

2.5.2 Physical swimming training

When the animals reached 70 days of age, they started swimming training, according to a methodology adapted from Nakao (Nakao et al., 2000), swimming 1 hour a day, 5 days a week for 8 weeks, without using overload. In which the first week of training corresponded to the adaptation period, in which the trained groups were initially acclimated to the aquatic environment, starting with 10 minutes and adding 10 minutes daily. From the second to the eighth week, the animals swam for 60

minutes/day, 5 days/week in an 85 cm long, 50 cm wide and 40 cm deep tank. The non-exercised groups remained during the same period in cages with water depth (4 cm), with a temperature of 32 ºC (± 1 °C), but without performing physical swimming effort. This procedure aimed to provide the same aquatic stress.

2.6 Behavioral tests

2.6.1 Open field (*OF*)

Open field device for the test consisted of a black square box with uniform light, measuring 60 x 60 x 60 cm, being subdivided into 9 quadrants measuring 9 x 9 cm delimited by a white line. The open field is a test used to evaluate anxiety behavior and exploratory activity in rats (Nava-Mesa, Lamprea, & Múnera, 2013).

Right after the 24-hour rest period, each animal was placed in the center of the open field, remaining in the apparatus for 10 minutes for free exploration. In this test, we evaluated the parameters of lifting behavior number (rearing), self-cleaning behavior time *(*grooming*)* and locomotion/ambulation (number of segment crossings by the animal with the four legs).

After the 24-hour rest period, each animal was placed in the center of the open field, remaining in the device for 10 minutes for free exploration. In this test, the parameters number of raising behavior (rearing), time of self-cleaning behavior (grooming) and locomotion/ambulation (number of times the animal passes to another square with its paws) were evaluated.

2.6.2 Elevated plus maze (EPM)

This test occurred with all experimental groups, where each animal was placed in the center of EPM, facing one of the closed arms and free exploration was allowed for five minutes. The sessions were recorded with a video camera installed on the

ceiling in a low light environment. Subsequently, the following parameters were analyzed: number of entries in the open arms and time spent, time spent in the central area, number of head dipping and stay time in closed arms (Handley & Mithani, 1984; Pellow & File, 1986).

2.6.3 Light-dark box (LDB)

The light-dark transition box made of wood, with total dimensions of 27 cm x 45 cm x 27 cm and consisted of two compartments, the light compartment having a larger dimension (27 cm x 27 cm x 27 cm) with the floor divided into 9 squares (9 cm x 9 cm), and the dark compartment was smaller (27 cm x 18 cm x 27 cm). In the partition between the two compartments, there is a central opening measuring 7 cm x 7 cm. This box also has a top cover, which is painted black in the extension that covers the dark compartment. In addition, a lamp (light fixture) was used to illuminate the clear compartment.

The animal is placed in the center of the clear compartment with its snout facing the central entrance. The following parameters were evaluated: length of stay in the light compartment, number of transitions (transitions between light and dark spaces) and number of lurking (observations of the animal from within dark to light environment), with a 5 minute total observation time (Bourin, Petit-Demoulière, Nic Dhonnchadha, & Hascöet, 2007).

2.6.4 Levels of malondialdehyde (MDA)

Macaíba pulp effect on cerebral malondialdehyde (MDA) levels. For this, the brain samples were suspended in a buffer of Tris- HCl 1:5 (w/v) and homogenized for 15 seconds on a cold plate. The resulting suspension was homogenized for 2 minutes with an automatic Potter homogenizer and centrifuged at 2500 g and 4°C for 10 min.

Supernatants were used to determine the content of MDA (Esterbauer & Cheeseman, 1990). The results were expressed as MDA tissue nmol g^{-1}.

2.7 Statistical Analysis

Behavioral data were analyzed using ANY-maze Video Tracking System software under license number 5REM-G7WG-W4Z5-ACZB. Then, the results were submitted to normality tests (D'Agostino-Pearson and Shapiro-Wilk), Analysis of Variance (TWO WAY - ANOVA), considering a significance level of 5% ($p < 0.05$).

3. Results

3.1 Behavioral Tests

3.1.1 Open field (OF)

In the data shown in Figure 1, we observed that the CT-EX group spent more time self-grooming compared to CT ($p = 0.0062$), MC ($p = 0.0030$) and MC-EX ($p = 0.0030$) (Figure 1 A). Using two-way ANOVA, we verified that these effects were influenced by the consumption of macaíba pulp [$F (1, 40) = 5,889$; $p = 0.0198$], by swimming exercise [$F (1, 40) = 4.198$; $p = 0.0471$], and by the interaction between diet and physical exercise [$F (1, 40) = 4,154$; $p = 0.0482$].

These effects were also observed when we analyzed the rearing. We found that MC-EX and MC had a higher number of rearing times when compared to CT and CT-EX ($p < 0.05$). We also observed that CT-EX had fewer rearing times compared to its control (CT) ($p = 0.0005$). As well as grooming, the observed effects were influenced by diet [$F (1, 40) = 52$; $p < 0.0001$], exercise [$F (1, 40) = 4.516$; $p = 0.0398$] and the interaction of both [$F (1, 40) = 10.3$; $p = 0.0026$].

FIGURE 1

Figure 1C shows the data corresponding to the analysis of locomotion/ambulation of the animals. We found that the groups that most explored the environment were MC-EX and MC compared to CT and CT-EX ($p < 0.05$). We verified that the consumption of macaíba pulp [$F (1.40) = 27.21$; $p < 0.0001$] contributed to greater exploitation in the groups that consumed it, demonstrating an anxiolytic effect in the groups that consumed the macaíba pulp.

Thus, for the tests performed in the OF apparatus, we verified that exercise induced an anxiogenic-like behavior, while the macaíba pulp contributed to induce anxiolytic-like behavior. And also, that the consumption of macaíba pulp by the exercised group managed to reverse exercise-induced anxiety.

3.1.2 Elevated plus maze (EPM)

Table 2 shows the results regarding the evaluation of the animals in the EPM. We verified that the diet influenced the number of entries [$F (1, 40) = 45.57$; $p < 0.0001$] and the time spent in the open arms [$F (1, 40) = 89.83$; $p < 0.0001$], the number of head dives [$F (1, 40) = 32.13$; $p < 0.0001$] and the time spent in the closed arms [$F (1, 40) = 15.98$; $p = 0.0003$]. And that exercise promoted an effect on the number of entries [$F (1, 40) = 17.26$; $p = 0.0002$] and stay time [$F (1, 40) = 9,869$; $p = 0.0032$] in the open arms. The interaction between diet and exercise was significant for the time spent in the closed arms [$F (1, 40) = 24.87$; $p < 0.0001$] and for the number of head dives [$F (1, 40) = 8.944$; $p = 0.0047$] of the animals.

TABLE 2

We observed that the animals in the MC-EX group moved more often to the open arms compared to MC ($p = 0.0010$), CT ($p < 0.0001$) and CT-EX ($p < 0.0001$). The data showed that MC had a greater number of entries in the open arms compared

to CT ($p = 0.0002$) and CT-EX ($p = 0.0738$). Among the control groups, the largest number of entries into open arms was presented by the exercised group ($p = 0.0252$).

The length of stay time of the animals in the open arms was also larger for MC-EX compared to MC ($p < 0.0001$), CT ($p < 0.0001$) and CT-EX ($p < 0.0001$). The MC group also had a longer stay in the open arms when compared to CT ($p = 0.0029$) and CT-EX ($p < 0.0001$). The comparison between the CT and CT-EX groups showed a length of stay time in the open arms of the sedentary control group ($p = 0.0349$).

The number of head dives was higher in the MC-EX group when compared to CT ($p = 0.0013$), MC ($p = 0.0439$) and CT-EX ($p < 0.0001$). MC also had a higher number of head dives compared to CT ($p = 0.0275$) and CT-EX ($p < 0.0001$). And among control groups, the number of head dives was higher for CT than for CT-EX ($p = 0.0111$).

Regarding the assessment of the animals' length of stay in the device closed arms, we saw that MC-EX and MC were the groups that remained for a shorter time compared to CT and CT-EX ($p < 0.05$).

The results obtained in the EPM confirm the findings of the tests in the OF in which anxiolytic effect of the consumption of Macaíba pulp is observed.

3.1.3 Light-dark box (LDB)

FIGURE 3

Based on the data presented in Figure 3, we verified that with respect to the length of stay time of the groups on the light side of the LDB, MC-EX and MC stayed longer compared to CT and CT-EX ($p < 0.0001$). We found that the consumption of macaíba pulp influenced this parameter evaluated [$F (1, 40) = 63.7$; $p < 0.0001$].

Regarding the number of peeps, we verified that it was higher only for MC-EX when compared to the other groups ($p < 0.0001$). And that there was an effect caused by diet [$F (1, 40) = 8,679$; $p = 0.0053$], exercise [$F (1, 40) = 20.9$; $p < 0.0001$], and an interaction between diet and exercise [$F (1, 40) = 28.5$; $p < 0.0001$].

For the evaluation of transitions between the light and dark sides of the LDB, we observed that the number of times the animals went from the dark to the light side was greater for MC-EX and MC compared to CT and CT-EX ($p < 0.05$). Regarding the evaluation of the effects of diet, exercise, and the interaction of both, we found that the diet had an influence on this parameter [$F (1, 40) = 8,047$; $p = 0.0071$].

In the LDB device, it was also possible to observe the effects of the diet in the groups with macaíba pulp with anxiolytic activity. Furthermore, in this device, we did not find any anxiogenic effects in the CT-EX group, which presented a behavior similar to its sedentary control.

3.1.4 Levels of malondialdehyde in the brain

The results show that the exercised group (CT-EX) presented a significant increase in cerebral MDA levels in relation to the sedentary control group (CT) ($p < 0.0001$). The administration of macaíba pulp extract associated with exercise was able to reduce the levels of cerebral MDA when we compared CT-EX with MC-EX ($p < 0.0001$), showing a beneficial effect in reducing cerebral oxidative stress (Figure 4). These effects were influenced by diet [$F (1, 40) = 32.73$; $p < 0.0001$], exercise [$F (1, 40) = 44.03$; $p < 0.0001$] and the interaction between diet and exercise [$F (1, 40) = 20.56$; $p < 0.0001$].

FIGURE 4

4. Discussion

In the present study, we investigated the relationship between a diet containing lipid compounds and antioxidants from macaíba pulp on lipid peroxidation. The investigation was conducted by quantifying MDA in brain tissue and evaluating behavioral parameters of anxiety in rats submitted to swimming exercises. Open-field, elevated plus-maze, and light-dark box apparatus were used to assess exploratory activity and anxiety behavior. Our results showed that the animals that consumed macaíba pulp had an anxiolytic effect and that the exercised group that consumed the pulp also managed to reverse the stress and intensify the anxiolytic behavior.

In our study, we observed that CT-EX presented an anxiogenic behavior expressed by the increase in grooming, less environmental exploration, and greater aversion to open spaces in the EPM apparatus. While the MC and MC-EX groups, mainly MC-EX, showed anxiolytic behavior verified by the decrease in grooming, increased rearing in the OF, greater exploration and permanence time in the open arms of the EPM, and longer permanence time on the light side of the LDB.

It is known that during exercise physiological processes promote an increase in the production of reactive oxygen species (ROS), which aim to maintain normal tone and contractility (Powers & Jackson, 2008). Thus, these processes present themselves as potential sources for the production of superoxide anion, hydrogen peroxide, and hydroxyl radical. The imbalance between the production of oxidizing substances and the body's antioxidant defenses, known as oxidative stress, can cause lipid peroxidation, leading to damage to cellular DNA and protein carbonylation, thus occasioning changes in cell functions and tissue damage (Deaton & Marlin, 2003; Goto & Radák, 2007; Halliwell, 2006) These changes occur due to free radicals modifying the permeability, fluidity, and integrity of cell membranes, and thus modifying their

functionality (Mahattanatawee et al., 2006). The central nervous system is sensitive to attacks caused by free radicals due to its relatively small total antioxidant capacity. Once lipid peroxidation begins, a spread of chain reactions occurs until end products are produced. Among these products are malonaldehyde (MDA), 4-hydroxy-2-nonenal (4-HNE), and F2-isoprostanes. These products are accumulated in biological systems (Cherubini, Ruggiero, Polidori, & Mecocci, 2005; Rahal et al., 2014).

Enzymatic defense systems act by detoxifying or repairing damage generated by free radicals. When this system is unable to eliminate the causative agents, damage can be generated, leading to impaired brain function. This impairment occurs because the brain tissue is very sensitive to oxidation, as it is one of the largest consumers of oxygen, and because it is rich in easily oxidizable lipids (Halliwell, 2006; Steenkamp et al., 2017). The increase in the oxidation state, as well as the increase in the pro-inflammatory process, damage the normal cell structure and thus inhibit the normal processes of cell survival, neurogenesis, and neuroplasticity, which trigger changes in anxiety behavior (Moylan et al., 2013).

Normally, the body tends to adapt to changes induced by exercise and acquires the ability to regulate changes in the body's enzymatic and non-enzymatic antioxidants. Furthermore, oxidants can modulate various cell signaling pathways and regulate gene expression in cells. In this context, diet can contribute to adaptation by decreasing lipid peroxidation, reactive oxygen species, and changes in the mitochondrial membrane potential through the consumption of antioxidant compounds (Cilla et al., 2018; Joseph et al., 2013; Mankowski, Anton, Buford, & Leeuwenburgh, 2015; Neha et al., 2019).

These mechanisms may justify the findings in our study, in which we verified an increase in the production of ROS demonstrated by the increase in the concentration of MDA in the brain tissue of the CT-EX group, since macaíba pulp is composed of fatty acids, which are structural components of the brain membranes. Also, its pulp has

antioxidant compounds such as carotenoids and flavonoids. This may explain why MDA levels were lower in the groups that consumed macaíba pulp and why the effects were even more expressive in MC-EX. According to Neha et al (2019), the intake of a polyphenolic diet may be related to the prevention of cardiovascular and neurodegenerative diseases, because it acts in a neuroprotective way, inhibiting oxidative stress, as observed in the present study. Thus, we suggest that the macaíba pulp contributed to stress reduction and, therefore, favored the body's higher adaptation to exercise, contributing to the anxiolytic effect. Similar results were obtained by Texeira et al. (2011) in a previous study using swimming exercise associated with a diet containing unsaturated fatty acids. Their study found that the group treated with soy oil and submitted to swimming presented lower anxiety levels. This finding was attributed to the effect of unsaturated fatty acids and the reduction of oxidative damage in the brain.

When Barbosa et al. (2018) evaluated the MDA concentration in rats undergoing treadmill exercise, they observed an increase of MDA in the brain tissue of the exercised control group compared to the sedentary group. However, when exercise was associated with goat milk fat, a source of acid-conjugated linoleic acid, this oxidative damage was reversed. Those results are similar to the findings obtained in our study, in which CT-EX showed an increase in MDA, but the consumption of macaíba pulp reversed and decreased MDA levels in MC-EX. These findings reinforce the antioxidant potential of the macaíba pulp, which showed antioxidant activity both in vitro and in vivo.

In addition, the pulp of macaíba is also composed of carotenoids, which have biological activities, including provitamin A activity related to the stimulation of the immune response, modulation of gap junction communication, cell cycle regulation and apoptosis, modulation of growth factors, and cell differentiation (Fiedor & Burda, 2014;

Ribeiro, Freitas, Silva, Carvalho, & Fernandes, 2018). Carotenoids have antioxidant activity, eliminating $DPPH^{\bullet}$, $NO^{\bullet}$, and $ABTS^{\bullet+}$, inhibiting the production of $O_2^{\bullet}$, increasing the activity of catalase, superoxide dismutase, glutathione reductase, and GSH, and reducing MDA levels (Ribeiro et al., 2018).

Carotenoids, which are lipophilic molecules, are located mainly in cell membranes. The inclusion of carotenoids may visibly affect the properties of membranes through changes in rigidity, mechanical strength, thickness, fluidity, or permeability, and these aspects are essential for their proper functioning (Fiedor & Burda, 2014). However, the absorption of carotenoids depends on factors such as the presence of lipids in the diet (Kim, Gordon, Ferruzzi, & Campbell, 2015; Kopec et al., 2014; Moran, Mohn, Hason, Erdman, & Johnson, 2018). As macaíba pulp is also composed of lipids, it is suggested that the absorption of carotenoids provided by its administration is facilitated. This process may be related to the decrease in lipid peroxidation, observed from the reduction of MDA in the brain verified in this research.

Thus, our results showed that diet plays an important role in the mechanism involving exercise and in neuronal and adaptive functionalities.

5. Conclusion

In the present study, we found that the consumption of macaíba pulp induces an anxiolytic effect that was accompanied by a reduction of lipid peroxidation in the brain of animals. In addition, this effect was enhanced when the animals were submitted to swimming exercise. Thus, the macaíba pulp presents itself as a potential food for human consumption. However, future studies with humans are necessary to verify its safety and effectiveness.

Conflicts of Interest

There are no conflicts to declare.

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Author contributions

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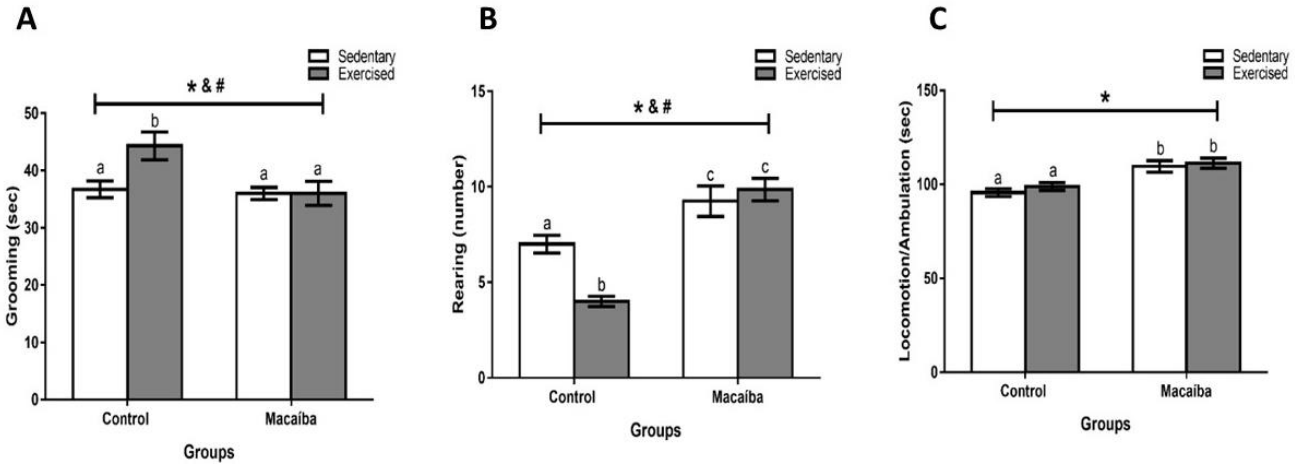

Fig. 1 Effect of the macaíba pulp on the open field test. (A) Self-cleaning behavior (grooming), (B) Rearing behavior number and (C) Locomotion/ambulation (number of segment crossings by the animal with the four legs). Sedentary control (CT) (n = 11); Sedentary Macaíba (MC) (n = 11); Control exercised (CT-EX) (n = 11); and Macaíba

exercised (MC-EX) (n = 11). Values are means, with standard deviations. Two-way ANOVA. Different letters indicate a significant difference (p <0.05). # - indicates effect of diet; & - indicates exercise effect; * - indicates the effect of the interaction between diet and exercise.

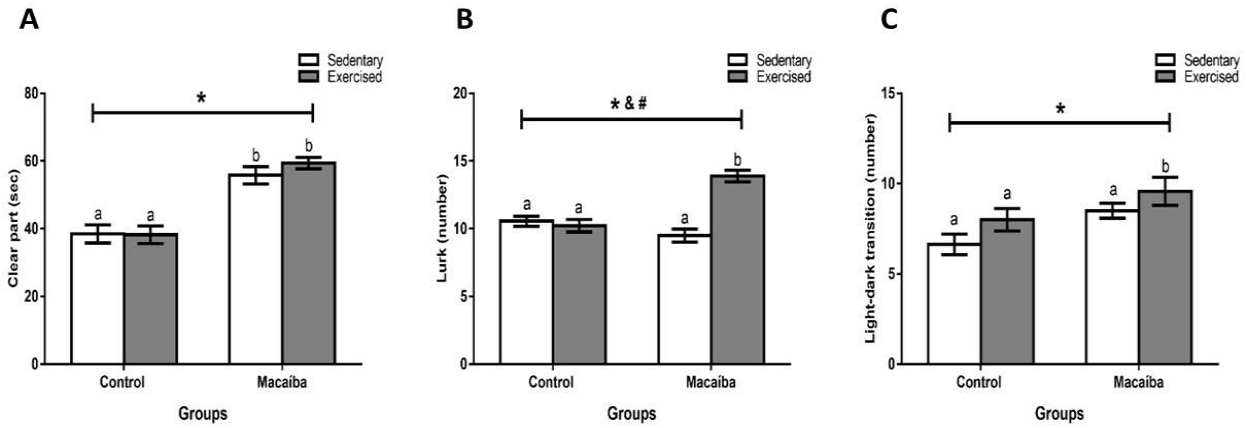

Fig 2. A -Time spent in the clear area (seconds), B - Lurking number, C - Number of transitions. Sedentary control (CT) (n = 11); Sedentary macaíba (MC) (n = 11); Control exercised (CT-EX) (n = 11); and Macaíba exercised (MC-EX) (n = 11). Values are means, with standard deviations. Two-way ANOVA. Different letters indicate a significant difference (p <0.05). # - indicates effect of diet; & - indicates exercise effect; * - indicates the effect of the interaction between diet and exercise.

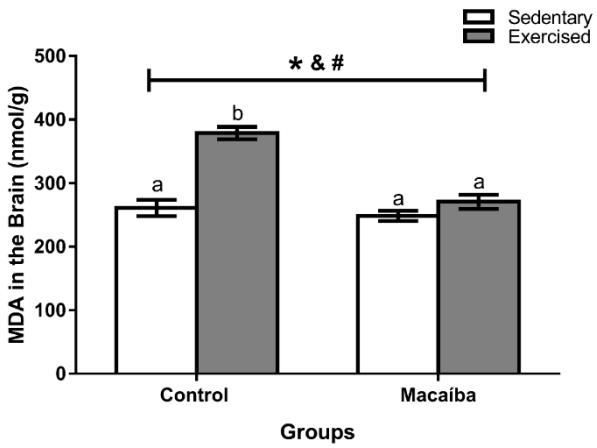

Fig. 3 Concentrations of malonaldehyde in brain tissue of adult rats. Sedentary control (CT) (n = 11); Sedentary Macaíba (MC) (n = 11); Control exercised (CT-EX) (n = 11) and Macaíba exercised (MC-EX) (n = 11). Values are means, with standard deviations. Two-way ANOVA. Different letters indicate a significant difference ($p < 0.05$).

Table 1 Nutritional composition of macaíba pulp.

Macronutrients and fibers	
Protein (g.100 g^{-1})	1.91(±0.1)
Carbohydrates (g.100 g^{-1})	35.94 (±1.65)
Lipids (g.100 g^{-1})	15.07 (±1.34)
Fibers (mg.100 g^{-1})	286.16 (±30)
Fatty acid profile (mg.100 g^{-1})	
C14:0 (myristic acid)	6.66
C16:0 (palmitic acid)	1075.36
C17:0 (marginal acid)	2.96
C18:0 (stearic acid)	99.18
C20:0 (arachidic acid)	8.25
C16:1n7 (palmitoleic acid)	206.81
C17:1n7c (heptadecenoic acid)	10.65
C18:1n9c (oleic acid)	8092.49
C18:1n11c (vaccenic acid)	503.48
C18:2n6c (linoleic acid)	1563.61
C18:3n3 (α-linolenic acid)	126.30
C20:1n9 (eicosenoic acid)	8.69
Saturated (g.100 g^{-1})	**1192.68**
Unsaturated (g.100 g^{-1})	**10512.03**
Phenolic and carotenoid constituents	
Total phenolics (mg GAE/100g)	133.13 (±3.14)
Total flavonoids (mg CE/100g)	39.38 (±0.00)
Yellow flavonoids (mg/100g)	0.63 (±0.05)
Total carotenoids (mg/100g)	48.00 (±0.00)
Antioxidant activies	
FRAP (µmol TE/g)	0.36 (±0.01)
ABTS (µmol TE/g)	4.43 (±2.25)
IC_{50} (mg/mL)	18.21 (±0.00)

GAE: Gallic acid equivalent; EC: Catechin equivalent; FRAP: Ferric reducing activity; ABTS: radical-scavenging capacity; TEAC: Trolox equivalent; SD: Standard deviation.

Table 2. Results of behavioral tests in the elevated plus labyrinth (EPM) of the control groups (CT and CT-EX) and treated with macaíba pulp (MC and MC-EX), submitted or not to the swimming exercise.

	Experimental Groups				Significance of analyzed effects		
Behavioral tests	**CT**	**CT-EX**	**MC**	**MC-EX**	**Diet**	**Exercise**	**Interaction**
Entries in open arms (number)	4.22 ±1.79^{a}	6.50 ± 2.88^{a}	8.30 ± 1.57^{b}	11.78 ± 2.68^{c}	P < 0.0001	P = 0.0002	ns
Time in open arms (seconds)	18.95 ± 8.57^{a}	12.71 ± 5.74^{b}	34.14 ± 13.62^{c}	61.63 ± 14.54^{d}	P < 0.0001	P = 0.0032	P < 0.0001
Head dive (number)	10.71 ± 2.06^{a}	6.25 ± 1.04^{b}	13.88 ± 4.94^{c}	16.50 ± 5.65^{d}	P < 0.0001	ns	P = 0.0047
Time in closed arms (seconds)	224.36 ± 30.60^{a}	223.25 ± 26.70^{a}	193.63± 23.93^{b}	189.07 ± 26.06^{b}	P = 0.0003	ns	ns

Sedentary control (CT) (n = 11); Sedentary Macaíba (MC) (n = 11); Control exercised (CT-EX) (n = 11); and Macaíba exercised (MC-EX) (n = 11). Values are means, with standard deviations. Two-way ANOVA. Different letters indicate a significant difference (p <0.05).

Artigo 2 - CONSUMPTION OF BRAZILIAN PALM FRUIT (*Acrocomia intumescens* Drude) IMPROVES BIOCHEMICAL PARAMETERS AND REDUCES CARDIOVASCULAR RISK IN EXERCISED RATS

(Periódico: Food Research International. Fator de impacto: 6.475)

CONSUMPTION OF BRAZILIAN PALM FRUIT (*Acrocomia intumescens* Drude) IMPROVES BIOCHEMICAL PARAMETERS AND REDUCES CARDIOVASCULAR RISK IN EXERCISED RATS

Abstract

Objective: Evaluate the effects of macaíba pulp on physical, biochemical, and oxidative stress parameters of exercised rats. **Methodology:** Forty-four male rats were divided into four groups, n = 11: sedentary control (CT), exercised control (CT-EX), sedentary macaíba (MC), and exercised macaíba (MC-EX). MC and MC-EX received 1000 mg/kg/day of macaíba pulp, and CT and CT-EX received distilled water, daily for eight weeks via gavage. The exercised animals underwent a week of adaptation, starting with 10 minutes to 60 minutes. From the second to the eighth week of training, the animals swam five days a week for 60 minutes. At the end of the treatment, the blood was collected to quantify the plasma concentrations of total cholesterol (TC), high density lipoprotein (HDL), low density (LDL) and very low density (VLDL), total cholesterol, glucose, urea, aspartate aminotransferase (AST) and alanine aminotransferase (ALT). With the lipoprotein values, the atherogenic index and coefficient and the coronary and cardiovascular risk were quantified. The liver was removed to assess malonaldehyde (MDA), total fat and TC concentrations. Mesenteric, retroperitoneal and epididymal fats were removed, weighed and the adiposity index quantified. **Results:** The groups treated with macaíba pulp demonstrated a reduction in TC, LDL, VLDL, glucose, urea, AST, ALT, cardiovascular risks, body fat, MDA, TC and total liver fat. Furthermore, MC and MC-EX showed increased HDL. **Conclusion:** We found that macaíba pulp was efficient in improving

biochemical parameters and reducing the lipid peroxidation marker generated by physical exercise, as well as reducing adipose tissue and cardiovascular risks.

Keywords: Malonaldehyde. Visceral fat. Diet. Supplementation.

1 Introduction

The palm tree of the species *Acrocomia intumescens* Drude, popularly known as macaíba, is part of the native Brazilian flora. It is part of the *Arecaceae* family of palms that comprises 252 genera and 2,522 species, found in tropical and subtropical regions (Barbosa da Silva et al., 2020; Dransfield et al., 2008).The literature reports that the oil extracted from *Acrocomia Aculeata*, a palm species belonging to the same family as Acrocomia *intumescens*, has shown anti-inflammatory and diuretic action *in vivo* (Lescano, Iwamoto, Sanjinez-Argandoña, & Kassuya, 2015).

Acrocomia intumescens has a fruit composed of two edible portions: the seed kernel and the pulp. The kernels are composed of antioxidants such as phenolics, flavonoids, carotenoids, and saturated and unsaturated fatty acids. The presence of these components has been linked to benefits on neurological health (De França Silva et al., 2021). The pulp is predominantly composed of unsaturated fatty acids and contains vitamin C, carotenoids, flavonoids, and minerals (Barbosa da Silva et al., 2020; Bora & Rocha, 2004; Coimbra & Jorge, 2011). However, little is known about the effects of pulp consumption *in vivo*.

Observing the potential of the macaíba pulp as a functional food, we hypothesize that associating a food source of antioxidants and essential fatty acids with physical exercise can bring about health benefits.

Exercise practice is capable of promoting an increase in heart rate and respiratory volume, leading to an increase in muscle oxygen demand and greater production of reactive oxygen species (ROS) (de Figueiredo et al., 2016). With an imbalance between the production of oxidizing substances and the body's antioxidant defenses, oxidative stress, can lead to lipid peroxidation, damage to cellular DNA, protein carbonylation, increased metabolic activity, changes in cell functions, origins, and tissue damage (Deaton & Marlin, 2003; Goto & Radák, 2007; Marin, Bolin, Campoio, Guerra, & Otton, 2013; Vollert et al., 2011).

The consumption of a diet with antioxidant components associated with moderate exercise has also been shown to benefit the quality of life, body weight, blood lipid profile, and the body's antioxidant defenses (Marin et al., 2013; Schwingshackl, Dias, & Hoffmann, 2014). Therefore, with the present study we aimed to evaluate the effects of the consumption of macaíba pulp on physical, biochemical, and oxidative stress parameters of exercised rats.

2 Materials and Methods

2.1 Acquisition of raw material and Macaíba pulp lyophilization

Macaíba palm fruit was obtained from the town of Areia, located at 6.963845 (latitude) and -35.749738 (longitude) in the state of Paraíba, in the Brazil Northeast region. The fruit is registered in the national system for the management of genetic heritage and associated traditional knowledge (SISGEN): ADD854A.

The Macaíba palm fruits were cleaned and pulped. After being pulped, they were frozen and subsequently subjected to the lyophilization process in lyophilizer L101 (Liotop®), 212Vca, -50 ºC for 48 hours. After obtaining the lyophilizate, the pulp was transferred to plastic packaging and vacuum-sealed and kept at -20 ºC.

2.3 Macronutrients characterization and fatty acid profile of the pulp

Lyophilized macaíba pulp was used for protein and carbohydrate determination by the methodology of Kjedhal, and carbohydrate as described by AOAC (2000), lipids by the methodology described by Folch, Less, and Stanley (1957), and lipid profile by Hartman and Lago's methodology (1973).

2.4 Analysis of bioactive compounds from macaíba pulp

2.4.1 Extraction

Macaíba pulp constituents were extracted with ethanol 80% (v/v) and evaluated for ABTS• scavenging capacity, ferric reducing activity (FRAP), and total flavonoids. For the total phenolic content, methanol was used for extractions, 1 g of macaíba pulp was weighed in a test tube and immediately afterward, 10 ml of solvent were added. The test tube was left at room temperature for 60 minutes and after filtration, the volume was completed to 10 mL with the extraction solvent and stored in a freezer (-18 °C) until analysis. All the extractions were performed in triplicate.

2.4.2 Determination of total phenolic compounds (TPC)

To determine the content of total phenolic compounds, the methodology described by Liu et al.(2002) was used with minor modifications. Briefly, 250 µL of the extract was mixed with 1250 µL of a 1:10 diluted Folin–Ciocalteau reagent. The solutions were mixed and left at room temperature (27°C) for 6 minutes. After incubation, 1000 ml of 7.5% sodium carbonate solution (Na_2CO_3) was added and stained in a 50 °C water bath for 5 minutes. The absorbance of the reaction mixtures was measured at 765 nm using a spectrophotometer (BEL Photonics, Piracicaba, São

Paulo, Brazil). The absorbance of the extract was compared with a gallic acid standard curve for estimating the concentration of TPC in the sample. The TPC was expressed as mg of gallic acid equivalents (GAE) per hundred grams of macaíba pulp based on dry weight (DW).

2.4.3 Determination of total flavonoids

The total flavonoid content was measured using the colorimetric assay developed by Zhishen, Mengcheng, and Jianming (1999). A known volume (0.5 mL) of the extract was added to a test tube and, at zero time, 150 µL of 5% $NaNO_2$ was added. After 5 min, 150 µL of 10% $AlCl_3$ was added, and, after 6min, 1 mL of 1 M NaOH, followed by the addition of 1.2 mL distilled water. Sample absorbance was read at 510 nm using a spectrophotometer (BEL Photonics, Piracicaba, São Paulo, Brazil). The absorbance of the extract was compared with a catechin standard curve for estimating the concentration of flavonoid content in the sample. The flavonoid contents were expressed as mg of catechin equivalents (QE) per hundred grams of macaíba pulp based on dry weight (DW).

2.4.4 Antioxidant activity – FRAP method

According to Benzie & Strain (1996), the FRAP method was performed with modifications proposed by Pulido, Bravo & Saura-Calixto (2000). In this assay, 3.6 mL of FRAP reagent (0.3 M, pH 3.6 acetate buffer, 10 mM TPTZ, and 20 mM ferric chloride) were mixed with 200 µL of extract diluted in distilled water, being incubated for 30 min at 37 ºC. The FRAP solution was used as a reference reagent, and absorbance was read at 593 nm. The results were expressed in µmol of Trolox equivalents per gram of macaíba pulp on a dry weight (DW) basis (µmol TE/g^{-1}).

2.4.5 Antioxidant activity – ABTS method $^{+}$

The ABTS method was carried out according to the methodology described by Surveswaran et al. (2007), with modifications. The ABTS radical was formed from the reaction of 140 mM potassium persulfate with 7 mM ABTS stock solution, kept in the dark and at room temperature for 16 h. For the analysis, ABTS radical was diluted in distilled water until a solution with the absorbance of 700 nm ± 0.02 nm at 734 nm was obtained. A 100 µL aliquot of each extract was then homogenized with 500 uL of the ABTS radical. The absorbance of the samples was read at 734 nm after 6 min of reaction. The results were expressed in µmol of Trolox equivalent per gram macaíba pulp on a dry weight (DW) basis (µmol TE/g^{-1}).

2.4 BIOLOGICAL TESTS

2.4.1 Animals and diets

Forty-eight Wistar rats (n = 44) at 70 days old, weighing 250-300 g, were used. The animals were maintained under standard conditions, with 12 hours light/dark cycle, relative humidity of 80 ± 5%, and controlled temperature of 22ºC ± 3ºC. They were fed a standard rodent diet and water *ad libitum.* They were randomly assigned to four groups, according to supplementation and exercise program: sedentary control group (CT - standard diet and distilled water) (n = 11), exercised control group (CT-EX - standard diet and distilled water) (n = 11), sedentary macaíba group (MC - standard diet and treated with macaíba pulp) (n = 11), exercised macaíba pulp group (MC-EX - standard diet and treated with macaíba pulp) (n = 11). Macaíba pulp was administered, 1000 mg/kg of body weight of the animal (dose established based in a previous trial), daily by gavage, before swimming exercise over a period of eight weeks, for the MC and MC-EX groups. At the end of the experiment, the animals were

euthanized using a guillotine. The entire experimental protocol was carried out upon approval by the Ethics Committee on the Use of Animals - CEUA/CSTR, nº 010/2019, following Animal Research: Reporting of In Vivo Experiments - The ARRIVE Guidelines.

2.4.2 Physical swimming training

When the CT-EX and MC-EX animals had reached the age of 70 days, swimming training began, according to a methodology adapted from Nakao (2000), swimming 1 hour a day, 5 days a week for 8 weeks, without using overload. The first week of training corresponded to an adaptation period, in which the trained groups were initially acclimated to the aquatic environment, starting with 10 minutes and adding 10 minutes daily. From the second to the eighth week, the animals swam for 60 minutes/day, 5 days/week in an 85 cm long, 50 cm wide, and 40 cm deep tank. The non-exercised groups remained during the same period in cages with water depth (4 cm), with a temperature of 32 ± 1°C, but without performing physical swimming effort. This procedure aimed to provide the same aquatic stress.

2.4.3 Food consumption and weight assessment

The animals were weighed weekly for weight monitoring. Feed intake was assessed weekly.

2.4.5 Murinometric parameters and body fat

The animals were weighed using a Balmak® scale, São Paulo, Brazil. Length, thoracic circumference, and abdominal circumference were measured using a tape

measure. Bodyweight and length were used to calculate the Body Mass Index (BMI) (Novelli et al., 2007).

The adiposity index was calculated from the adipose tissue, composed of visceral, retroperitoneal, and epididymal fat. These fats were removed and weighed separately (Nascimento et al., 2011).

2.4.6 Biochemical analysis

Blood collected after euthanasia was centrifuged in a Novatecnica® NT 810 centrifuge (22438 g for 10 minutes). After centrifugation, measurements of glucose (λ = 505 nm), total cholesterol (TC) (λ = 500 nm), triglyceride (TAG) (λ = 505 nm), and HDL-cholesterol (λ = 500 nm) were performed by enzymatic method using the Trinder procedure (Labtest-Diagnosis, St Louis, USA), using a Bel Photonics® spectrophotometer, Milano, Italy.

2.4.7 Determination of Atherogenic Index, Coronary Risk Index (CR1), and Cardiovascular Risk Index (CVR2)

The atherogenic index (AI), atherogenic coefficient (AC), cardiac hazard ratio 1 (CR1), and cardiac hazard ratio 2 (CVR2) were calculated from (Ahmadvand et al., 2016; Erejuwa et al., 2016; Ikewuch & Ikewuchi, 2009).

2.5 *Levels of malondialdehyde (MDA)*

Macaíba pulp effect was calculated for malonaldehyde (MDA) levels in heart and liver tissue. For this, the heart and liver samples were suspended in a buffer of Tris- HCl 1:5 (w/v) and homogenized for 15 seconds on a cold plate. The resulting suspension was homogenized for 2 minutes with an automatic Potter homogenizer and

centrifuged at 2500 g and 4°C for 10 min. Supernatants were used to determine the content of MDA (Esterbauer & Cheeseman, 1990). The results were expressed as MDA tissue nmol g -1.

2.7 Quantification of total fat of liver and feces

The feces were collected at the end of the experiment. The livers were removed after animals' euthanasia. Both of them were used to measure the total fat content using the Folch, Less & Stanley (1957) method.

2.7 Histological analysis of liver and soleus muscle

Fragments of liver and soleus muscle tissues were preserved in a 10% buffered formaldehyde solution until histological processing could take place. The tissues were subjected to a histological procedure and were embedded in histological paraffin and sectioned with a thickness of 4 µm. The sectioned tissues was stained with hematoxylin-eosin (HE) for microscopic analysis (Motic BA 200).

2.7 Statistical analysis

Data were submitted to normality analysis (D'Agostino-Pearson and Shapiro-Wilk), Analysis of Variance (Two-Way ANOVA), in the GraphPad Prism version 5.01 program (GraphPad Software Inc.), considering a significance level of 5% ($p < 0.05$).

3 Results

3.1 Nutritional composition of macaíba pulp and antioxidant activity

Macaíba pulp presented macronutrient values of 1.90 g.100 g-1 proteins, 35.90 g.100 g^{-1} carbohydrates, and 16.05 g.100 g-1 lipids, of which 89.81% (10512.03

mg.100) g-1) were unsaturated fatty acids, with a predominance of oleic acids (8082.49 mg.100 g-1), followed by linoleic acid (1563.61 mg.100 g-1) and 10.19% (1192.68 mg.100 g-1)) saturated fatty acids, with the highest content being palmitic acid (1075.36 mg.100 g-1). In addition to macronutrients, macaíba pulp is also composed of micronutrients, with 134.13 mg GAE/100mg total phenolics, 39.38 mg EC/100g total flavonoids, 0.64 mg/100g yellow flavonoids, and 287.16 mg/100g fiber.

The macaíba pulp was further shown to have an iron-reducing activity of 0.37 µmol TE/g, free radical scavenging capacity of 4.48 µmol TE/g, and IC50 of 18.22 mg/mL.

3.2 Body weight and feed intake

Figure 1A shows the mean values of the animals' body weights during the treatment period.

Based on the results obtained, there was a statistical difference between the groups in the second, fourth and eighth weeks of treatment. We found that the MC-EX group had lower weight when compared to CT ($p = 0.0002$), CT-EX ($p = 0.0001$) and MC ($p = 0.0008$) in the second week of treatment. Through the two-way ANOVA it was possible to see that the diet $F (1.40) = 11.12$; $p = 0.0018$], training [$F (1.40) = 6.348$; $p = 0.0158$] and the interaction between diet and swimming exercise [$F (1.40) = 6.865$; $p = 0.0124$] contributed to this result.

As for the fourth and eighth weeks, the MC-EX group also had lower body weight compared to CT ($p = 0.0050$; $p = 0.0065$, respectively) and CT-EX ($p = 0.0016$; $p= 0.0025$, respectively). The results show that the diet [$F (1.40) = 11.73$; $p = 0.0014$; [$F (1.40) = 13.48$; $p = 0.0007$, respectively] had an effect on body weight on the MC-EX group.

We also observed a reduction in body weight in the MC group compared to CT-EX ($p = 0.0254$). We verified the effect of diet on this result [$F(1.40) = 13.48$; $p = 0.0007$].

PLEASE INSERT FIGURE 1 HERE

With respect to average feed intake (Figure 1B). CT-EX was the group which consumed the most throughout the treatment period compared to the other groups ($p < 0.05$). MC-EX showed higher feed intake compared to CT during the second, fourth, sixth, and eighth weeks ($p < 0.05$), and it also showed higher feed intake during the fourth, sixth, and eighth weeks compared to the MC group ($p<0.05$).

We verified that in the first week there was an effect of diet and the interaction between diet and swimming exercise [$F(1.40) = 6.406$; $p = 0.0154$]. In the second, fourth and eighth weeks there was an effect of the diet [$F(1.40) = 7,005$; $p = 0.0116$; $F(1.40) = 5.184$; $p = 0.0282$; $F(1.40) = 26.84$; $p < 0.0001$, respectively], of the exercise [$F(1.40) = 26.45$; $p < 0.0001$; $F(1.40) = 37.71$; $p < 0.0001$; $F(1.40) = 65$; $p < 0.0001$, respectively] and the evidence of interaction between diet and exercise [$F(1.40) = 10.04$; $p=0.0029$; $F(1.40) = 4,931$; $p = 0.0321$; $F(1.40) = 4,693$; $p = 0.0363$, respectively]. In the third week of treatment, we verified an effect only from the swimming exercise [$F(1.40) = 7.222$; $p = 0.0104$]. In the sixth week we verified the influence of exercise $F(1.40) = 68.57$; $p < 0.0001$] and the interaction between diet and exercise $F(1.40) = 14.9$; $p = 0.0004$]. And in the seventh week there was an effect of both diet [$F(1.40) = 6.472$; $p = 0.0149$] and exercise $F(1.40) = 9.135$; $p = 0.0044$].

3.5 Evaluation of murinometric parameters, visceral fat, adiposity index, organ weight, and L length and muscle thickness

Table 2 shows the mean values corresponding to the physical parameters, visceral fat, organ and muscle weight, and muscle length and thickness.

PLEASE INSERT TABLE 2 HERE

We found that the MC and MC-EX groups presented a reduction in the measurement of waist circumference, compared to the CT and CT-EX ($p < 0.05$). There was also an effect of diet on this parameter [$F(1.40) = 41.58$; $p < 0.0001$].

The body mass index was lower in the exercised groups (CT-EX and MC-EX) ($p < 0.05$), with an effect of swimming exercise being detected [$F(1.40) = 14.18$; $p = 0.0005$] on the BMI.

As for the body fat content, a reduction was seen in the groups treated with macaíba pulp (MC and MC-EX). For the amount of mesenteric fat, this was lower in MC compared to CT ($p = 0.0093$) and CT-EX ($p < 0.0001$), and also lower for MC-EX when compared to CT ($p = 0.0025$) and CT-EX ($p < 0.0001$). In turn, it was verified that there was an influence of the diet [$F(1, 40) = 63.95$; $p < 0.0001$], from the swimming exercise [$F(1.40) = 11.75$; $p = 0.0014$] and evidence of a relation between diet and exercise [$F(1.40) = 17.06$; $p = 0.0002$]. The amount of retroperitoneal fat was lower in MC compared to CT-EX ($p = 0.0067$), and in MC-EX compared to CT ($p = 0.0174$) and CT-EX ($p = 0.0003$), with observed effect of diet [$F(1.40) = 14.25$; $p = 0.0005$]. In the amount of epididymal fat, there was also a reduction in the MC group compared to CT-EX ($p < 0.0001$), and in MC-EX compared to CT ($p = 0.0357$) and CT-EX ($p < 0.0001$). These findings were influenced by diet [$F(1.40) = 26.06$; $p < 0.0001$], exercise [$F(1.40) = 4.125$; $p = 0.0489$] and interaction between diet and exercise [$F(1.40) = 9.114$; $p = 0.0044$].

Based on the values of mesenteric, retroperitoneal and epididymal fat, we calculated the adiposity index; this was lower for CT ($p <0.0001$), MC ($p <0.0001$) and

MC-EX ($p < 0.0001$) when compared to CT-EX. There was a diet effect [$F(1.40) = 24.98$; $p < 0.0001$], on the swimming exercise [$F(1.40) = 10.32$; $p = 0.0026$] and the interaction between diet and exercise [$F(1.40) = 32.63$; $p < 0.0001$].

As for organ weights, there was a statistical difference for liver and heart. Where MC showed a reduction in liver weight compared to CT ($p = 0.0110$) and CT-EX ($p = 0.0022$), and greater reduction for MC-EX compared to CT-EX ($p = 0.0281$). In relation to liver weight, an effect of diet was observed [$F(1.40) = 12.23$; $p = 0.0012$]. Regarding the heart weight, this was higher in the exercised groups MC-EX and CT-EX relative to the sedentary groups ($p < 0.05$). The effect of the swimming exercise on the CT-EX and MC-EX groups was [$F(1.40) = 25.99$; $p < 0.0001$].

As for the assessment of the gastrocnemius and soleus muscles, concerning weight, length, and thickness, we found a statistical difference concerning the length and thickness of the gastrocnemius muscle. Higher values were found for the exercised groups (CT-EX and MC-EX) relative to the sedentary groups (CT and MC) ($p < 0.05$), with an effect of exercise on length [$F(1.40) = 21.83$; $p < 0.0001$] and thickness [$F(1.40) = 12.27$; $p = 0.0011$] of the gastrocnemius muscle.

3.6 Evaluation of biochemical parameters, determination of the atherogenic index, coronary risk index, and cardiovascular risk index

Data regarding biochemical and cardiovascular risk parameters are shown in Table 3.

PLEASE INSERT TABLE 3 HERE

For the biochemical parameters evaluated, there was a reduction in glucose, urea, AST, ALT, total cholesterol, LDL-c and VLDL-c, and an increase in HDL-c for the groups treated with macaiba pulp (MC and MC-EX) ($p<0.05$).

In these analyzed concentrations, it was seen that the diet had an influence on the glucose results [F (1.40) = 23.87; p < 0.0001], urea [F (1.40) = 107.5; p < 0.0001], AST [F (1.40) = 221.7; p < 0.0001], ALT [F (1.40) = 3990; p < 0.0001], total cholesterol [F (1.40) = 42.3; p < 0.0001], HDL-c [F (1.40) = 46.9; p < 0.0001], LDL-c [F (1.40) = 35.24; p < 0.0001] and VLDL-c [F (1.40) = 467.7; p < 0.0001]. It was also seen that the swimming exercise also had an influence on the blood levels of HDL-c [F (1.40) = 9.347; p = 0.0040] and LDL-c [F (1.40) = 7.252; p = 0.0103]. The interaction between diet and swimming exercise potentiated the effects on serum LDL-c concentration [F (1.40) = 8.1; p = 0.0070].

On the assessment of cardiovascular risks, it was seen that the exercised groups (CT-EX and MC-EX), as well as the sedentary group (MC) treated with macaíba pulp showed a reduction compared to the sedentary control group (CT) (p < 0.05). Diet and swimming exercise had effects on the atherogenic index [F (1.40) = 25.37; p < 0.0001; F(1.40) = 51.24; p < 0.0001, respectively], atherogenic coefficient [F (1.40) = 507.8; p < 0.0001; F(1.40) = 130.8; p < 0.0001, respectively], coronary risk [F (1.40) = 64.37; p < 0.0001; F(1.40) = 42.49; p < 0.0001, respectively] and cardiovascular risk [F (1.40) = 39.43; p < 0.0001; F (1.40) = 19.72; p < 0.0001, respectively]. Regarding the effect of the interaction between diet and swimming exercise, there was an increase in terms of the atherogenic index [F (1.40) = 37.18; p < 0.0001] and cardiovascular risk [F (1.40) = 22.54; p < 0.0001].

3.8 Malonaldehyde (MDA) concentration in heart and liver

MDA concentrations in the heart tissue (Figure 2A) were higher in the exercised groups compared to the sedentary ones (p < 0.05). Two-way analysis of variance

showed that there was an effect of diet [F (1.40) = 13.41, p = 0.0007] and exercise [F (1.40) = 67.17, p < 0.0001].

PLEASE INSERT FIGURE 2 HERE

Concerning MDA concentrations in the liver, with values expressed in Figure 2B, there were no statistical differences between groups (p > 0.05). However, when analyzed by two-way ANOVA, there was a statistical significance of the interaction of exercise with diet [F (1.40) = 4.262, p = 0.0455].

3.9 Percentage of total fat and total cholesterol and triglyceride levels in liver and feces

In Figure 3 shows the values corresponding to the total fat content and the levels of total cholesterol and triglycerides in the liver and feces.

PLEASE INSERT FIGURE 3 HERE

The total fat content in the liver was lower for MC and MC-EX compared to CT and CT-EX (p < 0.05), and it is evident that the diet had an effect [F (1.40) = 2836, p < 0.0001], of the exercise [F (1.40) = 17.13, p = 0.0002] and of the interaction [F (1.40) = 6.166, p = 0.0173] (Fig. 3A).

In feces, the fat content was higher for the sedentary control group compared to the others (p < 0.05). Effects of the diet [F (1.40) = 126.6, p < 0.0001], the exercise [F (1.40) = 38.3, p < 0.0001] and the interaction [F (1.40) = 669.8, p < 0.0001] (Fig. 3B) can be seen.

In the liver, total cholesterol values were higher for CT and lower for CT-EX, MC and MC-EX (p < 0.05) (Fig. 3 C and E), while triglyceride values were higher for CT-EX and MC-EX when compared to their controls (p < 0.05). There was an effect of the diet [F (1.40) = 53.82, p < 0.0001] and the interaction between diet and exercise [F (1.40) = 18.95, p < 0.0001] on the total cholesterol results. As for the triglyceride values,

diet [F (1.40) = 323.5, p < 0.0001], exercise [F (1.44) = 840.1, p < 0.0001] and their interactions [F (1.44) = 280.2, p < 0.0001] had an effect.

As for the levels of total cholesterol and triglycerides in feces, CT had higher levels of triglycerides and lower levels of total cholesterol compared to the others (p < 0.05) (Fig. 3 D and F). For total cholesterol, there was an effect of the diet [F (1.40) = 229.6, p < 0.0001], exercise [F (1.40) = 392.8, p < 0.0001], and the interaction between diet and exercise [F (1.40) = 82.31, p < 0.0001]. The same effect occurred for triglyceride values, where diet [F (1.40) = 163.2, p < 0.0001], exercise [F (1.40) = 81.16, p < 0.0001] and their interactions [F (1.40) = 15.46, p = 0.0003] influenced these values.

3.9 Hepatic and muscle histological analysis

PLEASE INSERT FIGURE 4 HERE

Figure 4 shows the histological sections of the liver and soleus muscle, where we verified that CT presented hepatic steatosis, while, in the other groups, the presence of steatosis was not verified.

Regarding the histological parameters for muscle tissue, striated muscle fibers containing one or two nuclei in the sarcomere with homogeneously colored cytoplasm were observed. In animals in the sedentary control groups, where the sarcomeres of the cells in this group showed a reduction in their constituents, a condition compatible with atrophy. In the other experimental protocols presented, CT-EX and MC-EX, muscle fibers remained homogeneous, maintaining fiber size and components.

4 Discussion

In the present study, we verified the effects of consumption of the fruit of a palm tree commonly consumed by Brazilians (macaíba, *Acrocomia intumescens* Drude), associated or not with regular physical exercise. The data showed that the consumption of the pulp of this fruit induced an improvement in the lipid profile, reduction in plasma glucose, liver enzymes, body fat and cardiovascular risk *in vivo*. When the consumption of macaíba was associated with exercise, there was a potentiation of the increase in HDL, reduction in LDL and coronary risk.

In the present study, the exercised groups consumed more, an effect that can be attributed to the compensatory mechanism. During continued physical exercise, the compensatory mechanism of energy intake is activated (MacLean, Blundell, Mennella, & Batterham, 2017; Stubbs et al., 2002). This ingestion is activated by changes in energy homeostasis and is regulated by hormones such as nesfatin-1, ghrelin, and peptide YY (PYY). The increase in ghrelin concentration during physical exercise can lead to an increase in food consumption (Favari et al., 2015; Li, Asakawa, Li, Cheng, & Inui, 2011; Mackelvie et al., 2007; Pałasz et al., 2012). So, for the exercised groups, no influence of diet was observed.

The results of this study demonstrated a relationship between the consumption of a diet containing unsaturated fatty acids and antioxidant compounds in the reduction of physical parameters such as body weight, waist circumference, and BMI, as well as a reduction in body fat and adiposity index in the groups that consumed macaíba pulp, being statistically higher for the MC-EX group. These findings can be explained by the mechanism in which G protein-coupled receptors, cell signalers, recognize fatty acids such as omega 3 and 9. These, in turn, can modulate the production of cyclic adenosine monophosphate (cAMP) from ion channels, from mitogen-activated protein kinases and the phospholipase C pathway, which are metabolic pathways related to

the reduction of fat deposits in adipose tissue. Furthermore, these receptors may have their expression increased in response to physical exercise (Gaspar et al., 2019; Hirasawa et al., 2005; Oh et al., 2010). Considering the presence of omega 3 and 9 fatty acids in the constitution of the pulp of macaíba, this may justify the results found in the present study.

In the exercised groups, there was an increase in heart weight and muscle length of the gastrocnemius muscle. The types of skeletal muscle fibers, whose characteristics are determined by the myosin heavy chain (MyHC) isoforms, can adapt to physiological demands in response to physical exercise, contributing to increased MyHC expression, promoting muscle hypertrophy (Smerdu & Perše, 2018), that may explain the increased weight of organs and muscles. In the present study, muscle atrophy can be explained by cage confinement.

In our study, it was found that in the groups treated with macaíba pulp and in the exercised control group, there was a plasma reduction in total cholesterol, LDL, total fat, and cholesterol in the liver, in contrast to an increase in HDL, and fecal fat and cholesterol, and reducing cardiovascular risks. Resistance training, as well as the presence of oleic acid in the diet, are related to the reduction of cholesterolemia and the promotion of LXR-dependent liver lipogenesis (liver X receptor). The transcriptional activity of LXRs is induced in response to elevated cellular cholesterol levels. LXRs bind to and regulate the expression of genes that encode proteins involved in the absorption, transport, efflux, excretion, and conversion of cholesterol to bile acids. LXR activation preferentially directs the incorporation of polyunsaturated fatty acids into phospholipids, inducing transcription of the remodeling enzyme lysophosphatidylcholine acyltransferase 3. The ability of the LXR pathway to couple cellular sterol levels with fatty acid saturation in membrane phospholipids has

implications for various physiological processes, including lipoprotein production, dietary lipid absorption, and intestinal stem cell proliferation (Wang & Tontonoz, 2018). In addition to lipids, antioxidant compounds can increase the activity of the cholesterol acyltransferase enzyme, which is the enzyme responsible for the esterification of cholesterol, allowing it to be selectively captured by the liver, decreasing its plasma levels (Lima & Couto, 2006).

In addition to being a source of essential fatty acids, macaíba is also a source of antioxidants. In the present study, we measured the concentration of MDA, a metabolite of lipid peroxidation, in the liver and heart. We found a lower MDA contraction in the heart in the exercised group fed with macaíba pulp compared to CT-EX. This result indicates a reduction in lipid peroxidation in this tissue. In liver MDA, the concentrations did not differ statistically, but we found an interaction effect between diet and exercise. Exercise-induced aerobic bioenergetic reactions in mitochondria and cytosol increase the production of reactive oxygen species (ROS), which can generate lipid peroxidation and increase the concentration of its metabolites, such as MDA. However, excess ROS generated by physical exercise can be eliminated by enzymatic and non-enzymatic antioxidants that act to protect the body from cell damage caused by oxidative stress (Mankowski, Anton, Buford, & Leeuwenburgh, 2015). As the macaíba pulp has in its composition nutrients with antioxidant characteristics such as flavonoids, phenolics, carotenoids, and unsaturated fatty acids, these may have contributed to these findings. Katsarou et al. (2016) also found that the presence of phenolic compounds in olive oil was able to decrease the oxidative and inflammatory state, with a reduction in MDA in the heart of hypercholesterolemic rats.

The appearance of hepatic steatosis in the sedentary control group was verified in our research. The steatosis found in animals was reversed when the animals

consumed macaíba or underwent physical activity. The appearance of fat in the liver may be related to the proportion of macronutrients and the number of amino acids containing sulfur and choline, lipotropic factor (Santos et al., 2015). This may explain the appearance of hepatic steatosis in the CT group. Based on the results presented in this study, we can infer that physical exercise, as well as the consumption of macaíba pulp containing fibers, fatty acids and antioxidants, may have favored the mobilization of hepatic fat through the aforementioned metabolic pathways, inhibiting the accumulation of fat in this organ.

In this sense, for a more complete understanding of the mechanisms related to the consumption of macaíba and its action *in vivo*, as a pre-clinical study, the results of the present study may contribute to future evaluations in clinical trials and recommendations aimed at individuals who practice exercise.

5 Conclusions

Based on the results obtained, it was found that the pulp of macaíba showed beneficial action whether consumed alone or in association with swimming exercise. The consumption of this fruit contributed to weight reduction, reduction of visceral fats, reduction of plasma concentrations of low-density lipoproteins, less cardiovascular risk and increasing high-density lipoproteins. Indeed, our study demonstrated that, when associated with exercise, the pulp of macaíba consumption induced a cardioprotective action.

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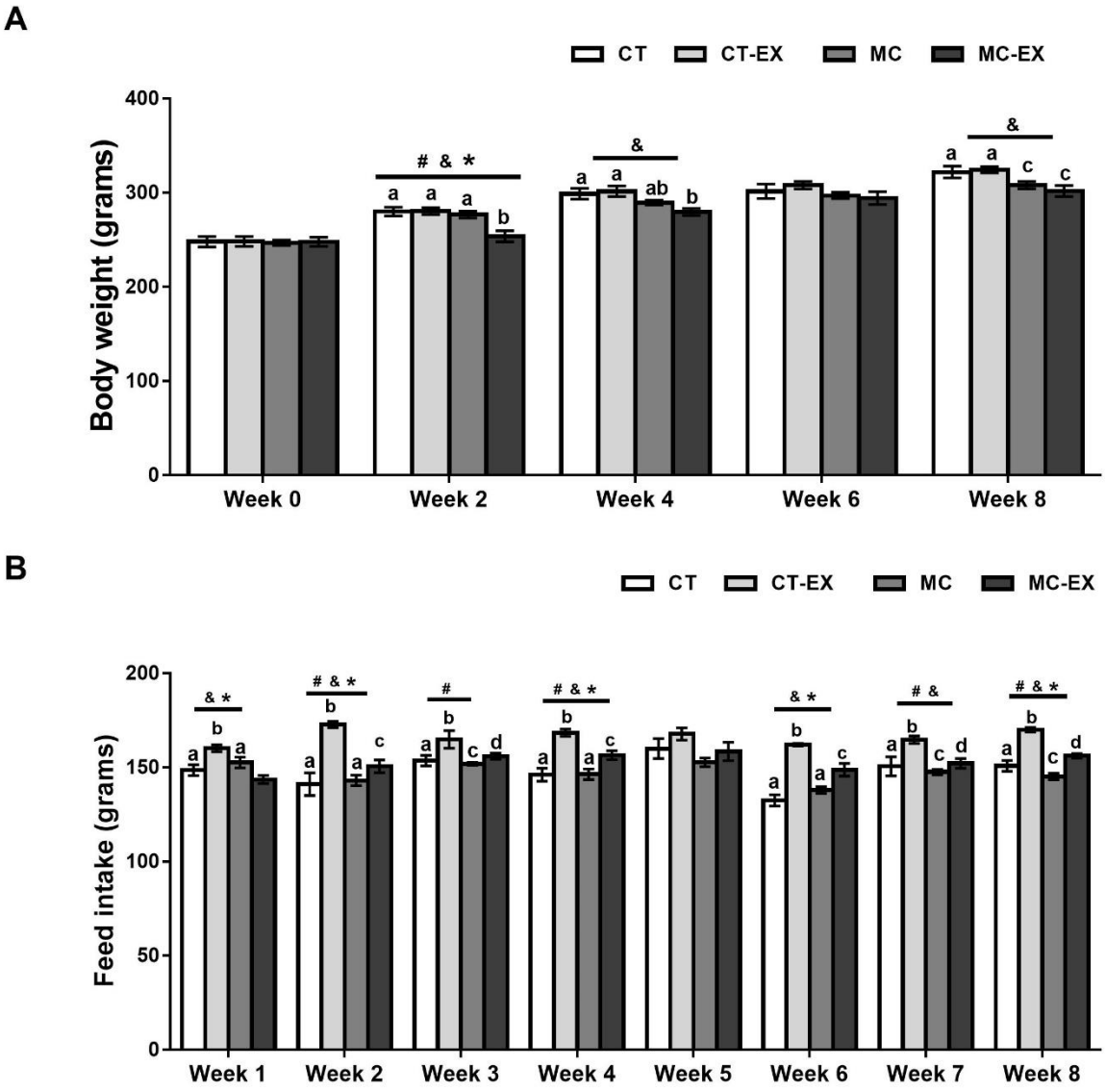

Figure 1. Body weight and feed intake. A - Body weight; B - Feed intake; CT- Sedentary control; CT-EX – exercised control; MC – sedentary treated with macaíba; MC-EX – exercised treated with macaíba. Data were expressed as mean ± SD, n = 11 per group. Results obtained from two-way ANOVA, different letters indicate difference between groups, $P < 0.05$; independent effects of diet, swimming exercise and the interaction between diet and exercise are expressed with $P < 0.05$. # - indicates effect of diet; & - indicates exercise effect; * - indicates the effect of the interaction between diet and exercise.

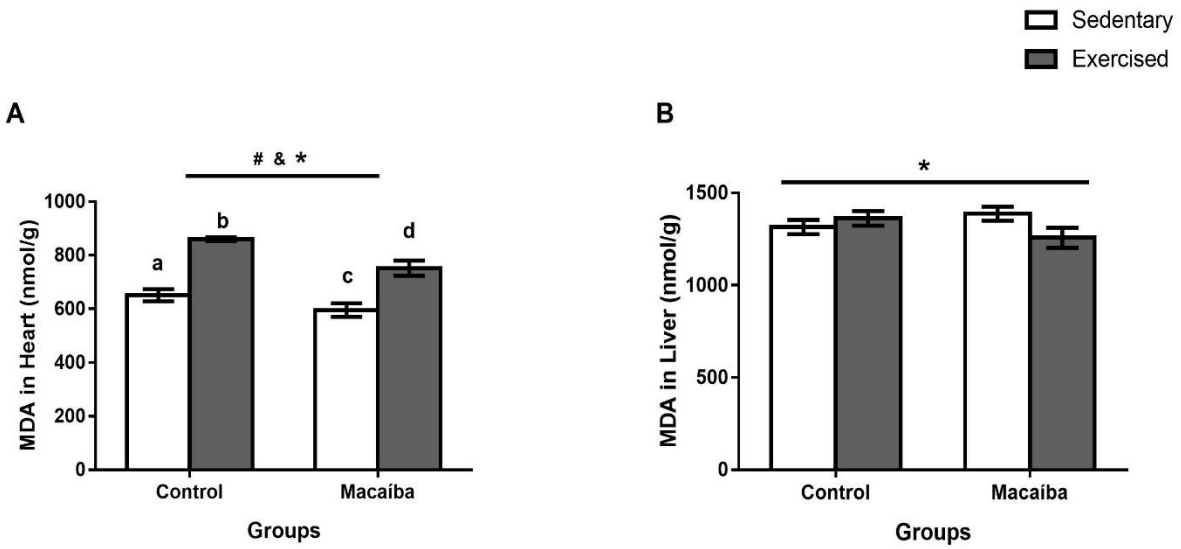

Figure 2. Malonaldehyde concentration in heart and liver. A - Malonaldehyde concentration in heart; B - Malonaldehyde concentration in liver; CT- Sedentary control; CT-EX – exercised control; MC – sedentary treated with macaíba; MC-EX – exercised treated with macaíba. MDA - Malonaldehyde. Data were expressed as mean ± SD, n = 11 per group. Results obtained from two-way ANOVA, different letters indicate difference between groups, $P < 0.05$; independent effects of diet, swimming exercise and the interaction between diet and exercise are expressed with $P < 0.05$. # - indicates effect of diet; & - indicates exercise effect; * - indicates the effect of the interaction between diet and exercise.

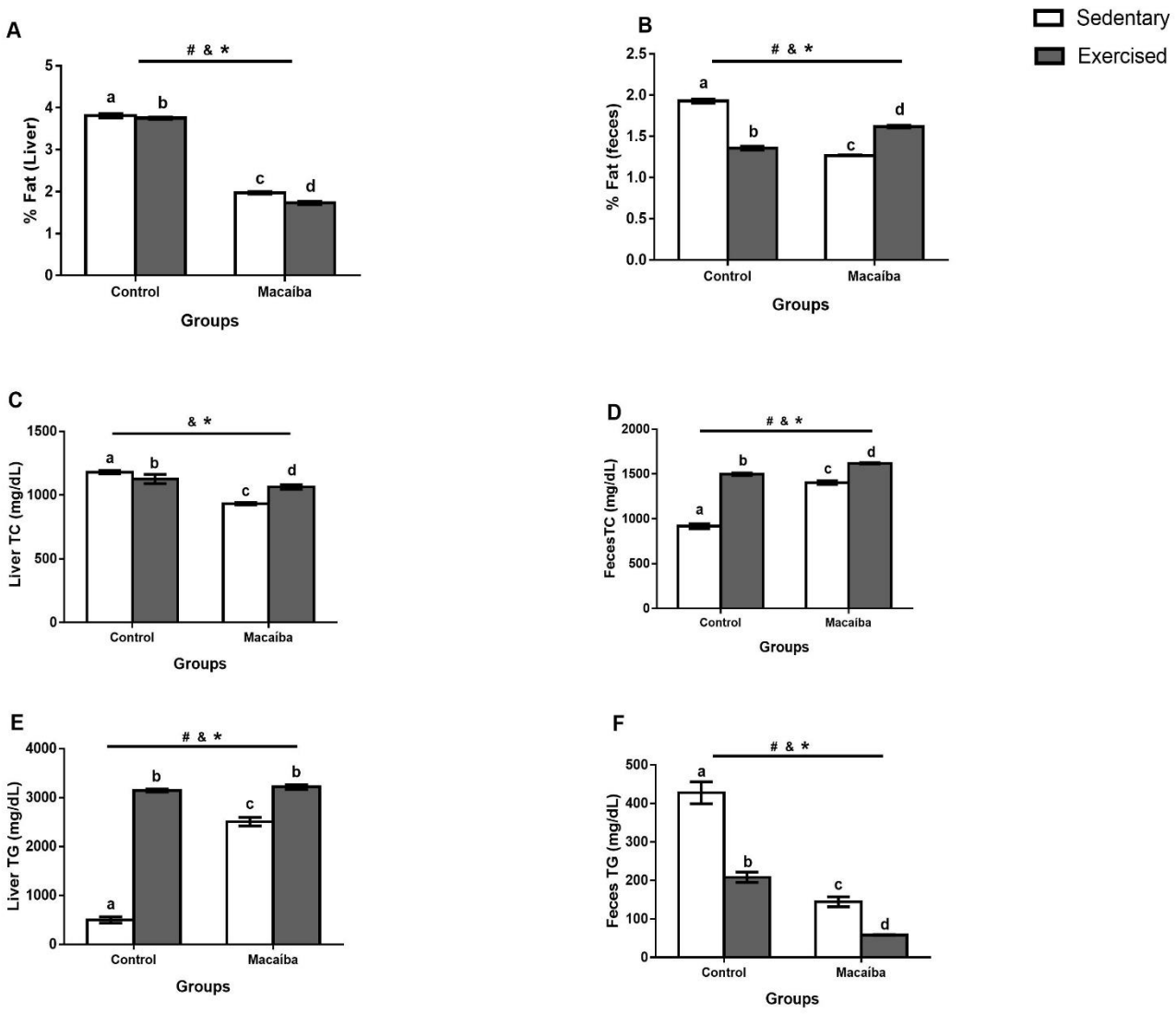

Figure 3. Percentage of total fat and total cholesterol and triglyceride levels in liver and feces. A - Total liver fat; B - Total feces fat; C - Total cholesterol levels in liver; D - total cholesterol levels in feces; E - Triglyceride levels in liver; F - Triglyceride levels in feces CT- Sedentary control; CT-EX – exercised control; MC – sedentary treated with macaíba; MC-EX – exercised treated with macaíba. Data were expressed as mean ± SD, n = 11 per group. Results obtained from two-way ANOVA, different letters indicate difference between groups, $P < 0.05$; independent effects of diet, swimming exercise and the interaction between diet and exercise are expressed with $P < 0.05$. # - indicates effect of diet; & - indicates exercise effect; * - indicates the effect of the interaction between diet and exercise.

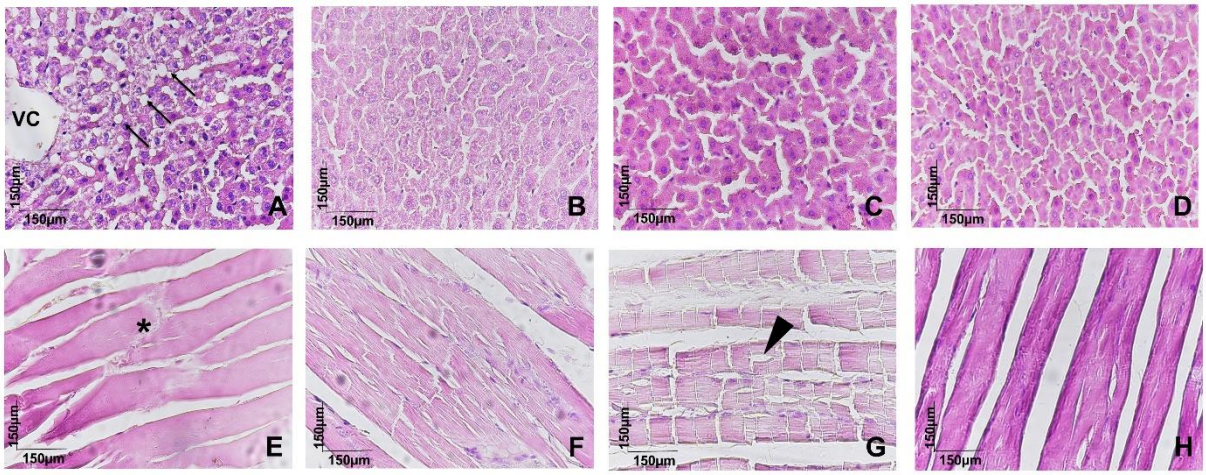

Figure 4. Hepatic and muscle histological analysis. The histological sections of the liver from the sedentary control group (A) showed hepatocytes arranged in cords with ample cytoplasm containing fat vacuoles inside them (hepatic steatosis), as shown in the arrow. In the other experimental protocols presented, exercised control (B), sedentary macaíba (C), and exercised macaíba (D), the hepatocytes were arranged in cords with regular cytoplasm and absence of steatosis. The histological sections of muscle tissue showed striated muscle fibers containing one or two nuclei in the sarcomere with homogeneously colored cytoplasm. In the animals in the sedentary control groups (E), we observed that the sarcomeres of the cells in this group presented a reduction in their constituents, a picture compatible with atrophy (*). The animals from the sedentary macaíba group (G) showed similar changes, however with the longitudinal breakage of the fiber structure (arrowhead), a picture of injury also compatible with muscle atrophy. In the other experimental protocols presented, exercised control (F), and exercised macaíba (H), the muscle fibers remained homogeneous and with the maintenance of the fiber size and its components.

Table 1. Composition of diet - Presence - Purina®

Ingredients (g kg^{-1})	
Moisture	130
Carbohydrates	405
Fat	45
Protein	230
Fiber	50
Total energy (Joules)	2.945

Adapted from Presence - Purina®

Table 2. Murinometric parameters, organ weight and muscle size and thickness data of the groups treated with Macaíba pulp (MC and MC-EX) and with standard diet (CT and CT-EX), submitted or not to swimming exercise.

	Experimental Groups				Analyzed Effects		
	CT	**CT-EX**	**MC**	**MC-EX**	**Diet**	**Exercise**	**Interaction (Diet and Exercise)**
Murinometric							
NL (cm)	23.65 ± 0.57	23.78 ± 0.58	23.13 ± 0.52	23.25 ± 0.71	$P = 0.0059$	ns	ns
AC (cm)	14.77 ± 0.75^{a}	15.10 ± 0.33^{a}	13.94 ± 0.29^{b}	13.99 ± 0.49^{b}	$P < 0.0001$	ns	ns
TC (cm)	13.75 ± 0.46	14.01 ± 0.45	13.57 ± 0.11	13.86 ± 0.34	ns	$P = 0.0175$	ns
BMI (g/cm^2)	0.58 ± 0.02^{a}	0.55 ± 0.03^{b}	0.59 ± 0.04^{a}	0.55 ± 0.03^{b}	ns	$P = 0.0005$	ns
Weight of visceral fats (g)							
Mesenteric fat	3.11 ± 0.47^{a}	3.97 ± 0.44^{b}	2.67 ± 0.32^{c}	2.59 ± 0.23^{c}	$P < 0.0001$	$P = 0.0014$	$P = 0.0002$
Retroperitoneal fat	2.20 ± 0.29^{a}	2.40 ± 0.32^{a}	2.02 ± 0.27^{b}	1.87 ± 0.36^{b}	$P = 0.0005$	ns	ns
Epididymal fat	2.17 ± 0.35^{a}	2.63 ± 0.31^{a}	1.98 ± 0.32^{b}	1.89 ± 0.21^{b}	$P < 0.0001$	$P = 0.0489$	$P = 0.0044$
Adiposity index (%)	2.14 ± 0.20^{a}	2.64 ± 0.23^{a}	2.18 ± 0.14^{b}	2.04 ± 0.16^{b}	ns	ns	ns
Organ weight (g)							
Brain	1.84 ± 0.03	1.84 ± 0.07	1.78 ± 0.08	1.82 ± 0.08	ns	ns	ns
Liver	9.38 ± 0.62^{a}	9.52 ± 0.39^{a}	8.76 ± 0.65^{b}	8.99 ± 0.48^{b}	$P = 0.0012$	ns	ns
Heart	1.15 ± 0.09^{a}	1.27 ± 0.13^{b}	1.11 ± 0.05^{a}	1.28 ± 0.09^{b}	ns	$P < 0.0001$	ns
Kidneys	2.03 ± 0.18	2.11 ± 0.10	1.98 ± 0.16	1.99 ± 0.13	ns	ns	ns
Muscle weight (g)							
Gastrocnemius	1.93 ± 0.19	2.00 ± 0.08	1.93 ± 0.10	2.01 ± 0.20	ns	ns	ns
Solear	0.14 ± 0.02	0.15 ± 0.01	0.15 ± 0.02	0.15 ± 0.01	ns	ns	ns

Length of muscles (mm)							
Gastrocnemius	36.80 ± 0.68[a]	38.28 ± 1.24[b]	36.17 ± 1.76[a]	38.58 ± 1.59[b]	ns	$P < 0.0001$	ns
Solear	28.80 ± 2.33	27.81 ± 2.31	28.33 ± 1.87	28.60 ± 1.44	ns	ns	ns
Muscle thickness (mm)							
Gastrocnemius	13.38 ± 1.64[a]	14.24 ± 0.27[b]	13.25 ± 0.83[a]	14.57 ± 0.90[b]	ns	$P < 0.0001$	ns
Solear	4.53 ± 0.50	4.49 ± 0.16	4.08 ± 0.79	4.49 ± 0.30	ns	ns	ns

CT- Sedentary control; CT-EX – exercised control; MC – sedentary treated with macaíba; MC-EX – exercised treated with macaíba. NL - Nasoanal Length, AC - Abdominal Circumference, TC - Thoracic Circumference, BMI - Body Mass Index. Data were expressed as mean ± SD, n = 11 per group. Results obtained from two-way ANOVA, different letters indicate difference between groups, $P < 0.05$; independent effects of diet, swimming exercise and the interaction between diet and exercise are expressed with $P < 0.05$.

Table 3. Biochemical data of the groups treated with Macaíba pulp (MC and MC-EX) and with standard diet (CT and CT-EX), submitted or not to swimming exercise.

	Experimental Groups				Analyzed Effects		
	CT	**CT-EX**	**MC**	**MC-EX**	**Diet**	**Exercise**	**Interaction (Diet and Exercise)**
Biochemical							
Glucose (mg/dl)	95.11 ± 3.66^{a}	99.7 ± 5.87^{a}	89.00 ± 7.54^{b}	88.91 ± 5.19^{b}	$P < 0.0001$	ns	ns
Urea (mg/dl)	39.65 ± 13.30^{a}	48.09 ± 8.10^{b}	16.63 ± 3.45^{c}	18.72 ± 3.93^{c}	$P < 0.0001$	ns	ns
Creatinine (mg/dl)	0.36 ± 0.04	0.36 ± 0.05	0.36 ± 0.03	0.36 ± 0.02	ns	ns	ns
AST (u/l)	188.46 ± 9.65^{a}	179.73 ± 28.85^{a}	113.35 ± 4.35^{b}	115.95 ± 3.71^{b}	$P < 0.0001$	ns	ns
ALT (u/l)	57.98 ± 1.13^{a}	55.88 ± 1.55^{a}	28.39 ± 0.60^{b}	29.00 ± 2.18^{b}	$P < 0.0001$	ns	ns
TC (mg/dl)	72.50 ± 11.55^{a}	75.00 ± 8.37^{a}	56.67 ± 9.83^{b}	55.42 ± 5.10^{b}	$P < 0.0001$	ns	ns
TG (mg/dl)	24.85 ± 8.96	25.82 ± 9.66	21.36 ± 2.90	22.86 ± 3.93	ns	ns	ns
HDL-c (mg/dl)	34.75 ± 4.80^{a}	37.73 ± 4.97^{b}	43.18 ± 4.69^{c}	49.13 ± 4.91^{d}	$P < 0.0001$	$P = 0.0040$	ns
LDL-c (mg/dl)	25.10 ± 6.54^{a}	17.50 ± 5.54^{b}	13.05 ± 2.16^{c}	13.26 ± 2.17^{c}	$P < 0.0001$	$P = 0.0103$	$P = 0.0070$
VLDL-c (mg/dl)	7.52 ± 0.42^{a}	7.27 ± 0.20^{b}	4.33 ± 0.63^{c}	4.33 ± 0.52^{c}	$P < 0.0001$	ns	ns
Cardiovascular Risks							
Atherogenic Index	0.75 ± 0.18^{a}	0.25 ± 0.14^{b}	0.33 ± 0.05^{b}	0.29 ± 0.09^{b}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Atherogenic Coefficient	1.37 ± 0.12^{a}	0.99 ± 0.09^{b}	0.66 ± 0.10^{c}	0.36 ± 0.08^{d}	$P < 0.0001$	$P < 0.0001$	ns
Coronary Risk	1.67 ± 0.12^{a}	1.34 ± 0.06^{b}	1.28 ± 0.18^{b}	1.09 ± 0.14^{b}	$P < 0.0001$	$P < 0.0001$	ns
Cardiovascular Risk	0.74 ± 0.19^{a}	0.44 ± 0.06^{b}	0.38 ± 0.06^{c}	0.39 ± 0.06^{c}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$

CT- Sedentary control; CT-EX – exercised control; MC – sedentary treated with macaíba; MC-EX – exercised treated with macaíba. TC - total cholesterol; TG – triglycerides; HDL-c – high density lipoprotein; LDL-c – low density lipoprotein; VLDL-c- very low lipoprotein; AST - aspartate aminotransferase; ALT - alanine aminotransferase. Data were expressed as mean ± SD, n = 11 per group. Results obtained from two-way ANOVA, different letters indicate difference between groups, $P < 0.05$; independent effects of diet, swimming exercise and the interaction between diet and exercise are expressed with $P < 0.05$.

5 CONSIDERAÇÕES GERAL

Nossos resultados demonstraram que o consumo de polpa de macaíba induziu efeito ansiolítico, cardioprotetor, modulou parâmetros bioquímicos com aumento de HDL, redução de VLDL, LDL e de colesterol total, além de contribuir na redução de gorduras viscerais e perda de peso. Quando este consumo foi associado com o exercício de natação, verificamos potencialização na redução de risco cardiovascular e índice aterogênico, redução de gordura hepática e de aumento no efeito ansiolítico.

Com base no que foi explanado e nos resultados obtidos, verificamos a necessidades de se investigar em trabalhos futuros seus efeitos metabólicos em processos inflamatórios, assim como seus efeitos e segurança em diferentes fases da vida.

ANEXO C: Certidão de Aprovação do Comitê de Ética Animal

Universidade Federal de Campina Grande
Centro de Saúde e Tecnologia Rural
Comissão de Ética no Uso de Animais
Av. Santa Cecília, s/n, Bairro Jatobá, Rodovia
Patos,
CEP: 58700-970, Cx postal 64, Tel. (83) 3511-3045

Universidade Federal de Campina Grande

A(o): Dr (ª). **Juliana Késsia Barbosa Soares**

Protocolo CEUA/CSTR Nº **010/2019**

CERTIDÃO

Certificamos para os devidos fins que o projeto intitulado **"Efeito da suplementação com polpa de macaíba (acrocomia intumescens) sobre parâmetros bioquímicos, estresse oxidativo e comportamento em ratos exercitados"**, coordenado pelo (a) pesquisador (a) acima citado (a), obteve parecer consubstanciado pelo regulamento interno deste comitê, sendo **APROVADO**, em caráter de ***Reuniao ordinária no dia 11 de Abril de 2019***, estando a luz das normas e regulamento vigentes no pais atendidas as pesquisas para especificações cientificas.

Patos, 17 de Abril de 2019.

Rosália Severo de Medeiros
Coordenadora do CEP/CEUA/UFCG/Patos