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Camille Gaube Guex

ATIVIDADE RADIOPROTETORA, PERFIL TOXICOLÓGICO E EFEITO CONTRA A TOXICIDADE DA CICLOFOSFAMIDA DO EXTRATO DE TUCUMÃ: ESTUDOS *IN VITRO* E *IN VIVO*

Santa Maria, RS
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Tese apresentada ao Curso de Doutorado de Pós-Graduação em Farmacologia da Universidade Federal de Santa Maria (UFSM, RS), como requisito para obtenção do título de **Doutora em Farmacologia**.

Orientadora: Prof.ª Dr.ª Liliane de Freitas Bauermann

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Aprovada em 04 de agosto de 2020:

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Santa Maria, RS
2020

Para meus pais, irmãos
e amigos, por todo apoio,
dedico este trabalho.

E, também,

“Para todos que já tiveram
um momento de fraqueza.
Não vai doer para sempre,
então não deixe isso afetar
o que há de melhor em você.”
(J.A. Redmerski)

Agradecimentos

- Quem estará na jornada ao seu lado?
- E isso importa?
- Mais do que a própria viagem.
- Ernest Hemingway -

Agradeço, primeiramente, aos meus pais e irmãos, que nunca mediram esforços para que eu chegasse até aqui. Pai e mãe, obrigada pelas oportunidades e pelo privilégio de poder estudar.

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Ressentimento do mundo

Para Carlos Drummond de Andrade

Enquanto no mundo
tem gente pensando
que sabe muito,
eu apenas sinto.
Muito.

- David Cohen -

RESUMO

ATIVIDADE RADIOPROTETORA, PERFIL TOXICOLÓGICO E EFEITO CONTRA A TOXICIDADE DA CICLOFOSFAMIDA DO EXTRATO DE TUCUMÃ: ESTUDOS *IN VITRO* E *IN VIVO*

AUTORA: Camille Gaube Guex
ORIENTADORA: Liliane de Freitas Bauermann

O câncer é uma doença multifatorial caracterizada pela perda de controle da divisão celular. Os tratamentos existentes para o câncer incluem a radioterapia e a quimioterapia, que fazem uso de radiação ionizante e quimioterápicos para induzir a morte celular, respectivamente. Entretanto, os efeitos adversos decorrentes dos tratamentos podem tornar-se graves e até levar à interrupção dos mesmos. Produtos naturais estão ganhando grande destaque para atenuar esses efeitos devido aos seus compostos bioativos. Nesse cenário, o tucumã é um fruto amazônico de grande interesse farmacológico por possuir importantes substâncias bioativas, que podem atuar na redução dos danos induzidos pela radiação e quimioterapia. Dessa maneira, decidimos investigar o potencial efeito do extrato bruto de tucumã (EBT) em atenuar os efeitos causados pela radiação em um modelo *in vitro*. Na sequência, foi investigada a potencial toxicidade aguda e de doses repetidas do EBT e sua atividade contra a toxicidade da ciclofosfamida (CFF), em modelos *in vivo*. Além disso, realizamos a caracterização fitoquímica do extrato através de HPLC-PAD, GC-MS e UHPLC-MS. Essas análises revelaram a presença de polifenóis, flavonoides, catequinas, carotenoides e ácidos graxos saturados e insaturados. Para o estudo *in vitro*, queratinócitos e fibroblastos foram tratados com o EBT (por 24h) e expostos à radiação na dose 2Gy. Após 24h, foram realizadas análises de marcadores apoptóticos e do estresse oxidativo, mortalidade celular e fatores de crescimento. Para a toxicidade aguda, foram utilizadas ratas *Wistar* divididas em controle e teste, as quais as últimas foram tratadas com o EBT na dose única de 2000 mg/kg. Para o estudo de toxicidade de doses repetidas foram utilizados ratos machos e fêmeas que, excetuando pelo grupo controle, foram tratados com o EBT nas doses 200, 400 e 600 mg/kg, via oral, durante 28 dias. Parâmetros fisiológicos, comportamentais, hematológicos, bioquímicos, histopatológicos e marcadores do estresse oxidativo foram analisados. Para o terceiro estudo, foram utilizados ratos *Wistar* divididos em tratamento e pré-tratamento. Nos primeiros, a administração da CFF antecedeu o tratamento oral com o EBT; enquanto que no pré-tratamento, o contrário ocorreu. A CFF foi administrada (via IP) na dose de 150 mg/kg (divididas em três dias) e o EBT foi administrado nas doses de 100, 200 e 400 mg/kg, via oral, por 14 dias. Parâmetros fisiológicos, hematológicos, bioquímicos e marcadores do estresse oxidativo foram analisados. Em relação aos resultados do estudo *in vitro*, a radiação alterou os níveis dos marcadores apoptóticos e do estresse oxidativo avaliados, além dos fatores de crescimento. O pré-tratamento com o extrato de tucumã foi capaz de atenuar os parâmetros citados, variando de acordo com o tipo de célula. Em referência ao estudo de toxicidade aguda, durante o período de experimento não houve sinais de toxicidade, nem mortes foram registradas. Entretanto, quando administrado repetidamente e na dose mais alta (600 mg/kg), foi observada toxicidade renal em machos, evidenciada pela análise histológica. Nos demais parâmetros, tanto de machos quanto de fêmeas, não foram encontrados sinais de toxicidade e nem mortalidade foi registrada. Assim, o EBT pode ser considerado seguro quando administrado em doses baixas. Após exposição à CFF, foram observadas alterações nos pesos corporais, dos órgãos e nos pesos relativos. Além disso, as análises hematológicas e bioquímicas também apresentaram alterações, variando para cada estudo. As administrações orais de EBT não foram capazes de atenuar significativamente esses parâmetros. Entretanto, o EBT melhorou os níveis de MDA e carbonilação, assim como da atividade da SOD, todos afetados pela CFF. O pré-tratamento mostrou os melhores resultados em atenuar os efeitos tóxicos da CFF, especialmente na melhora dos marcadores de dano oxidativo. Dessa maneira, os resultados encontrados são promissores, indicando que o tucumã é um fruto com potenciais atividades biológicas, porém deve ser utilizado em doses baixas.

Palavras-chave: Radioterapia. Quimioterapia. Toxicidade. *Astrocaryum aculeatum*. *Wistar*.

ABSTRACT

RADIOPROTECTIVE ACTIVITY, TOXICOLOGIC PROFILE AND EFFECT AGAINST CYCLOPHOSPAMIDE TOXICITY OF TUCUMÃ EXTRACT: *IN VITRO* AND *IN VIVO* STUDIES

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ADVISOR: Liliane de Freitas Bauermann

Cancer is a multifactorial disease characterized by loss of control of cell division. Nowadays, it is a serious health problem worldwide, leading to many deaths every year. Current treatments for cancer include radiation therapy and chemotherapy, which use ionizing radiation and different drugs to induce cell death, respectively. However, the adverse effects associated with these treatments can become serious and lead to treatment interruption. Natural products are gaining attention to act as alternative therapies to attenuate these effects due to their bioactive compounds. In this scenario, tucumã is an Amazonian fruit with great pharmacological interest due the presence of important bioactive substances, which can act by reducing the damage induced by radiation and chemotherapy. Therefore, we decided to investigate the potential effect of tucumã crude extract (TCE) in mitigating the effects caused by radiation in an *in vitro* model. Afterward, the acute and repeated dose toxicity of TCE and its activity against the toxicity of cyclophosphamide (CPP) were investigated, all *in vivo* studies. In addition, we perform the phytochemical characterization of the extract by HPLC-PAD, GC-MS and UHPLC-MS. These analyses revealed the presence of polyphenols, flavonoids, catechins, carotenoids and saturated and unsaturated fatty acids. For the *in vitro* study, keratinocytes and fibroblasts were treated with TCE (for 24h) and exposed to radiation at a dose of 2 Gy. After 24h, analyses of apoptotic markers and oxidative stress, cell mortality and growth factors were performed. For acute toxicity, female *Wistar* rats were divided into control and test groups, in which those in the test group were treated with TCE in a single dose of 2000 mg/kg. For the repeated dose toxicity study, male and female rats were used and, except for the control group, were treated with TCE at doses of 200, 400 and 600 mg/kg, orally, for 28 days. Physiological, behavioral, hematological, biochemical and histopathological parameters and markers of oxidative stress were analyzed. For the third study, *Wistar* rats were used and divided into treatment and pre-treatment. In the treated one, CPP administration preceded oral treatment with TCE; while in pre-treatment, the opposite occurred. CPP was administered (via IP) at a dose of 150 mg/kg (divided into three days) and TCE was administered at doses of 100, 200 and 400 mg/kg, orally, for 14 days. Physiological, hematological and biochemical parameters and markers of oxidative stress were analyzed. Regarding the results of the *in vitro* study, radiation altered the levels of the apoptotic markers and oxidative stress, as well as of growth factors. The pretreatment with the tucumã extract was able to attenuate the aforementioned parameters, varying according to the cell type. In reference to the acute toxicity study, during the experiment period there were no signs of toxicity nor deaths were recorded. However, when administered repeatedly and at the highest dose (600 mg/kg), renal toxicity was observed in males, as evidenced by histological analysis. In the other parameters, for both males and females, no signs of toxicity were found and no mortality was recorded. Thereby, TCE can be considered safe when administered in low doses. After exposure to CPP, changes in body weights, organs and relative weights were observed. In addition, hematological and biochemical analyses also showed changes, varying for each study. Oral administrations of TCE were not able to significantly attenuate these parameters. However, TCE has improved levels of MDA and carbonylation, as well as SOD activity, all affected by CPP. The pretreatment showed the best results in reducing the toxic effects of CPP, especially in the improvement of oxidative damage markers. Therefore, the results found in this work are promising, indicating that tucumã is a fruit with potential biological activities, however it should be used in low doses.

Keywords: Radiotherapy. Chemotherapy. Toxicity. *Astrocaryum aculeatum*. *Wistar*.

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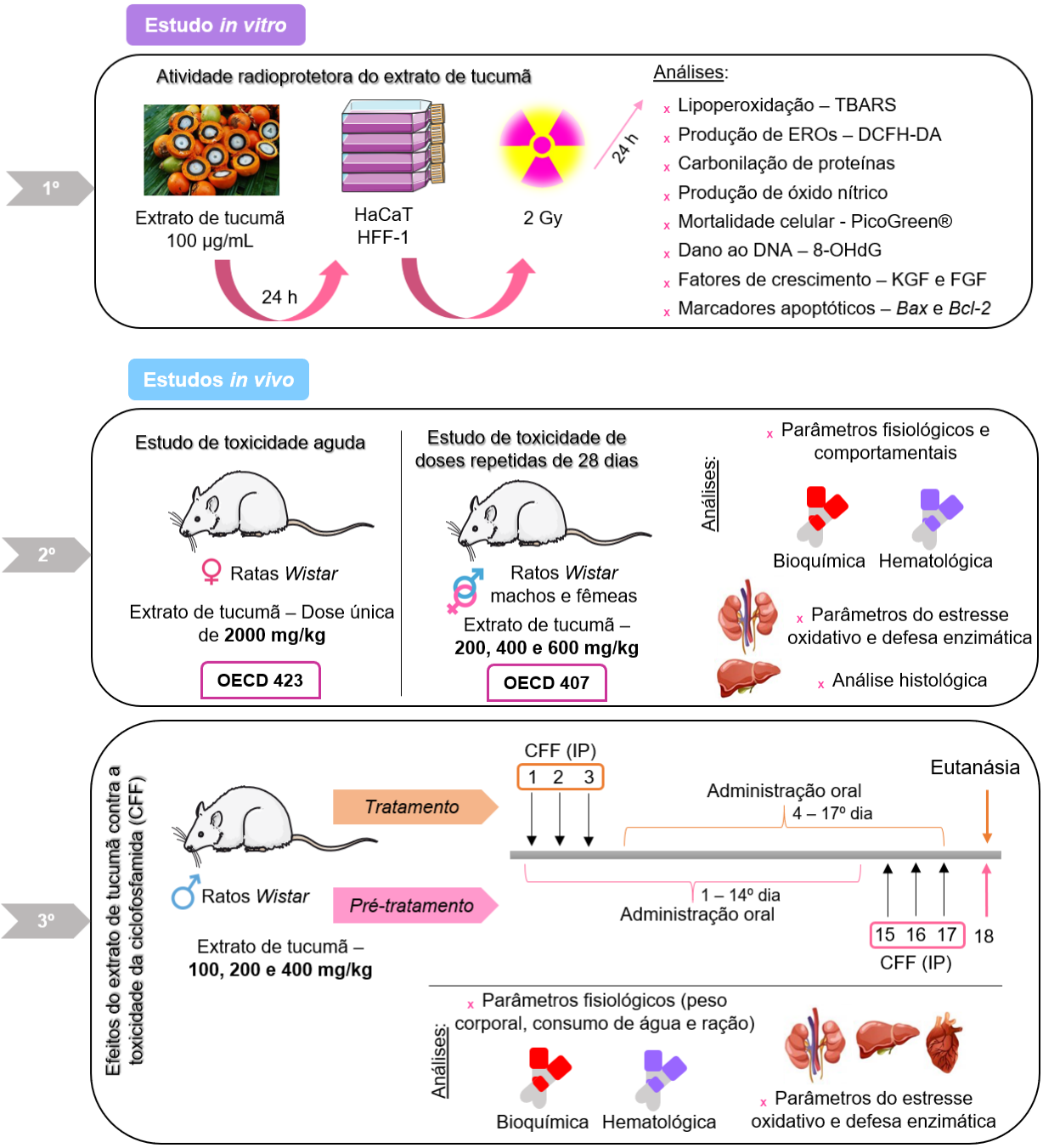
LISTA DE ABREVIAÇÕES E SIGLAS

ANOVA	Análise de variância, do inglês *analysis of variance*
ANVISA	Agência Nacional de Vigilância Sanitária
Ca-DTPA	Pentetato de cálcio trissódico
CAT	Catalase
CEUA	Comissão de Ética no Uso de Animais
CETP	Extrato bruto da polpa do tucumã, do inglês *crude extract of tucumã pulp*
CFF	Ciclofosfamida
CONCEA	Conselho Nacional de Controle de Experimentação Animal
CO_2	Dióxido de carbono
DCFH-DA	2'-7'- diclorofluoresceína diacetato
DMEM	*Dulbecco's Modified Eagle Medium*
DNA	Ácido desoxirribonucleico, do inglês *deoxyribonucleic acid*
DPPH	2,2-difenil-1-picrilhidrazil
EBT	Extrato bruto do tucumã
ERs	Espécies reativas
EROs/ROS	Espécies reativas de oxigênio / do inglês *reactive oxygen species*
e^{-aq}	Elétrons livres hidratados
FBS	Soro fetal bovino, do inglês *fetal bovine serum*
FDA	Administração de Alimentos e Drogas, do inglês *Food and Drug Administration*
GEP	Gerência de Ensino e Pesquisa
GPx	Glutationa peroxidase
GR	Glutationa redutase
GST	Glutationa S-transferase
Gy	Gray
$H^{\bullet}$	Radical hidrogênio
H_2O_2	Peróxido de hidrogênio
INCA	Instituto Nacional de Câncer
IP	Intraperitoneal
LPO	Lipoperoxidação
MDA	Malondialdeído
OECD	Organização para Cooperação Econômica e Desenvolvimento, do inglês *Organisation for Economic Cooperation and Development*
$OH^{\bullet}$	Radical hidroxila
RI	Radiação ionizante
SisGen	Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado
SOD	Superóxido dismutase
TBARS	Substâncias reativas ao ácido tiobarbitúrico, do inglês *thiobarbituric acid reactive substances*
TPE	Extrato da polpa de tucumã, do inglês *tucumã pulp's extract*
UV	Ultravioleta
WHO	Organização Mundial da Saúde, do inglês *World Health Organization*
Zn-DTPA	Pentetato de zinco trissódico

SUMÁRIO

APRESENTAÇÃO

A tese ora apresentada encontra-se na forma de três artigos científicos: o primeiro mostra a potencial atividade radioprotetora *in vitro* do extrato de tucumã; enquanto o segundo demonstra a avaliação das toxicidades aguda e de doses repetidas *in vivo*. O terceiro artigo traz os efeitos do extrato contra a toxicidade induzida pela ciclofosfamida. Os esquemas a seguir sintetizam os três estudos que compõem esta tese.



1 INTRODUÇÃO

O câncer é caracterizado como uma massa anormal de células, resultante de uma proliferação celular excessiva, persistente e descontrolada (ALBERTS et al., 2011; JUNQUEIRA & CARNEIRO, 2005). A perda do controle da divisão celular torna as células cancerosas capazes de invadirem outras estruturas orgânicas, podendo espalhar-se para outras partes do organismo através dos sistemas sanguíneo e linfático (RUDDON, 2007). Atualmente, o câncer é um dos problemas de saúde pública mais complexos que o sistema de saúde enfrenta, visto sua relevância epidemiológica, social e econômica (INSTITUTO NACIONAL DE CÂNCER - INCA, 2019). No Brasil, as neoplasias são a segunda causa de óbito na população, o que representou 16,6% do total de óbitos ocorridos em 2015 (INCA, 2019). Mundialmente, foram registradas 9,6 milhões de mortes em decorrência do câncer, no ano de 2018 (WORLD HEALTH ORGANIZATION - WHO, 2020).

Os tratamentos convencionais utilizados para o combate ao câncer são a cirurgia, a radioterapia e a quimioterapia. Essas modalidades terapêuticas podem ser utilizadas combinadas ou não, dependendo da suscetibilidade dos tumores a cada uma delas e à sequência mais adequada de sua administração (INCA, 2019). A radioterapia utiliza a radiação ionizante para induzir a morte celular. As lesões nas células tumorais podem ocorrer diretamente em suas moléculas de DNA ou indiretamente através da produção de espécies reativas (ERs), em consequência da hidrólise (DOBRZUŃSKA, 2013). As ERs provocam danos em estruturas celulares, como o DNA, enzimas e constituintes da membrana (LECCIA et al., 1993; NAMBIAR et al., 2011), levando a efeitos nocivos para o organismo. Além disso, os danos também podem atingir as células saudáveis dos tecidos vizinhos, tornando-se uma das limitações decorrentes desse tratamento. Esses efeitos incluem náusea, diarreia, supressão no sistema sanguíneo, alopecia, sintomas neurológicos, distúrbios do comportamento, radiodermites, entre outros (CITRIN et al., 2010).

Já a quimioterapia consiste no uso de quimioterápicos - isolados ou em combinação - com diferentes mecanismos citotóxicos, capazes de causar danos ao DNA. O objetivo principal desse tratamento é interferir no processo de crescimento e divisão das células neoplásicas (DA COSTA, 2012). Entretanto, a citotoxicidade inespecífica dos quimioterápicos é uma grande limitação da quimioterapia, pois produzem efeitos adversos como náuseas e vômitos, perda de peso, desidratação,

leucopenia, trombocitopenia, anemia, mucosite, alopecia, ulceração na boca, esofagite, entre outros. Esses efeitos estão associados à interrupção do tratamento e redução de dose, o que resulta em pior resposta e resistência aos quimioterápicos (DA COSTA, 2012).

Logo, a busca por compostos que possam atenuar os efeitos adversos tanto da radioterapia quanto da quimioterapia tem se intensificado nos últimos anos. Grande atenção tem se dado às plantas e seus derivados, pois são conhecidos por possuírem substâncias bioativas com potenciais propriedades farmacológicas, além de serem facilmente implementados à dieta. O Brasil é o país de maior biodiversidade do planeta, sendo a utilização de produtos naturais para tratar, curar ou prevenir doenças uma das práticas mais antigas entre a população (VEIGA JUNIOR et al., 2005). Entretanto, antes de validar a potencial atividade de uma planta ou extrato, é necessário que ensaios de segurança sejam realizados. Na atualidade, existem diversos protocolos que podem ser seguidos a fim de comprovar o uso seguro de uma substância. Neles, diferentes tipos de testes de toxicidade são descritos, como a toxicidade aguda e de doses repetidas, as quais, quando realizadas em condições adequadas, podem servir de base para futuros estudos sobre as atividades biológicas de dada substância.

Nesse contexto, o tucumã (*Astrocaryum aculeatum*) é uma palmeira encontrada na Amazônia central e ocidental, podendo desenvolver-se em ambientes degradados e de vegetação secundária (LEITÃO, 2008; YUYAMA et al., 2008). Seu fruto é rico em carotenoides, compostos fenólicos, fibras e proteínas, sendo amplamente consumido pela população (FERREIRA et al., 2008). As substâncias bioativas encontradas nas plantas têm um importante papel na prevenção e/ou tratamento de doenças crônicas, bem como participam do desenvolvimento de fármacos (BRASIL, 2012). Atividades como antioxidante e anti-inflamatória são relacionadas à presença desses constituintes, tornando o tucumã um potencial aliado na terapia contra os efeitos adversos da radiação ionizante e dos quimioterápicos. Além disso, até o presente momento, não há estudos que mostrem a toxicidade oral *in vivo* do tucumã. Assim sendo, o presente estudo teve como objetivo investigar a potencial atividade radioprotetora do extrato de tucumã em culturas celulares expostas à radiação ionizante, além de avaliar as toxicidades oral aguda e de doses repetidas e o potencial efeito protetor do extrato contra a toxicidade da ciclofosfamida em ratos *Wistar*.

1.2 OBJETIVOS

Avaliar a potencial atividade radioprotetora *in vitro* do extrato bruto do tucumã (*Astrocaryum aculeatum*), bem como investigar suas toxicidades aguda e de doses repetidas e o seu potencial efeito protetor contra a toxicidade da ciclofosfamida *in vivo*.

1.2.1 Objetivos específicos

- *In vitro:*
  - → Avaliar o potencial efeito radioprotetor do extrato bruto do tucumã (EBT) em culturas celulares de fibroblastos e queratinócitos e linfócitos expostas à radiação na dose de 2 Gy, através das análises de:
    - Marcadores do estresse oxidativo;
    - Produção de óxido nítrico;
    - Viabilidade e mortalidade celular;
    - Fatores de crescimento e
    - Marcadores da rota apoptótica.
- Analisar a capacidade antioxidante do EBT e realizar sua caracterização fitoquímica através de análises espectrofotométricas e cromatográficas.
- *In vivo:*
  - → Avaliar as toxicidades aguda (OECD 423) e de doses repetidas (OECD 407) do EBT em ratos *Wistar* através de:
    - Parâmetros fisiológicos e comportamentais;
    - Análises hematológicas, bioquímicas e histopatológicas e
    - Análises de marcadores do estresse oxidativo.
  - → Investigar o potencial efeito protetor do EBT contra a toxicidade induzida pela ciclofosfamida em ratos *Wistar* por meio de:
    - Parâmetros fisiológicos;
    - Análises hematológicas e bioquímicas e
    - Análises de marcadores do estresse oxidativo.

2 REVISÃO BIBLIOGRÁFICA

2.1 CÂNCER: CONCEITOS GERAIS

A carcinogênese é definida como proliferações clonais, nas quais eventos genéticos, epigenéticos, além de fatores ambientais promovem alterações na fisiologia celular, que levam à transição de um estado celular normal para um estado maligno (DALZIEL et al., 2014). O câncer é caracterizado como uma doença multicausal crônica, na qual ocorre o crescimento descontrolado das células teciduais através do excesso de proliferação e deficiência nas taxas de morte celular, resultando na formação de um agrupamento de clones de células neoplásicas (QIAN et al., 2011). Através de mecanismos biológicos específicos e complexos, essas células são capazes de sobreviver e crescer continuamente, além de colonizar outros tecidos com o auxílio dos sistemas sanguíneo e linfático (VISCARDI, 2018). O desenvolvimento do câncer envolve três principais etapas: a iniciação, a promoção e a progressão tumoral (INCA, 2019). Ademais, o câncer pode desenvolver-se em qualquer região do corpo humano, variando de acordo com a complexidade da doença e uma série de fatores internos e externos que podem influenciar nas etapas de formação (VISCARDI, 2018).

Nos últimos anos, o câncer tornou-se um grave problema de saúde pública. Em 2018, foram estimados 18,1 milhões de novos casos de câncer e, mundialmente, é a segunda causa de morte, sendo estimadas 9,6 milhões de mortes no mesmo ano (WHO, 2020). Além disso, o número de novos casos e mortes continua a crescer devido ao aumento da expectativa de vida e das transições epidemiológicas e demográficas (WHO, 2020). No Brasil, esse fato é uma realidade: para 2018 e 2019, a estimativa foi de cerca de 600 mil novos casos de câncer no país para cada um desses anos (INCA, 2019). Os principais tratamentos convencionais utilizados atualmente incluem cirurgia para remoção total ou parcial do órgão ou tecido afetado, radioterapia e quimioterapia.

2.2 TRATAMENTOS CONVENCIONAIS DO CÂNCER

As principais modalidades de tratamento do câncer são a quimioterapia, a radioterapia e a cirurgia, podendo haver a combinação entre elas. A escolha para utilizar um ou mais tratamentos varia de acordo com o tipo e localização da neoplasia

desenvolvida, seu estágio de desenvolvimento, da resposta das células neoplásicas e do organismo ao tratamento, incluindo desde a diminuição da neoplasia até efeitos colaterais do paciente (U.S. NATIONAL INSTITUTES OF HEALTH, 2018; PADMA, 2015). Atualmente, poucas são as neoplasias malignas tratadas com apenas uma dessas modalidades terapêuticas (INCA, 2019).

A cirurgia consiste na remoção total ou parcial do órgão ou tecido afetado; a radioterapia utiliza altas doses de radiação para induzir a morte das células neoplásicas, podendo ser indicada antes ou depois da cirurgia. Já a quimioterapia se dá através do uso de fármacos combinados para atingir as células neoplásicas e induzir sua morte (U.S. NATIONAL INSTITUTES OF HEALTH, 2018; PADMA, 2015).

2.3 RADIOTERAPIA

A radioterapia é o tratamento que utiliza a radiação ionizante (RI) para levar à morte celular. As alterações físico-químicas no meio intracelular exposto à radiação ionizante irão determinar a gravidade dos efeitos biológicos, conforme o organismo ou tecido irradiado (BIRAL, 2002; ONCOLOGY NURSING SOCIETY, 2006).

A radioterapia é frequentemente utilizada para alcançar um controle local ou regional de doenças malignas, tanto individual ou em combinação com outras terapias, como quimioterapia ou cirurgia (CITRIN et al., 2010). A sua finalidade é alcançar uma resposta terapêutica favorável ao destruir e/ou inibir o crescimento de células tumorais do organismo, impedindo sua multiplicação por mitose e/ou determinando a sua morte celular, e preservando, ao máximo, os tecidos normais (GOMES, 2010; SCHNEIDER, 2013). Em muitos países, é utilizada para o tratamento de câncer de mama, carcinoma de células escamosas de cabeça e pescoço e também tem mostrado reduzir o risco de recorrência da doença (GAO et al., 2015 TAYLOR & KIRBY, 2015).

Em uma sessão de radioterapia, a dose absorvida no tumor é de 2 Gy, enquanto que 4 Gy é a dose letal – dose capaz de provocar a morte de 50% dos seres humanos irradiados no corpo todo - cerca de 30 dias após a exposição. Entretanto, durante o processo de radiação terapêutica, muitos tecidos normais podem ser afetados, como o tórax, coração, pele e mucosa, entre outros, prejudicando a qualidade de vida do paciente (GAO et al., 2015). Essa exposição pode resultar em extensos efeitos adversos, incluindo toxicidades agudas autolimitadas, sintomas

crônicos leves ou disfunção severa de órgãos (CITRIN et al., 2010). Além desses, esse tratamento também pode desencadear náusea, diarreia, supressão no sistema sanguíneo, alopecia (perda de pelos), sintomas neurológicos, distúrbios do comportamento e radiodermites. Doses iguais ou maiores que 2 Gy podem causar linfopenia e significante apoptose e parada mitótica nas células-tronco hematopoiéticas e células progenitoras da medula óssea (SURYAVANSHI et al., 2015). Altas doses de RI estão associadas ao desenvolvimento do estresse oxidativo, que tem sido implicado em morbidade tardia. Ainda, para doses iguais ou maiores que 10 Gy, os efeitos incluem danos nos sistemas nervoso e cardiovascular, levando o indivíduo à morte em poucos dias (OKUNO, 2013). Esses efeitos são um grande desafio na clínica, já que são fatores limitantes na continuidade do tratamento. Assim, os efeitos biológicos decorrentes da exposição à RI variam de acordo com a dose absorvida, a taxa de exposição (crônica ou aguda) e da forma de exposição (corpo inteiro ou localizada).

Devido aos sérios efeitos adversos decorrentes do tratamento com a radioterapia, tem-se investido na busca por compostos que possam atenuar esses efeitos. Os compostos mencionados são conhecidos como radiomodificadores e os produtos naturais têm sido uma importante fonte de novos estudos.

2.3.1 Radiomodificadores

Os radiomodificadores podem atuar como radioprotetores ou radiossensibilizadores, ou até como ambos em tecidos diferentes, dependendo de diversos fatores metabólicos. Um radioprotetor se caracteriza por ser administrado antes da exposição à RI, protegendo o tecido normal, enquanto que um radiossensibilizador é capaz de sensibilizar o tecido tumoral à RI. Devido à alta toxicidade apresentada pelos compostos sintéticos, tem-se aumentado a busca por agentes naturais que possam atuar como radiomodificadores e que apresentem menos efeitos tóxicos aos usuários (SOUZA *et al*., 2000).

A radioproteção é alvo de grande interesse no meio onde se aplica a RI, principalmente por meio de mecanismos químicos. Muitos fármacos descobertos para o tratamento de doenças em humanos são derivados de plantas (BRASIL, 2012), por isso, tem-se buscado nesses compostos a possibilidade de produtos não tóxicos para o uso de radioproteção. Um protetor químico eficiente deve ser capaz de proteger

contra os efeitos danosos da RI durante procedimentos terapêuticos, bem como durante acidentes nucleares e voos espaciais. De acordo com JAGETIA, 2007: "*um radioprotetor ideal deve ser barato, não produzir efeitos tóxicos em uma ampla variação de doses, ser administrado oralmente e ter uma absorção rápida, apresentar um fator de redução de dose considerável e atuar através de múltiplos mecanismos*".

As plantas e produtos naturais são conhecidos por possuírem essas características. Ainda, os produtos naturais que apresentam atividades anti-inflamatória, antitumoral, antioxidante, antimicrobiana e sequestrador de radicais livres são potenciais radioprotetores (JAGETIA, 2007). Além dessas qualidades, o agente deve ser seletivo em proteger tecidos normais da radioterapia e agir sem proteger o tumor, ao contrário, nenhum benefício no índice terapêutico será atingido (CITRIN et al., 2010). Embora exista um número de compostos que apresentam essas características, os únicos compostos aprovados pela Administração de Alimentos e Drogas dos EUA (*Food and Drug Administration* - FDA) são a amifostina (WR-2721, [ácido S-2-(3 aminopropilamino) etilfosforotioico]), azul da prússia, Ca-DTPA/Zn-DTPA (pentetato de cálcio trissódico/pentetato de zinco trissódico) e iodeto de potássio (DOS SANTOS, 2013). Souza et al. (2000) demonstraram que essas substâncias, utilizadas como radioprotetores, apresentam efeitos adversos graves, como náusea, vômito, hipotensão, hipocalcemia e febre em doses clinicamente efetivas, além de possuírem alta hepatoxicidade e neurotoxicidade. Assim, é necessária a busca por compostos que possam atenuar os danos causados pela exposição à RI. Atualmente, tem-se dado destaque aos produtos de origem natural, por possuírem importantes compostos fitoquímicos com potenciais atividades farmacológicas.

2.3.2 Produtos naturais como radioprotetores

Os produtos naturais já fazem parte da dieta de grande parcela da população por possuírem compostos com importantes atividades biológicas. Atividades como antioxidante e anti-inflamatória oferecem às plantas um potencial alvo para a proteção contra a RI, atuando como um radioprotetor. Na literatura, alguns estudos já investigaram os potenciais efeitos radioprotetores de produtos naturais.

Srinivasan et al. (2007) trataram hepatócitos de ratos com licopeno (carotenoide natural) em três diferentes doses e, então, os submeteram à radiação-γ

(1, 2 e 4 Gy). O pré-tratamento com licopeno foi capaz de reduzir a peroxidação lipídica e a formação de radicais livres que induzem a quebra da fita de DNA, indicando um potencial uso aos pacientes de radioterapia por proteger células normais do dano da radiação.

Em estudo realizado por Sunada et al. (2014), células de ovários de hamster chinês foram previamente tratadas com rutina, flavonoide amplamente encontrado na natureza, e foram, então, expostas à RI na dose de 1 Gy. Foi observado que o tratamento com a rutina foi capaz de reduzir a troca de cromátides-irmãs induzida pela radiação e aumentar a sobrevivência celular com concentrações que não induziram a toxicidade celular. O aumento na sobrevivência celular pode estar relacionado à habilidade da rutina em inibir a formação de radicais livres, protegendo, assim, o DNA contra danos induzidos pela RI.

Além disso, em um estudo *in vivo*, camundongos receberam silibilina (pertencente à família dos flavonoides), via oral, durante 3 dias antes de serem expostos à radiação de corpo inteiro (7,5 Gy), resultando em significante proteção à morte induzida por radiação e dano ao DNA em leucócitos (TIWARI et al., 2010).

Por apresentarem propriedades de grande interesse farmacológico, os produtos naturais são uma importante fonte na busca por novos agentes que possam atuar na redução e/ou prevenção dos efeitos nocivos apresentados após exposição à RI.

2.4 QUIMIOTERAPIA

A quimioterapia é a forma de tratamento sistêmico do câncer, que utiliza os medicamentos conhecidos como quimioterápicos administrados em intervalos regulares (INCA, 2019). Ainda de acordo com o INCA (2019), existem cinco finalidades da quimioterapia: **a)** prévia ou neoadjuvante: indicada para a redução de tumores locais e da extensão da área cirúrgica; **b)** adjuvante ou profilática: indicada após o tratamento cirúrgico curativo; **c)** curativa: curar pacientes com neoplasias malignas para os quais representa o principal tratamento; **d)** para controle temporário de doença: indicada para o tratamento de tumores sólidos, avançados ou recidivados ou neoplasias hematopoiéticas de evolução crônica; e **e)** paliativa: sem finalidade curativa, tem o intuito de melhorar a qualidade da sobrevida do paciente.

O grande desafio no tratamento é a citotoxicidade inespecífica dos quimioterápicos e seus metabólitos, o que torna um fator limitante da quimioterapia. Por agirem em células de rápida divisão, como as do tecido hematopoiético, do epitélio de revestimento do aparelho gastrintestinal, do tecido germinativo e folículo piloso, esses medicamentos causam efeitos adversos hematológicos e não-hematológicos (SOLIMANDO, 2008; CHU & DEVITA, 2009). A supressão das células do sistema hematopoiético costuma ocorrer em torno de sete dias após o início do tratamento, havendo recuperação completa em cerca de 21 dias (SOLIMANDO, 2008; CHU & DEVITA, 2009). Além disso, por possuírem efeito emetogênico, as náuseas e vômitos consequentes do tratamento podem levar à perda de peso, desidratação, desequilíbrio eletrolítico e desnutrição (DA COSTA, 2012).

Assim, os efeitos adversos aos quimioterápicos são a principal causa de atraso e redução de dose da quimioterapia. Esse cenário pode resultar na interrupção do tratamento, o que implica em pior resposta, além de resistência aos quimioterápicos (DA COSTA, 2012).

2.4.1 Agentes quimioterápicos

Os agentes quimioterápicos induzem a morte das células cancerígenas, principalmente através de danos à molécula de DNA. Baseando-se em aspectos estruturais do DNA foram desenvolvidos fármacos que são análogos de bases do DNA para interferir na replicação do material genético (HEIDELBERGER et al., 1957). Além disso, fármacos para inibir a síntese de DNA ou lesionar diretamente a molécula de DNA, como os agentes alquilantes, bem como para agirem na inibição do fuso mitótico ou na inibição da topoisomerase também foram desenvolvidos. Assim, os principais quimioterápicos são os alquilantes, antimetabólitos, antibióticos e os inibidores mitóticos (DA COSTA, 2012).

Como exemplo de quimioterápico antimetabólito, podemos citar a capecitabina, que é um pró-fármaco do fluorouracil, hidrolisado no fígado e tecidos; sendo indicado nas metástases do câncer de mama. As reações adversas associadas ao seu uso incluem diarreia, perda de apetite, náuseas, vômitos e mucosite (HERTZ, 2014). A doxorrubicina é um antibiótico antineoplásico que age na inibição da síntese do DNA e RNA, intercalando entre as bases, inibindo a topoisomerase. Podem ocorrer

calafrios, reações alérgicas, infecções, dor no tórax, distensão abdominal, ulceração na boca, esofagite e desidratação associadas ao seu uso (HERTZ, 2014).

O paclitaxel é um alcaloide da família dos taxanos que age impedindo a migração dos microtúbulos - por aumentar a ação da tubulina - inibindo a divisão celular. Possui reações adversas sérias como leucopenia, trombocitopenia, anemia, bradicardia durante a infusão, episódios cardiovasculares severos, neuropatia periférica e mialgia/artralgia (HERTZ, 2014). A ciclofosfamida (CFF) é um agente alquilante citotóxico**.** Seu mecanismo de ação se dá pelo entrecruzamento das cadeias de DNA e RNA e inibição da síntese de proteínas das células. É utilizada no tratamento de linfoma, leucemia, câncer de mama, na esclerose múltipla, no lúpus eritematoso sistêmico, além de ser um agente imunossupressor para transplante de órgãos (MOHAMMADI et al., 2014). No entanto, sua utilidade clínica é restrita devido a vários efeitos adversos associados à toxicidade reprodutiva, hepatotoxicidade, nefrotoxicidade, cardiotoxicidade, mielossupressão e imunossupressão em pacientes e modelos animais (EKELEME-EGEDIGWE et al., 2019; YOO et al., 2020). Estudos mostram que o estresse oxidativo tem um importante papel na toxicidade induzida pela CFF (FAHMY et al., 2016). Esse estresse oxidativo resulta em sérios danos celulares associados à peroxidação lipídica e alterações de proteínas e ácidos nucleicos (FAHMY et al., 2016).

Como é possível observar, os quimioterápicos causam danos às células e tecidos normais, apresentando sérios efeitos adversos. Por isso, a busca por compostos que possam preservar a eficácia do quimioterápico e atenuar seus efeitos adversos é o objetivo de muitos estudos, com grande destaque aos produtos naturais.

2.4.2 Produtos naturais como coadjuvantes na quimioterapia

Assim como os produtos naturais podem ter um papel fundamental na atenuação dos efeitos adversos desencadeados pela radioterapia, na quimioterapia eles também podem agir beneficamente. Muitos estudos têm mostrado a potencial atividade de plantas na redução dos efeitos adversos pelos quimioterápicos. Um dos fármacos quimioterápicos amplamente utilizado para a indução experimental de toxicidade é a CFF, acima citada.

Em 2016, no estudo realizado por Kumar & Venkatesh, foi demonstrada a propriedade imunoprotetora da lectina de cebola (*Allium cepa*) em ratos

imunossuprimidos com a CFF. A lectina foi capaz de restaurar a contagem de células linfoides e promover a resposta imune dos animais tratados. O potencial efeito antihepatotóxico dos polissacarídeos extraídos da *Mongifera indica* (fruto conhecido popularmente como manga) em ratos induzidos com a CFF foi demonstrado por Fahmy et al. (2016). Nesse estudo, o tratamento com os polissacarídeos restaurou as atividades das enzimas alanina aminotransferase e aspartato aminotransferase, importantes biomarcadores de danos hepáticos, além de reduzir os níveis de malondialdeído e aumentar a atividade das enzimas antioxidantes glutationa S-transferase e superóxido dismutase.

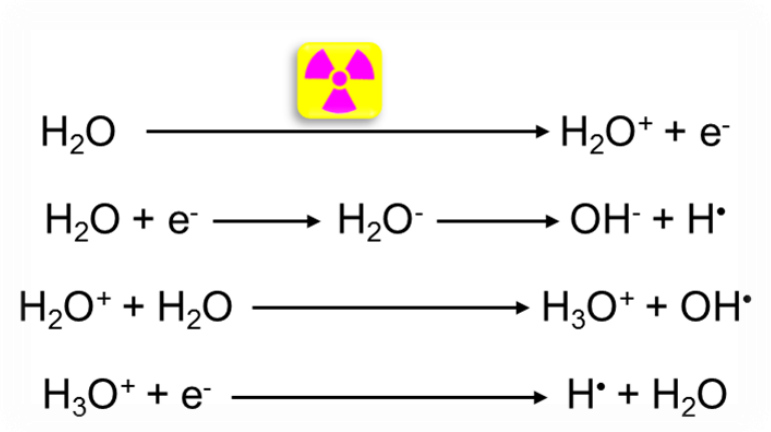
No estudo de Nafees et al. (2015) foi avaliado o efeito protetor da rutina (flavonoide que ocorre em muitos vegetais) na hepatotoxicidade induzida pela CFF. Nesse trabalho, os animais foram pré-tratados com a rutina e, então, a indução foi realizada. Os efeitos hepatoprotetores da rutina foram associados à regulação positiva das atividades das enzimas antioxidantes e à regulação negativa dos marcadores de toxicidade sérica. Liu et al. (2016) demonstraram a atividade nefroprotetora da paeoniflorina (*Paeonia lactiflora*) em camundongos induzidos com a CFF. O tratamento com a paeoniflorina diminuiu os níveis urinários de ácido úrico e creatinina, os níveis séricos e renais de citocinas, bem como atenuou as alterações histológicas dos tecidos renais causadas pela CFF.

Como já mencionado, os produtos naturais, por seus compostos com propriedades de grande interesse farmacológico, são importantes aliados no combate aos efeitos adversos da quimioterapia.

2.5 PROCESSO OXIDATIVO E SISTEMA ANTIOXIDANTE

A produção de radicais livres é um processo contínuo e fisiológico, que possui importantes funções biológicas, podendo ocorrer no citoplasma, na membrana plasmática, nas mitocôndrias, entre outros. Porém, quando em excesso, pode levar a sérios danos no sistema biológico. Um dos fatores que leva ao aumento da geração de espécies reativas de oxigênio (EROs) e outros radicais livres, é a exposição à RI, devido à radiólise da água (figura 1).

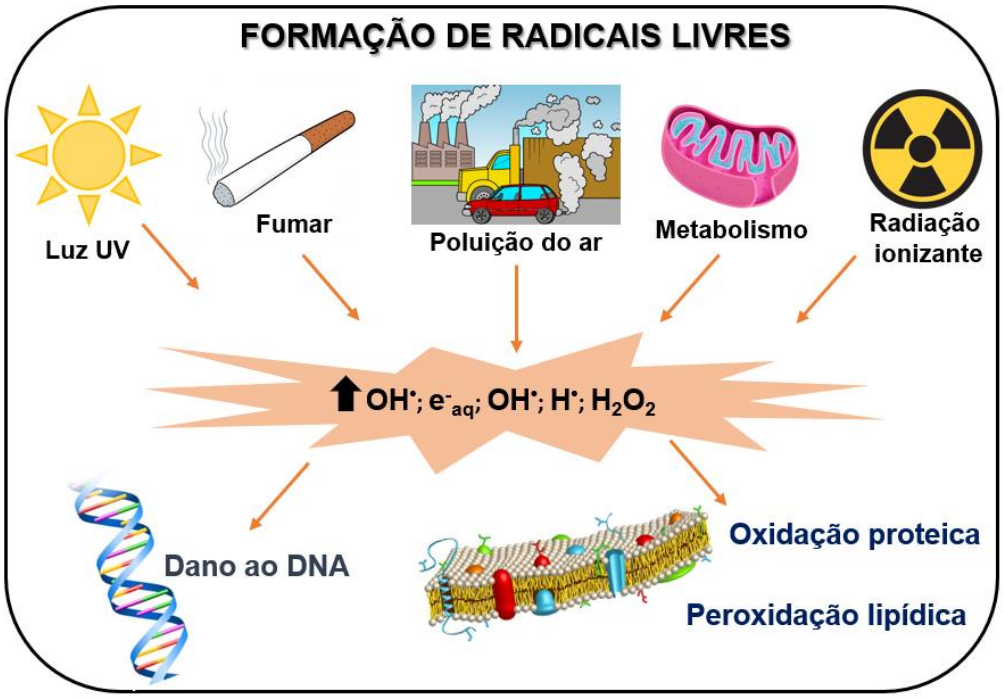
Figura 1 – Radiólise da água.



Fonte: Figura adaptada de ANDRADE & BAUERMANN, 2010.

Os principais produtos da radiólise da água são: elétrons livres hidratados (e^-_{aq}); radical hidroxil ($OH^•$); radical hidrogênio ($H^•$) e peróxido de hidrogênio (H_2O_2), sendo a vida média desses radicais extremamente curta (10^{-6} a 10^{-10} segundos) (10^{-6} a 10^{-10} segundos) (BITELLI, 2006; SHIRAZI et al., 2007; DARTNELL, 2011). Além disso, os metabólitos da CFF, a fosfaramida e a acroleína, também são responsáveis pela produção de radicais livres de oxigênio altamente reativos (OMOLE et al., 2018). A acroleína forma adutos com a lisina e reage com a glutationa, levando à geração de estresse oxidativo (IQUBAL et al., 2019). A ação das EROs no organismo pode causar danos ao DNA e também oxidar lipídios e proteínas (BARREIROS et al., 2006), sendo o OH• a espécie mais reativa por ser capaz de reagir com todas as biomoléculas produzindo derivados que não podem ser regenerados pelo metabolismo celular. O H_2O_2, por não ser um radical livre, possui vida longa e é capaz de atravessar as membranas celulares, apresentando-se potencialmente tóxico para as células (BARBOSA et al., 2010). A figura 2 representa alguns fatores que influenciam na geração excessiva de radicais livres.

Figura 2 – Fatores que influenciam na geração de radicais livres e seus danos biológicos.



Fonte: Autora.

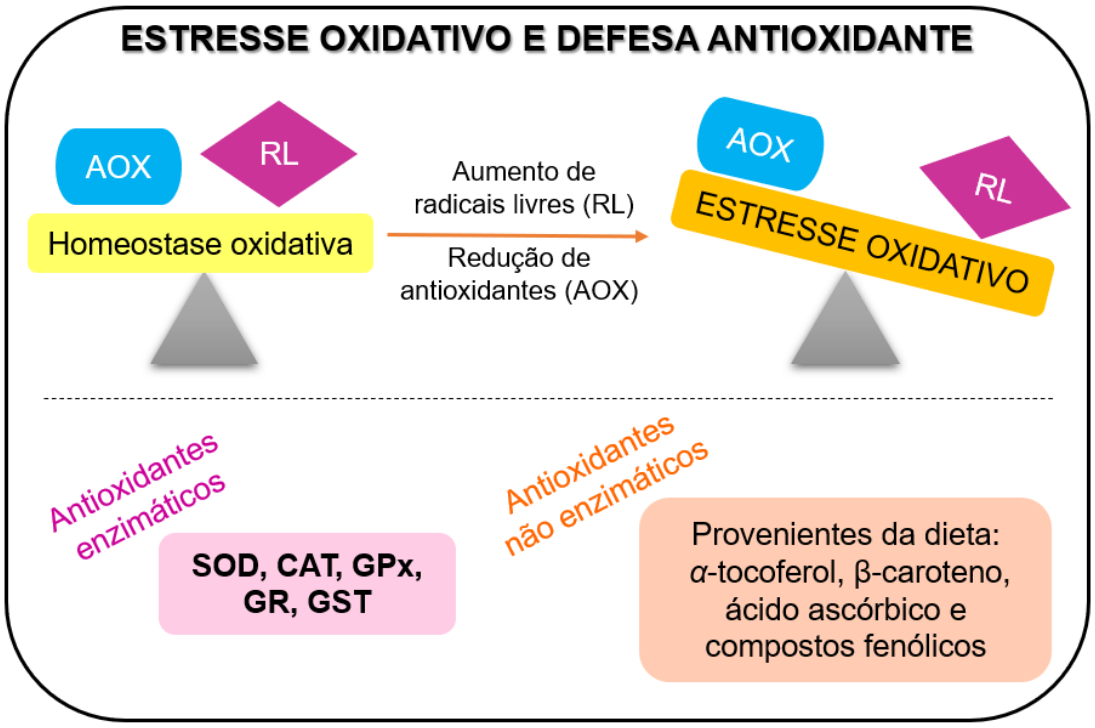
A fim de evitar os danos causados pelos radicais livres, o organismo desenvolveu mecanismos para neutralizar ou atenuar esses danos. Para se proteger contra a ação dos radicais livres, a célula pode atuar como detoxificadora do agente, impedindo a sua formação e, assim, o ataque a lipídios, proteínas e bases do DNA; ou com a função de reparar a lesão ocorrida, que está relacionado com a remoção dos danos da molécula de DNA e a reconstituição de membranas celulares (BIANCHI & ANTUNES, 1999). O sistema de defesa antioxidante do sistema biológico pode ser classificado em enzimático ou não-enzimático. O mecanismo antioxidante enzimático faz parte da proteção primária e intrínseca do organismo, nas quais as enzimas superóxido dismutase (SOD), catalase (CAT), glutationa peroxidase (GPx), glutationa redutase (GR) e glutationa S-transferase (GST) estão incluídas. A partir da ação destas enzimas, o organismo é capaz de manter a concentração das EROs dentro dos limites fisiológicos normais (RIBEIRO et al., 2005).

O sistema de defesa não-enzimático é constituído por substâncias antioxidantes que podem ter origem endógena ou dietética. Como exemplo de antioxidantes endógenos pode-se citar: glutationa reduzida, peptídeos de histidina e

proteínas ligadas ao ferro (transferrina e ferritina) (BARREIROS et al., 2006). Além desses, o organismo utiliza aqueles provenientes da dieta como o *α*-tocoferol (vitamina E), β-caroteno, ácido ascórbico (vitamina C) e compostos fenólicos, com destaque aos flavonoides e poliflavonoides (BARREIROS et al., 2006). Esses últimos compostos estão presentes naturalmente em frutas, vegetais, chás obtidos de algumas espécies vegetais e vinhos, sendo sua ação antioxidante já conhecida na literatura (DREOSTI, 2000; MANACH et al., 2004). Quando há um desequilíbrio entre compostos oxidantes e antioxidantes, a partir da geração excessiva de radicais livres ou em detrimento da velocidade de remoção destes, ocorre o estresse oxidativo (BARBOSA et al., 2010). A figura 3 representa o equilíbrio oxidativo e a formação do estresse oxidativo, assim como mostra os principais antioxidantes enzimáticos e não enzimáticos.

Por possuírem capacidade antioxidante, os compostos naturais são alvo de grande estudo sobre suas possíveis atividades biológicas, podendo ser eficaz contra os danos causados pela RI e pela quimioterapia.

Figura 3 – Geração de estresse oxidativo e principais antioxidantes enzimáticos e não enzimáticos.

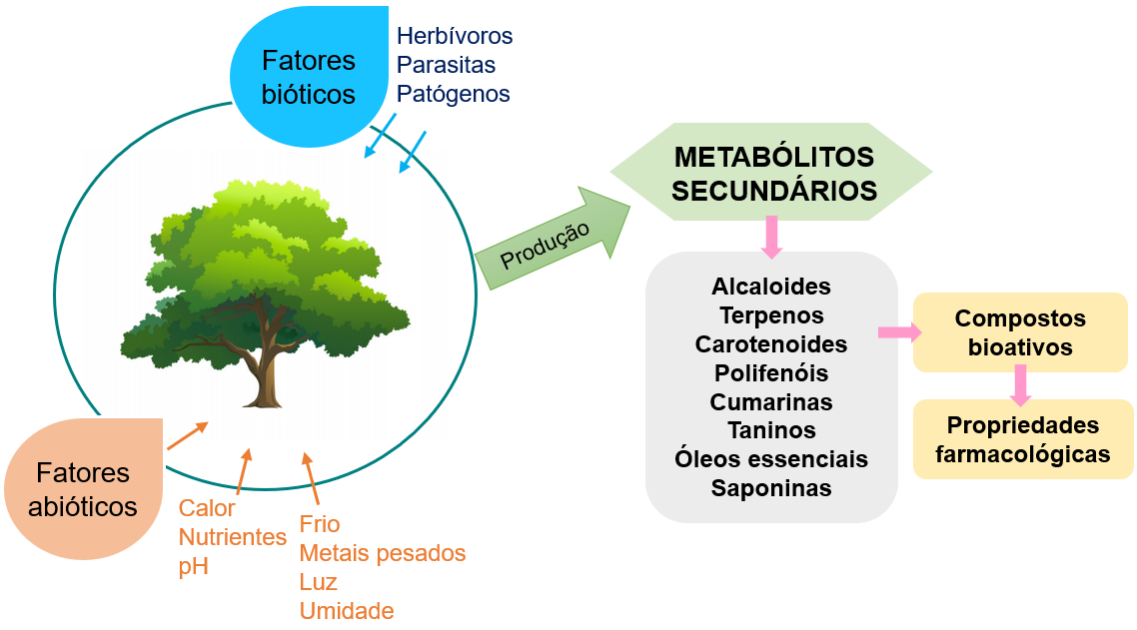


Fonte: Autora.

2.6 METABÓLITOS SECUNDÁRIOS DE PLANTAS

As plantas são conhecidas por produzirem metabólitos primários e secundários. Os primeiros diferenciam-se dos segundos por serem essenciais à vida da planta. Os metabólitos secundários são biossintetizados para diferentes finalidades, como a regulação do crescimento, interações intra e interespecíficas, proteção contra radiação UV (ultravioleta) e defesa contra predadores e infecções (VERPOORTE, 1998; WILLS et al., 2000; GOBBO-NETO & LOPES, 2007). São eles quem dão às plantas suas propriedades bioativas de interesse farmacológico, que atuam como potenciais derivativos de novas drogas. Dentre os principais compostos derivados desse metabolismo estão: polifenóis, alcaloides, cumarinas, antraquinonas, taninos, óleos essenciais e saponinas (SANTOS et al., 2010). Ainda, os carotenoides, também presentes em plantas, apresentam importante papel na diminuição do risco de doenças crônicas, devido às suas atividades biológicas. A figura 4 mostra alguns fatores bióticos e abióticos que influenciam na produção de metabólitos secundários pelas plantas.

Figura 4 – Fatores que influenciam na produção de metabólitos secundários pelos vegetais.



Fonte: Autora.

Os compostos fenólicos são produtos do metabolismo secundário e exercem funções fisiológicas essenciais (SOARES, 2002). Suas propriedades antioxidante, anti-inflamatória e antimicrobiana os tornam um importante potencial terapêutico, sendo os fenólicos de maior importância os flavonoides, as antocianinas e os taninos (MIDDLETON et al., 2000). Os flavonoides são compostos polifenólicos amplamente encontrados no reino vegetal e seu consumo está associado a frutas, vegetais e cereais integrais. Possuem importantes efeitos biológicos como anti-inflamatórios (GOMES et al., 2008), antioxidantes (BUTTERFIELD et al., 2002; LIU et al., 2008), sequestro de radicais livres (GALVANO et al., 2007), ação antiviral e antibacteriana (HAVSTEEN, 2002), inibição da agregação plaquetária (CURIN et al., 2006) e promoção da saúde cardiovascular (CHEN et al., 2008; LIN et al., 2008).

Os carotenoides são pigmentos lipossolúveis de coloração amarela a vermelha, encontrados no reino vegetal, animal e em microrganismos, podendo ser classificados em carotenos e xantofilas. Os carotenos correspondem àqueles que são formados apenas por hidrocarbonetos, ou seja, possuem apenas carbono e hidrogênio em sua estrutura, como o β-caroteno. Já as xantofilas, agregam os compostos que possuem oxigênio na molécula, como a β-criptoxantina (RIBEIRO, 2011).

Além disso, os carotenoides estão presentes nos óleos de frutas oleaginosas e são conhecidos pela sua propriedade provitamínica A, nutriente importante para o desenvolvimento dos tecidos epiteliais e para o funcionamento do ciclo de regeneração visual dos fotorreceptores (OLSON, 1989; BURRI, 1997; AMBRÓSIO et al., 2006). Além disso, podem apresentar atividades biológicas como antioxidante, fotoprotetora e anticâncer (CARDOSO, 1997; GOMES, 2007). Carotenoides também reduzem os riscos de deleção genômica ao prevenir danos celulares e diminuir os níveis de espécies reativas de oxigênio no meio intracelular (MALDONADO-ROBLEDO et al., 2003). Assim, por apresentarem importantes compostos bioativos, novos estudos que validem a eficácia e segurança do uso desses produtos naturais são de extrema necessidade. Nesse sentido, os ensaios toxicológicos desempenham um papel essencial na avaliação de segurança de uma dada substância.

2.7 ESTUDOS TOXICOLÓGICOS

O uso inadequado de plantas medicinais e seus derivados pela população pode desencadear uma série de efeitos adversos à saúde, muito embora o usuário acredite

que, por ser um produto natural, não seja capaz de ser nocivo (FISHER et al. 2000; CARRASCO et al*.,* 2009). Por isso, os testes de avaliação toxicológica e segurança do uso de extratos e derivados vegetais não farmacêuticos, que são comercializados livremente, são essenciais para que seu uso seja seguro pela população (DOYAMA et al*.,* 2005; CARRASCO et al*.,* 2009).

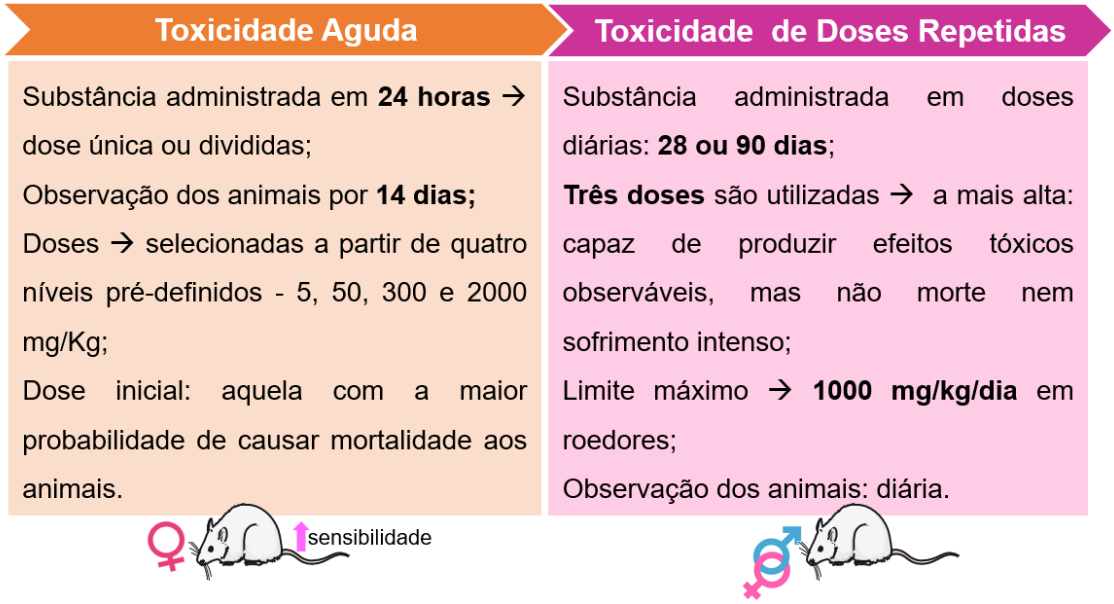
A toxicologia é a ciência que estuda os efeitos nocivos provocados por um agente tóxico aos sistemas biológicos. Através dos testes de toxicidade são avaliadas as alterações que ocorrem no organismo vivo quando expostos a uma determinada substância em condições adequadas. Essas alterações podem ser reversíveis ou não, com efeito molecular, celular ou tecidual e até causar a morte do indivíduo (TAHRAOUI, 2010; VALADARES, 2006). No presente, existem protocolos para a realização dos ensaios toxicológicos aceitos pela comunidade científica. Dentre esses, podemos citar a *Organisation for Economic Cooperation and Development* (OECD) e a *Food and Drug Administration* (FDA), que são guias mundialmente aceitos. No Brasil, a Agência Nacional de Vigilância Sanitária (ANVISA) é responsável pela regulamentação desses testes (BRASIL, 2013; TUROLLA & NASCIMENTO, 2006).

Para os ensaios toxicológicos *in vivo*, a espécie de animal mais indicada é o rato, embora outras espécies de roedores também possam ser utilizadas. Visto que o potencial tóxico de uma dada substância depende da dose, da via de administração e do tempo de exposição existem diferentes estudos para avaliação da toxicidade. Um deles é o estudo da toxicidade aguda, que tem por finalidade analisar os efeitos provocados de uma substância administrada em uma ou mais doses durante um período não superior a 24 horas, seguido de observação dos animais por 14 dias após a administração (ANVISA, 2013). As doses a serem utilizadas nesse teste são selecionadas a partir de quatro níveis pré-definidos (5, 50, 300 e 2000 mg/kg), sendo que a dose inicial deve ser aquela que tem a maior probabilidade de causar mortalidade aos animais (OECD, 2001). Para esse teste é recomendado, preferencialmente, o uso de fêmeas, pois apresentam maior sensibilidade que os machos aos efeitos das substâncias.

Já o estudo de toxicidade de doses repetidas tem como objetivo definir o perfil toxicológico da substância através da administração de doses diárias, podendo ocorrer por um período de 28 ou 90 dias (ANVISA, 2013; OECD, 2008). Nesse teste, três doses são utilizadas, sendo que a mais alta deve ser escolhida baseada na

capacidade da substância produzir efeitos tóxicos observáveis, mas não morte nem sofrimento intenso, não excedendo o limite máximo de 1000 mg/kg/dia em roedores (ANVISA, 2013). A observação dos animais é diária e os parâmetros analisados incluem: consumo de água e ração, alterações na massa corporal, anormalidades motoras, comportamentais, morbidade e mortalidade, modificações bioquímicas, hematológicas, análises macroscópica e microscópica dos órgãos (OECD, 2008). A figura 5 resume os principais tópicos propostos para a realização dos testes de toxicidade aguda e de doses repetidas.

Figura 5 – Recomendações para a realização dos ensaios de toxicidade aguda e de doses repetidas.



Fonte: Autora – baseado nas diretrizes da OECD (2001; 2008) e ANVISA (2013).

Dessa maneira, o teste de toxicidade aguda é a fase inicial para investigação do potencial tóxico de uma substância e, a partir dele, é possível sugerir as doses dos testes de toxicidade subsequentes. Juntos, fornecem informações relevantes sobre os riscos que dada substância pode causar à saúde animal e humana. Além disso, são essenciais para a determinação de doses seguras que possam ser utilizadas em estudos adicionais acerca das potenciais atividades biológicas de plantas e seus extratos, por exemplo. Dentre as espécies com importantes atividades benéficas ao

organismo e com poucos estudos sobre sua toxicidade em modelos *in vivo*, está o tucumã (*Astrocaryum aculeatum*), pertencente à família Arecaceae.

2.8 FAMÍLIA ARECACEAE

As palmeiras, pertencentes à família Arecaceae, estão entre as espécies de maior longevidade no reino vegetal e as plantas vasculares mais abundantes nos trópicos (HENDERSON et al., 2000); são plantas monocotiledôneas, geralmente arborescentes e terrestres. A família Arecaceae é constituída por 252 gêneros e aproximadamente 2600 espécies (DRANSFIELD et al., 2008). No Brasil, encontram-se 37 gêneros e 282 espécies, sendo 122 endêmicas (LEITMAN et al., 2015) e, para a região Amazônica, são reconhecidos 35 gêneros e 150 espécies (HENDERSON, 1995). As palmeiras possuem importante diversidade morfológica, apresentando como sinapomorfias a presença de corpos de sílica em forma de chapéu, o fruto indeiscente e o endosperma com hemicelulose (DRANSFIELD et al., 2008). Hábito, tipo de folha, inflorescência, flor e fruto são as características que apresentam grande peso no reconhecimento dos diversos táxons.

As folhas são dispostas em espiral, geralmente pinadas, por vezes flabeladas. O caule é o estipe, solitário ou cespitoso e as raízes são fasciculadas, podendo apresentar raízes acima do nível do solo, auxiliando na fixação e complementando o sistema radicular (LORENZI et al., 1996). A inflorescência é do tipo espádice, com flores geralmente de pequeno tamanho, atraindo grande número de polinizadores por estarem agrupadas em grande quantidade nas inflorescências (FAVRETO, 2010). A inflorescência é envolta por uma grande bráctea que a protege até o seu desenvolvimento. O fruto é do tipo drupa, geralmente fibroso, raramente como baga, e possui endosperma com óleos ou carboidratos, que em algumas plantas pode ser ruminado (JUDD et al., 2009).

2.8.1 Espécie *Astrocaryum aculeatum* (tucumã)

A Amazônia é conhecida por sua rica biodiversidade, com inúmeras plantas de grande potencial econômico e nutricional. Dentre estas, a espécie *Astrocaryum aculeatum*, popularmente conhecida como tucumã ou tucumã-do-amazonas, é uma palmeira encontrada principalmente no estado do Amazonas, mas também no Pará,

Roraima, Rondônia, Acre, Peru e na Colômbia (CAVALCANTE, 1991). Essa espécie apresenta crescimento monopodial, arborescente, estipe ereto e solitário, e monoico (CAVALCANTE, 1996). Sua altura é de 8 a 20 m, podendo alcançar 25 m (figura 6) (LEITÃO, 2008). Os frutos são produzidos durante o ano todo, sendo o pico da produção nos meses de junho a janeiro. Sua frutificação é tardia, quando a planta já tem ao menos 7 anos e altura entre 6 e 9 metros, com produção de cerca de 50 kg de frutos por ano (SHANLEY & MEDINA, 2005). As palmeiras estão frequentemente associadas a ambientes degradados e de vegetação secundária (capoeiras), savanas, pastagens e roçados, sendo excepcionalmente tolerantes a solos pobres e degradados (FOOD AND AGRICULTURE ORGANIZATION, 1987).

Figura 6 – População de tucumã (*Astrocaryum aculeatum*).

Fonte: Figura adaptada do Instituto Nacional de Pesquisas da Amazônia.

Os frutos normalmente são elipsoides e alaranjados quando maduros, com a casca representando 17,2% do seu peso e possuindo cor verde-amarelada. A polpa tem cor que varia do amarelo ao alaranjado, com peso variando de 20 a 100 g, sendo 22,4% de porção comestível (AGUIAR et al., 1980; FERRÃO, 1999; LIMA et al., 1986) e sua consistência é pastoso-oleosa com característica fibrosa (FERREIRA et al., 2008) (figura 7). A extração de óleo da polpa e das amêndoas pode ser utilizada como insumo para produção de biocombustível (BARBOSA et al., 2009; LIRA, 2012) ou para uso na alimentação humana e na indústria de cosméticos, sendo que o endocarpo é

utilizado na fabricação de artesanatos (KAHN & MOUSSA, 1999; RAMOS et al., 2011; LOPES et al., 2012).

Figura 7 – Aspecto geral dos frutos da espécie *Astrocaryum aculeatum* (tucumã).

Fonte: Figura adaptada de <http://www.palmpedia.net/wiki/Astrocaryum_aculeatum> Acesso em: 22 abr. 2020.

A polpa do fruto é consumida ao natural, utilizada na elaboração de vinhos, produção de sorvetes, cremes e patês, e ainda como recheio de sanduíche e em tapioca, sendo estes muito apreciados nos “cafés regionais” (LEITÃO, 2008).

Na polpa do tucumã já foram encontrados carotenoides (62,65 ug/g de polpa fresca), sendo 21 formas diferentes, nas quais o *trans*-β-caroteno representa 75%; também flavonoides como catequina e quercetina, além de ácido ascórbico (58±4 mg/100g) (DE ROSSO & MERCADANTE, 2007; GONÇALVES, 2008). Assim, o tucumã é uma fonte notável de carotenoides com atividades de pró-vitamina A, apresentando β-caroteno (92,0 µg/g), α-caroteno (2,5 µg/g), γ-caroteno (2,1 µg/g), sendo que 100g de sua polpa pode contribuir com aproximadamente 153,4% das necessidades diárias de vitamina A (1000 µg/RE) para um homem adulto (LEITÃO, 2008). Por ser uma importante fonte de carotenoides, pode ser utilizada como alternativa contra a hipovitaminose A (AMBRÓSIO et al., 2006).

Além disso, apresenta alto valor nutricional, principalmente devido ao seu alto teor em lipídios e carboidratos, sendo fonte de calorias e fibras (FERREIRA et al., 2008). Na composição química do fruto do tucumã encontra-se, em média, 46% de

umidade, 30% de lipídios, 9% de fibras, 5% de proteínas e 3% em minerais (SILVA, 2012). O valor nutricional estimado é de 247 kcal/100g de tucumã. Constituintes bioativos como as vitaminas, os carotenoides e os compostos fenólicos, presentes nas frutas, são associados à prevenção de patologias crônicas como o câncer, doenças cardiovasculares, diabetes tipo II, *Alzheimer,* entre outras (CARTER et al., 2010; LIU, 2013; WANG et al., 2011; WANG et al., 2014).

Alguns autores já evidenciaram diferentes atividades biológicas apresentadas pela espécie *A. aculeatum*. Em estudo realizado por Jobim et al. (2014), foi demonstrado que os extratos da polpa e da casca do tucumã apresentaram significante atividade antibacteriana contra três bactérias Gram-positivas e atividade antifúngica contra *Candida albicans*. Sagrillo et al. (2015) também realizaram estudo utilizando os extratos da polpa e da casca do tucumã *in vitro*. Nesse estudo, foi verificada a atividade citoprotetora dos extratos em culturas de linfócitos expostas ao H_2O_2, nas quais os extratos foram capazes de aumentar a viabilidade celular através da modulação de apoptose. O estudo *in vivo* de Carneiro et al. (2017) evidenciou a atividade antigenotóxica do óleo de tucumã através do teste do micronúcleo em células do sangue periférico de camundongos.

Até o momento, poucos estudos *in vivo* sobre a toxicidade e os potenciais efeitos biológicos do tucumã foram realizados, assim, novos estudos sobre o fruto são de extrema importância para confirmar sua segurança e eficácia.

3 MATERIAIS E MÉTODOS E RESULTADOS

Os itens *materiais e métodos* e *resultados* da presente tese estão apresentados sob a forma de artigos científicos. A apresentação dos artigos está baseada nas versões submetidas a cada revista científica.

4 ARTIGO 1 – TUCUMÃ (*Astrocaryum aculeatum*) EXERT RADIOPROTECTIVE EFFECTS ON HUMAN KERATINOCYTES AND FIBROBLASTS CELLS EXPOSED TO IONIZING RADIATION

DELINEAMENTO EXPERIMENTAL: Para este estudo, foram utilizadas linhagens celulares de fibroblastos (HFF-1) e queratinócitos (HaCaT) humanas. As células foram mantidas em garrafas de cultivo celular em meio DMEM por 24 h em estufa (37ºC / CO_2 5%). Decorrido esse tempo, as células foram tratadas com o extrato de tucumã (casca + polpa) na concentração de 100 µg/mL e mantidas em estufa por mais 24 h. Após, foram expostas à radiação ionizante no setor de Radioterapia do Hospital Universitário de Santa Maria (HUSM), sob responsabilidade do Físico Médico Tadeu Baumhardt. A dose de radiação utilizada foi de 2 Gy e o aparelho utilizado foi o Acelerador Linear Elekta (Elekta®, Estocolmo, Suécia), que emite raios-X. Para simular a densidade do tecido humano e assegurar que a dose de radiação fosse igualmente distribuída em todas as células (não pode existir ar nesse processo), a solução balanceada de Hanks foi adicionada às garrafas. Além disso, as garrafas foram vedadas com Parafilm M® e imersas em água em uma caixa de acrílico para receber a radiação. Esses processos também foram realizados com as garrafas do grupo controle não irradiado (0 Gy) para reproduzir o mesmo estresse em todas as células. Após esse processo, as garrafas foram mantidas em estufa 24h e, então, as seguintes análises foram realizadas: DCFH-DA (diclorofluoresceína diacetato), óxido nítrico, mortalidade celular (PicoGreen®), TBARS (substâncias reativas ao ácido tiobarbitúrico) e carbonilação de proteínas. Através de kits comerciais foram analisados os níveis de 8-OHdG (8-hidroxi-2'-deoxiguanosina), dos fatores de crescimento de fibroblastos e queratinócitos, além dos marcadores apoptóticos *Bax* e *Bcl-2*. Esse estudo foi aprovado pela Gerência de Estudo e Pesquisa (GEP) do HUSM sob o número 050/2017. O artigo a seguir traz o trabalho na íntegra e foi submetido à *revista Physica Medica*.

Tucumã (*Astrocaryum aculeatum*) exert radioprotective effects on human keratinocytes and fibroblasts cells exposed to ionizing radiation

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Abstract

Purpose: Tucumã (*Astrocaryum aculeatum*) is a fruit native from Brazil with biological activities, including antioxidant, which is important for the searching of new radioprotectors. Therefore, the aim of the study was to evaluate the potential radioprotector effect of the tucumã crude extract (TCE) *in vitro* on keratinocytes and fibroblasts cells exposed to ionizing radiation. **Methods*:*** For the investigation of *in vitro* radioprotective effects of TCE, keratinocytes and fibroblasts were treated with the extract (100 µg/mL) 24 h prior to exposition to radiation (2 Gy). After the exposition, analyses about citogenetic and oxidative damage were performed. **Results:** After exposition to radiation, the lipoperoxidation, ROS production and carbonyl protein was increased in both kerotinocytes and fibroblasts cells. Pretreatment with TCE was able to attenuate these increased levels. Besides, the biomarker of DNA damage 8-OHdG had its level increased after irradiaton and the treatment with TCE was able do reduce it. The apoptotic markers *Bax* and *Bcl-2* also were altered by irradiation, and TCE pretreatment restaured its levels. Pretreatment with TCE was able to attenuate the levels of oxidative markers and genotoxicity induced by radiation. **Conclusions:** Our results shows that tucumã extract may present a potential radioprotector effect in keratinocytes and fibroblasts cells.

Keywords: Arecaceae. Radioprotector. Crude extract. Oxidative damage. 8-OhdG.

1 Introduction

Humans are often exposed to radiation, whether natural or artificial. Many people have been submitted to ionizing radiation in radiotherapy sessions, which it is effective in treating malignancies such as cancer. The therapeutic purpose of radiotherapy is to target tumor cells by destroying and/or preventing their growth, preserving the integrity of normal tissues [1]. However, in patients undergoing radiotherapy it is frequently associated the development of myelosuppression, thrombocytopenia and hemorrhage [2]. In addition, radiation exposure may induce changes in cell macromolecules, affecting their functions [3]. Although the beneficial effects, the toxicity of radiation in normal tissues remains a major problem, hence the search for agents that can reduce these adverse effects. These agents, known as radioprotectors, may act by scavenging the free radicals formed during the water radiolysis [4], thus preventing DNA damage [5] and should not have cumulative or irreversible toxicity and should be easily administered [6].

Natural products have gained attention for their important biological activities. In addition, they present low toxicity in relation to the synthetic radioprotectors [7] and have better routes of administration [8]. The use of natural compounds such as lycopene [9], polyphenols [10] like curcumin, resveratrol [8] and rutin [11], among others, and also the extract of the species *Dracaena cochinchinensis* ("Dragon's blood") have already been analyzed regarding its possible radioprotective activity [2].

In this scenario, tucumã *(Astrocaryum aculeatum)* is a plant belonging to the Arecaceae family, distributed in the central region of the Amazon basin from south to north. In Brazil, tucumã grows in Acre, Amazonas, Pará, Rondônia and Roraima [12]. It is a palm tree reaching up to 25 meters in height, with fruits produced all year round. Flavonoids like catechin and quercetin have already been identified in its pulp, besides it presents important nutritional properties including vitamin B2 and fibers [13]. De Rosso & Mercadante (2007) [14] showed the presence of 21 different forms of carotenoids in tucumã fruit, including trans-β-carotene, the percursor of provitamin A.

Bioactive constituents like vitamins, carotenoids and phenolic compounds are associated with the prevention of chronic pathologies such as cancer, cardiovascular diseases, type II diabetes, Alzheimer's disease, among others [15,16]. Moreover, its antioxidant property has already been described in study by Sagrillo *et al.* (2015) [12], in which the tucumã extract had a strong antioxidant activity, an important action against radiation induced damages. Previous investigations also showed

cytoprotective and genoprotective effect of tucumã on lymphocyte cultures exposed to H_2O_2 using spectrophotometric, fluorimetric and immunoassays [12]. Therefore, we postulated here that tucumã extract could have an antioxidant and genoprotective effect in keratinocytes and fibroblasts exposed to ionizing radiation.

2 Materials and methods

2.1 Obtention of tucumã crude extract

Tucumã fruit was obtained from a composite sample representing a mixture of progenies from a native forest near Manaus City (Amazonas State, Brazil), located in the Amazonian region (3.08ºS, 60.01ºW). The ethanolic extract was performed from the tucumã fruit (peel and pulp) according to Sagrillo *et al.* (2015) [12]. Briefly, the fruits were crushed and placed separately into sealed amber glass jars containing an absolute ethanol solution at a ratio of 1:5 (w/v). The extraction was performed over four days at room temperature. The homogenate was filtered and the ethanol was removed using a rotary evaporator at reduced pressure; the fruit extract was lyophilized to obtain the tucumã crude extract (TCE).

2.2 Cell culture

The HFF-1 human fibroblast cell line was cultured in Dulbecco's Modified Eagle Media (DMEM) with 10% fetal bovine serum (FBS), while the HaCaT human keratinocyte cell line was cultured in DMEM with 15% FBS, both supplemented with 1% penicillin/streptomycin and amphotericin B. The cells were cultured at 37 °C with 5% CO_2 and were expanded by obtaining the optimal amount for the experiments. Cell suspension was placed in standard 25 cm^2 culture flask (2.5 x 10^5 cells). Further, the cells were incubated at 37 °C in humidified atmosphere with 5% CO_2 for 24 hours in order to produce the assays.

2.3 Treatment of the cells

Twenty four hours prior to irradiation, the TCE was added to the normal cells at concentration of 100 µg/mL. In the study performed by Sagrillo *et al.* (2015) was observed that this concentration did not have any toxic effect to the cells. TCE was dissolved in DMEM with 10% or 15% FBS according to the cell type and filtered for sterilization. The control groups were treated only with the DMEM media.

2.4 In vitro irradiation

Irradiation was performed using an Elekta linear accelerator, Precise System model (Elekta®, Stockholm, Sweden), generating 6-MV photon radiation, beam rates of 600 MU/min (equivalent dose 2 Gy at standard measured conditions: SAD = 100 cm). The area of the irradiation field was 35 cm × 35 cm, SSD (Source Skin Distance) = 97cm and, to homogenize it, each dose of irradiation was divided into two fields (Gantry at 0° and 180°). The dose of 2 Gy was based on the common fractionation dose for radiotherapy. The samples were kept at 37ºC for 1 h soon after irradiation to facilitate the repair (right or wrong repair). Non-irradiated control groups (0 Gy) were treated at equal conditions as irradiated groups. The irradiation procedure was performed by the physicist responsible in the radiotherapy sector of the Universitary Hospital of Santa Maria and the study was approved by the "*Gerência de Ensino e Pesquisa*" of the hospital under the no. 050/2017.

2.5 2'-7'-dichlorofluorescein diacetate (DCFDA) ROS production assay

Twenty four hours of the irradiation, the effect of TCE treatment on the oxidative metabolism of the cells was analyzed. The ROS level was determined using the non-fluorescent cell permeating compound 2'-7'-dichlorofluorescein diacetate (DCFDA) assay, where the DCFDA is hydrolyzed by intracellular esterases to DCFH, which is trapped within the cell. This non-fluorescent molecule is then oxidized to fluorescent dichlorofluorescein (DCF) by cellular oxidants. After the designated treatment time, the cells were treated with DCFDA (10 mM) for 60 minutes at 37ºC. In the assay, $1x10^5$ cells from each sample were used to measure ROS levels [17]. The fluorescence was measured at an excitation of 488 nm and an emission of 525 nm. DCF production is proportional to ROS molecules present in the samples.

2.6 Lipoperoxidation assay

Lipoperoxidation (LPO) estimation was performed using TBARS (thiobarbituric acid reactive substances) method, which the colorimetric reaction of the malondialdehyde (MDA), a product of LPO, with thiobarbituric acid (TBA), is quantified. This reaction produces a colored compound that absorbs maximally at 532 nm as described by Jentzsch *et al.* (1996) [18]. TBARS levels were measured using a standard curve of MDA and the values are expressed in nmol MDA/mg of protein.

2.7 Spectrophotometric assay of protein carbonyl

The spectrophotometric assay of protein carbonyls in cells supernatants was assayed according to Reznick & Packer (1994) [19] with slight modifications. The total protein-bound carbonyl content was determined by derivatizing the protein carbonyl adducts with DNPH, which forms a stable dinitrophenyl hydrazone product that is then monitored spectrophotometrically at 370 nm with a multimode microplate reader.

Diluted samples (500 µL) were mixed with 100 µL of DNPH solution (10 mM in 2.5 M HCl) for 1 h at 37 ºC in the dark while mixing every 15 min. Following reaction, proteins were precipitated with 250 µl of denaturation buffer (SDS 3%), 1000 µL of etanol and 1000 µL of hexane. The mixture was centrifuged at 3000 rpm for 15 min to obtain a pellet, and the supernatant was removed. The pellet was dissolved in 500 µl of denaturation buffer (SDS 3%) and taken to the dry bath at 45 ºC for 20 min. The concentration of protein carbonyls was expressed as nanomoles of carbonyl moieties per milligram of protein (nmol/mg protein).

2.8 Nitric oxide (NO) assay

Twenty four hours post irradiation, NO in the culture medium was examined as an indicator of NO production by Griess reagent (Sigma-Aldrich, St. Louis, MO, USA). The Griess reagent (1% sulfanilamide, 0.1% N-1-napthylethylenediamine dihydrochloride in 5% phosphoric acid) was mixed with culture medium at 1:1 ratio and incubated at room temperature for 15 min. The absorbance at 540 nm was measured with ELISA reader [20].

2.9 Cell mortality by PicoGreen® fluorometric assay

Cell mortality was evaluated by quantification of double-strand DNA (dsDNA) levels present in supernatant using a protocol previously described by Batel *et al.* (1999) [21]. The method is based on the ability of the specific fluorochrome dye (PicoGreen®) to make a very stable complex with dsDNA in alkaline conditions instead of ssDNA, proteins, SDS and urea. This selectivity characteristic used to follow DNA denaturation with decreasing fluorimetric signal intensity proportionate to increasing ssDNA and mononucleotide content. Initially we evaluated the potential toxic effect on DNA denaturation 24h after exposed the cells to radiation.

The fluorescence was measured at an excitation of 485 nm, and an emission of 520 nm was recorded at room temperature (SpectraMax M2/M2e Multi-mode Plate

Reader, Molecular Devices Corporation, Sunnyvale, CA, USA). Expression of results to DNA strand break calculations were made in relation to control values, blank values were measured in wells containing only the samples. Results were expressed as a percentage of dsDNA (dsDNA, ng/mL).

2.10 Immunoassays

Cell culture supernatants were immediately separated by centrifugation at 3000 rpm for 10 min and stored at −80°C for further analysis. To quantify DNA damage by 8-OHdG (8-hydroxy-2′-deoxyguanosine) and other cell proliferation markers: KGF (keratinocyte growth fator) and FGF (fibroblast growth fator) was used commercial immunoassay kits (Quantikine Human Kits - R&D Systems, Minneapolis, USA). Apoptotic markers (*Bax* and *Bcl-2*) were also quantified using kits from Cayman Chemical Company, Ann Arbor, USA. All analyses were performed at the same laboratory follow manufactures instructions.

2.11 Statistical analysis

The results were analyzed using one-way analysis of variance (ANOVA), followed by Tukey *post-hoc* test (*GraphPad Prism Software, Inc., version 6*). Data are expressed as mean + S.D. and the differences between groups were considered to be statistically significant when $p < 0.05$.

3 Results

3.1 Effects of tucumã crude extract on ROS production

The dose of 2 Gy was not able to increase significantly the ROS production in keratinocyte cells, although we did observed a slight rise when compared to the control group. The pretreatment with TCE was able to reduce the ROS level in comparison to the group that was only exposed to radiation and TCE group (figure 1). In fibroblast cells, the ROS levels decreased in both groups exposed to IR when compared to the TCE group (figure 2).

3.2 Effects of tucumã crude extract on lipoperoxidation

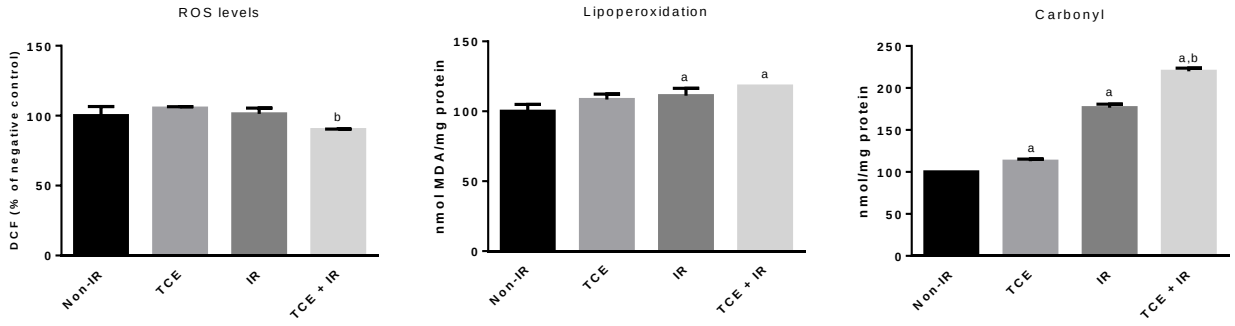
MDA had its levels increased significantly in the groups exposed to radiation both in fibroblast and keratinocyte cells. Treatment with TCE was not able to reduce

the levels significantly, although was observed that in fibroblast cells an attenuation occurred when compared to the IR group. However, in fibroblasts cells no differences were found between the groups (figures 1 and 2).

3.3 Effects of tucumã crude extract on protein carbonyl

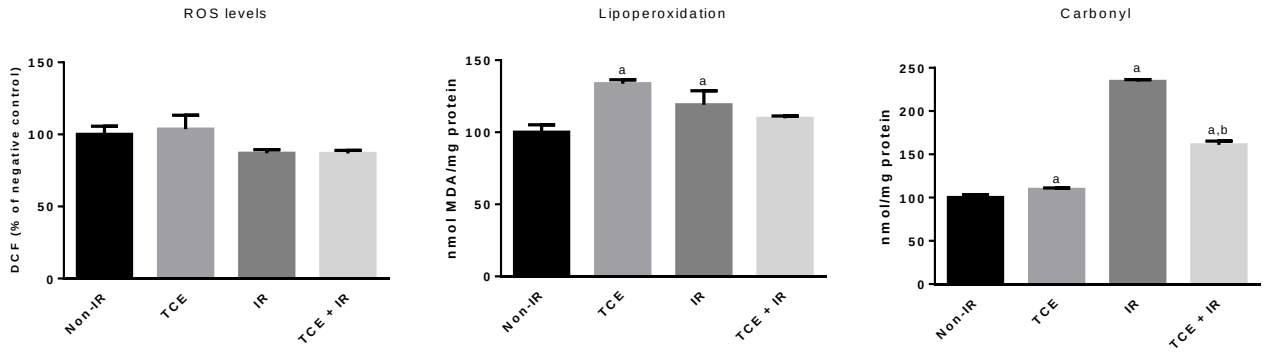
The radiation (2 Gy) induce the increase of protein carbonylation in both keratinocyte and fibroblast cells when compared to the control group. In fibroblast cells, the treatment with TCE was able to reduce its levels significantly in comparison to the irradiated group (figures 1 and 2).

Fig. 1. Effects of pretreatment with tucumã crude extract (24h) followed by exposure to radiation (2 Gy) on ROS production, lipoperoxidation and protein carbonylation in keratinocytes cells.



One-way ANOVA followed by Tukey *post hoc*. Columns represents the mean + S.D. [a]Significantly different from non-IR group; [b]Significantly different from IR group: $p < 0.05$.

Fig. 2. Effects of pretreatment with tucumã crude extract (24h) followed by exposure to radiation (2 Gy) on ROS production, lipoperoxidation and protein carbonylation in fibroblasts cells.

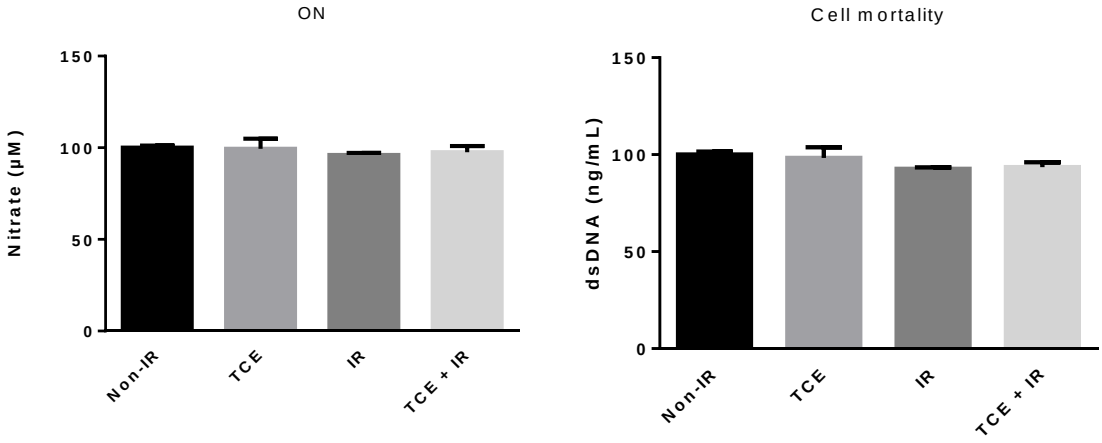


One-way ANOVA followed by Tukey *post hoc*. Columns represents the mean + S.D. [a]Significantly different from non-IR group; [b]Significantly different from IR group: $p < 0.05$.

3.4 Effects of tucumã crude extract on nitric oxide production and cell mortality

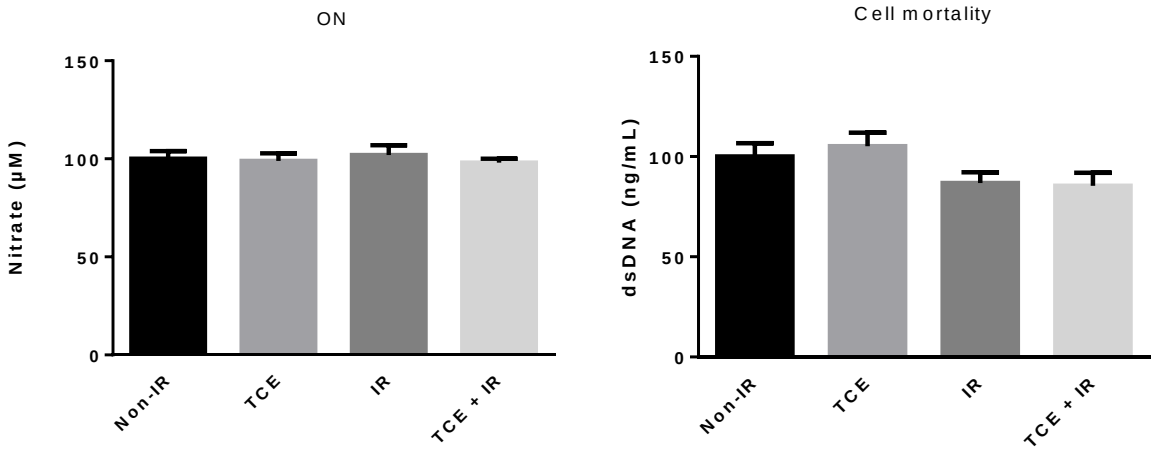
Nitric oxide production did not presented alterations between the groups analyzed both in keratinocytes and fibroblasts cells. Also, no differences were found in DNA double-strand denaturation in both cell types (figures 3 and 4).

Fig. 3. Effects of pretreatment with tucumã crude extract (24h) followed by exposure to radiation (2 Gy) on nitric oxide production and DNA double-strand denaturation in keratinocytes cells.



One-way ANOVA followed by Tukey *post hoc*. Columns represents the mean + S.D. No differences were observed.

Fig. 4. Effects of pretreatment with tucumã crude extract (24h) followed by exposure to radiation (2 Gy) on nitric oxide production and DNA double-strand denaturation in fibroblasts cells.

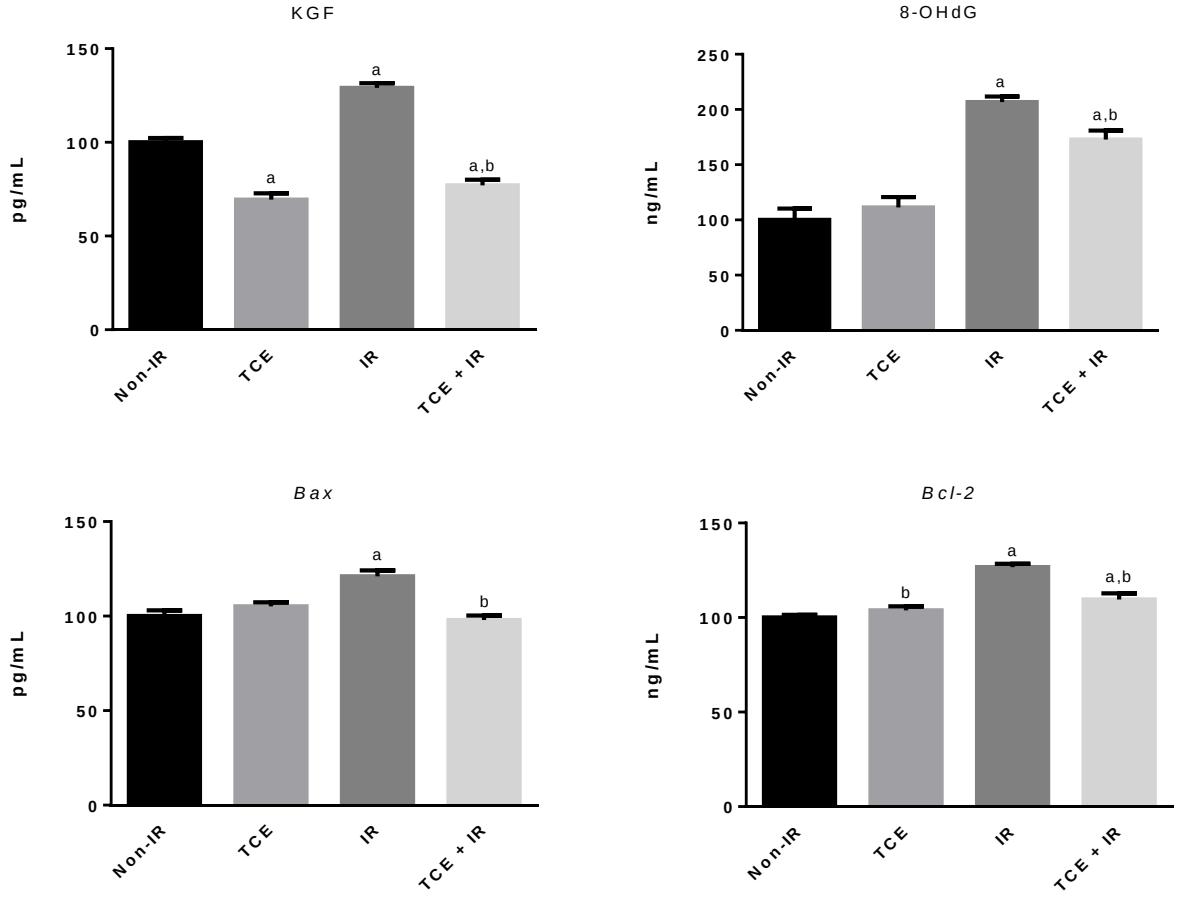


One-way ANOVA followed by Tukey *post hoc*. Columns represents the mean + S.D. No differences were observed.

3.5 Effects of tucumã crude extract on KGF, FGF, 8-OHdG, Bax and Bcl-2

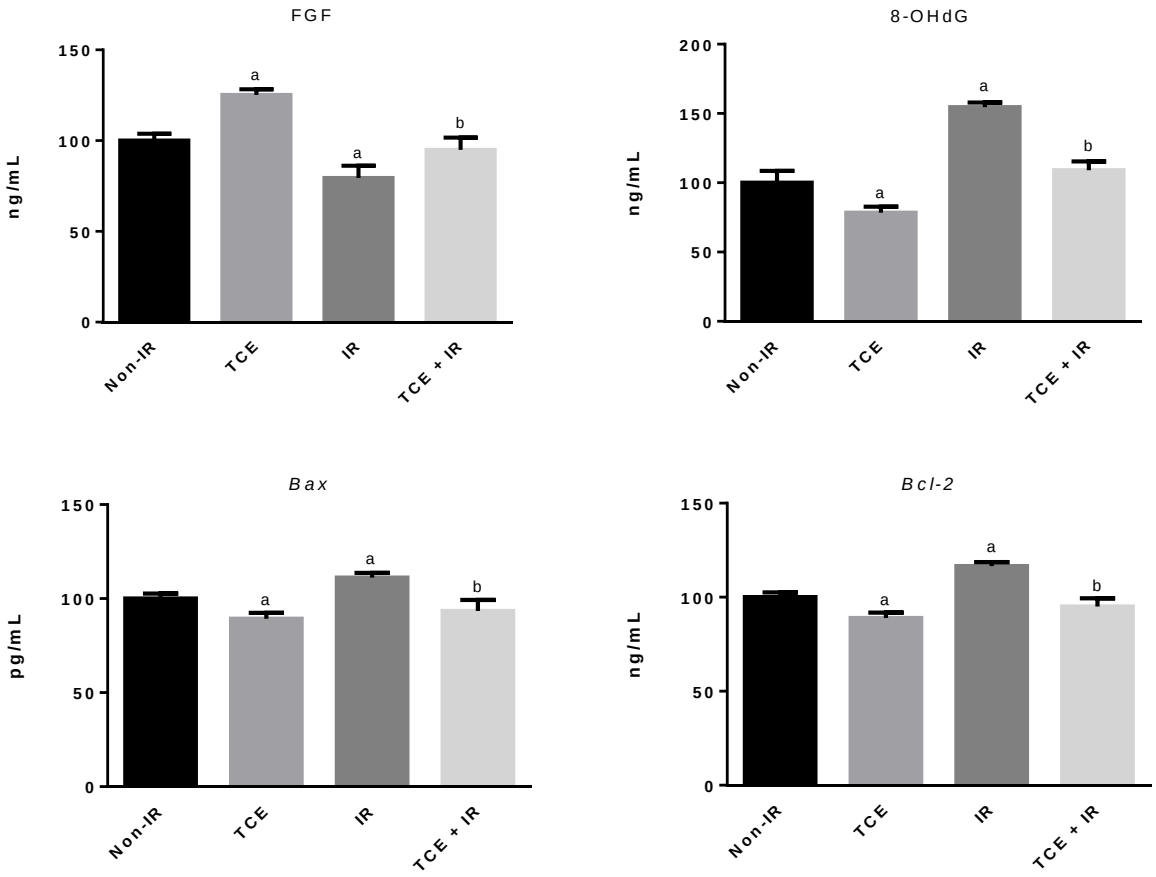
On keratinocytes cells was observed elevated levels of KGF, 8-OHdG, *Bax* and *Bcl-2* in the group exposed to radiation and the pretreatment with TCE decreased significantly the levels of all parameters (figure 5). On the other hand, we did not found an elevated level of FGF in fibroblast cells in the irradiated group. The other parameters (8-OHdG, *Bax* and *Bcl-2*), like in the keratinocytes, had its levels increased and the pretreatment with tucumã was able to attenuate them (figure 6).

Fig. 5. Effects of pretreatment with tucumã crude extract (24h) followed by exposure to radiation (2 Gy) on KGF, 8-OHdG, *Bax* and *Bcl-2* parameters in keratinocytes cells.



One-way ANOVA followed by Tukey *post hoc*. Columns represents the mean + S.D. Columns represents the mean + S.D. [a]Significantly different from non-IR group; [b]Significantly different from IR group: $p < 0.05$.

Fig. 6. Effects of pretreatment with tucumã crude extract (24h) followed by exposure to radiation (2 Gy) on FGF, 8-OHdG, *Bax* and *Bcl-2* parameters in fibroblasts cells.



One-way ANOVA followed by Tukey *post hoc*. Columns represents the mean + S.D. Columns represents the mean + S.D. [a]Significantly different from non-IR group; [b]Significantly different from IR group: $p < 0.05$.

4 Discussion

In this study, we performed the investigation of radioprotection properties of pretreatment with tucumã crude extract with *in vitro* cultures followed by exposition to radiation through citogenetic and oxidative damage analyses. Although the target of radiotherapy is the cancer cells, the healthy cells, especially those with a high rate of proliferation, also suffer the toxic effects of the radiation, which may result in serious side effects for patients [22]. Since radiotherapy is the treatment of choice for a several types of cancer, search for efficient radioprotectors are needed to protect normal tissues from the radiation-induced damage.

Natural compounds from plants like lycopene, a natural carotenoid, and poliphenols like resveratrol, curcumin and rutin, among other flavonoids, already have been reported about its radioprotective effects [9, 10, 8, 11, 23]. Compounds with antioxidant characteristics are known to possess potential protection against radiation by scavenging free radicals. In this context, tucumã is a fruit native from Brazil, with important biological activities, including antioxidant [12]. Exposure to ionizing radiation is known to induce oxidative stress through generation of ROS resulting in an imbalance of the pro-oxidant and antioxidant systems in the cells, which may contribute to the cell death [24]. The DNA molecule is one of the main targets of radiation and can be attacked by direct or indirect mechanisms. Indirect mechanism involves the production of ROS, which is the result of the interaction of radiation with the water present in the cells, process known as water radiolysis [25]. By interacting with biological tissue, ROS play an important role in the pathogenesis of radiation-induced tissue injury, by producing toxic free radicals that cause damage to DNA or proteins and also lead to lipid peroxidation [9]. One of the final products of lipid peroxidation, malondialdehyde (MDA), is an important biological marker of oxidative damage and can be easily quantified [26].

In our study, the MDA levels in the cells that were exposed to radiation significantly increased when compared to the control group. This may be due to the attack of free radicals on the membrane, which possess fatty acid as component [27]. However, the concentration of extract tested was not able to decrease its levels, possibly due to the fact that was not enough to quench all free radicals generated. On the fibroblasts cells was possible to observe a slight reduction, demonstrating that may be in other concentrations, tucumã can exert an effective activity on reduction of lipoperoxidation. Over production of ROS due to exposition to ionizing radiation can lead to oxidative damage, which induce cytotoxicity like chromosomal damage and gene mutations [28]. We did not observe elevated levels of ROS in the radiation group. Dong *et al.* (2011) [29] showed that the increase of ROS production occurred only 4 days after irradiation, suggesting that direct radiolysis of water molecules constitutes a relatively smaller portion of ROS generated by irradiation, being the enzyme-linked production more expressive. The protein carbonylation was significantly increased in the irradiation group. Pretreatment with tucumã decreased the carbonyl levels of fibroblast cells, showing a great activity of the extract, since carbonyl is an important biomarker of oxidative damage.

The excess of ROS can lead to oxidation of the DNA bases, such as oxidize deoxyguanosine (dG) to form 8-hydroxy-guanosine (8-oxodG). 8-hydroxy-2′-deoxyguanosine (8-OHdG) is considered the major type of DNA damage and it is an excellent biomarker for evaluation of cellular oxidative stress due to its implication in nucleobase mutations [30]. In our study, treatment with TCE reduce the levels of 8-OHdG both in fibroblast and keratinocyte cell, which was significantly increased by the irradiation. Molecules able to scavenging free radicals can act as radioprotectors by reducing DNA lesions [31].

Plants with high content of polyphenols and flavonoids are important in the search for new radioprotectors. In study performed by Sagrillo *et al.* (2015) [12] was determined the high content of polyphenols and flavonoids in the extract of tucumã's pulp and the strong antioxidant capacity. It is reported that aromatic compounds containing hydroxyl groups are important scavengers, which is the case of flavonoids [32]. Flavonoids can undergo electron and/or hydrogen atom transfer to sites on DNA damaged by excess of ROS [33]. They can donate hydrogen atom from their hydroxyl groups and stabilize the phenoxy radical formed by delocalization of the unpaired electron within the aromatic structure [34]. It was also observed that tucumã pulp presents a high content of carotenoids [12]. Srinivasan *et al*. (2009) [9] investigated the radioprotector effect of lycopene, a dietary carotenoid synthesized by plants, on human lymphocytes. It was observed that pretreatment protected the lymphocytes from γ-radiation-induced damage by reducing peroxidation of membrane lipids and free radicals induced DNA strand break formation. Carotenoids can scavenge singlet oxygen ($^1O_2$) and other excited species by quenching energy from $^1O_2$, being the carotenoid converted to the energy rich triplet state [9]. The energy absorbed by the carotenoid is dissipated by vibrations and rotations and their interactions with the environment. After this process carotenoid remains able to quench new excited species.

Radiation-induced damage to DNA can include single-strand breaks, base damage or even double-strand breaks, considered the most deleterious effect [23]. Once damaged, DNA can trigger apoptotic pathway through a chain reaction of signals [35], including p53 and Bcl-2 family. Cell death promote by p53 protein is normally by the activation of pro-apoptotic protein like *Bax* and anti-apoptotic proteins like *Bcl-2* [36]. Some compounds have already been reported that were able to decrease radiation-induced apoptotic lymphocytes by increasing the expression of *Bcl-2* and

reducing the expressions of *Bax* like the black tea and apigenin [37,38]. The radiation exposed group showed an elevated level of *Bax* and pretreatment with tucumã was able to reduce its level, however was not accompanied by the increase in *Bcl-2* level. We observed a low level of cell death when exposed to the radiation at 2 Gy in both keratinocyte and fibroblast by the PicoGreen® test. Dong *et al.* (2011) [29] also found no evidence of apoptosis on irradiation in normal human fibroblast, suggesting that apoptosis does not appear to be a common radiation response in these cells.

KGF and FGF are responsible for regulation of epithelial proliferation, differentiation, and migration and also plays an important role in epithelial wound repair [39]. Its detection is common in altered epithelial cells, such as in inflammatory and tumorigenic processes. Also, in the study by Hille *et al.* (2010) [39] was observed that cultures exposed to ionizing radiation showed a stimulated proliferation of KGF. In accordance, we found an elevated concentration of KGF in the group exposed to IR and the TCE was able to decrease its concentration. Better response/tolerance of normal tissue during radiotherapy it is an advantage for the patients, given the lack of products that can act as radioprotectors. On the other hand, FGF had its levels decreased in the radiation group, may be indicating that this type of cell is more sensitive to the radiation effects.

5 Conclusion

In summary, since the radiation dose is directly connected to amount of DNA damage produced, change in radiation dose should be reflected in a proportional change both in production of oxidative stress and damage to DNA. The radiation dose of choice (2 Gy) was able to induce oxidative stress, however not enough to observe DNA damage, since was not found an elevated level of cell mortality. It is well known that substances with strong antioxidant capacity are important in the search for new radioprotectors. Treatment with TCE showed a high activity against radiation-induced damage, whereas the attenuation in biomarkers of oxidative stress in keratinocyte and fibroblast cells, which it is important since the excess of ROS can damage the cells DNA. However, more studies are needed to better confirm the potential effect radioprotector of the tucumã crude extract.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

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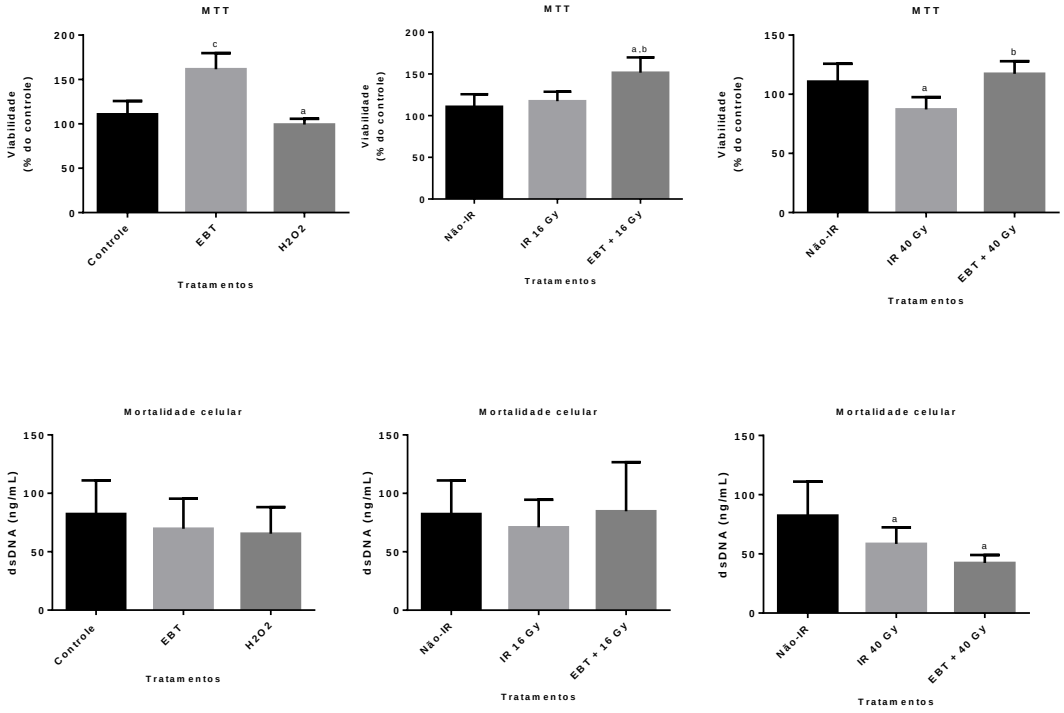
ESTUDO ADICIONAL *IN VITRO*

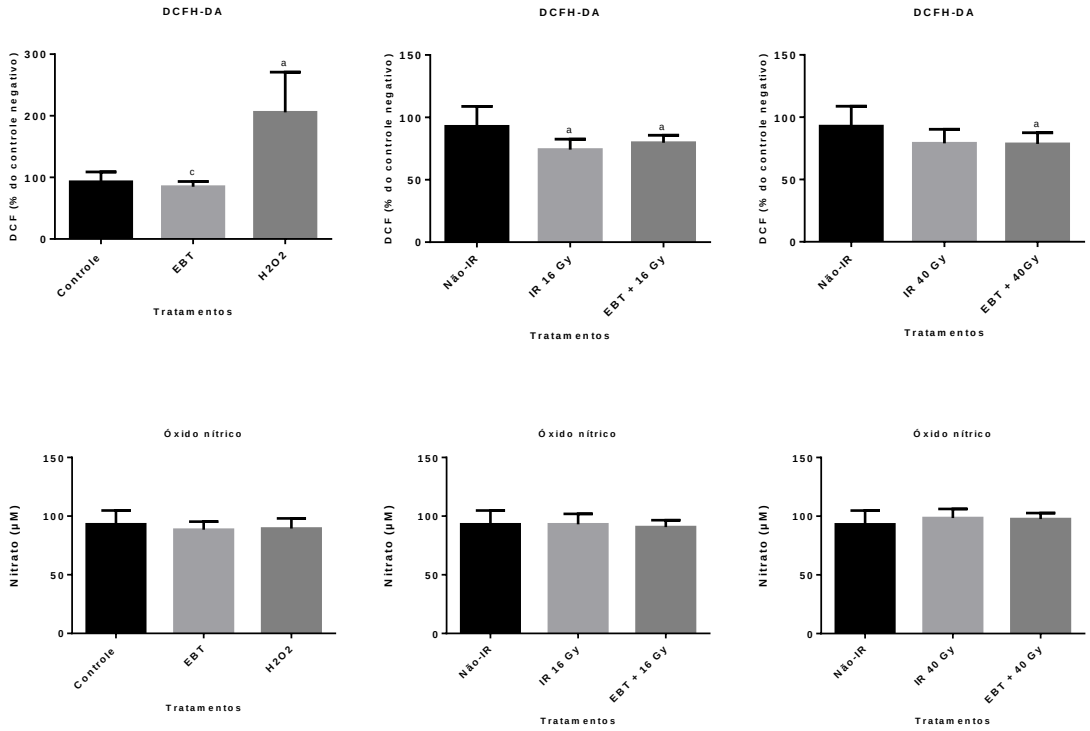
A fim de obter melhores resultados com a exposição de células à radiação ionizante, um novo estudo foi conduzido. Algumas modificações foram feitas: foram utilizadas células de linfócitos isoladas de sangue periférico, doses maiores de RI e o tempo para a realização dos testes após a exposição foi reduzido. Diferente do primeiro estudo, nesse, as células foram dispostas em placas de ELISA de 96 poços e expostas à radiação nas doses de 16 e 40 Gy. A concentração do extrato de tucumã foi a mesma que no primeiro experimento: 100 µg/mL. As doses de radiação foram divididas em duas etapas: 8 Gy + 8 Gy para atingir 16 Gy e 20 Gy + 20 Gy para atingir 40 Gy. As células foram primeiramente tratadas com o EBT e após 8h foram expostas à primeira dose de radiação; após 10h foram expostas à segunda. Durante esse processo foram mantidas em estufa nas condições adequadas. As análises foram realizadas 2 horas após a última exposição à RI e foram as seguintes: MTT, ON, PicoGreen e DCFH-DA. *Sangue total periférico:* foi obtido de amostras descartadas do Laboratório de Análises Clínicas (LEAC) da Universidade Franciscana (UFN), com aprovação no Comitê de Ética em Pesquisas com Seres Humanos da UFN sob nº CAAE 31211214.4.0000.5306, com ausência de identificação. A separação celular foi obtida através de gradiente de separação utilizando o Histopaque® (1:1) com o sangue total. As amostras foram centrifugadas a 1000 rpm por 30 minutos. Após, as células mononucleadas de sangue total periférico foram dispostas em tubos falcon com meio RPMI 1640 com 10% de soro fetal bovino e suplementado com 1% de estreptomicina e penicilina e centrifugadas a 1800 rpm por 8 minutos. Após a centrifugação, as células foram suspensas em meio RPMI. Os resultados encontram-se a seguir.

Efeitos do pré-tratamento com EBT em linfócitos irradiados nas doses de 16 e 40 Gy na viabilidade e mortalidade celular (MTT e PicoGreen®):



As colunas representam a média ± D.P. ANOVA de uma-via seguida de Tukey. Os valores foram significativos quando $p < 0,05$. [(a)]Diferente do grupo controle; [(b)]Diferente do grupo IR; [(c)]Diferente do grupo H_2O_2.

Efeitos do pré-tratamento com EBT em linfócitos irradiados nas doses de 16 e 40 Gy nos níveis de espécies reativas e óxido nítrico:



As colunas representam a média ± D.P. ANOVA de uma-via seguida de Tukey. Os valores foram significativos quando $p < 0,05$. [(a)]Diferente do grupo controle; [(b)]Diferente do grupo IR; [(c)]Diferente do grupo H_2O_2.

Como é possível observar, mesmo com as possíveis limitações que ocorreram no primeiro estudo excluídas, a exposição à radiação não foi capaz de causar danos às células – não foram observadas diferenças apenas no grupo irradiado em relação ao grupo controle. Sendo assim, decidimos investigar as possíveis atividades biológicas do extrato do tucumã *in vivo*, visto os poucos estudos/artigos publicados que existem sobre o assunto até o presente momento. Por não haver estudo *in vivo* demonstrando a possível toxicidade da espécie, nosso primeiro estudo investigou as toxicidades aguda e de doses repetidas do extrato. Após esses estudos, foi possível definir doses seguras para investigarmos o potencial efeito protetor (tratamento e pré-tratamento) do extrato contra a toxicidade induzida pela CFF em ratos *Wistar* (artigos 2 e 3).

5 ARTIGO 2 – TUCUMÃ (*Astrocaryum aculeatum*) EXTRACT: PHYTOCHEMICAL CHARACTERIZATION, ACUTE AND SUBACUTE ORAL TOXICITY STUDIES IN *Wistar* RATS

O artigo científico a seguir foi publicado na revista científica *Drug and Chemical Toxicology* em 25/05/2020. Fator de impacto (2018) de 1,946. <http://www.tandfonline.com/> https://doi.org/10.1080/01480545.2020.1777151

DELINEAMENTO EXPERIMENTAL: Ratos *Wistar* machos e fêmeas foram obtidos no Biotério Central da UFSM. Os animais foram mantidos em condições adequadas (ciclo claro/escuro de 12h; umidade e temperatura controladas) e aclimatizados por 10 dias antes do início dos experimentos. Para a toxicidade aguda foram utilizadas 12 ratas, divididas em grupo controle e grupo teste (n = 6/grupo). Os animais do grupo teste receberam o extrato de tucumã na dose única de 2000 mg/kg, enquanto que o grupo controle recebeu apenas água (veículo). Os tratamentos foram via oral (gavagem), pela manhã; e o experimento teve duração de 14 dias. Para a toxicidade de doses repetidas, 20 ratos machos e 20 fêmeas foram utilizados, divididos em 4 grupos (n = 5/grupo): controle (apenas água) e três doses de extrato de tucumã (200, 400 e 600 mg/kg) – administrados via oral (gavagem). O experimento durou 28 dias e os animais foram tratados diariamente, pela manhã. Em ambos os experimentos, os animais foram observados diariamente para identificação de algum sinal atípico para a espécie. Parâmetros fisiológicos e comportamentais foram registrados ao longo do experimento. Após a eutanásia, sangue e órgãos foram coletados para as posteriores análises.

Tucumã (*Astrocaryum aculeatum*) extract: phytochemical characterization, acute and subacute oral toxicity studies in *Wistar* rats

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Tucumã (*Astrocaryum aculeatum*) extract: phytochemical characterization, acute and subacute oral toxicity studies in *Wistar* rats

Abstract

Natural products are often used by the population to treat and/or prevent several disorders. Tucumã is an Amazonian fruit widely consumed by local population and no *in vivo* toxicity studies regarding its safety are available in the literature to date. Therefore, the phytochemical characterization, acute and repeated dose 28-day oral toxicities of crude extract of tucumã's pulp (CETP) in *Wistar* rats were evaluated. For the CETP preparation, tucumã pulp was crushed and placed into sealed amber glass jars containing absolute ethanol solution for extraction. CETP phytochemical analyses evidenced the presence of carotenoids, flavonoids, unsaturated and satured fatty acids, and triterpenes. In the acute toxicity, female rats from the test group were treated with CETP at single dose of 2000 mg/kg. For the repeated dose toxicity, CETP was administered to male and female rats at doses of 200, 400 and 600 mg/kg, for 28 days. Body weight was recorded during the experiment and blood, liver and kidney were collected for further analysis. No mortality or toxicity signs were observed during the studies. CETP was classified as safe (category 5, OECD guide), in acute toxicity. In repeated dose study was observed alterations in some biochemical parameters, as well as in oxidative damage and enzymatic activity. Histopathological findings showed renal damage in male rats at higher dose. The data obtained suggest that CETP did not induced toxicity after exposure to a single or repeated doses in female rats. However, in males may be considered safe when given repeatedly in low doses.

Keywords: Carotenoids; Tucumã; Arecaceae; Toxicity; Acute; Repeated doses.

1. Introduction

Plants are commonly used by human populations and often for medicinal purposes, such as in the cure and/or prevention of diseases. In developed and underdeveloped countries, it is estimated that 70 to 80% of the population uses complementary or alternative medicine (Araújo *et al*. 2017). Natural products have been targeted by many studies aiming to obtain molecules with therapeutic potential and are known to be important in the development of new drugs (Menegati *et al*. 2016). They represent an alternative treatment that is easily accessible, has low cost and is believed to be safe and without adverse effects because it is "natural" (Traesel *et al*. 2014). To validate the potential role of natural products in improving the treatment of diseases and use as a functional food, studies on toxicity and safety are necessary.

Brazil has great biodiversity, particularly in its Amazon biome, which is characterized by a dense rainforest with hot and humid climate and heavy rains that are frequent all year round (Matos *et al*. 2019). With such broad diversity, the Amazon offers to local populations several different species of fruit. For example, *Astrocaryum aculeatum* Meyer, popularly known as "tucumã", "tucumã-do-amazonas" or "tucumã-açu", is a palm tree belonging to the Arecaceae family that can be found in degraded environments among secondary vegetation in the Amazonian ecosystem (Maia *et al*. 2014). The fruits of this species are the most consumed part of the palm tree, followed by its roots, seeds, palm heart, leaves, flowers and flower sap (Agostini-Costa 2018).

The tucumã fruits are widely consumed by local population *in natura*, in sandwiches and tapioca, desserts and ice cream (Oliveira *et al.* 2018) and they are traditionally used to treat the respiratory system, infections, infestations and is also associated with digestive system disorders (Agostini-Costa 2018; Macía *et al*. 2011). Many fruits and oleaginous plants extracted from the Amazon are especially rich in compounds with high antioxidant capacity, such as carotenoids, anthocyanins and polyphenols (De Rosso & Mercadante 2007). According to Agostini-Costa (2018) there are several wild species, which have been used for ethno-

pharmacological purposes and are traditionally consumed, that need to be better evaluated, including *A. aculeatum* fruits. In fact, *in vitro* pharmacological activities have already been described in the literature, such as antimicrobial (Jobim *et al*. 2014) and cytoprotective (Sagrillo *et al*. 2015) action. However, *in vivo* studies regarding this species' safety and toxicity are still needed to ensure its use by the population. Moreover, toxicity studies are essential to define safe doses for further investigations regarding biological activities. Therefore, due to the lack of data in the current literature, we decided to assess the oral toxicity of tucumã extract in *Wistar* rats through acute and repeated doses over a 28-day period.

2. Materials and methods

2.1. Botanical material and extract preparation

This study is registered in the *Sistema Nacional de Gestão de Patrimônio Genético e do Conhecimento Tradicional Associado* (SisGen) under the number A75395F. Tucumã fruit was collected directly from native palm trees found in primary and secondary forests, pastures and home gardens, being obtained from a composite sample representing a mixture of progenies from Manaus City (Amazonas State, Brazil), located in the Amazonian region (3º6'26''S, 60º1'34''W). Tucumã usually blooms from June to January and produces fruit from February to August; we obtained the fruits (5 kg) from Manaus city in August, 2017. According to Sagrillo *et al.* (2015), the tucumã ethanolic extract was prepared from tucumã pulp that was crushed and placed separately into sealed amber glass jars containing an absolute ethanol solution at a ratio of 1:5 (w/v) for extraction. The extraction was performed over four days at room temperature with daily agitation. The homogenate was filtered through Whatman No. 1 paper and then collected; the ethanol was removed using a rotary evaporator at reduced pressure, 25 ºC at 115 rpm. Following this procedure, the pulp extract was lyophilized and stored at -20 ºC until the use. We obtained 109 g of dried tucumã pulp extract.

2.2. Determination of total phenolics content, total flavonoids content and radical-scavenging activity – 2, 2 diphenyl-2-pricrylhydrazyl (DPPH) assay

The total phenolic content and flavonoids content determinations in crude extract of tucumã pulp (CETP) was performed according to Chandra & Mejia (2004) and Woisky & Salatino (1998), respectively, with slight modifications. The radical scavenging activity of CETP was quantified in the presence of DPPH• stable radical, according to a method slightly modified (Choi *et al*. 2002). These assays were carried out as described in Guex *et al.* (2018).

2.3. Quantification of CETP compounds by high-performance liquid chromatography-photodiode array (HPLC-PDA)

2.3.1. Carotenoid analysis

CETP was diluted in MeOH/MTBE mixture [70:30 (v/v)]. Solutions were filtered (0.22 μm) and analyzed in a liquid chromatograph (CBM-20A Prominence, Shimadzu LC) coupled to a photodiode array (PDA) detector. Samples (20 μL) were separated using a Zorbax ODS C18 25 mm x 4.6 mm x 5 μm (Agilent Technologies). Mobile phase was a linear gradient of a MeOH/MTBE mixture from 95:5 (v/v) to 70:30 (v/v) over 30 min, followed by a 50:50 (v/v) ratio for 20 min, and maintaining this proportion for 15 min with the column temperature set to 29°C and flow rate at 0.9 mL/min (De Rosso & Mercadante 2007). Carotenoids were identified by comparing their retention times and PDA spectra with pure standards and literature data. Carotenoids were quantified by HPLC−PDA, using a six-point analytical curve of (all-E)-β-carotene. All other carotenoid contents were estimated using the curve of (all-E)-β-carotene (y = 19095x – 6503.8). Analytical curve was linear (r=0.9998); the limit of detection (LoD) and limit of quantification (LoQ) for (all-E)-β-carotene in the extract were, 0.089 and 0.25 ppm, respectively.

2.3.2. Phenolic compounds analysis

CETP was analyzed by HPLC with photodiode array (PDA) detector using a reverse-phase C-18 Hypersil Gold column (5 µm particle size, 150 mm, 4.6 mm) at 38°C. Injection volume was 20 µL and the mobile phases were composed of 5% (v/v) methanol in acidified water (0.1% (v/v) of formic acid) as solvent (A) and 0.1% (v/v) of formic acid in acetonitrile as solvent B, following the method described by Quatrin *et al.* (2019).

The absorption spectra were recorded from 200 to 800 nm and phenolic compounds were identified by comparison with the retention time of authentic standards and the spectral data obtained from UV–visible absorption spectra. The chromatograms for quantification purposes were obtained at 280 nm, for hydroxybenzoates, at 320 nm for hydroxycinnamates, and at 360 nm for flavonols. Stock solutions of standard references were prepared in the initial mobile phase and were diluted in eight equidistant points within the concentration range of LOQ–60 mg L^{-1}. Hydroxybenzoate derivatives were quantified as equivalents of catechin, hydroxycinnamate derivatives were quantified as equivalents of trans-ferulic acid and flavonol derivatives were quantified as equivalents of quercetin using the following calibration curve for catechin: $y = 19861x + 21544$ ($r=0.996$); trans-ferulic acid: $y = 165905x + 113049$ ($r=0.997$) and quercetin: $y = 64434x – 373423$ ($r=0.895$). The LoD and LoQ for catechin, trans-ferulic acid and quercetin were, respectively, 0.026 and 0.078 ppm; 0.011 and 0.033 ppm; and 0.146 and 0.444.

2.4. Phytochemical characterization by gas chromatography–mass spectrometry (GC-MS)

CETP (5 mg) was solubilized in *n*-hexane (1 mL). This sample was analyzed by GC-MS on a Shimadzu QP2010 Plus (Shimadzu Corporation, Kyoto, Japan) system equipped with an AOC-20i auto sampler operating in the electron ionization (EI) mode at 70 eV under the following conditions: DB-5 capillary column (30 m × 250 mm × 0.25 µm); carrier gas helium (99.9%) at a constant flow of 1.4 mL/min; sample injection volume of 1 µL at 5 mg/mL (split

ratio of 4); injector temperature 280 °C; ion source temperature at 250°C. The oven temperature was programmed to increase from 60-330 °C at 2-25 min, in linear velocity. Mass spectra were recorded with a scan interval of 0.3 s within the mass range 29–700 Da. The identification of the hexane fraction components was based on Computer matching by libraries such as FFNSC1.3, NIST 11 and Wiley 7 and SciFinder.

2.5. Animals

Male and female *Wistar* rats (weighing 80 - 140 g) obtained from animal house of Universidade Federal de Santa Maria (UFSM) were used for the studies. Rats were housed by sex in polypropylene cages with environmental enrichment and acclimatized for ten days before the beginning of the experiment. Animals were maintained under a 12:12h light/dark cycle at controlled temperature (22 ± 2 ºC) with food and water *ad libitum*. All experimental procedures were conducted in compliance with the ethical principles of *Conselho Nacional de Controle de Experimentação Animal* (CONCEA) and approved by the Ethics and Animal Welfare Committee of UFSM (CEUA numbers 6572091017 and 2489120418).

2.6. Acute toxicity

Acute toxicity was performed according to OECD guideline 423, with slight modifications (OECD, 2001). Female *Wistar* rats, nulliparous and non-pregnant were used for the study. The animals were divided into two groups (n = 6 *per* group): control and test. The animals were fasted overnight for 4 hours and weighed before the administration of the substances. The CETP was dissolved in distilled water right before the administration. Animals from the test group received CETP at single dose of 2000 mg/kg, while the control group received only the vehicle (distilled water) (3 mL/kg), both treatments were via oral, by gavage, in the morning. The food was provided to the animals after 3 hours of the substance administration. Body weight of the animals was recorded daily, during the treatment period.

After administration, animals were observed individually during the first 30 minutes and then daily for 14 days. As described in our previous study (Guex *et al*., 2018) and with OECD (2001) recommendations, the observations included mortality and changes in skin and fur, eyes and mucous membranes, and respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern. Attention was directed to observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. If one or more of these signs were observed in the animals, they no longer were seen as objects of study and would have their euthanasia anticipated to avoid pain and suffering. At the end of the treatment, animals were fasted overnight and euthanized with intraperitoneal injection of xylazin (10 mg/kg) and ketamine (100 mg/kg) anesthesia, followed by blood collection by cardiac puncture for hematologic and biochemical analyses. After euthanasia, liver and kidney were removed for macro and microscopic evaluation and the relative organ weights were calculated as (organ weight/body weight) x 100. An aliquot of the organs was separated for homogenates to analyze enzymatic activity and oxidative damage.

2.7. Repeated dose 28-day toxicity

Male and female (nulliparous and non-pregnant) *Wistar* rats were used for the repeated dose 28-day oral toxicity, which was conducted according to OECD guideline 407 (OECD, 2008). The animals were divided into 4 groups according to gender ($n = 5$ *per* group/*per* gender) as follows: I. control group: was administered the vehicle (distilled water 3 mL/kg); groups II, III and IV. was administered CETP at doses of 200, 400 and 600 mg/kg, respectively. The administration was via oral, by gavage, and the treatment was daily in the morning for 28 days. Body weight of the animals was recorded daily, during the treatment period. During the experiment, the food consumption and water intake were recorded for each group of animals every four days.

Animals were observed for signs of abnormalities during the treatment period as described at item 2.7.1. At the end of the treatment, animals were fasted overnight and euthanized as described at item 2.7.1., followed by blood collection by cardiac puncture for hematologic and biochemical analyses. After euthanasia, liver and kidney were removed for macroscopic and microscopic evaluation. Liver and kidneys were weighed and the relative organ weights were calculated as (organ weight/body weight) x 100. An aliquot of the organs was separated for homogenates to analyze enzymatic activity and oxidative damage.

2.8. Open field

Spontaneous exploratory behavior was evaluated in the open field test by measures of locomotion and exploration one day prior to euthanasia, after the last exposure to CETP at different doses (200, 400 and 600 mg/kg/day). This test is often used in order to identify signs of anxiety and/or motor disturbance. The animals were placed individually in the central area of the open field, which is constructed with plywood, measuring 45×45 cm, with 30 cm walls and the base divided into nine squares with tape markers (3×3). During 5 minutes was recorded: (a) latency (the time taken to animal leave the starting square), (b) crossings (number of times the line of a square is crossed with all four paws), (c) rearings (number of times the animal stands on its hind paws), (d) grooming (frequency of grooming activity), and (e) number of defecation boli (Jänicke & Coper 1996). At the end of each test the apparatus was cleaned with ethanol 30%. One day before the test, the animals were acclimatized in the open field, where they were allowed to explore the apparatus for 5 minutes.

2.9. Histopathology

After euthanasia, liver and kidney samples were removed, fixed in 10% buffered formalin and processed routinely by paraffin embedding and light microscopic examination.

Sections (6 μm thick) were stained by hematoxylin and eosin (H.E) with the use of the standard techniques. Blinded histological analysis was performed by a trained histologist.

2.10. Hematological and biochemical parameters

Blood without anticoagulant was allowed to clot before centrifugation (3200 *g* x 10 min) to obtain serum, which was utilized for the assessment of glucose (GLU), blood urea nitrogen (BUN) and creatinine (CRE) levels, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT) and butyrylcholinesterase (BChE) activities, serum total protein (TP), albumin (ALB), total cholesterol (CHOL), low density lipoprotein (LDL), high density lipoprotein (HDL) and triglycerides (TG) levels, using commercial kits (Diagnostic Kits Laboratory Bioclin/Quibasa, Minas Gerais, Brazil) and an automatic biochemical analyzer (BS-120 Mindray®, Shenzhen, China).

Hematological analyses were performed on EDTA-coated tubes, and the BC-VET 2800 (Mindray®) was used to determine the hematocrit (HCT), total hemoglobin concentration (HGB), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular volume (MCV). The total red blood cells (RBC), white blood cells (WBC), and platelet (PLT) were also counted. Blood smears were prepared from each sample and were stained with Romanowsky dye to examine the differential leukocyte count and morphological evaluation of blood cells.

2.11. Protein carbonyl and lipid peroxidation

Hepatic and renal tissues were homogenized in tris/HCl buffer 10 mM (pH = 7.4). Protein carbonyls were measured spectrophotometrically following reaction with DNPH (2,4-dinitrophenylhydrazine). The test was performed in triplicate and the absorbance reading at 370 nm (Reznick & Packer 1994). Lipoperoxidation (LPO) was estimated using thiobarbituric acid

reactive substances (TBA-RS) method, which the colorimetric reaction of the malondialdehyde (MDA) with thiobarbituric acid (TBA) is quantified. This reaction produces a colored compound that absorbs maximally at 532 nm as described by Buege & Aust (1978). The protein concentration of each sample was determined using Lowry assay (Lowry *et al*. 1951).

2.12. Enzymatic activity

Hepatic and renal tissues were used to assess the activity of CAT and SOD enzymes. Catalase (CAT) activity was determined by measuring the decrease in hydrogen peroxide 0.01 M (H_2O_2) absorption. It is based on the consumption of H_2O_2 by CAT and loss of absorbance at 240 nm according to Aebi (1984). Superoxide dismutase (SOD) activity was determined as described by Sun & Zigman (1978) with slight modifications. It is based on its ability to inhibit the auto-oxidation of epinephrine determined in absorbance at 480 nm. The reaction mixture contained 0.965 mL of glycine buffer (pH = 10.2), 0.05 or 0.01 mL of the homogenate and 0.02 mL of epinephrine (60 mM, pH = 2.0) in HCl (1M) was used to initiate the reaction.

2.13. Statistical analysis

Results are expressed as mean ± standard deviation (S.D.). Differences among groups were determined by Student's *t*-test and one-way analysis of variance, followed by Tukey *post-hoc* test. Mann-Whitney, Kruskal-Wallis and Dunn's were used as statistic tests when the requirements to perform a parametric test were not satisfied (*GraphPad Prism Software, Inc., version 6*). Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Total phenolics content, total flavonoids content and DPPH assay

The concentration of total polyphenols in CETP was 3.00 ± 0.30 mg/g gallic acid equivalent (GAE), whilst the flavonoid content showed a concentration of 2.76 ± 0.10 mg/g rutin equivalent (RE). Antioxidant activity showed an IC_{50} of 5.22 ± 0:33 µg/mL (IC_{50} = Concentration required to inhibit 50% of DPPH radical).

3.2. Carotenoid and phenolic compounds quantification by HPLC-PAD

Through the analysis by HPLC-PAD was possible to determine the carotenoids and phenolic compounds content. Lutein and β-carotene were the major identified carotenoids, amounting to 21.1% and 26.2% of total carotenoid content (1.20 mg/100 g), respectively. Regarding the phenolic compounds, hydroxybenzoate derivatives and tannins were the major constituents found (3139.43 mg of catechin equivalents /100 g) followed by flavonol derivatives (163.17 mg of quercetin equivalents/100 g) and hydroxycinnamate derivatives (8.62 mg of trans-ferulic acid equivalents/100 g) (figure 1).

[Figure 1 near here]

3.3. Phytochemical characterization by GC-MS

The identified compounds in CETP *n*-hexane fraction by GC-MS are described in Table **1**. The major constituents are 1,3-diolein (**26**), 11-octadecenoic acid (**3**), glycerol 1-oleate 3-stearate (**27**), 1-monoolein (**14**), 1-palmitoyl-3-oleoylglycerol (**24**), cycloartenol (**22**), palmitic acid (**1**) and linoleic acid (**4**), corresponding to 80.6% of the total area of present compounds. This way, we observed that the plant material is rich in unsaturated fatty acids, besides to present saturated fatty acids and triterpenes. We also verified that not only this species is little studied, chemically, as well as the *Astrocaryum* genus and the family Arecaceae to which it belongs (SciFinder, 2019).

[Table 1 near here]

3.4. Acute toxicity

After administration of a single dose of 2000 mg/kg of CETP or vehicle, the animals were observed for 14 days. The body weights were recorded during the experiment and no difference was observed between the groups, the same was found in organ and relative organ weights (table 2). Moreover, no signs of toxicity or behavioral changes were observed. During the treatment no mortality was recorded and at necropsy no abnormality was found in the liver and kidneys. Regarding the biochemical and hematological analysis no significant differences were observed between the groups as well as in the MDA levels, SOD and CAT activities. Histopathological analysis of liver and kidney were performed in both groups and it was possible to observe that the tissues maintained their normal physiological aspect and there were no significant morphological differences between the control group and group treated with CETP at single dose (data not shown).

[Table 2 near here]

3.5. Repeated dose 28-day toxicity

Repeated oral administration of CETP at 200, 400 and 600 mg/kg doses did not induce any sign of toxicity to the animals and no mortality was recorded during the experiment. At necropsy, the macroscopic evaluation did not show any abnormality in the liver and kidneys. The body weights of the animals exhibited a normal trend, liver and kidney weights presented alterations in different groups and relative organ weight did not showed significant changes between the groups (table 3).

[Table 3 near here]

Food and water intake were significant different between the treated groups in females, whilst in males no difference was observed (figure 2). Hematological parameters (table 4) and open field test did not present significant differences. In hepatic tissue the CAT activity, MDA and carbonyl levels did not show significant alterations between the groups and the same was

observed on carbonyl levels, CAT and SOD activities of renal tissue. In males rats, the hepatic SOD activity was elevated in the 200 and 400 mg/kg groups compared with the control. While in females, the renal MDA levels were reduced in the animals treated with CETP 400 mg/kg in comparison to the control group (data not shown).

[Figure 2 near here]

[Table 4 near here]

Regarding the biochemical parameters, in females ALT levels were reduced in the group treated with CETP at 400 mg/kg when compared to the control group and the HDL levels increased in the 600 mg/kg in comparison to the 200 mg/kg group. In males, total proteins were reduced in the 200 mg/kg in relation to the control and 400 mg/kg groups. In addition, HDL levels were increased in the animals treated with CETP at 400 and 600 mg/kg when compared to the control, LDL levels reduced on the 400 and 600 mg/kg groups in relation to the animals of the group 200 mg/kg (table 5).

[Table 5 near here]

3.5.1. Histopathology

Histopathological analysis of liver and kidney were performed in male and female rats from the control group and groups exposed to CETP for 28 days at different doses (200, 400 and 600 mg/kg). In females, the tissues maintained their physiological aspect and there were no significant morphological differences between the control group and groups treated with CETP. On the other hand, in males, the treatment with CETP at highest dose (600 mg/kg) caused degeneration in renal tissue, which showed loss of normal morphology in the glomerular area in comparison to the control group. No changes were observed on hepatic tissue of animals treated with CETP at different doses when compared to the control (figures 3 and 4).

[Figure 3 near here]

[Figure 4 near here]

4. Discussion

Plant consumption has increased worldwide for medicinal purposes, use as a functional food and as nutritional supplements (Kohler & Baghdadi-Sabeti 2011). Despite the often-unknown biological effects of the great diversity of molecules present in crude plant extracts, these substances are commonly used (Konan *et al*. 2007). However, even though plants are a source of important pharmacological activities, they may also present adverse effects, often associated with misuses by the population. Thus, natural products are currently the focus of several studies regarding safety and efficacy assessments.

The tucumã is a Brazilian fruit widely consumed by local population, presenting great nutritional value, mainly due to its high content of lipids, carbohydrates and proteins (Silva *et al.* 2018). Several natural compounds are known to possess antioxidant capacity, which is associated with the metabolites found in their structure. Our study showed a high content of polyphenols and flavonoids, in addition to antioxidant capacity, as demonstrated by the DPPH method. Our results are in agreement with those of the study performed by Sagrillo *et al.* (2015), in which a high content of polyphenols and flavonoids and strong antioxidant capacity were found in tucumã pulp extract. Moreover, a HPLC-PAD analysis revealed that the phenolic fraction of CETP was mostly composed by catechin and other hydroxybenzoate derivatives. Corroborating with Gonçalves *et al*. (2010), who identified flavonoids such as catechin and quercetin as bioactive compounds in this fruit. Due to their high antioxidant capacity, these compounds are associated with reduced risk of developing certain diseases, like heart and cardiovascular diseases, cancer and disorders associated with oxidative stress (Wu *et al*. 2019; Złotek *et al*. 2019). In tucumã fruit 21 different types of carotenoids have been identified, among which all-trans-β-carotene (47.36 μ/g), the precursor of vitamin A, represents 75% of all carotenoids (De Rosso & Mercadante 2007). Corroborating these studies, the present investigation showed that the major carotenoid found in this plant was β-carotene followed by lutein. Carotenoids are pigments found in colored fruits and green leafy vegetables. Its

consumption has been associated with health benefits such as reduced risk of age-related macular degeneration and cataract, some cancers and coronary heart disease (Eggersdorfer & Wyss 2018). The phytochemical characterization of tucumã fruit is not entirely clear in the literature. We performed a GC-MS analysis, which showed mainly the presence of unsaturated fatty acids, saturated fatty acids and triterpenes. The therapeutic potential of unsaponifiable components like phytosterols, triterpenes and unsaturated fatty acids in the prevention of cardiovascular diseases and atherosclerosis shows the importance of extracting and identifying these compounds (Huang *et al*. 2019). The consumption of saturated fatty acids, such as palmitic acid, is related to an increase in HDL and LDL, hence, it is associated with cardiovascular risk factors. On the other hand, linoleic acid, an unsaturated fatty acid, reduces the incidence of cardiovascular diseases, in addition to reducing the risk of Diabetes Mellitus (Silva *et al*. 2018).

In this study, we evaluated the toxicity of tucumã pulp extract through acute and repeated doses (28 days) in oral toxicity studies. Toxicity tests have an important role in the assessment of the toxic characteristics of a substance. After exposure to CETP in a single dose of 2000 mg/kg, no mortality or signs of toxicity were observed. Moreover, none of the analyses performed showed significant differences between groups. Therefore, the oral toxicity of this crude extract can be classified as category 5, in which acute lethal toxicity is greater than 2000 mg/kg, according to the Harmonised Overall System OECD.

Treatment with CETP at different doses for 28 days did not induce mortality nor did it triggered signs of toxicity in the animals. The open field test is one of the most popular ethological tests to evaluate anxiety-like behavior and locomotor activity in rodents (Kuniishi *et al.*, 2017). In our study there was no difference between groups regarding the parameters evaluated in the test. Changes in body and organ weights are a sensitive indicator of damage caused by the substance tested (Berenguer-Rivas *et al*. 2013), as is alteration to relative organ

weight. During the experiment period, both male and female animals showed a normal trend in body weight. The increase observed in liver and kidney weights of females from the group that received 200 mg/kg was accompanied by an increase in body weight, and no difference was found in relative organ weight, which suggests that CETP did not induce damages to the animals.

In addition, the consumption of water and food were different between the female groups treated with CETP, however no biological importance was attributed to this, since it was in accordance with the weights of each group. Moreover, no alteration was observed in renal and hepatic tissues through a histopathological analysis in female rats, demonstrating low toxicity of this extract after exposure to repeated doses in this sex. However, the alterations observed in the renal tissue of the group of male rats that received the highest dose (600 mg/kg) may indicate possible toxicity of the extract, which suggests toxicity depends on sex and dose, since this alteration was only observed in males. Sex differential has an important role in toxicological assessment due to the variability in responses to different toxic agents in the species, in which male animals are more suscetible to determined liver and kidney toxicants than females (Olayode *et al*. 2019). Some factors such as the rate of absorption and elimination of the toxic agent, the nature of the test agent and its metabolites and the interaction of the test agent with organ specific target enzymes may be involved on the variability (Olayode *et al*. 2019). In fact, the *in vitro* study performed by De Souza *et al*. (2013) showed that tucumã extract exhibited a relative genotoxic effect in mononuclear cells from human peripheral blood at higher concentrations (> 500 μg/mL) and longer time exposure. This indicates that duration of treatment and concentration of the extract are essential to determine safety and that at higher concentrations, both *in vitro* and *in vivo*, the species may induce toxicity. Therefore, it is important to consider variables that may influence treatment response, such as bioavailability and the absorption of compounds.

Histopathological alterations in renal tissue were not accompanied by alterations in hematological and biochemical parameters. Hematological parameters are important for assessing physiological and pathological status in humans and animals, since the hematopoietic system is very susceptible to toxic substances (Li *et al*. 2010; Khan *et al*. 2011). Exposure to CETP did not cause alterations in any of the hematological parameters analyzed. Clinical biochemistry assessments usually provide useful information on toxic effects especially regarding liver and kidney dysfunction. Enzymes ALT, AST, ALP and GGT are important biomarkers of liver health and an increase in their serum levels are indicative of organ damage. In this study, the treatment with CETP at 400 mg/kg reduced levels of ALT in females without altering other parameters, corroborating that no hepatic injury was induced by the extract. Similarly, renal biomarkers (CRE and BUN) were not altered after CETP administration at different doses, corroborating the absence of liver and renal toxicity. Total protein levels were altered in males from different groups; however, this change had no clinical significance since the values found were within the normal range for healthy rats of this age and sex (Lima *et al.* 2014).

Serum cholinesterase or butyrylcholinesterase can be found in the central and peripheral nervous system and in the liver. Decreased enzyme activity is observed when hepatocellular impairment occurs, since it is synthesized in the liver, which makes it an important biochemical marker of organ damage (Santarpia *et al*. 2013). Our experiment showed no significant differences between groups, indicating no organ damage. The liver plays an essential role in lipid metabolism, stages of lipid synthesis and transportation, therefore, an abnormal lipid profile may be an indication of severe liver dysfunction (Ghadir *et al*. 2010). Ferreira *et al*. (2008) showed that another species of tucumã (*A. vulgare*) is composed of fatty acids, which may be involved in a reduction of plasma cholesterol, triglycerides, and LDL, as well as an increase of HDL concentration. In our study, treatment with CETP increased HDL levels of

males and females and decreased LDL levels of males. Stearic acids are known to act moderately on reducing LDL levels and increasing HDL (Silva *et al*. 2018). Thus, the results found in this study may be important for future investigations on the use of tucumã extract to treat dyslipidemias, since stearic acid was shown to be present.

The development of chronic diseases is associated with oxidative stress, which is characterized by an imbalance between the antioxidant system and reactive species production. Malondialdehyde (MDA) is one of the final products generated by lipid peroxidation and is an important biological marker of oxidative damage (Mansour *et al.* 2008). Furthermore, protein carbonylation can lead to alteration in protein functions, which may result in the etiology or progression of various diseases (Levine 2002). Enzymes like CAT and SOD are part of an enzymatic defense strategy, which is responsible for neutralizing reactive species, therefore preventing oxidative damage. In this study, CETP was shown to decrease MDA levels in the renal tissue of females and increase SOD activity in the hepatic tissue of males, suggesting that the tucumã fruit may play an important role in preventing oxidative damage. These results may be attributed to the phenolic compounds found in the extract, which are known to possess high antioxidant capacity. Therefore, our results indicate that lower doses of tucumã extract are safe and have possible biological activities that may benefit the prevention and/or treatment of disorders.

5. Conclusion

In summary, the tucumã extract may be considered as non-toxic, since there were no critical alterations in biological and behavioral aspects, no mortality nor signs of toxicity after administration of either single or repeated doses. Hematological and biochemical parameters and hepatic histopathology corroborates the low toxicity of the extract. However, renal histopathological analyses showed potential toxic action of the extract in male rats at the highest

dose when administered repeatedly. These results show sex-dependent toxicity, in which males were more susceptible than females. Moreover, CETP did not increase oxidative damage nor did it reduce enzymatic activity. Therefore, the extract of the tucumã pulp may be considered safe when used at a low dose. However, further studies such as subchronic and chronic oral toxicities are needed to ensure the safe use of this fruit extract.

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Disclosure of interest

The authors report no conflict of interest.

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Table 1. Chemical composition of the CETP *n*-hexane fraction by GC-MS.

N°	Compound	Formula	Exact mass	CAS	SI	RT min	%A	References
1	Palmitic acid	$C_{16}H_{32}O_2$	256.24	57-10-3	96	31.54	**4.93**	Silva et al, 2018
2	Palmitic acid, ethyl ester	$C_{18}H_{36}O_2$	284.27	628-97-7	93	32.15	0.85	Andrade et al, 1998*
3	11-Octadecenoic acid	$C_{18}H_{34}O_2$	282.26	506-17-2	94	34.93	**16.79**	Bony et al, 2012*
4	Linoleic acid	$C_{18}H_{32}O_2$	280.24	60-33-3	92	34.98	**4.84**	Silva et al, 2018
5	Stearic acid	$C_{18}H_{36}O_2$	284.27	57-11-4	89	35.27	1.63	Silva et al, 2018
6	Oleic acid, ethyl ester	$C_{20}H_{38}O_2$	310.29	111-62-6	92	35.38	3.40	Lu et al, 2008*
7	13,16-Docosadienoic acid	$C_{22}H_{40}O_2$	336.30	7370-49-2	88	35.45	0.92	Shen et al, 2012*
8	Stearic acid, ethyl ester	$C_{20}H_{40}O_2$	312.30	111-61-5	83	35.83	0.20	Azmat et al, 2010*
9	Oleic alcohol	$C_{18}H_{36}O$	268.27	143-28-2	84	40.52	0.28	Mancio et al, 2017*
10	2-Palmitoylglycerol	$C_{19}H_{38}O_4$	330.28	23470-00-0	95	41.03	1.15	Sreeja et al, 2018**
11	Octadecanoic acid, 4-methoxy-, methyl ester	$C_{20}H_{40}O_3$	328.29	41015-55-8	66	43.26	0.69	Sarkar et al, 2017**
12	13-Heptadecyn-1-ol	$C_{17}H_{32}O$	252.25	56554-77-9	67	43.36	0.17	Al-Qudah et al,2010**
13	Diolein isomer	$C_{39}H_{72}O_5$	620.54	-		43.50	0.30	-
14	1-Monoolein	$C_{21}H_{40}O_4$	356.29	111-03-5	92	43.81	**7.62**	Ribeiro et al, 2018*
15	Linolein, 2-mono-	$C_{14}H_{26}O$	210.19	3443-82-1	88	43.89	2.32	Paganuzzi, 1999
16	1-Monostearin	$C_{21}H_{42}O_4$	358.31	123-94-4	83	44.14	0.26	Paganuzzi, 1999
17	Campesterol	$C_{28}H_{48}O$	400.37	474-62-4	83	51.10	0.43	Lognay et al, 1995*
18	Ergost-25-ene-3,5,6,12-tetrol	$C_{28}H_{48}O_4$	448.36	56052-97-2	70	51.21	0.26	Qiu et al, 2007**
19	Stigmasterol	$C_{29}H_{48}O$	412.37	83-48-7	63	51.49	0.13	Santos et al, 2013
20	Cycloeucalenol acetate	$C_{32}H_{52}O_2$	468.40	10376-42-8	85	51.94	1.57	Ramu et al, 2015**
21	γ-Sitosterol	$C_{29}H_{50}O$	414.39	83-47-6	93	52.21	2.73	Shanthi, 2013**
22	Cycloartenol	$C_{30}H_{50}O$	426.38	469-38-5	92	53.13	**5.00**	Bereau et al, 2003*
23	1,3-Dipalmitoylglycerol	$C_{35}H_{68}O_5$	568.50	502-52-3	63	59.62	1.18	Siew et al, 2000
24	1-Palmitoyl-3-oleoylglycerol	$C_{37}H_{70}O_5$	594.52	3343-30-4	87	62.87	**7.45**	Ray et al, 2014**
25	Diolein isomer	$C_{39}H_{72}O_5$	620.54	-		63.15	0.63	-
26	1,3-Diolein	$C_{39}H_{72}O_5$	620.54	2465-32-9	89	67.24	**23.26**	Ribeiro et al, 2018*
27	Glycerol 1-oleate 3-stearate	$C_{39}H_{74}O_5$	622.55	18266-27-8	89	67.63	**10.71**	Ray et al, 2014**

Note: (CAS) chemical abstracts service; (SI) similarity index based to Wiley 7, NIST 11 and FFNSC1.3 spectral libraries; (RT) retention time; (%A) relative area. *Not described to *A. aculeatum*, but described to Arecaceae. ** Not described to Arecaceae.

Table 2. Body and organ weights, and relative organ weight (%) after acute administration of CETP 2000 mg/kg in female rats.

Parameters	Study group	
	Control	Test
Initial body weight (g)	188.50 ± 10.46	199.20 ± 7.75
Final body weight (g)	219.20 ± 11.94	234.30 ± 11.98
Liver (g)	11.37 ± 1.64	12.27 ± 1.28
Kidney (g)	1.75 ± 0.14	1.86 ± 0.08
Relative organ weight (%)		
Liver	5.57 ± 0.58	5.67 ± 0.49
Kidney	0.86 ± 0.05	0.86 ± 0.04

Results are expressed as mean ± S.D. Student's *t*-test/Mann-Whitney (n = 6). The values were significantly different when $p < 0.05$. No differences were observed.

Table 3. Body, organ and relative organ weights (%) after repeated doses (28 days) administration of CETP at 200, 400 and 600 mg/kg in female and male rats.

Parameters	Study group			
	Control	200 mg/kg	400 mg/kg	600 mg/kg
Female				
Initial body weight (g)	102.60 ± 10.83	110.40 ± 7.70	121.60 ± 11.39	117.40 ± 9.09
Final body weight (g)	197.00 ± 12.81	217.40 ± 12.22	211.40 ± 14.94	205.80 ± 12.44
Liver (g)	10.64 ± 0.98	11.65 ± 0.35	10.51 ± 0.87	10.31 ± 0.90[b]
Kidney (g)	1.68 ± 0.10	1.81 ± 0.08	1.56 ± 0.05[b]	1.59 ± 0.06
Relative organ weight (%)				
Liver	6.61 ± 0.40	6.86 ± 0.34	6.24 ± 0.59	6.19 ± 0.67
Kidney	1.05 ± 0.06	1.05 ± 0.03	0.94 ± 0.05	0.96 ± 0.09
Male				
Initial body weight (g)	137.60 ± 14.10	130.20 ± 10.28	147.20 ± 3.27	142.00 ± 4.74
Final body weight (g)	283.20 ± 22.33	298.00 ± 16.45	298.40 ± 22.32	313.20 ± 21.76
Liver (g)	14.18 ± 1.50	15.41 ± 0.93	15.18 ± 1.17	15.58 ± 1.03
Kidney (g)	2.46 ± 0.32	2.43 ± 0.12	2.43 ± 0.23	2.34 ± 0.13
Relative organ weight (%)				
Liver	7.30 ± 0.24	7.47 ± 0.30	7.01 ± 0.25	7.13 ± 0.21
Kidney	1.14 ± 0.08	1.12 ± 0.06	1.12 ± 0.07	1.07 ± 0.06

Results are expressed as mean ± S.D. One-way ANOVA/Kruskal-Wallis followed by Tukey test/Dunn's (n = 5). The values were significantly different when $p < 0.05$. [(b)]Compared to the 200 mg/kg group.

Table 4. Hematological parameters of female and male rats after 28 days treatment with CETP at doses of 200, 400 and 600 mg/kg.

	Study groups			
Parameters	Control	CETP 200 mg/kg	CETP 400 mg/kg	CETP 600 mg/kg
Female				
RBC (10^6/µL)	7.73 ± 0.48	7.68 ± 0.29	7.88 ± 0.43	7.73 ± 0.54
HGB (g/dL)	15.12 ± 0.95	15.32 ± 0.80	15.60 ± 0.69	14.86 ± 1.03
HCT (%)	47.80 ± 2.78	48.20 ± 2.37	50.08 ± 2.83	47.92 3.65
MCV (fL)	61.14 ± 1.11	62.78 ± 1.58	63.62 ± 2.63	62.04 ± 2.60
CHCM (g/dL)	31.50 ± 0.23	31.72 ± 0.54	31.10 ± 1.01	31.00 ± 0.60
PLT (10^3/µL)	1376.00 ± 104.90	1353.00 ± 168.90	1432.00 ± 209.80	1626.00 ± 233.20
PP (mg/dL)	7.44 ± 0.47	7.40 ± 0.40	7.28 ± 0.22	7.36 ± 0.51
WBC (10^6/µL)	7.42 ± 1.72	7.58 ± 0.82	6.74 ± 1.14	8.66 ± 2.26
Neutrophils (%)	15.60 ± 5.17	15.20 ± 4.65	16.40 ± 3.36	23.00 ± 5.47
Lymphocytes (%)	80.20 ± 5.26	83.00 ± 4.84	80.60 ± 4.82	72.20 ± 6.09
Monocytes (%)	2.20 ± 1.64	1.20 ± 1.30	1.00 ± 1.41	1.20 ± 1.64
Eosinophils (%)	1.80 ± 1.64	0.40 ± 0.89	2.00 ± 1.22	1.60 ± 1.81
Basophils (%)	00.00 ± 00.00	00.00 ± 00.00	00.00 ± 00.00	00.00 ± 00.00
Male				
RBC (10^6/µL)	8.13 ± 0.70	7.60 ± 0.18	7.69 ± 0.08	7.91 ± 0.42
HGB (g/dL)	16.16 ± 1.37	14.82 ± 0.42	15.52 ± 0.21	15.84 ± 0.74
HCT (%)	51.00 ± 4.33	46.60 ± 2.03	46.50 ± 0.66	48.42 ± 2.12
MCV (fL)	61.22 ± 1.55	61.30 ± 1.60	60.52 ± 1.30	61.26 ± 2.04
CHCM (g/dL)	32.12 ± 0.67	32.13 ± 0.70	33.34 ± 0.70	32.66 ± 0.66
PLT (10^3/µL)	1570.00 ± 223.30	1627.00 ± 216.10	1460.00 ± 85.60	1529.00 ± 153.20
PP (mg/dL)	7.08 ± 0.10	6.56 ± 0.29	7.04 ± 0.26	6.56 ± 0.38
WBC (10^6/µL)	9.10 ± 1.65	7.96 ± 1.05	8.72 ± 0.98	8.96 ± 0.82
Neutrophils (%)	25.00 ± 4.12	27.00 ± 4.24	21.20 ± 3.34	24.20 ± 7.72
Lymphocytes (%)	73.20 ± 6.22	69.60 ± 5.22	74.80 ± 4.08	71.00 ± 6.28
Monocytes (%)	2.00 ± 2.34	1.00 ± 1.00	2.80 ± 2.16	1.80 ± 1.48
Eosinophils (%)	1.20 ± 1.09	2.40 ± 1.81	1.20 ± 0.83	3.00 ± 3.67
Basophils (%)	00.00 ± 00.00	00.00 ± 00.00	00.00 ± 00.00	00.00 ± 00.00

Results are expressed as mean ± S.D. One-way ANOVA/Kruskal-Wallis followed by Tukey test/Dunn's (n = 5). The values were significantly different when $p < 0.05$. No significant differences were observed.

Table 5. Biochemical parameters of female rats after 28 days treatment with CETP at doses of 200, 400 and 600 mg/kg.

	Study groups			
Parameters	Control	200 mg/kg	400 mg/kg	600 mg/kg
Female				
ALT (U/L)	60.50 ± 6.45	48.50 ± 6.55	35.75 ± 2.63[a]	43.20 ± 6.41
AST (U/L)	89.80 ± 12.34	75.20 ± 12.52	74.20 ± 14.48	77.20 ± 10.08
ALP (U/L)	76.83 ± 8.34	50.98 ± 15.01	69.38 ± 16.95	73.62 ± 13.05
GGT (U/L)	2.00 ± 3.46	1.00 ± 1.73	0.80 ± 1.30	1.80 ± 1.64
BUN (mg/dL)	42.80 ± 4.43	35.60 ± 5.45	39.80 ± 2.49	38.20 ± 4.26
CRE (mg/dL)	0.62 ± 0.04	0.68 ± 0.04	0.68 ± 0.08	0.72 ± 0.15
TP (g/dL)	6.78 ± 0.20	6.60 ± 0.31	6.56 ± 0.47	7.08 ± 1.04
ALB (g/dL)	3.04 ± 0.20	3.08 ± 0.16	2.96 ± 0.21	2.97 ± 0.23
BChE (U/L)	621.30 ± 141.70	765.50 ± 93.51	637.90 ± 271.70	549.80 ± 99.13
GLU (mg/dL)	238.40 ± 30.96	260.30 ± 54.24	238.80 ± 48.05	235.00 ± 58.13
CHOL (mg/dL)	87.75 ± 14.52	87.20 ± 8.16	84.00 ± 7.77	90.00 ± 6.21
LDL (mg/dL)	6.64 ± 0.78	5.87 ± 1.09	6.70 ± 0.64	7.14 ± 0.96
HDL (mg/dL)	25.91 ± 2.51	25.43 ± 1.31	26.99 ± 3.87	31.45 ± 1.99[b]
TG (mg/dL)	248.80 ± 67.29	208.60 ± 41.81	189.00 ± 39.89	155.00 ± 37.06
Male				
ALT (U/L)	51.00 ± 8.86	40.80 ± 2.77	52.25 ± 4.64	48.40 ± 9.58
AST (U/L)	81.00 ± 12.36	69.20 ± 3.27	77.20 ± 3.34	73.80 ± 6.30
ALP (U/L)	694.00 ± 135.60	551.00 ± 134.90	594.80 ± 152.00	708.50 ± 15.89
GGT (U/L)	1.20 ± 0.83	1.20 ± 1.30	1.60 ± 1.14	2.20 ± 1.48
BUN (mg/dL)	33.60 ± 4.98	38.60 ± 5.77	39.80 ± 6.87	41.00 ± 4.74
CRE (mg/dL)	0.66 ± 0.05	0.68 ± 0.04	0.74 ± 0.08	0.68 ± 0.04
TP (g/dL)	6.66 ± 0.18	6.12 ± 0.28[a]	6.78 ± 0.31[b]	6.58 ± 0.29
ALB (g/dL)	2.88 ± 0.13	2.70 ± 0.10	2.90 ± 0.14	2.96 ± 0.19
BChE (U/L)	300.20 ± 76.39	220.60 ± 69.00	269.10 ± 35.47	246.90 ± 27.61
GLU (mg/dL)	283.40 ± 53.55	310.20 ± 44.91	326.00 ± 45.70	290.80 ± 73.82
CHOL (mg/dL)	83.40 ± 5.59	78.80 ± 6.61	80.40 ± 11.22	74.20 ± 3.83
LDL (mg/dL)	9.14 ± 2.39	11.84 ± 2.62	6.47 ± 0.14[b]	6.42 ± 1.07[b]
HDL (mg/dL)	17.20 ± 5.19	23.24 ± 3.83	26.27 ± 3.66[a]	27.05 ± 2.20[a]
TG (mg/dL)	200.80 ± 12.07	194.40 ± 49.70	230.00 ± 99.85	204.00 ± 63.35

Results are expressed as mean ± S.D. One-way ANOVA/Kruskal-Wallis followed by Tukey test/Dunn's (n = 5). The values were significantly different when $p < 0.05$. [(a)]Different from the control. [(b)]Different from the 200 mg/kg group.

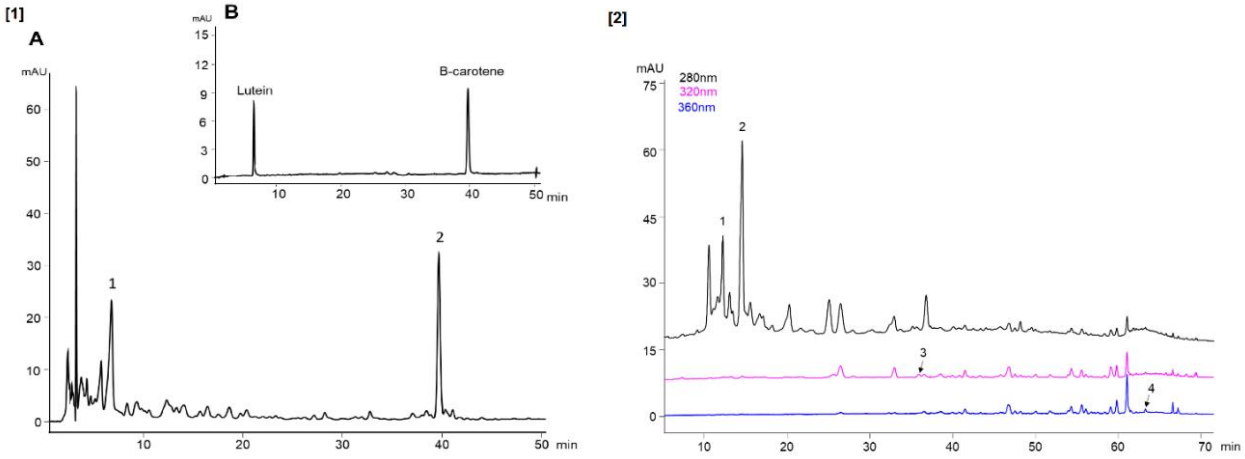

Figure 1

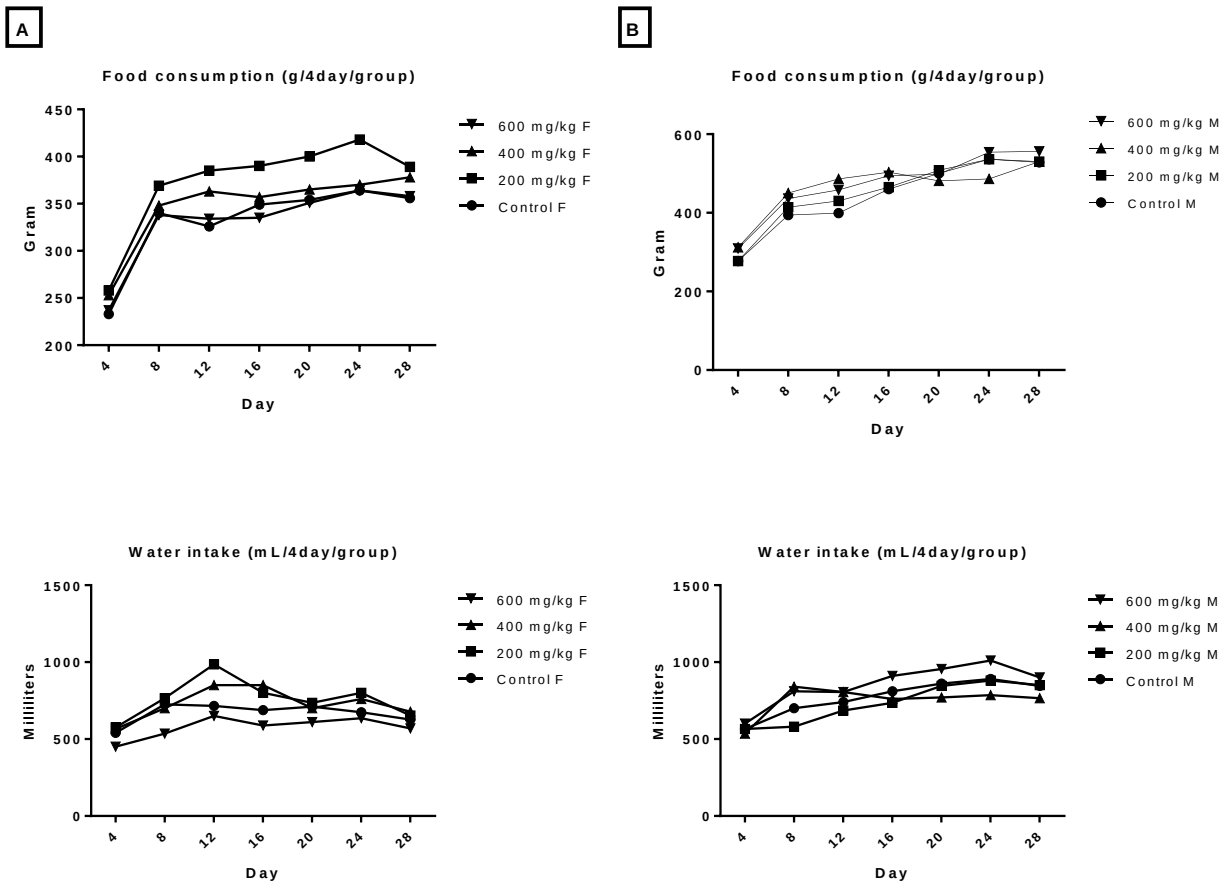

Figure 2

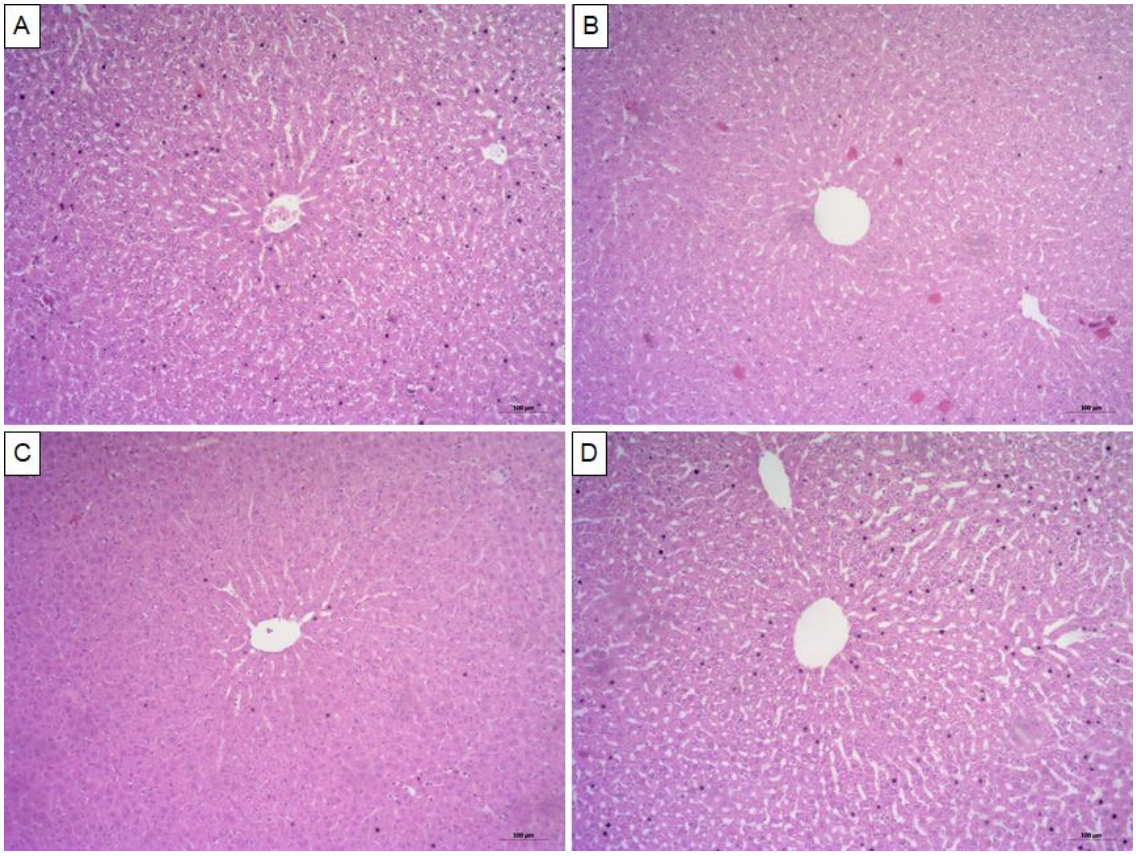

Figure 3

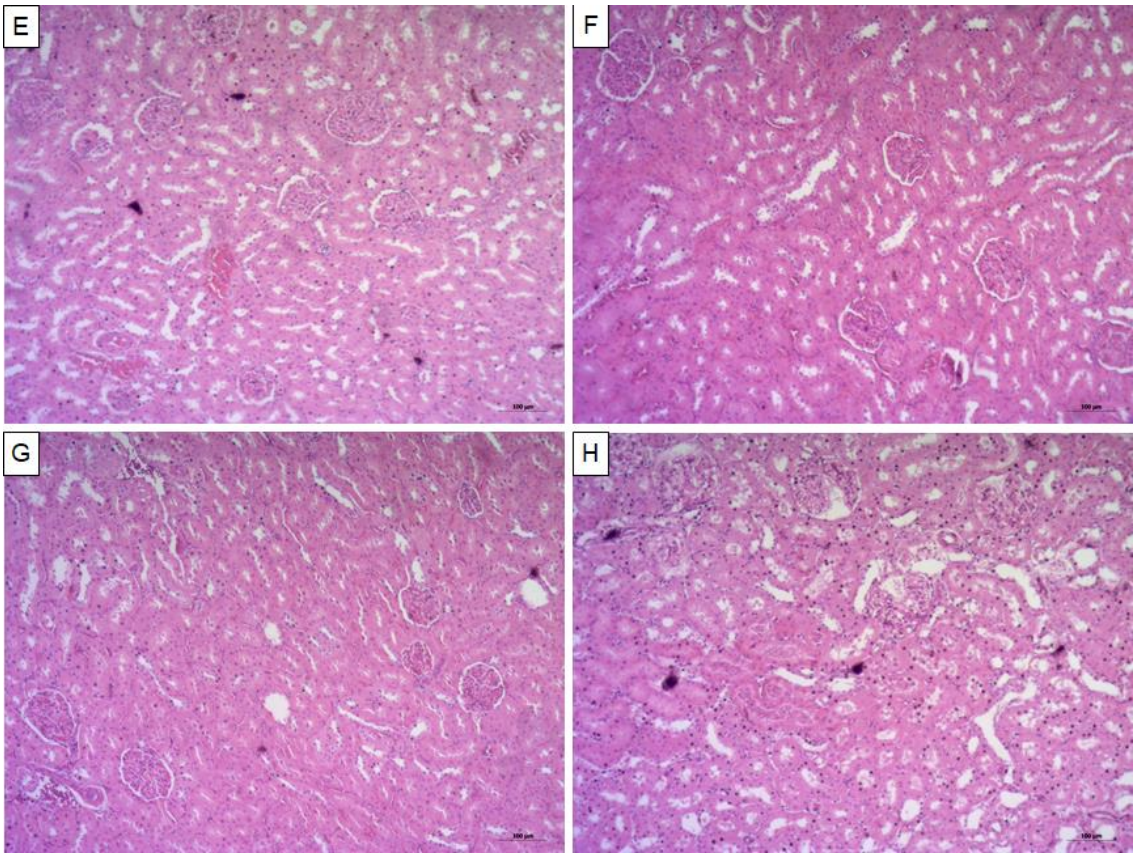

Figure 4

Legends for figures

Figure 1. Representative chromatogram of CETP carotenoids **[1]**: HPLC-PDA CETP chromatogram acquired at 450 nm (A); chromatogram of carotenoids standards at 450 nm (B). Peak 1: Lutein; Peak 2: β-carotene. **[2]** Chromatograms acquired at 280 nm, 320 nm and 360 nm. Peak 1: 4-OH benzoic acid; peak 2: Catechin; peak 3: Trans-ferrulic acid; peak 4: Quercetin.

Figure 2. Mean food and water consumption of treated rats and controls measured every 4 days during the study. (A) Female; (B) Male.

Figure 3. Photomicrographs of histological sections from liver tissue in female rats from **(A)** control group and **(B)** CETP 600 mg/kg treated-group; Photomicrographs of histological sections from liver tissue in male rats from **(C)** control group and **(D)** CETP 600 mg/kg treated-group; note the morphology of cells with normal appearance. (Hematoxylin/eosin, 100X, scale bar = 100 μm).

Figure 4. Photomicrographs of histological sections from kidney tissue in female rats from **(E)** control group and **(F)** CETP 600 mg/kg treated-group - note the morphology of cells with normal appearance. Photomicrographs of histological sections from kidney tissue in male rats from **(G)** control group and **(H)** CETP 600 mg/kg treated-group – note the loss of normal morphological structure at the highest dose of CETP. (Hematoxylin/eosin, 100X, scale bar = 100 μm).

SUPPLEMENTARY MATERIAL

Tucumã (*Astrocaryum aculeatum*) extract: phytochemical characterization and acute and subacute oral toxicity studies in *Wistar* rats

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Phytochemical analyses

Table 1S. Total polyphenols content and total flavonoids in CETP expressed on mg acid galic equivalent (GAE)/g dry fraction and mg rutin equivalent (RE)/g dry fraction, respectively. 50% Inhibitory Concentration (IC_{50}) determined by DPPH method.

Table 2S. Carotenoid quantification of CETP.

Table 3S. Phenolic compounds quantification of CETP.

Figure 1S. Structures described from CETP n-hexane fraction.

Acute oral toxicity

Table 4S. Hematological parameters not altered after acute administration of CETP 2000 mg/kg in female rats.

Table 5S. Biochemical parameters not altered after acute administration of CETP 2000 mg/kg in female rats.

Table 6S. Activities of CAT, SOD and MDA content on hepatic and renal tissues after acute administration of CETP 2000 mg/kg in female rats.

Figures 2S and 3S. Photomicrographs of histological sections from liver and kidney tissues in female rats after exposition to CETP in a single dose of 2000 mg/kg.

Repeated dose 28-day oral toxicity (subacute)

Table 7S. Parameters assessed in the open field test in female and male rats exposed to CETP at doses of 200, 400 and 600 mg/kg during 28 days.

Table 8S. MDA and carbonyl content; activities of CAT and SOD on hepatic and renal tissues after repeated administration of CETP (200, 400 and 600 mg/kg) in female rats.

Table 9S. MDA and carbonyl content; activities of CAT and SOD on hepatic and renal tissues tissue after repeated administration of CETP (200, 400 and 600 mg/kg) in male rats.

Figure 4S. Body weights of the animals during the 28 days of treatment at different doses of CETP (200, 400 and 600 mg/kg).

Table 10S. Food and water consumption of animals in different groups every four days of treatment.

PHYTOCHEMICAL ANALYSES

Table 1S. Total polyphenols content and total flavonoids in CETP expressed on mg acid galic equivalent (GAE)/g dry fraction and mg rutin equivalent (RE)/g dry fraction, respectively. 50% Inhibitory Concentration (IC_{50}) determined by DPPH method.

Astrocaryum aculeatum (Tucumã)	
PT (mg GAE/g)	3.00 ± 0.30
FT (mg RE/g)	2.76 ± 0.10
IC_{50} Tucumã (µg/mL)	5.22 ± 0.33
IC_{50} Ascorbic acid (µg/mL)	2.76 ± 0.35

Data are expressed as mean ± standard deviation (S.D).

Table 2S. Carotenoids quantification of CETP.

	Quantification (mg/100 g)*	%
Lutein	0.246 ± 0.002	21.1
β-carotene	0.305 ± 0.001	26.2
Non-identified carotenoids	0.664 ± 0.008	52.7
Total carotenoids	1.215 ± 0.013	100

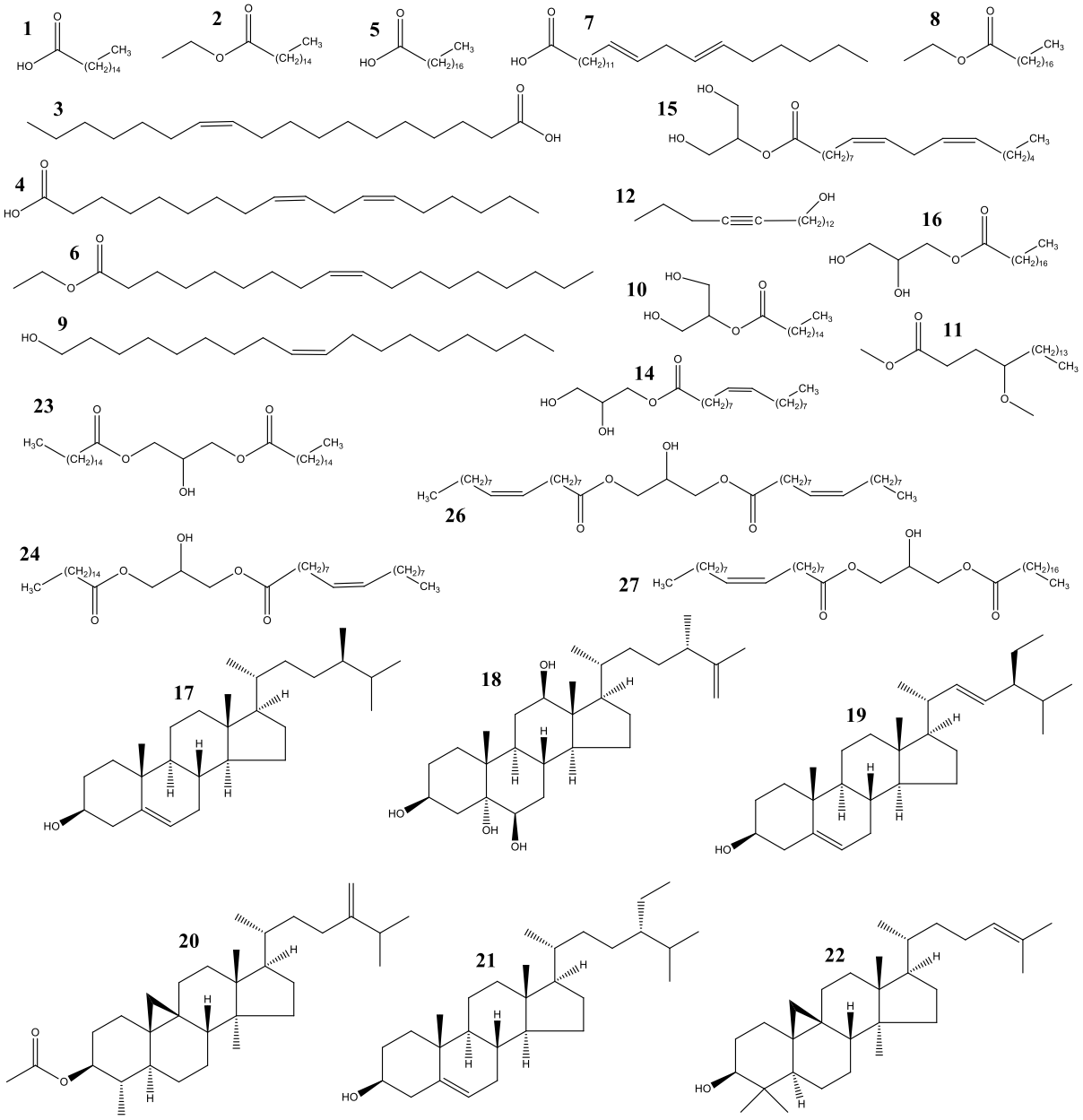
Results express as mean ± standard deviation. *Carotenoids were quantified as equivalents of β-carotene.

Table 3S. Phenolic compounds quantification of CETP.

	λ (nm)	Quantification mg/100g*
Hydroxybenzoate derivatives	280	3139.43 ± 113.56
Hydroxycinnamate derivatives	320	8.62 ± 1.61
Flavonol derivatives	360	163.17 ± 6.22
Total phenolic compounds		3314.76 ± 126.38

Results express as mean ± standard deviation; *Hydroxybenzoate derivatives were quantified as equivalent to catechin, hydroxycinnamate derivatives were quantified as equivalent to trans-ferulic acid and flavonols derivatives were quantified as equivalent to quercetin.

Figure 1S. Structures described from CETP *n*-hexane fraction.



ACUTE ORAL TOXICITY

Table 4S. Hematological parameters not altered after acute administration of CETP 2000 mg/kg in female rats.

Parameters	Study group	
	Control	Test
RBC (10^6/µL)	7.71 ± 0.27	7.68 ± 0.72
HGB (g/dL)	13.40 ± 0.69	13.83 ± 1.10
HCT (%)	41.88 ± 1.84	42.85 ± 2.19
MCV (fL)	54.28 ± 1.49	56.02 ± 2.57
CHCM (g/dL)	31.88 ± 0.43	32.20 ± 1.04
PP (mg/dL)	7.00 ± 0.25	6.93 ± 0.27
PLT (10^3/µL)	837.17 ± 127.64	807.00 ± 168.57
WBC (10^6/µL)	5.77 ± 1.62	4.38 ± 2.43
Neutrophils (%)	22.83 ± 5.78	22.50 ± 7.06
Lymphocytes (%)	74.17 ± 4.75	73.00 ± 7.56
Monocytes (%)	1.83 ± 0.75	2.33 ± 1.03
Eosinophils (%)	2.33 ± 0.81	2.00 ± 0.63
Basophils (%)	0.00 ± 0.00	0.00 ± 0.00

Results are expressed as mean ± S.D. Student's *t*-test/Mann-Whitney (n = 6). The values were considered to be significantly different when $p < 0.05$. No significant differences were observed.

Table 5S. Biochemical parameters not altered after acute administration of CETP 2000 mg/kg in female rats.

Parameters	Study group	
	Control	Test
ALB (g/dL)	2.90 ± 0.36	2.81 ± 0.41
ALT (UI/L)	43.50 ± 9.31	38.33 ± 9.85
AST (UI/L)	109.50 ± 26.62	92.00 ± 21.77
ALP (UI/L)	49.90 ± 15.44	46.10 ± 8.91
CRE (mg/dL)	0.58 ± 0.04	0.58 ± 0.04
BUN (mg/dL)	40.17 ± 3.18	37.50 ± 6.28

Results are expressed as mean ± S.D. Student's *t*-test/Mann-Whitney (n = 6). The values were considered to be significantly different when $p < 0.05$. No significant differences were observed.

Table 6S. Activities of CAT, SOD and MDA content on hepatic and renal tissues after acute administration of CETP 2000 mg/kg in female rats.

Parameters	Study group	
	Control	Test
Liver		
MDA	1.01 ± 0.18	1.20 ± 0.14
CAT	25.76 ± 3.01	26.37 ± 3.68
SOD	5.96 ± 3.28	8.74 ± 1.67
Kidney		
MDA	0.77 ± 0.12	0.70 ± 0.12
CAT	22.24 ± 2.84	21.54 ± 2.95
SOD	15.38 ± 2.28	14.01 ± 3.31

Results are expressed as mean ± S.D. Student's *t*-test/Mann-Whitney (n = 6). The values were considered to be significantly different when $p < 0.05$. No differences were observed. MDA expressed in nmol MDA/mg prot. CAT expressed in U CAT/mg prot. SOD expressed in U SOD/mg prot.

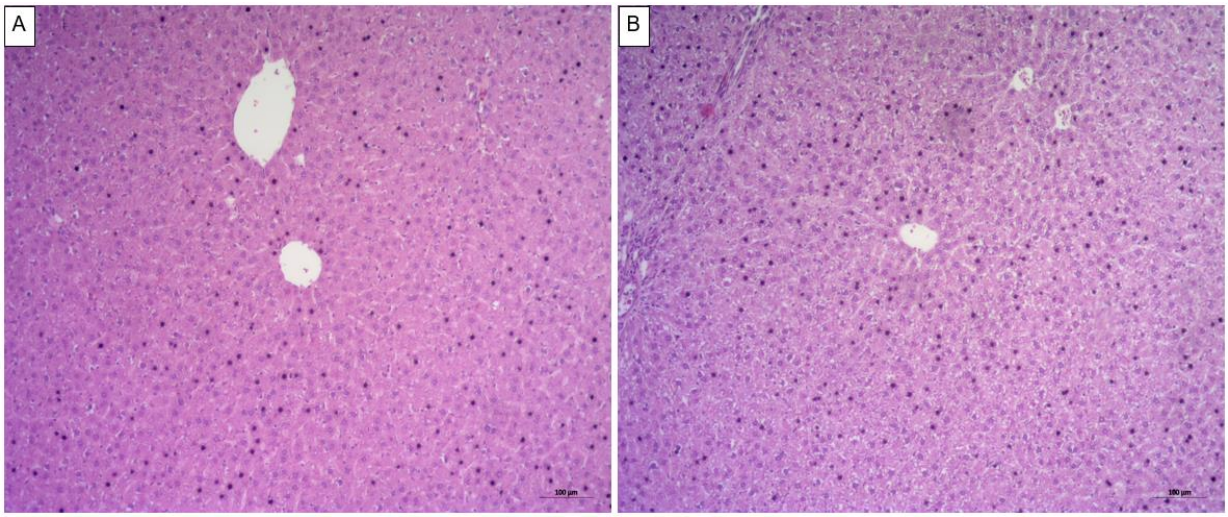


Figure 2S. Photomicrographs of histological sections from liver tissue in female rats after exposition to CETP in a single dose of 2000 mg/kg. **(A)** control group and **(B)** test group showing morphology of cells with normal appearance. (Hematoxylin/eosin, 100X, scale bar = 100 µm).

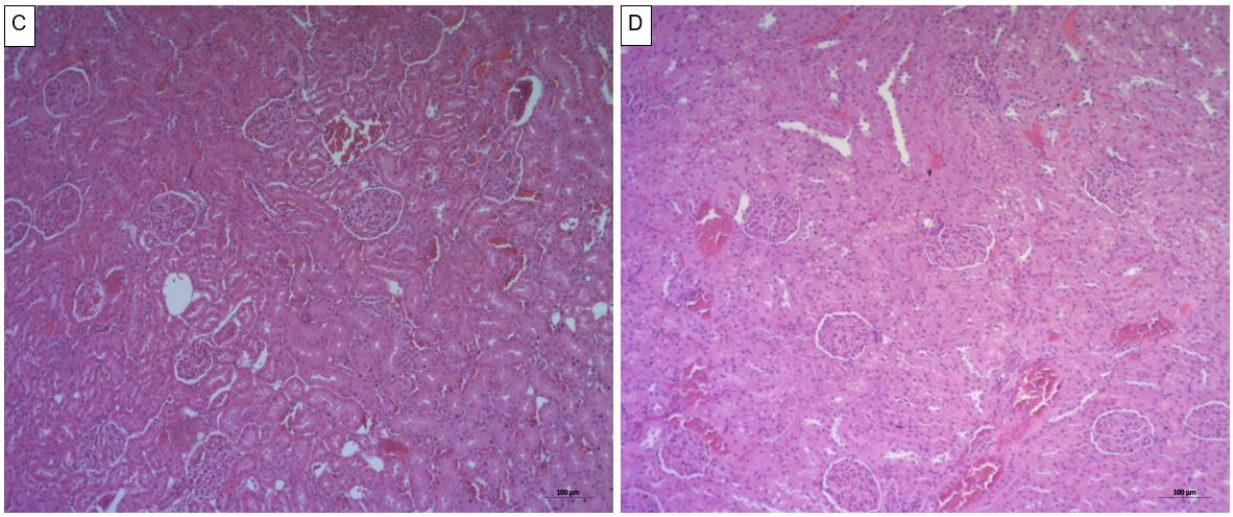


Figure 3S. Photomicrographs of histological sections from kidney tissue in female rats after exposition to CETP in a single dose of 2000 mg/kg. **(C)** control group and **(D)** test group showing morphology of cells with normal appearance. (Hematoxylin/eosin, 100X, scale bar = 100 µm).

REPEATED DOSE 28-DAY ORAL TOXICITY

Table 7S. Parameters assessed in the open field test in female and male rats exposed to CETP at doses of 200, 400 and 600 mg/kg during 28 days.

Parameters	Study group			
	Control	CETP 200 mg/kg	CETP 400 mg/kg	CETP 600 mg/kg
Female				
Latency (s)	1.40 ± 0.54	1.40 ± 0.54	1.40 ± 0.54	1.40 ± 0.54
Crossings (nº)	36.33 ± 14.22	27.60 ± 9.31	33.00 ± 11.98	26.80 ± 10.64
Rearings (nº)	8.00 ± 2.64	6.00 ± 2.82	9.00 ± 5.78	8.00 ± 3.16
Grooming activity (nº)	8.00 ± 1.87	6.00 ± 4.06	3.60 ± 1.51	5.60 ± 2.96
Number of defecation boli	2.80 ± 1.92	1.80 ± 2.04	1.80 ± 1.48	2.60 ± 2.07
Immobility period (s)	17.40 ± 29.88	29.75 ± 29.40	29.60 ± 27.27	30.50 ± 34.80
Male				
Latency (s)	1.30 ± 0.44	1.40 ± 0.54	1.40 ± 0.54	1.40 ± 0.54
Crossings (nº)	29.60 ± 12.92	37.20 ± 9.41	34.67 ± 12.10	37.60 ± 6.69
Rearings (nº)	10.20 ± 6.61	6.80 ± 2.77	4.20 ± 3.27	6.60 ± 4.21
Grooming activity (nº)	5.60 ± 4.09	5.00 ± 2.12	6.80 ± 2.38	3.20 ± 1.30
Number of defecation boli	2.60 ± 1.34	3.20 ± 1.30	2.60 ± 2.40	3.00 ± 2.00
Immobility period (s)	26.00 ± 28.22	39.20 ± 27.34	67.50 ± 15.00	61.80 ± 34.87

Results are expressed as mean ± S.D. One-way ANOVA/Kruskal-Wallis followed by Tukey test/Dunn's (n = 5). The values were considered to be significantly different when $p < 0.05$. No differences were observed.

Table 8S. MDA and carbonyl content; activities of CAT and SOD on hepatic and renal tissues after administration of repeated doses of CETP (200, 400 and 600 mg/kg) in female rats.

Parameters	Study groups			
	Control	CETP 200 mg/kg	CETP 400 mg/kg	CETP 600 mg/kg
Liver				
MDA	1.96 ± 0.32	1.34 ± 0.47	1.69 ± 0.36	1.40 ± 0.64
Carbonyl	1.45 ± 0.33	1.84 ± 0.24	2.05 ± 0.63	2.21 ± 0.37
CAT	15.07 ± 2.26	14.57 ± 2.24	15.65 ± 4.59	17.96 ± 2.24
SOD	11.06 ± 4.96	8.78 ± 4.76	9.97 ± 6.47	7.66 ± 3.20
Kidney				
MDA	0.49 ± 0.13	0.36 ± 0.07	0.32 ± 0.05[a]	0.35 ± 0.03
Carbonyl	2.03 ± 0.57	1.57 ± 0.71	1.51 ± 0.35	1.23 ± 0.45
CAT	11.85 ± 2.36	11.21 ± 2.94	13.15 ± 1.72	14.60 ± 1.44
SOD	7.68 ± 3.12	10.93 ± 3.70	14.93 ± 6.04	7.69 ± 3.32

Results are expressed as mean ± S.D. One-way ANOVA/Kruskal-Wallis followed by Tukey test/Dunn's (n = 5). The values were considered to be significantly different when $p < 0.05$. [(a)]Different from the control group. MDA expressed in nmol MDA/mg prot. Carbonyl expressed in nmol carbonyl/mg prot. CAT expressed in U CAT/mg prot. SOD expressed in U SOD/mg prot.

Table 9S. MDA and carbonyl content; activities of CAT and SOD on hepatic and renal tissues tissue after administration of repeated doses of CETP (200, 400 and 600 mg/kg) in male rats.

Parameters	Study groups			
	Control	CETP 200 mg/kg	CETP 400 mg/kg	CETP 600 mg/kg
Liver				
MDA	1.45 ± 0.59	1.29 ± 0.38	1.77 ± 0.29	1.71 ± 0.27
Carbonyl	1.45 ± 0.44	2.42 ± 0.49	1.83 ± 0.77	2.03 ± 0.24
CAT	14.87 ± 1.02	14.74 ± 2.48	14.02 ± 1.38	13.64 ± 1.00
SOD	7.15 ± 4.17	17.44 ± 6.49[a]	20.15 ± 3.23[a]	13.25 ± 3.68
Kidney				
MDA	0.26 ± 0.05	0.31 ± 0.05	0.27 ± 0.10	0.32 ± 0.04
Carbonyl	1.32 ± 0.43	1.40 ± 0.25	1.58 ± 0.51	1.54 ± 0.21
CAT	13.79 ± 1.70	13.14 ± 2.36	14.81 ± 0.71	14.87 ± 1.54
SOD	9.70 ± 7.13	12.96 ± 4.43	13.05 ± 6.34	9.86 ± 1.12

Results are expressed as mean ± S.D. One-way ANOVA/Kruskal-Wallis followed by Tukey test/Dunn's (n = 5). The values were considered to be significantly different when $p < 0.05$. [(a)]Different from the control group. MDA expressed in nmol MDA/mg prot. Carbonyl expressed in nmol carbonyl/mg prot. CAT expressed in U CAT/mg prot. SOD expressed in U SOD/mg prot.

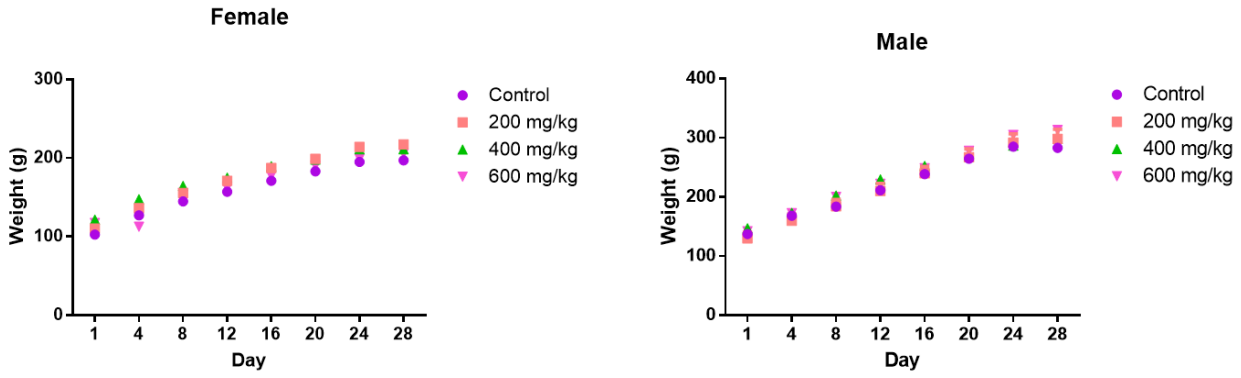


Figure 4S. Body weights of the animals during the 28 days of treatment at different doses of CETP (200, 400 and 600 mg/kg).

Table 10S. Food and water consumption of animals in different groups every four days of treatment.

	Study group			
	Control	CETP 200 mg/kg	CETP 400 mg/kg	CETP 600 mg/kg
Female				
Food consumption (g/4day)	331.70 ± 45.23	372.70 ± 52.73[a,c]	347.70 ± 42.82	331.00 ± 43.07
Water intake (mL/4day)	668.40 ± 65.47	759.00 ± 129.20[c]	729.00 ± 101.20[c]	576.90 ± 68.28
Male				
Food consumption (g/4day)	442.00 ± 92.46	451.40 ± 90.37	464.00 ± 71.22	472.10 ± 84.66
Water intake (mL/4day)	772.90 ± 113.60	734.30 ± 130.20	751.40 ± 99.40	855.70 ± 134.50

Results are expressed as mean ± S.D. One-way ANOVA/Kruskal-Wallis followed by Tukey test/Dunn's (n = 5). The values were considered to be significantly different when $p < 0.05$. [(a)]Different from the control. [(c)]Different from the 600 mg/kg group.

6 ARTIGO 3 – POTENTIAL EFFECTS OF *Astrocaryum aculeatum* EXTRACT AGAINST CYCLOPHOSPHAMIDE-INDUCED HEMATOLOGIC AND ORGAN TOXICITY IN RATS

DELINEAMENTO EXPERIMENTAL: Para este experimento, foram utilizados 72 ratos *Wistar* machos provenientes do Biotério Central da UFSM. Os animais foram mantidos em condições adequadas e aclimatizados por 10 dias antes do início do experimento. Nesse trabalho, investigamos o potencial efeito do extrato de tucumã em atenuar os efeitos tóxicos da ciclofosfamida (CFF). Analisamos tanto o tratamento quanto o pré-tratamento com o extrato de tucumã. Para isso, o experimento foi dividido em dois protocolos:

1) **Tratamento**	2) **Pré-tratamento**
I) Salina 0,9% + Água filtrada	**VII)** Água filtrada + Salina 0,9%
II) CFF* + Água filtrada	**VIII)** Água filtrada + CFF*
III) CFF + Levamisol 2,5 mg/kg	**IX)** Levamisol 2,5 mg/kg + CFF
IV) CFF + EBT** 100 mg/kg	**X)** EBT** 100 mg/kg + CFF
V) CFF + EBT 200 mg/kg	**XI)** EBT 200 mg/kg + CFF
VI) CFF + EBT 400 mg/kg	**XII)** EBT 400 mg/kg + CFF

*CFF = ciclofosfamida 150 mg/kg; **EBPT = extrato bruto de tucumã

n = 6 animais/por grupo → 3 animais por caixa

A CFF foi administrada intraperitonealmente (ip) em três dias na dose de 50 mg/kg (total = 150 mg/kg); o grupo controle recebeu salina 0,9% via ip e a droga padrão escolhida foi o levamisol (via oral). Através do estudo de toxicidade de doses repetidas foi possível observar toxicidade renal do extrato em ratos machos na dose mais alta (600 mg/kg). Por isso, as doses de extrato utilizadas neste tratamento foram de 100, 200 e 400 mg/kg, administradas via oral, durante 14 dias. No decorrer do tratamento foram registrados o peso corporal e consumo de água e ração. Ao final do experimento, os animais foram eutanasiados e sangue e órgãos foram coletados para as posteriores análises. O artigo a seguir traz o trabalho na íntegra e foi submetido à revista *Chemico-Biological Interactions*.

Potential effects of *Astrocaryum aculeatum* **extract against cyclophosphamide-induced hematologic and organ toxicity in rats**

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Abstract

Cyclophosphamide (CPP) is a cytotoxic alkylating agent widely used for the treatment of various types of cancer, rheumatic arthritis and lupus. However, its adverse effects like hepatotoxicity, nephrotoxicity and myelosuppression are often associated with the treatment interruption. Natural products are a great alternative to attenuate these toxic effects. Tucumã is a fruit from Amazon region widely consumed by local population with important bioactive compounds. Therefore, we aimed to characterize the tucumã pulp extract (TPE) by UHPLC-ESI-HRMS and to investigate its potential benefit against CPP toxic effects. The spectrometric analysis showed the presence of fatty acids (major compounds), flavonoids, catechins, phenylpropanoid-derivative and organic acids in the TPE. The animals were exposed to CPP at dose of 150 mg/kg via i.p. and divided into treated or pretreated with TPE at 100, 200 and 400 mg/kg, orally. Physiological aspects, hematological and biochemical parameters, markers of oxidative stress and enzyme antioxidant activity were assessed. Results show that CPP induced toxicity to the animals, indicate by reduced body weight during the experiment and a lower relative organ weight of kidney, liver, thymus and spleen. Administration of TPE was not able to attenuate efficiently these effects, as well as in biochemical and hematological analyses. However, TPE reduced the malondialdehyde and carbonyl content, while increased the superoxide dismutase activity. In summary, the pretreatment with TPE showed the better results, indicating that extract can act by attenuating the oxidative damage. Thereby, further studies are necessary to better elucidate the mechanisms of action by which the tucumã extract acts and its biological activities in experimental models *in vivo*.

Keywords: Cancer. Arecaceae. Tucumã. Fatty acids. Flavonoids. *Wistar*.

1 Introduction

Cytotoxic drugs are often used as chemotherapy treatment to several types of cancers and for long-term immunosuppressive therapy [1]. Cyclophosphamide (CPP) is a cytotoxic alkylating agent widely used for the treatment of leukemia, multiple myeloma, lymphomas, rheumatic arthritis, lupus and as immunosuppressant for bone marrow transplantation [2]. The use of these drugs is limited due to its serious adverse effects like hepatotoxicity, nephrotoxicity, urotoxicity, cardiotoxicity and myelosuppression [3]. Studies have been reported the important role of oxidative stress in the toxicity induced by CPP. Increased generation of reactive oxygen species (ROS) is associated with myelossupression, renal damage and hepatotoxicity [4,5,6]. Oxidative stress causes severe cellular damage associated with lipid peroxidation and alterations of proteins and nucleic acids, which can lead to the impairment of cell functions and cytolysis [7,8,9].

In this scenario, antioxidant dietary compounds play an important role in stress oxidative prevention, since they can act as free radical scavengers. Fruits and vegetables are known for their antioxidant compounds like phenolic compounds, carotenoids, anthocyanins and tocopherols [10]. Therefore, it is important to consider antioxidant natural products as a effective approach against various disorders and diseases induced by oxidative stress [3], including in the attenuation of CPP-induced toxicity.

The Amazon region is known for its great diversity of fruit species, which can be included the species *Astrocaryum aculeatum.* Popularly known as “tucumã” or “tucumã-do-Amazonas”, it belongs to the Arecaceae family and is a plam tree native from tropical South America. Its fruits are widely consumed by local population in different forms: consumed *in natura*, in sandwiches and tapioca, desserts and ice cream [11]. In our previous study, tucumã extract phytochemical characterization showed the presence of carotenoids, flavonoids, triterpenes and unsaturated and satured fatty acids [12], which are responsible for provide health benefitis.

Therefore, given the importance of plants as therapeutic support for the treatment or prevention of many disorders, the present study aimed to investigate the potential benefitial effect of tucumã fruit extract on attenuation of blood, liver and kidney toxicity induced by CPP in *Wistar* rats.

2 Methods

2.1 Chemicals and reagents

Kits for all biochemical parameters were donated from Bioclin/Quibasa (Minas Gerais, Brazil). Cyclophosphamide was obtained from *Hospital Universitário de Santa Maria* (Genuxal®, Baxter Healthcare S/A, Germain). Levamisole was obtained commercially (Ascaridil®, Janssen-Cillag Farmacêutica, Brasil). All others chemicals and reagents were of analytical grade.

2.2 Plant collection and extract preparation

Tucumã fruit was collected directly from native palm trees found in primary and secondary forests from a composite sample representing a mixture of progenies from Manaus City (Amazonas State, Brazil), located in the Amazonian region (3º6'26''S, 60º1'34''W). The tucumã pulp (5 kg) was obtained in June, 2018 and the ethanolic extract was prepared according to our previous study [12]. We obtained 107 g of dried tucumã pulp extract (TPE). The present study is registered in the *Sistema Nacional de Gestão de Patrimônio Genético e do Conhecimento Tradicional Associado* (SisGen) under the number AADC663.

2.3 Qualitative chemical analysis by UHPLC-ESI-HRMS

The TPE was dissolved in methanol (HPLC grade) at 1 mg/mL then filtered through PTFE (0.22 µm). The sample was analyzed by UHPLC-ESI-HRMS/Orbitrap (Accela-Exactive Plus, Thermo Scientific®). The chromatographic analysis of the extract was performed on an ACE® analytical column (3.0 × 150 mm, 3 µm) placed in an oven maintained at 40 °C using 0.1% formic acid aqueous as solvent A and acetonitrile with 0.1% formic acid as solvent B under the following gradient elution program: 10-100% of solvent B in 30 min in linear gradient, 400 µL/min flow rate. Electrospray ionization method was used for mass spectrometry under the following conditions: spray voltage = 3.5 kV in negative mode, temperature of the capillary at 300 °C and mass range: *m/z* 150-1500.

2.4 Experimental animals

Male *Wistar* rats weighing 150 – 200 g were obtained from Animal House of Universidade Federal de Santa Maria (UFSM). The animals were randomly housed in

polypropylene cages with environmental enrichment at different experimental groups. The rats were acclimatized for ten days before the commencement of the experiments and maintained under controlled temperature (22 ± 2 ºC) with a constant 12:12h light-dark cycle, with food and water *ad libitum*. This study was conducted in accordance with the ethical principles for animal experimentation of the *Conselho Nacional de Controle de Experimentação Animal* (CONCEA). All the experimental procedures were approved by the Ethics and Animal Welfare Committee of UFSM under the number 1039171218.

2.5 Experimental groups

The study was divided in two experiments: the first one consisted in the effects of TPE after the induction of immunosuppression (treatment) and the second was the pretreatment with TPE followed by the induction. The animals were divided into 6 groups (*n* = 6 per group) for each experiment (supplementary table 1). For the immunosuppression induction, cyclophosphamide (CPP) was diluted in 0.9% saline and administered via intraperitoneal in three days at dose of 50 mg/kg (totaling 150 mg/kg). The TPE doses of 100, 200 and 400 mg/kg were chosen according to our research group toxicity study [12]. Levamisole (LEV - drug reference standard) was administered at dose of 2.5 mg/kg and the control groups received only the vehicle (filtered water or saline via ip). The oral administration was by gavage (10 mL/kg) and both experiments lasted 17 days; supplementary figure 1 details the experimental procedures. At the end of the experiment, animals were fasted overnight and anesthetized, followed by blood collection for hematologic and biochemical analyses. After euthanasia, liver, kidney, heart, lungs, thymus and spleen were removed for macroscopic evaluation, weighed and the relative organ weights were calculated as (organ weight/body weight) x 100. An aliquot of the liver, kidney and heart was separated for homogenates to analyze enzymatic activity and oxidative damage.

2.6 Blood analysis

The serum was used for the following biochemical analyses: glucose (GLU), blood urea nitrogen (BUN) and creatinine (CRE) levels, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), butyrylcholinesterase (BChE) and lactate dehydrogenase

(LDH) activities, serum total protein (TP), albumin (ALB), total cholesterol (CHOL), triglycerides (TG), calcium (Ca) and phosphorus (P) levels, sodium (Na^+) and potassium (K^+) ions using commercial kits (Diagnostic Kits Laboratory Bioclin/Quibasa, Minas Gerais, Brazil) and an automatic biochemical analyzer (BS-120 Mindray®, Shenzhen, China).

Hematological analyses were performed on EDTA-coated tubes, and the BC-VET 2800 (Mindray®) was used to determine the hematocrit (HCT), total hemoglobin concentration (HGB), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular volume (MCV). The total red blood cells (RBC), white blood cells (WBC), and platelet (PLT) were also counted. Blood smears were prepared from each sample and were stained with Romanowsky dye to examine the differential leukocyte count and morphological evaluation of blood cells.

2.7 Assessment of oxidative stress markers

Kidney, liver and heart tissues were homogenized in tris/HCl buffer 50 mM (pH = 7.4) for perform the oxidative damage and enzymatic activity analyses. Protein carbonyls (PC) were measured spectrophotometrically following reaction with DNPH (2,4-dinitrophenylhydrazine), performed in triplicate and the absorbance reading at 370 nm [14].

Lipoperoxidation (LPO) was estimated using thiobarbituric acid reactive substances (TBA-RS) method, which the colorimetric reaction of the malondialdehyde (MDA) with thiobarbituric acid (TBA) is quantified. This reaction produces a colored compound that absorbs maximally at 532 nm as described by Buege & Aust (1978) [15]. The protein concentration of each sample was determined using Lowry assay [16].

2.8 Assessment of antioxidant enzyme activity

Superoxide dismutase (SOD) activity was determined as described by Sun & Zigman (1978) [17] with slight modifications. The test is based on its ability to inhibit the auto-oxidation of epinephrine determined in absorbance at 480 nm. The reaction mixture contained 0.965 mL of glycine buffer (pH = 10.2), 0.05 or 0.01 mL of the homogenate and 0.02 mL of epinephrine (60 mM, pH = 2.0) in HCl (1M) was used to initiate the reaction.

2.9 Statistical analysis

Data are expressed as the mean ± S.D. A one-way analysis of variance (ANOVA), followed by Tukey *post-hoc* test was used to determine the significance of data. Kruskal-Wallis and Dunn's were used as statistic tests when the requirements to perform a parametric test were not satisfied. Values were considered statistically significant at $p < 0.05$ (*GraphPad Prism Software, Inc., version 6*).

3 Results

3.1 Qualitative chemical analysis by UHPLC-ESI-HRMS

The TPE spectrometric analysis detected fatty acids as major compounds besides flavonoids, catechins, phenylpropanoid-derivative and organic acids (supplementary table 2). *Astrocaryum aculeatum* is chemically studied poorly in the literature which makes dereplication a little more difficult when suggesting compounds based on the previous isolation or identification already mentioned for this taxon. Therefore, most of the suggested compounds have not been described for this family. The TPE chromatogram is shown in the figure 1.

[Figure 1 near here]

3.2 Physiological aspects of the animals

The body weight of the animals from treatment did not present a normal trend during the experiment as shown in figure 2. In this experiment, only animals from the control group continued to gain weight until the end of the experiment. The final body weight was significant decreased in all treated animals, except for the CPP+TPE200 group, when compared to the control group. On the other hand, in the pretreated animals no differences between the groups were observed. The water and food intake were also reduced on treated animals from all the groups, except for the CPP+TPE200 group, in comparison to the control group. As found in body weight of the pretreated animals, no differences between these groups were observed in water and food intake (figure 1).

In relation to organ weights, a significant reduction was found in kidney, liver, and thymus of the treated animals compared to the animals from control. Kidneys weight were reduced on all treated groups, except in the CPP+TPE200 group; while liver weight reduced in all groups, except in the CPP group. Thymus weight was

reduced in all treated groups (supplementary table 3). Pretreated animals showed a reduction in thymus and spleen weights in all groups when compared to the control group. Relative organ weight was also significant decreased in both experiments. In the treatment, kidney relative weight reduced in the LEV+CPP group, while liver and thymus reduced in all groups, when compared to control. Regarding pretreated animals, it was observed a reduced thymus and spleen relative weights in all pretreated groups when compared to the control group (supplementary table 4).

[Figure 2 near here]

3.3 Blood analysis

3.3.1 Hematological parameters

Regarding the hematological analysis of treatment groups, it was observed a significant decrease in RBC, PP and lymphocytes levels when compared to the control group. RBC levels were reduced on CPP and CPP+LEV groups; PP reduced in CPP+LEV group; while lymphocytes were reduced on groups CPP, CPP+LEV and CPP+TPE100. On the other hand, PLT and neutrophils levels were increased significantly in CPP, CPP+LEV and CPP+TPE100 groups in comparison to the control group (table 1). Concerning the pretreatment analyses, RBC, HGB, HCT and WBC levels were significant decreased in all pretreated groups when compared to the control group. Levels of MCV were decreased on CPP, LEV+CPP, TPE200+CPP and TPE400+CPP groups compared to the control. In the PLT levels were observed a reduction in all groups, except in the highest TPE dose group (400 mg/kg) in comparison to the control group (table 2).

[Table 1 near here]

[Table 2 near here]

3.3.2 Biochemical parameters

The biochemical analyses from the treatment experiment showed a significant decrease on ALB, ALT, ALP, BUN and CRE levels in relation to the control group. The ALB levels decreased in all groups, except in the group treated with TPE at 200 mg/kg; ALT activity was decrease in all treated groups; while ALP reduced only in the TPE 200 mg/kg group. Levels of CRE was found to be reduced on CPP, CPP+LEV and

CPP+TPE200 groups, whilst BUN levels decreased in all treated animals. Moreover, levels of BUN from the CPP+LEV and CPP+TPE100 groups were also decreased when compared with the CPP group (table 3). Levels of TG and DHL also showed significant alterations: TG levels were reduced on the groups treated with TPE at 100 and 400mg/kg doses when compared to the control group, while the DHL increased in the group treated with levamisole when compared to the CPP group (supplement. table 5).

In the pretreatment experiment, was observed a significant reduction on ALT and AST activity: AST decreased on CPP group; while ALT was decreased in all pretreated groups, both in comparison to the control group (table 3). Other biochemical parameters showed significant changes: GLU levels were increased in the LEV+CPP and TPE100+CPP groups and DHL levels were reduced in CPP and TPE100+CPP groups. P, K and Ca levels were decreased on the LEV+CPP group; all these changes were found when compared to the control group (supplement. table 5).

[Table 3 near here]

3.4 Oxidative stress and antioxidant enzyme activity markers

The groups from the treatment experiment showed no differences in the MDA levels of hepatic, renal and heart tissues. Regarding the PC levels, in renal tissue was observed a significant increase in the group treated with CPP when compared to the control group. The levels of the groups treated with levamisole and TPE (100, 200 and 400 mg/kg) reduced in comparison to the CPP group. In cardiac tissue, the reduction of carbonyl levels was observed In the animals treated with TPE at 200 and 400 mg/kg compared to the CPP group. No differences were observed in hepatic tissue.

Pretreated animals showed significant decrease in MDA levels of hepatic tissue of the animals treated with the three doses of TPE, whilst in renal tissue the decrease was observed in the 200 mg/kg group, both compared to the CPP group. In the cardiac tissue, MDA levels increased in the CPP group in comparison to the control. In renal tissue, PC was found to be increased in CPP group when compared to the control and its levels decreased in animals pretreated with TPE (100, 200 and 400 mg/kg) in relation to the CPP group. Pretreatment with TPE at doses of 200 and 400 mg/kg was able to reduce the carbonyl levels in cardiac tissue in comparison to the CPP group. In the liver no differences were observed.

Regarding enzyme activity, SOD reduced in hepatic and cardiac tissues in the treated animals from CPP group when compared to the control. In the pretreated animals, the SOD activity reduced in all organs of CPP group in relation to the control. Pretreatment with TPE at 200 mg/kg enhanced SOD activity in hepatic tissue when compared to CPP group. All data are presented in table 4.

[Table 4 near here]

4 Discussion

The incidence of different types of cancer is increasing daily and the chemotherapy is one of the treatments often used [18]. However, the serious side reactions of multi-organ damage associated with these antitumour drugs is a limiting factor, which can lead to treatment interruption [19]. Therefore, the search for alternative therapies that can reduce the adverse effects of these drugs has increased worldwide. Natural products has gained attention since they are a source of bioactive compounds with important pharmacological activities such as antioxidant, anti-inflammatory and anti-apoptotic [20].

In this study we investigated the effects of the tucumã pulp extract after ciclophosphamide-induced toxicity. To the best of our knowledge, few studies have been published on chemical components related to tucumã fruit. Plants produce phytochemicals by primary or secondary metabolism for protection against threats, besides their role in the plant growth and reproduction [21]. Our present spectrometric analysis of TPE showed almost all putative compounds have already been described for antioxidant activity, mainly flavonoids [22], catechins [23] and phenylpropanoid-derivative [24]. Moreover, in our previous study, analyses by HPLC and GC-MS revealed the presence of the carotenoids β-carotene and lutein and triterpenes, respectively [12]. Recently, attention has been given to the role of diet in human health. Studies have linked the high consume of vegetables with a reduction in the risk of various chronic disorders such as cancer, diabetes and heart diseases. Some constituents like carotenoids, polyphenols and flavonoids are associated with these health benefits. The antioxidant capacity of these compounds plays an important role in prevention of chronic diseases related to oxidative stress, due to their free radical-scavenging activity and improve in the response to cell stress [25].

Recording physiological parameters is a valid marker of toxicity of substances. Changes in body and organ weight in animals are associated with wellbeing and physiological impact of toxic effect of substances [26]. The weight loss and reduced consume of water and food in treated animals indicates the toxic effects of CPP, since its influence on the animals appetite. The oral treament with levamisole and TPE was not able to attenuate CPP effects on body weight of the animals. This scenario was not observed in the pretreated animals, suggesting that pretreatment with TPE is more efficient in prevent CPP toxic effects. As immune organs, spleen and thymus participate in specific immunity and their index may reflect the immune function [27]. Pretreated rats had thymus and spleen weights reduced, besides lower relative organ weight, indicating a possible alteration in the immune activity. In treated animals the significant changes were observed in liver and kidneys weights and relative organ weights. TPE nor levamisole were able to reverse this CPP-induced organ atrophies.

In relation to the hematopoietic system, is well known that it is one of the most sensitive parameters for assessing the toxicity of drugs in humans and animals [28]. The red blood cells were more affected in pretreated animals, as in the treated ones only lymphocytes and neutrophils showed changes. These results can be associated with spleen atrophy, given its participation in the regulation of hematopoiesis [29]. On the other hand, the renal and hepatic biomarkers were more susceptible to the toxic effects of CPP on treated animals. This is also in accordance with the results found in kidney and liver weights and index. Biochemical parameters assessments are essential to analyze the impact of the substances toxic effects in specific organs like liver and kidney which are responsible for metabolising and excreting toxic substances [30]. All together, this results suggest that the first experiment is a better model for induction of hepatotoxicity or nephrotoxicity by CPP. While the second one affected more efficiently the blood cells, indicating a better model for development of immunossupression. However, the treatments with TPE or levamisole did not restore any of the hematological or biochemical parameters assessed. The activity of vegetables depends on different factors such as the presence of bioactive compounds and its abundance. For instance, the effects of different flavonoids may be antagonistic, in some cases acting as immunosuppresive and in others as immunostimulating [31]. The metabolism and absorption of these phytochemicals can also interfere in the animals response. The carotenoids, for example, are absorbed as lipids and there are nutrients that can interfere in its bioavailability, which is the case

of oil [32]. The fact that tucuma fruit is very rich in fatty acids may have interfered with carotenoids bioavailability, influencing in tucumã extract activity.

The beneficial effects of TPE may be associated with the improvement in the oxidative stress status. The toxicity induced by CPP is due its metabolites, phosphoramide and acrolein, which produce highly reactive oxygen free radicals, besides interfering in the tissue antioxidant defense system [33]. LPO and PC are useful as a biomarkers of tissue oxidative stress, moreover PC is a good indicator of PC aggregation and protein oxidation [34]. Our results show that CPP-treatment elevated oxidative stress in different organs and TPE was able to attenuate the MDA and carbonyl levels, specially in the pretreated animals. Moreover, SOD activity was found significant reduced after exposition to CPP and administration of TPE improved its activity. Improvement in antioxidant response corroborates the potential protective effect of TPE against CPP adverse effects. The diet is an important adjuvant in protect the body against the effects of increased ROS generation resulting in oxidative stress [35]. The bioactive compounds found in the extract could be associated with the positive results observed after exposition to TPE. These compounds can absorb free radicals and inhibit the initiation chain or interrupt the propagation of oxidative reactions promoted by the radicals [32]. By neutralizing the free radicals produced by CPP metabolites, the cell membrane is protected from the potential damage. This is in accordande with study performed by Sagrillo *et al.* (2015) [13] which reported the strong antioxidant capacity of the tucumã extracts. Moreover, our previous study showed that TPE extract has a strong antioxidant capacity [12], which comes in line with the results from biomarkers of oxidative stress and enzyme antioxidant activity. Unfortunately, a great number of fruits consumed in the Amazonian region are yet few studied. As far as we know, *in vivo* studies regarding tucumã extract activities were not available in the literature to date.

5 Conclusion

In summary, our study showed that TPE was not able to reverse efficiently all the toxic effects caused by CPP. However, the data suggest that TPE has a benefit against toxic complications of CPP by attenuating oxidative stress and improving the enzyme antioxidant response. This may be associated with its strong antioxidant capacity and antioxidant content. Besides, the results indicate that the pretreatment

with TPE showed a better response in attenuate the toxic effects of CPP. Furthermore, this study shows new findings about *Astrocaryum aculeatum*, indicating that its fruit extract has pharmacological potential. Hence, further studies are essential to understand the mechanisms by which tucumã extract can act and validate its biological activities in *in vivo* models.

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Conflict of interests

The authors declare that there are no conflicts of interest.

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Figure 1. UHPLC-ESI-HRMS chromatogram of *Astrocaryum aculeatum* extract.

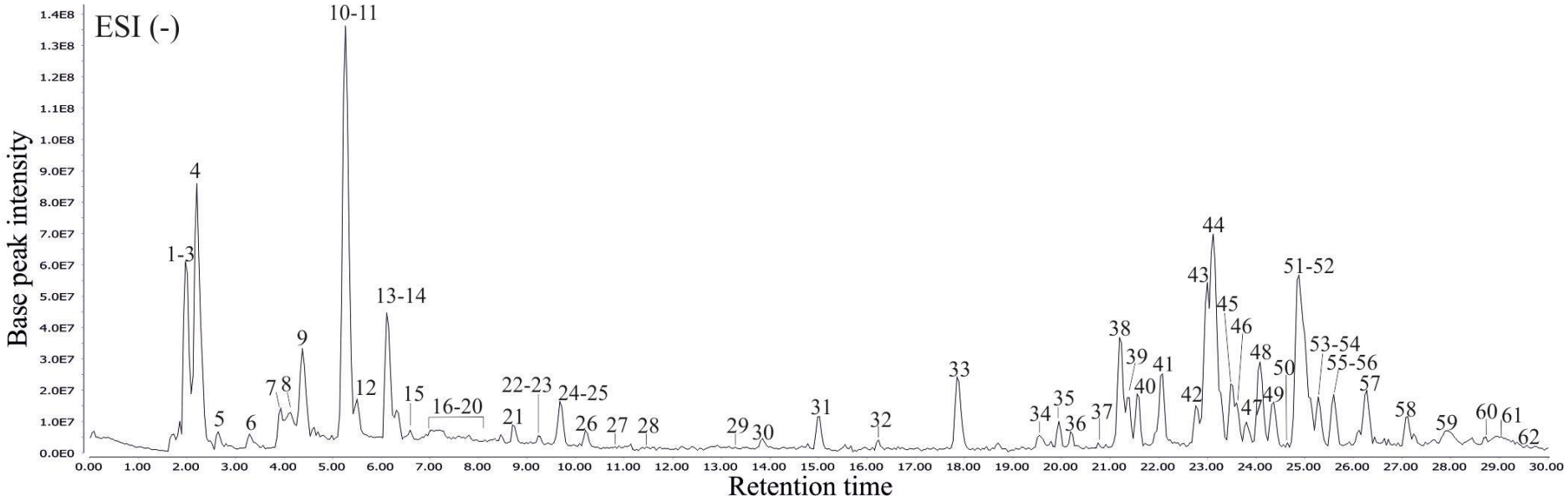

Figure 2. Body weight of the animals and water intake and food consumption every four days of experiment. **(A)** Treatment; **(B)** Pretreatment.

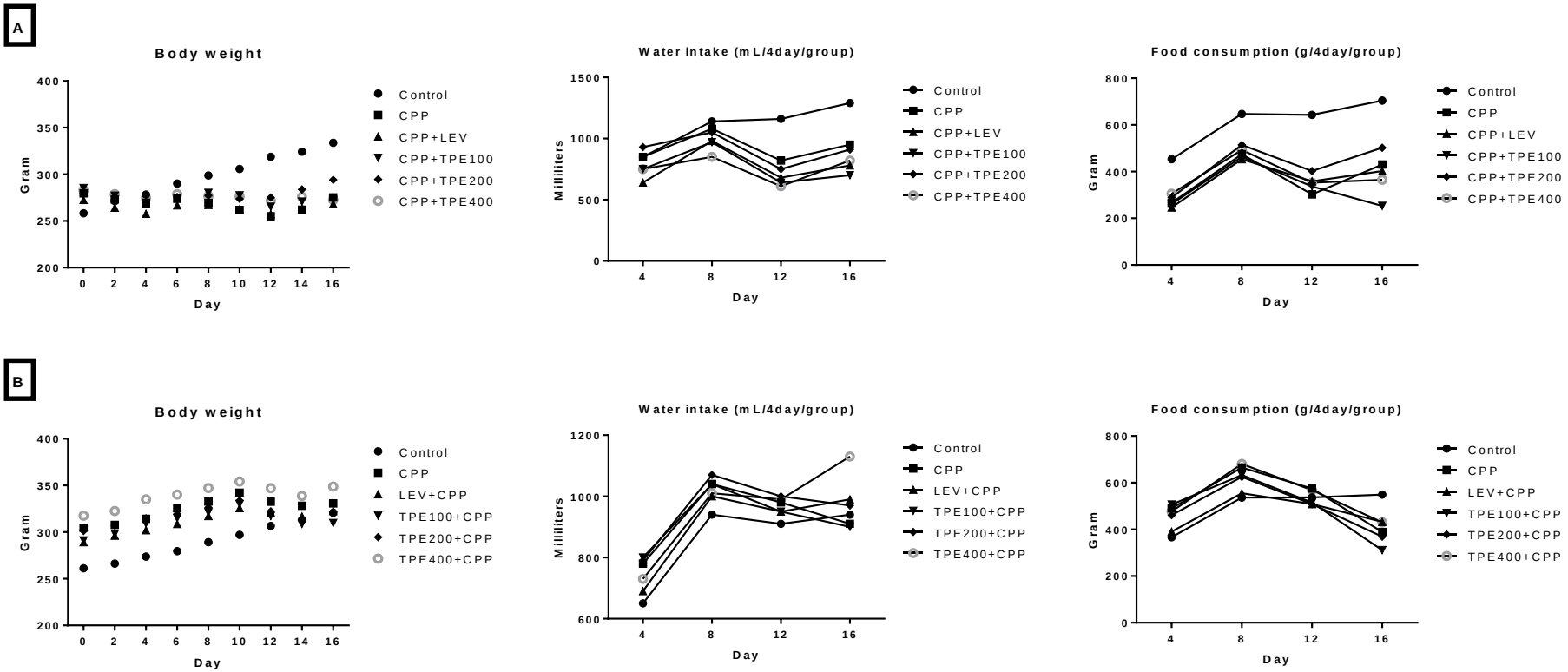

Table 1. Hematological parameters from animals after exposition to CPP and treatment with TPE at 100, 200 and 400 mg/kg.

Parameters	Experimental groups					
	Control	CPP	CPP+LEV	CPP+TPE100	CPP+TPE200	CPP+TPE400
RBC (10^6/µL)	7.84 ± 0.31	6.98 ± 0.28[a]	7.05 ± 0.27[a]	7.47 ± 0.54	7.30 ± 0.32	7.30 ± 0.30
HGB (g/dL)	14.65 ± 0.67	13.77 ± 0.72	13.65 ± 0.84	14.14 ± 0.71	13.84 ± 0.71	13.98 ± 0.57
HCT (%)	41.45 ± 1.40	39.97 ± 2.02	38.50 ± 2.22	40.12 ± 2.56	40.74 ± 2.65	39.97 ± 1.72
MCV (fL)	53.00 ± 2.42	57.38 ± 3.65	54.58 ± 1.65	53.74 ± 1.08	55.86 ± 2.96	54.80 ± 0.53
CHCM (g/dL)	35.28 ± 1.40	34.47 ± 2.13	35.38 ± 0.31	35.24 ± 0.36	34.02 ± 2.02	34.95 ± 0.42
PLT (10^3/µL)	894.67 ± 428.66	1438.00 ± 282.08[a]	1367.00 ± 103.67[a]	1424.00 ± 135.60[a]	1362.00 ± 184.99	1293.00 ± 164.50
PP (mg/dL)	7.10 ± 0.16	6.83 ± 0.19	6.43 ± 0.36[a]	6.56 ± 0.21	6.48 ± 0.48	6.63 ± 0.48
WBC (10^6/µL)	10.11 ± 1.40	7.01 ± 0.63	6.93 ± 1.09	7.48 ± 1.19	6.78 ± 1.32	6.02 ± 0.63[a]
Neutrophils (%)	14.67 ± 3.01	48.80 ± 7.29[a]	53.50 ± 12.85[a]	50.40 ± 6.38[a]	45.20 ± 9.14	41.83 ± 7.78
Lymphocytes (%)	82.00 ± 5.36	44.60 ± 7.63[a]	40.50 ± 12.85[a]	44.00 ± 4.18[a]	50.00 ± 8.67	52.40 ± 8.56
Monocytes (%)	3.33 ± 2.58	2.33 ± 1.21	3.50 ± 1.22	2.20 ± 1.64	2.80 ± 0.83	2.00 ± 1.09
Eosinophils (%)	0.00 ± 0.00	2.00 ± 1.09	2.00 ± 1.09	1.40 ± 1.34	1.40 ± 0.89	2.00 ± 2.09
Basophils (%)	0.00 ± 0.00	0.33 ± 0.81	0.33 ± 0.51	1.40 ± 0.89	0.80 ± 1.09	0.50 ± 0.83

Data are expressed as mean ± S.D. One-way ANOVA/Kruskal-Wallis followed by Tukey test/Dunn's (n = 6). The values were considered to be significantly different when $p < 0.05$. [a]Different from the control.

Table 2. Hematological parameters from animals after pretreatment with TPE at 100, 200 and 400 mg/kg and exposition to CPP.

Parameters	Experimental groups					
	Control	CPP	CPP+LEV	CPP+TPE100	CPP+TPE200	CPP+TPE400
RBC (10^6/µL)	8.12 ± 0.71	7.11 ± 0.30[a]	6.64 ± 0.58[a]	7.30 ± 0.22[a]	7.08 ± 0.32[a]	7.02 ± 0.28[a]
HGB (g/dL)	18.26 ± 1.37	15.07 ± 0.56[a]	14.32 ± 1.44[a]	15.42 ± 0.58[a]	14.63 ± 0.52[a]	14.58 ± 0.29[a]
HCT (%)	49.28 ± 3.94	40.23 ± 1.08	38.10 ± 3.59	42.37 ± 1.41	40.22 ± 1.55	39.37 ± 1.26
MCV (fL)	60.14 ± 2.52	56.67 ± 1.01[a]	57.48 ± 1.61[a]	58.05 ± 1.02	56.32 ± 1.24[a]	56.12 ± 0.67[a]
CHCM (g/dL)	37.00 ± 0.58	37.38 ± 0.49	37.53 ± 0.50	36.33 ± 0.57[b]	36.33 ± 0.23[b]	37.02 ± 0.48
PLT (10^3/µL)	651.40 ± 188.58	261.33 ± 217.78[a]	255.33 ± 154.55[a]	118.00 ± 89.37[a]	216.50 ± 170.09[a]	356.16 ± 227.73
PP (mg/dL)	6.80 ± 0.28	6.53 ± 0.30	6.60 ± 0.57	6.13 ± 0.16[a]	6.60 ± 0.30	6.43 ± 0.19
WBC (10^6/µL)	9.64 ± 2.42	1.35 ± 0.98[a]	1.31 ± 0.70[a]	0.60 ± 0.28[a]	0.85 ± 0.68[a]	1.73 ± 1.27[a]
Neutrophils (%)	13.80 ± 4.14	30.67 ± 15.38	37.83 ± 11.60	19.50 ± 16.78	25.67 ± 17.91	28.67 ± 19.63
Lymphocytes (%)	85.60 ± 4.45	63.00 ± 17.03	56.00 ± 13.13	78.50 ± 18.39	69.83 ± 20.90	67.83 ± 18.28
Monocytes (%)	0.60 ± 0.54	0.66 ± 1.21	4.83 ± 3.12	1.83 ± 1.60	3.83 ± 5.45	3.50 ± 2.51
Eosinophils (%)	0.00 ± 0.00	0.33 ± 0.51	0.33 ± 0.81	0.16 ± 0.40	0.66 ± 1.63	0.00 ± 0.00
Basophils (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Data are expressed as mean ± S.D. One-way ANOVA/Kruskal-Wallis followed by Tukey test/Dunn's (n = 6). The values were considered to be significantly different when $p < 0.05$. [a]Different from the control.

Table 3. Biochemical parameters from animals exposed to CPP and treated or pretreated with TPE 100, 200 and 400 mg/kg.

Parameters	Experimental groups					
	Control	CPP	CPP+LEV	CPP+TPE100	CPP+TPE200	CPP+TPE400
Treatment						
ALB (g/dL)	2.78 ± 0.16	2.31 ± 0.34[a]	2.08 ± 0.18[a]	2.02 ± 0.13[a]	2.58 ± 0.21	2.28 ± 0.14[a]
ALT (U/L)	62.83 ± 8.32	40.80 ± 5.84[a]	38.00 ± 8.64[a]	34.60 ± 9.12[a]	45.33 ± 11.27	36.80 ± 6.90[a]
AST (U/L)	88.83 ± 8.32	76.60 ± 9.65	89.20 ± 6.45	76.40 ± 5.89	73.80 ± 10.08	75.40 ± 7.19
ALP (U/L)	167.50 ± 19.32	126.20 ± 24.08	134.00 ± 26.57	141.60 ± 38.94	111.00 ± 20.89[a]	121.60 ± 31.48
GGT (U/L)	4.66 ± 3.32	7.16 ± 2.71	2.33 ± 1.63	4.00 ± 2.34	3.83 ± 3.25	4.33 ± 3.26
BUN (mg/dL)	60.00 ± 4.73	44.00 ± 3.67[a]	35.50 ± 3.01[a,b]	33.60 ± 4.03[a,b]	44.83 ± 6.94[a]	36.83 ± 2.04[a]
CRE (mg/dL)	0.63 ± 0.05	0.46 ± 0.08[a]	0.45 ± 0.05[a]	0.44 ± 0.05[a]	0.58 ± 0.04	0.48 ± 0.04
TP (g/dL)	6.71 ± 0.14	6.53 ± 0.28	6.41 ± 0.31	6.36 ± 0.20	6.30 ± 0.41	6.26 ± 0.24
	Control	CPP	LEV+CPP	TPE100+CPP	TPE200+CPP	TPE400+CPP
Pretreatment						
ALB (g/dL)	2.84 ± 0.13	2.73 ± 0.08	2.60 ± 0.14	2.58 ± 0.23	2.55 ± 0.15	2.55 ± 0.22
ALT (U/L)	55.80 ± 7.72	39.67 ± 4.50[a]	36.17 ± 8.72[a]	34.00 ± 8.07[a]	37.83 ± 5.87[a]	40.00 ± 7.69[a]
AST (U/L)	85.80 ± 8.84	57.67 ± 1.86[a]	58.50 ± 4.63	58.50 ± 4.55	62.17 ± 13.69	66.50 ± 13.34
ALP (U/L)	168.40 ± 35.51	108.00 ± 23.63	146.80 ± 28.67	109.30 ± 23.27	107.80 ± 23.79	111.00 ± 52.84
GGT (U/L)	1.00 ± 1.22	1.33 ± 1.03	3.50 ± 1.64	2.66 ± 1.86	3.33 ± 2.16	3.00 ± 1.09
BUN (mg/dL)	50.60 ± 3.78	44.00 ± 3.68	41.33 ± 4.76	46.67 ± 12.56	44.00 ± 3.63	43.33 ± 4.45
CRE (mg/dL)	0.60 ± 0.00	0.61 ± 0.04	0.60 ± 0.00	0.53 ± 0.05	0.56 ± 0.08	0.66 ± 0.08
TP (g/dL)	6.54 ± 0.35	6.21 ± 0.34	6.25 ± 0.45	6.06 ± 0.23	6.08 ± 0.23	6.10 ± 0.15

Results are expressed as mean ± S.D. One-way ANOVA/Kruskal-Wallis followed by Tukey test/Dunn's (n = 6). The values were considered to be significantly different when $p < 0.05$. [a]Different from the control. [b]Different from the CPP group.

Table 4. Carbonyl and MDA content and SOD activity from animals exposed to CPP and treated and pretreated with TPE 100, 200 and 400 mg/kg.

		Experimental groups					
	Treatment	Control	CPP	CPP+LEV	CPP+TPE100	CPP+TPE200	CPP+TPE400
Kidney	MDA	0.53 ± 0.12	0.86 ± 0.22	0.60 ± 0.19	0.66 ± 0.23	0.66 ± 0.20	0.72 ± 0.11
	Carbonyl	1.24 ± 0.35	2.25 ± 0.12[a]	1.22 ± 0.47[b]	1.11 ± 0.26[b]	1.22 ± 0.36[b]	1.21 ± 0.41[b]
	SOD	13.68 ± 1.71	7.88 ± 1.17[a]	11.54 ± 3.76	11.17 ± 1.69	11.31 ± 1.47	11.50 ± 1.58
Liver	MDA	0.83 ± 0.17	1.34 ± 0.61	1.22 ± 0.48	1.24 ± 0.47	0.70 ± 0.18	0.66 ± 0.12
	Carbonyl	1.33 ± 0.18	1.51 ± 0.53	1.36 ± 0.57	1.41 ± 0.25	1.29 ± 0.24	1.48 ± 0.13
	SOD	19.71 ± 3.09	14.97 ± 1.97	16.21 ± 4.37	15.29 ± 1.52	16.53 ± 2.72	16.45 ± 4.30
Heart	MDA	2.67 ± 0.67	3.00 ± 0.56	2.91 ± 0.56	2.94 ± 0.43	2.42 ± 0.52	2.85 ± 0.40
	Carbonyl	4.05 ± 0.86	5.80 ± 1.43	4.82 ± 1.04	3.86 ± 0.83	3.06 ± 1.40[b]	2.93 ± 0.67[b]
	SOD	9.85 ± 0.91	6.29 ± 1.79[a]	8.75 ± 1.73	7.37 ± 2.01	7.66 ± 2.16	9.17 ± 1.54
	Pretreatment	Control	CPP	LEV+CPP	TPE100+CPP	TPE200+CPP	TPE400+CPP
Kidney	MDA	1.93 ± 0.36	2.15 ± 0.46	1.82 ± 0.40	1.80 ± 0.24	1.39 ± 0.37[b]	1.60 ± 0.46
	Carbonyl	2.95 ± 0.70	4.49 ± 0.90[a]	3.15 ± 0.24[b]	2.87 ± 0.48[b]	2.75 ± 0.46[b]	2.96 ± 0.59[b]
	SOD	12.27 ± 2.69	8.83 ± 1.25[a]	11.47 ± 1.19	10.15 ± 1.55	11.43 ± 1.12	10.96 ± 1.10
Liver	MDA	0.60 ± 0.11	0.87 ± 0.13	0.69 ± 0.18	0.48 ± 0.09[b]	0.45 ± 0.13[b]	0.43 ± 0.12[b]
	Carbonyl	1.96 ± 0.34	3.10 ± 0.52[a]	2.35 ± 0.58	1.82 ± 0.42[b]	2.39 ± 0.54	2.19 ± 0.27[b]
	SOD	16.38 ± 3.54	11.12 ± 2.01[a]	13.55 ± 1.16	13.93 ± 1.54	15.53 ± 1.43[b]	13.44 ± 1.52
Heart	MDA	1.90 ± 0.38	3.45 ± 0.33[a]	2.73 ± 0.41	2.50 ± 0.85	2.38 ± 0.82	2.43 ± 0.64
	Carbonyl	1.59 ± 0.38	2.76 ± 0.35[a]	2.35 ± 0.46	2.37 ± 0.21	2.26 ± 0.56	2.47 ± 0.51
	SOD	8.75 ± 1.34	6.70 ± 0.86[a]	8.27 ± 1.47	8.16 ± 0.71	8.37 ± 1.05	8.18 ± 0.63

Data are expressed as mean ± S.D. One-way ANOVA/Kruskal-Wallis followed by Tukey test/Dunn's (n = 6). The values were considered to be significantly different when $p < 0.05$. [a]Different from the control. [b]Different from the CPP group. MDA expressed in nmol MDA/mg prot. SOD expressed in U SOD/mg prot. Carbonyl content expressed in nmol carbonyl/mg protein.

SUPPLEMENTARY MATERIAL

Potential effects of *Astrocaryum aculeatum* extract against cyclophosphamide-induced hematologic and organ toxicity in rats

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The following data are available as supplementary material:

Table S1. Groups division for the treatment and pre-treatment experiments.

Figure S1. Representation of experimental procedures.

Table S2. Qualitative chemical analysis of *Astrocaryum aculeatum* by UHPLC-ESI-HRMS in negative mode ionization.

Table S3. Water intake and food consumption every four days of experiment; Body weight, organ weights and relative organ weights of the TPE-treated animals.

Table S4. Water intake and food consumption every four days of experiment; Body weight, organ weights and relative organ weights of the TPE-pretreated animals.

Table S5. Biochemical parameters from animals exposed to CPP and treated or pretreated with TPE 100, 200 and 400 mg/kg.

Table S1. Groups division for the treatment and pretreatment experiments.

Group	*Treatment*	*Group*	*Pretreatment*
I	Control	**VII**	Control
II	CPP + Vehicle	**VIII**	Vehicle + CPP
III	CPP + Levamisole	**IX**	Levamisole + CPP
IV	CPP + TPE100	**X**	TPE100 + CPP
V	CPP + TPE200	**XI**	TPE200 + CPP
VI	CPP + TPE400	**XII**	TPE400 + CPP

Figure S1. Representation of experimental procedures.

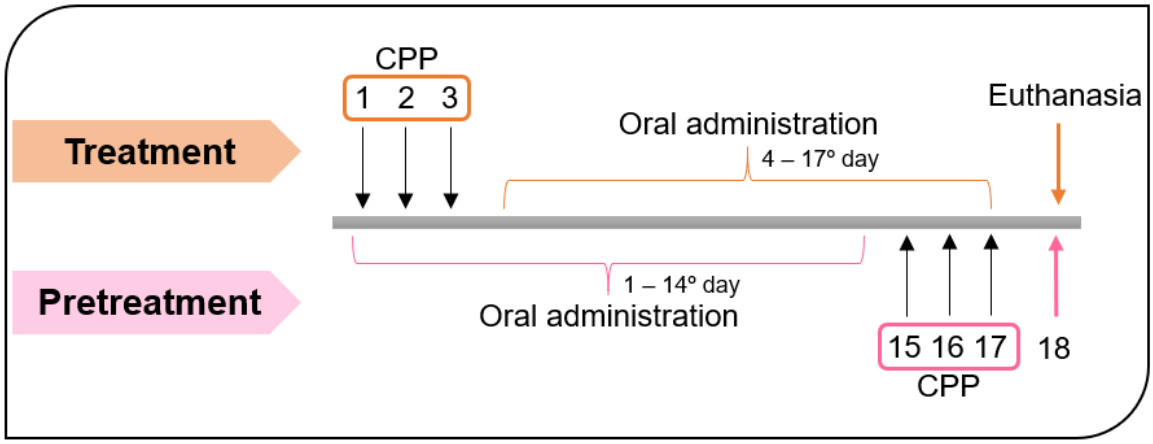

Table S2. Qualitative chemical analysis of *Astrocaryum aculeatum* by UHPLC-ESI-HRMS in negative mode ionization.

N°	RT (min)	Putative formula	*m/z* error (ppm)	Compound type	Putative compound_CAS (References)	Relative area
1	1.97	$C_6H_{10}O_7$	193.0339 (-4.97)	organic acid	galacturonic acid_685-73-4[1]	3.69E7
2	1.97	-	215.0313	n.i.		1.78E8
3	2.09	$C_6H_{10}O_6$	177.0390 (-5.00)	carbohydrate	glucosone_1854-25-7[2]	9.87E6
4	2.21	-	191.0177 (-2.63)	n.i.		3.61E8
5	2.65	$C_{16}H_{28}O_{11}$	395.1543	carbohydrate	1-(3-methylbutanoyl)-6-apiosylglucose_467242-31-5[3]* or methyl 3',4'-isopropylidene-β-lactoside_98169-70-1[4]* or 1-O-acylglycoside_86491-59-0[5]* or nonioside O_1273182-78-7[6]*	2.03E6
6	3.29	$C_{14}H_{24}O_{10}$	351.1276 (-4.31)	carbohydrate	dactylorhin C_256459-23-1[7]*	6.77E6
7	3.93	$C_{16}H_{28}O_{11}$	395.1543		1-(3-methylbutanoyl)-6-apiosylglucose_467242-31-5[3]* or methyl 3',4'-isopropylidene-β-lactoside_98169-70-1[4]* or 1-O-acylglycoside_86491-59-0[5]* or nonioside O_1273182-78-7[6]*	1.44E7
8	4.13	$C_{16}H_{28}O_{11}$	395.1538 (-3.77)		1-(3-methylbutanoyl)-6-apiosylglucose_467242-31-5[3]* or methyl 3',4'-isopropylidene-β-lactoside_98169-70-1[4]* or 1-O-acylglycoside_86491-59-0[5]* or nonioside O_1273182-78-7[6]*	1.45E7
9	4.38	$C_{30}H_{26}O_{12}$	577.1336 (-1.78)	flavonoid	69 hits*	8.72E7
10	5.26	$C_6H_8O_6$	289.0702 (-3.58)	catechin	catechol_154-23-4[8]	1.32E9
11	5.26	$C_{16}H_{16}O_8$	335.0762 (-1.48)	PPD	4-O-caffeoylshikimic acid_180842-65-3[9] or 5-O-caffeoylshikimic acid_73263-62-4[9] or caffeylshikimic acid_6082-44-6[10] or 3-O-caffeoylshikimic acid_180981-12-8[9]	3.00E7
12	5.50	$C_{30}H_{26}O_{12}$	577.1335 (-1.85)	polyphenol	n.i. (102 hits)*	4.46E7
13	6.11	$C_6H_8O_6$	289.0702 (-3.48)	catechin	catechol_154-23-4[8]	1.32E9
14	6.11	$C_{16}H_{16}O_8$	335.0764 (-0.97)	PPD	4-O-caffeoylshikimic acid_180842-65-3[9] or 5-O-caffeoylshikimic acid_73263-62-4[9] or caffeylshikimic acid_6082-44-6[10] or 3-O-caffeoylshikimic acid_180981-12-8[9]	4.02E8
15	6.64	$C_{30}H_{26}O_{12}$	577.1335 (-1.85)	polyphenol	n.i. (102 hits)*	1.47E7
16	7.04	$C_{26}H_{40}O_{15}$	591.2285 (-0.68)	phenolic glicoside	ligurobustoside M_583058-06-4[11]	3.75E6
17	7.20	$C_{26}H_{28}O_{14}$	563.1399 (-0.42)	flavonoid	flavonoid_1800284-87-0[12]*	2.05E7
18	7.24	$C_{27}H_{30}O_{16}$	609.1455 (-0.09)	flavonoid	rutin_153-18-4[8]	5.01E5
19	7.40	$C_{27}H_{30}O_{15}$	593.1496 (-1.70)	flavonoid	afroside_35109-95-6[13] or scolimoside_20633-84-5[13]	2.04E7

20	8.17	$C_{27}H_{30}O_{17}$	593.1497 (-1.62)	flavonoid	afroside_35109-95-6[13] or scolimoside_20633-84-5[13]	1.99E7
21	8.74	$C_{17}H_{30}O_{10}$	393.1758 (-0.62)	glycosides	8 hits*	3.44E7
22	9.27	C27H28O15	591.1338 (-2.08)	flavonoid	16 hits*	1.39E6
23	9.27	-	621.1428	n.i.	-	1.36E7
24	9.68	-	431.2383	n.i.	-	2.12E7
25	9.68	$C_9H_{16}O_4$	187.0965 (-2.86)	fatty acids	azelaic acid_123-99-9[14]*	1.66E8
26	10.16	-	431.2384	n.i.	-	1.05E7
27	10.85	-	649.1917	n.i.	-	2.34E6
28	11.66	$C_{15}H_{10}O_7$	301.0342 (-1.99)	flavonoid	quercetin_117-39-5[8]	2.00E6
29	13.44	$C_{15}H_{10}O_6$	285.0394 (-1.70)	flavonoid	kaempferol_520-18-3[8]	4.63E6
30	13.85	$C_{18}H_{34}O_5$	329.2324 (-1.18)	fatty acids	23 hits*	1.46E7
31	15.02	-	169.0852	n.i.	-	4.81E7
32	16.23	$C_{11}H_{14}O_3$	193.08560 (-4.51)	PPD	2,6-dimethoxy-4-(2-propen-1-yl)-phenol_6627-88-9[15]	5.77E7
33	17.85	-	249.1513	n.i.	-	8.77E7
34	19.50	$C_{29}H_{48}O_{11}$	571.30933	glycosides	crenulatoside C_871734-11-1[16]* or Crenulatoside B_871734-10-0[16]*	5.14E5
35	19.94	-	265.1466	n.i.	-	4.13E7
36	20.22	-	297.1519	n.i.	-	1.98E7
37	20.80	$C_{18}H_{36}O_4$	315.2525 (-3.09)	fatty acids	9,10-dihydroxy-octadecanoic acid_120-87-6[14]*	4.17E7
38	21.19	-	311.1673	n.i.	-	1.44E8
39	21.35	-	465.2967	n.i.	-	6.42E6
40	21.55	$C_{23}H_{24}O_5$	379.1546 (0.11)	polyphenol	46 hits*	1.34E6
41	22.07	-	571.2869	n.i.	-	9.99E7
42	22.76	$C_{28}H_{44}O_{11}$	555.2817 (2.05)	terpene	forskoditerpenoside C_1041183-11-2[17]* or tricalysioside I_874332-43-1[18]* or tricalysioside K_874308-03-9[18]*	3.56E7
43	23.08	-	325.1816	n.i.	-	1.11E8
44	23.12	-	741.0518	n.i.	-	1.31E8
45	23.48	-	325.1831	n.i.	-	8.53E7
46	23.60	$C_{30}H_{46}O_{11}$	581.2958 (-0.58)	terpene	12 hits*	4.31E7
47	23.80	$C_{23}H_{38}O_8$	441.2504 (3.61)	n.i.	-	4.12E6
48	24.08	$C_{18}H_{34}O_3$	297.2418 (-3.87)	fatty acids	ricinoleic acid_141-22-0[19] or isomer	3.65E7
49	24.32	$C_{28}H_{50}O_{11}$	561.3255 (-3.60)	terpene glycoside	pelargoside A1_1447303-92-5[20]*	7.80E6
50	24.60	$C_{18}H_{34}O_3$	297.2418 (-4.00)	fatty acids	ricinoleic acid_141-22-0[19] or isomer	1.28E7
51	24.88	$C_{18}H_{34}O_4$	313.2368 (-3.59)	fatty acids	1,10-dibutyl ester-decanedioic acid_109-43-3 or isomer[21]*	5.70E7
52	24.88	$C_{18}H_{32}O_3$	295.2263 (-3.29)	fatty acids	dimorphecolic acid_18104-44-4[22]* or densipolic acid_7121-47-3[23]* or vernolic acid_503-07-1[24]*	3.78E7
53	25.20	$C_{18}H_{34}O_4$	313.2366 (-4.20)	fatty acids	1,10-dibutyl ester-decanedioic acid_109-43-3 or isomer[21]*	5.39E7

54	25.20	$C_{18}H_{32}O_3$	295.2262 (-3.86)	fatty acids	dimorphecolic acid_18104-44-4[22]* or densipolic acid_7121-47-3[23]* or vernolic acid_503-07-1[24]*	3.38E7
55	25.60	$C_{18}H_{34}O_4$	313.2366 (-3.94)	fatty acids	1,10-dibutyl ester-decanedioic acid_109-43-3 or isomer[21]*	1.69E7
56	25.60	$C_{18}H_{32}O_3$	295.2263 (-3.59)	fatty acids	dimorphecolic acid_18104-44-4[22]* or densipolic acid_7121-47-3[23]* or vernolic acid_503-07-1[24]*	2.87E7
57	26.28	-	339.1982	n.i.	-	6.86E7
58	27.12	-	563.3399	n.i.	-	1.43E7
59	28.00	$C_{16}H_{32}O_3$	271.2264 (-3.06)	fatty acids	3-hydroxy palmitic acid_2398-34-7[25]* or isomer	2.34E7
60	28.73	$C_{18}H_{34}O_3$	297.2417 (-4.27)	fatty acids	ricinoleic acid_141-22-0[19] or isomer	4.98E7
61	29.30	$C_{16}H_{32}O_3$	271.2264 (-3.36)	fatty acids	3-hydroxy palmitic acid_2398-34-7[25]* or isomer	2.46E7
62	29.73	$C_{18}H_{34}O_3$	297.2428 (-0.44)	fatty acids	ricinoleic acid_141-22-0[19] or isomer	8.31E6

Note: (PPD) phenylpropanoid-derivative; (*) Not reported to *Astrocaryum and* / or Arecaceae; (AO) compound described for antioxidant activity; (CAS) chemical abstracts service available at https://www.cas.org/products/scifinder

Table S3. Water intake and food consumption every four days of experiment; Body weight, organ weights and relative organ weights of the TPE-treated animals.

Parameters	Experimental groups					
	Control	CPP	CPP+LEV	CPP+TUC100	CPP+TUC200	CPP+TUC400
Food consumption (g/4day)	611.80 ± 109.40	368.00 ± 99.73[a]	364.80 ± 88.17[a]	328.30 ± 97.30[a]	426.80 ± 104.70	378.80 ± 80.41[a]
Water intake (mL/4day)	1110.00 ± 185.70	925.00 ± 117.30	770.00 ± 151.90[a]	765.00 ± 143.90[a]	910.00 ± 123.30	757.50 ±106.90[a]
Initial body weight (g)	258.30 ± 25.07	279.50 ± 12.11	272.50 ± 17.49	285.30 ± 17.24	279.20 ± 14.77	283.00 ± 21.59
Final body weight (g)	333.70 ± 28.97	275.30 ± 15.42[a]	268.00 ± 18.99[a]	272.20 ± 15.01[a]	294.00 ± 15.94	272.50 ± 31.13[a]
Kidneys (g)	2.66 ± 0.20	2.29 ± 0.07[a]	2.17 ± 0.19[a]	2.24 ± 0.26[a]	2.36 ± 0.17	2.15 ± 0.19[a]
Liver (g)	15.55 ± 2.29	13.00 ± 0.44	11.51 ± 1.23[a]	11.97 ± 0.76[a]	12.53 ± 1.38[a]	11.33 ± 1.21[a]
Heart (g)	1.14 ± 0.07	1.01 ± 0.08	0.96 ± 0.07	0.96 ± 0.07	1.07 ± 0.10	1.00 ± 0.10
Spleen (g)	0.86 ± 0.09	1.07 ± 0.14	0.99 ± 0.16	0.90 ± 0.19	0.98 ± 0.08	0.97 ± 0.23
Thymus (g)	1.02 ± 0.17	0.57 ± 0.06[a]	0.55 ± 0.05[a]	0.68 ± + 0.16[a]	0.53 ± 0.07[a]	0.51 ± 0.08[a]
Lung (g)	1.55 ± 0.20	1.56 ± 0.17	1.39 ± 0.16	1.69 ± 0.54	1.50 ± 0.12	1.50 ± 0.30
Relative organ weight (%)						
Kidneys	0.89 ± 0.03	0.85 ± 0.01	0.80 ± 0.06[a]	0.81 ± 0.08	0.84 ± 0.02	0.80 ± 0.04
Liver	5.20 ± 0.39	4.81 ± 0.17	4.35 ± 0.33[a]	4.22 ± 0.67[a]	4.45 ± 0.34[a]	4.06 ± 0.26[a]
Heart	0.38 ± 0.02	0.37 ± 0.02	0.35 ± 0.02	0.35 ± 0.02	0.38 ± 0.04	0.36 ± 0.02
Spleen	0.29 ± 0.04	0.40 ± 0.06	0.37 ± 0.06	0.33 ± 0.08	0.35 ± 0.03	0.35 ± 0.08
Thymus	0.34 ± 0.03	0.23 ± 0.03[a]	0.20 ± 0.02[a]	0.24 ± 0.06[a]	0.17 ± 0.05[a]	0.16 ± 0.06[a]
Lung	0.52 ± 0.04	0.58 ± 0.06	0.52 ± 0.04	0.61 ± 0.19	0.53 ± 0.05	0.54 ± 0.07

Data are expressed as mean ± S.D. One-way ANOVA/Kruskal-Wallis followed by Tukey test/Dunn's (n = 6). The values were considered to be significantly different when $p < 0.05$. [a]Different from the control.

Table S4. Water intake and food consumption every four days of experiment; Body weight, organ weights and relative organ weights of the TPE-pretreated animals.

Parameters	Experimental groups					
	Control	CPP	CPP+LEV	CPP+TUC100	CPP+TUC200	CPP+TUC400
Food consumption (g/4day)	497.00 ± 87.53	529.80 ± 117.90	471.30 ± 74.18	491.30 ± 133.40	490.80 ± 106.50	538.80 ± 110.20
Water intake (mL/4day)	860.00 ± 140.70	927.50 ± 111.80	907.50 ± 146.60	922.50 ± 100.10	957.50 ± 119.30	965.00 ± 168.40
Initial body weight (g)	261.20 ± 47.81	304.70 ± 14.64	289.30 ± 18.90	291.00 ± 20.24	301.30 ± 22.15	317.50 ± 9.39[a]
Final body weight (g)	339.80 ± 22.93	326.20 ± 12.26	322.50 ± 15.76	317.20 ± 21.98	321.50 ± 42.50	348.70 ± 14.90
Kidneys (g)	2.62 ± 0.16	2.59 ± 0.12	2.50 ± 0.28	2.28 ± 0.15	2.45 ± 0.20	2.60 ± 0.12
Liver (g)	14.00 ± 1.64	14.21 ± 0.84	13.28 ± 1.20	12.53 ± 0.70	12.57 ± 1.04	13.79 ± 0.99
Heart (g)	1.30 ± 0.15	1.22 ± 0.04	1.17 ± 0.11	1.16 ± 0.05	1.14 ± 0.13	1.18 ± 0.06
Spleen (g)	0.92 ± 0.13	0.52 ± 0.05[a]	0.55 ± 0.06[a]	0.50 ± 0.08[a]	0.47 ± 0.05[a]	0.53 ± 0.07[a]
Thymus (g)	0.76 ± 0.15	0.44 ± 0.14[a]	0.43 ± 0.12[a]	0.36 ± 0.14[a]	0.46 ± 0.16[a]	0.44 ± 0.13[a]
Lung (g)	1.59 ± 0.43	1.51 ± 0.13	1.85 ± 0.20	1.45 ± 0.11	1.49 ± 0.22	1.55 ± 0.16
Relative organ weight (%)						
Kidneys	0.85 ± 0.03	0.79 ± 0.02	0.80 ± 0.05	0.76 ± 0.01	0.77 ± 0.04	0.76 ± 0.04
Liver	4.51 ± 0.22	4.38 ± 0.09	4.26 ± 0.26	4.05 ± 0.20	4.07 ± 0.15	4.06 ± 0.19
Heart	0.42 ± 0.03	0.38 ± 0.02	0.37 ± 0.02	0.37 ± 0.01	0.36 ± 0.01	0.36 ± 0.01
Spleen	0.29 ± 0.02	0.16 ± 0.01[a]	0.17 ± 0.02[a]	0.16 ± 0.02[a]	0.14 ± 0.01[a]	0.15 ± 0.02[a]
Thymus	0.24 ± 0.03	0.13 ± 0.04[a]	0.14 ± 0.04[a]	0.11 ± 0.04[a]	0.14 ± 0.05[a]	0.12 ± 0.03[a]
Lung	0.51 ± 0.13	0.46 ± 0.03	0.55 ± 0.02	0.47 ± 0.07	0.46 ± 0.03	0.45 ± 0.03

Data are expressed as mean ± S.D. One-way ANOVA/Kruskal-Wallis followed by Tukey test/Dunn's (n = 6). The values were considered to be significantly different when $p < 0.05$. [a]Different from the control.

Table S5. Biochemical parameters from animals exposed to CPP and treated or pretreated with TPE 100, 200 and 400 mg/kg.

Parameters	Experimental groups					
Treatment	Control	CPP	CPP+LEV	CPP+TPE100	CPP+TPE200	CPP+TPE400
BChE (U/L)	204.30 ± 42.61	231.10 ± 14.27	233.20 ± 38.74	218.10 ± 38.72	225.30 ± 52.51	267.20 ± 36.50
GLU (mg/dL)	270.80 ± 25.62	227.80 ± 36.89	224.30 ± 26.72	233.20 ± 42.51	257.50 ± 83.20	222.50 ± 37.78
CHOL (mg/dL)	57.00 ± 7.51	58.17 ± 6.43	55.00 ± 8.24	53.00 ± 6.36	54.50 ± 5.32	56.50 ± 4.08
TG (mg/dL)	173.00 ± 40.12	99.83 ± 16.02	93.00 ± 21.18	82.40 ±14.64[a]	93.33 ± 25.75	94.50 ± 38.39[a]
P (mg/dL)	7.43 ± 0.44	7.51 ± 0.44	7.33 ± 0.50	7.36 ± 0.37	7.56 ± 0.52	7.03 ± 1.08
K^+ (mmol/L)	5.20 ± 0.27	4.76 ± 0.32	5.11 ± 0.21	5.02 ± 0.40	5.08 ± 0.44	4.63 ± 0.28
Ca (mg/dL)	11.50 ± 0.42	11.52 ± 0.38	11.12 ± 0.25	11.38 ± 0.32	11.35 ± 0.56	11.23 ± 0.44
Na^+ (mmol/L)	150.10 ± 17.31	136.70 ± 10.44	143.90 ± 2.99	145.10 ± 3.57	146.00 ± 7.17	148.00 ± 2.70
LDH (U/L)	360.80 ± 127.10	253.00 ± 126.80	474.30 ± 58.80[b]	220.90 ± 121.50	284.10 ± 115.80	161.20 ±55.60[a]
Pretreatment	Control	CPP	LEV+CPP	TPE100+CPP	TPE200+CPP	TPE400+CPP
BChE (U/L)	230.90 ± 27.47	241.40 ± 25.56	225.90 ± 80.68	206.00 ± 43.57	211.40 ± 28.37	214.10 ± 36.03
GLU (mg/dL)	173.00 ± 17.22	204.50 ± 24.16	220.70 ± 36.80[a]	224.80 ± 21.66[a]	207.50 ± 18.75	212.00 ± 14.93
CHOL (mg/dL)	67.20 ± 6.57	71.50 ± 8.55	81.67 ± 20.17	64.33 ± 7.03	64.83 ± 11.43	77.83 ± 11.50
TG (mg/dL)	161.60 ± 43.17	150.80 ± 36.32	98.50 ± 26.74	147.00 ± 55.57	95.50 ± 19.44	116.70 ± 54.42
P (mg/dL)	7.02 ± 0.41	6.20 ± 0.47	5.96 ± 0.45[a]	6.33 ± 0.54	6.56 ± 0.42	6.78 ± 0.83
K^+ (mmol/L)	5.28 ± 0.21	4.76 ± 0.29	4.16 ± 1.09[a]	4.70 ± 0.72	4.73 ± 0.33	4.78 ± 0.51
Ca (mg/dL)	11.40 ± 0.60	11.02 ± 0.50	10.47 ± 0.44[a]	11.00 ± 0.34	10.68 ± 0.33	10.67 ± 0.32
Na^+ (mmol/L)	147.70 ± 2.26	145.30 ± 2.63	143.00 ± 6.58	145.60 ± 3.17	149.40 ± 2.56	145.90 ± 3.76
LDH (U/L)	393.40 ± 185.00	79.72 ± 21.31[a]	116.90 ± 62.76	93.45 ± 35.94[a]	170.00 ± 96.79	152.10 ± 116.30

Data are expressed as mean ± S.D. One-way ANOVA/Kruskal-Wallis followed by Tukey test/Dunn's (n = 6). The values were considered to be significantly different when $p < 0.05$. [a]Different from the control. [b]Different from the CPP group.

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7 DISCUSSÃO

O câncer é uma doença caracterizada pelo crescimento anormal de células, que pode ocorrer em diferentes regiões do corpo. Essa doença tornou-se um sério problema de saúde pública e é responsável por um grande número de mortes mundialmente (WHO, 2020). Além disso, as estimativas são de que os números de novos casos de câncer continuem crescendo. Na atualidade, podemos citar três modalidades de tratamento disponíveis: a cirurgia, a radioterapia e a quimioterapia. Elas podem ser utilizadas combinadas ou não, porém a grande maioria dos cânceres exige alguma combinação entre elas (INCA, 2019). A radioterapia consiste no uso de RI para induzir a morte celular. Pode agir direta ou indiretamente: diretamente quando atinge a molécula de DNA e indiretamente quando interage com as moléculas de água, o que leva à formação de espécies reativas (ER). Essas ER, quando em excesso, podem atacar lipídeos, proteínas e o DNA. Por outro lado, a quimioterapia utiliza os fármacos, conhecidos como quimioterápicos, para levar à morte celular. Atualmente, existem muitas opções de quimioterápicos, cada qual com seu mecanismo de ação. Entretanto, as duas modalidades terapêuticas não são seletivas somente às células tumorais, assim, os tecidos e órgãos saudáveis também são afetados por elas. Tanto na radioterapia quanto na quimioterapia, os efeitos adversos decorrentes podem se tornar graves ao longo do tratamento. Esses efeitos incluem desde náusea, vômitos e desidratação até linfopenia, trombocitopenia e neuropatia periférica, entre outros. Muitas vezes, esses efeitos estão associados à redução de dose e interrupção do tratamento.

O tratamento terapêutico para os efeitos adversos decorrentes da radioterapia e quimioterapia são limitados e, no caso da radioterapia, também podem apresentar efeitos tóxicos aos pacientes. Por isso, tem-se buscado nos produtos naturais a possibilidade de terapias alternativas para atenuar esses efeitos. Nesse contexto, o tucumã (*Astrocaryum aculeatum*) foi o fruto de escolha deste trabalho, por possuir compostos fitoquímicos que justificam suas possíveis atividades biológicas. Esse fruto é de origem Amazônica e é amplamente consumido pela população local. É um fruto ainda pouco estudado, tanto em termos de caracterização fitoquímica quanto em estudos sobre suas atividades *in vivo*. Alguns estudos já demonstraram suas

atividades *in vitro* como citoprotetora, antimicrobiana e anti-inflamatória (SAGRILLO et al. 2015; JOBIM et al., 2014; CABRAL et al., 2020).

Pelo fato de muitos estudos já mostrarem o potencial biológico que as plantas e seus derivados têm em mitigar os efeitos adversos da RI e dos quimioterápicos, nosso estudo investigou a potencial atividade do extrato de tucumã contra os efeitos deletérios da radiação em um modelo experimental *in vitro*. Para tanto, foram utilizadas linhagens celulares de fibroblastos e queratinócitos humanos. As células foram tratadas com o EBT (100 µg/mL) e, após 24 h, foram expostas à RI na dose de 2 Gy. Após 24 h da exposição, as análises sobre mortalidade celular, parâmetros do estresse oxidativo, fatores de crescimento de fibroblastos e queratinócitos e marcadores apoptóticos foram realizadas. Foi possível observar o aumento da lipoperoxidação e carbonilação proteica, importantes marcadores de dano oxidativo. Além disso, a 8-OHdG também teve seus níveis elevados pela exposição à RI, indicando dano ao DNA. O pré-tratamento com o EBT foi capaz de atenuar esses danos, além de restaurar os níveis dos marcadores de apoptose *Bax* e *Bcl-2*, que também foram alterados pela RI. Os fatores de crescimento de queratinócitos e fibroblastos também foram afetados pela exposição à RI e o pré-tratamento com o extrato reparou seus níveis. Substâncias com forte capacidade antioxidante são importantes candidatos na busca por novos radioprotetores. Além disso, compostos como polifenóis, flavonoides e carotenoides mostram importantes mecanismos para estabilizar as espécies reativas. Sagrillo et al. (2015) demonstraram a presença desses compostos no EBT, além da sua forte capacidade antioxidante. Esses resultados sugerem que o tucumã é um potencial alvo de estudo para agir como um radioprotetor.

Nosso estudo *in vitro* apresentou algumas limitações metodológicas, as quais tentamos excluir em um segundo estudo. Entretanto, também não obtivemos os resultados esperados. Dessa maneira, decidimos investigar os potenciais efeitos biológicos do fruto em modelos experimentais *in vivo*. Para isso, 10 kg de fruto foram obtidos de Manaus (Amazonas) e o extrato bruto foi obtido através de maceração seguida de extração em uma solução de etanol absoluto e água por quatro dias, com agitação diária. Ao final, o material foi filtrado, submetido à rota evaporação e liofilizado. O conteúdo total de polifenóis e flavonoides foi analisado através de espectrofotometria, a qual revelou uma grande concentração desses compostos no extrato. Além disso, analisamos a capacidade antioxidante do extrato através do

método do DPPH, o qual evidenciou a forte atividade do mesmo. Esses resultados estão de acordo com os encontrados no estudo de Sagrillo et al. (2015).

A espécie *A. aculeatum* ainda é pouco estudada no que diz respeito aos seus constituintes fitoquímicos. Sendo assim, análises por HPLC-PAD, GC-MS e UHPLC-ESI-HRMS foram realizadas para identificar os constituintes presentes no EBT. Através do HPLC-PAD foi possível determinar a presença de compostos fenólicos (taninos, derivados de flavonóis) e carotenoides (β-caroteno e luteína). A análise por GC-MS demonstrou que o EBT é rico em ácidos graxos insaturados e saturados e triterpenos, incluindo os ácidos palmítico e linoleico. A análise espectrométrica por UHPLC-ESI-HRMS confirmou a presença de ácidos graxos como os principais constituintes do EBT e de outros compostos como ácidos orgânicos, flavonoides e catequinas. Esses resultados são importantes, visto que é uma espécie ainda pouco estudada. Os compostos identificados no EBT são conhecidos por possuírem forte capacidade antioxidante, o que lhes confere importante ação contra os danos do estresse oxidativo. O dano oxidativo está relacionado ao maior risco de desenvolvimento de câncer, doenças cardiovasculares e desordens inflamatórias (SILVA et al., 2010). Os compostos antioxidantes atuam absorvendo radicais livres e inibindo a cadeia de iniciação ou interrompendo a cadeia de propagação das reações oxidativas promovidas pelos radicais (SILVA et al., 2010). Polifenóis, flavonoides, catequinas e carotenoides apresentam forte capacidade antioxidante, efeitos cardioprotetor e anti-inflamatório, além de redução do risco de catarata, entre outros (SILVA et al., 2010; STRACK & SOUZA; 2012). Logo, a inclusão de frutas e vegetais na dieta está associada à redução do risco de desenvolvimento de doenças crônicas. Dessa maneira, estudos envolvendo o fruto do tucumã são essenciais para confirmar sua eficácia e segurança.

Até a finalização deste trabalho, estudos sobre a possível toxicidade da espécie não estavam disponíveis na literatura. Assim, iniciamos nossa pesquisa com os estudos de toxicidades orais aguda e de doses repetidas para que, posteriormente, pudéssemos investigar a potencial atividade do EBT em ratos contra a toxicidade da ciclofosfamida. As plantas e seus derivados muitas vezes são percebidos como agentes “livres de riscos à saúde” justamente por serem naturais. Porém, ao contrário do que se acredita, eles podem trazer riscos à saúde do usuário. Por isso, os testes toxicológicos são essenciais para validar a eficácia e segurança de uso de uma dada substância. Muitos protocolos existem para padronizar esses ensaios e evitar o uso

desnecessário de animais. Neste trabalho, utilizamos os protocolos 423 e 407 da OECD para realização dos testes de toxicidade aguda e de doses repetidas, respectivamente. No teste de toxicidade aguda, após a administração da dose única de 2000 mg/kg do EBT em ratas, nenhuma mortalidade foi observada, assim como nenhum sinal de toxicidade. Não foram observadas alterações nos pesos corporais dos animais nem nas análises hematológicas, bioquímicas e histopatológicas. Além disso, o tratamento com o extrato não interferiu nos marcadores de estresse oxidativo analisados. Assim, o extrato pode ser classificado na categoria 5, na qual a toxicidade letal aguda é superior a 2000 mg/kg, de acordo com o Sistema Geral Harmonizado da OECD.

Para o estudo de toxicidade de doses repetidas, ratos machos e fêmeas foram tratados com o EBT durante 28 dias. As doses do extrato foram de 200, 400 e 600 mg/kg. No decorrer do experimento, nenhuma morte foi registrada e também não houve sinais de toxicidade. O teste do campo aberto, importante para avaliar o comportamento tipo ansiedade e atividade locomotora, foi realizado e não evidenciou nenhuma alteração nos animais. O peso corporal e o consumo de água e ração não tiveram alterações que indicassem danos à saúde dos animais. O sistema hematopoiético é um dos mais afetados pela toxicidade de uma substância, tornando-se um importante marcador biológico. Além disso, AST e ALT são importantes biomarcadores de danos hepáticos, assim como CRE e URE são para danos renais. Assim, o aumento de seus níveis pode indicar a presença de toxicidade. No nosso estudo, os parâmetros hematológicos e bioquímicos não apresentaram alterações.

As análises histológicas do tecido hepático não apresentaram alteração em nenhuma das doses testadas, tanto em machos quanto em fêmeas. Em contrapartida, no tecido renal dos ratos machos tratados com a dose mais alta (600 mg/kg) foram encontradas alterações morfológicas, indicando uma possível toxicidade do extrato. Nas fêmeas nenhuma alteração foi observada. Esses resultados indicam uma dependência de dose e de gênero, visto que a alteração foi encontrada somente na dose mais alta e em machos. Segundo Olayode et al. (2019), os machos são mais sensíveis que as fêmeas em relação às lesões hepáticas e renais. O estudo realizado por Souza et al. (2013) evidenciou que o tratamento *in vitro* com o extrato de tucumã provocou genotoxicidade quando exposto às células por mais tempo e nas concentrações mais altas. Esses resultados sugerem que a genotoxicidade do tucumã é dependente de concentração e de tempo de exposição. Assim, no presente estudo,

também podemos inferir que a toxicidade do EBT foi dose e tempo dependentes, além de evidenciar a toxicidade gênero-dependente. Isso se dá devido ao fato de a toxicidade ter sido observada apenas em machos, na dose mais alta e quando administrado repetidamente. Muitas variáveis podem influenciar na resposta dos animais ao extrato como a presença de compostos bioativos e sua biodisponibilidade assim como seu metabolismo e absorção (SOUZA et al., 2013). A absorção e biodisponibilidade dos compostos depende da fonte alimentícia. Os carotenoides, por exemplo, são absorvidos de forma semelhante aos lipídios, porém existem nutrientes que podem interferir no processo de absorção quando ingeridos juntamente. De fato, a absorção de β-caroteno é afetada pela concentração de gordura da dieta (AMBRÓSIO et al., 2006). Pelo fato de ser um fruto rico em ácidos graxos, essa característica pode ter aumentado a biodisponibilidade dos carotenoides nos animais. Sendo assim, o EBT, quando administrado repetidamente e em doses altas, pode apresentar toxicidade, sendo indicado seu uso em doses mais baixas.

Os ensaios toxicológicos são essenciais para definir as doses seguras de uma substância. Após nossos estudos, foi possível determinar as doses a serem utilizadas no experimento que avaliou o potencial efeito protetor do EBT contra a toxicidade da ciclofosfamida (CFF). Para esse estudo, as doses escolhidas do extrato foram de 100, 200 e 400 mg/kg. Para induzir a toxicidade hematológica e de órgãos, a CFF foi o quimioterápico de escolha na dose de 150 mg/kg, via intraperitoneal. Os animais foram tratados oralmente com o EBT ou com o fármaco padrão (levamisol). O tratamento oral durou 14 dias e, ao todo, o experimento teve 17 dias de tratamento. Esse experimento foi dividido em dois, no qual o primeiro consistiu em induzir o dano com CFF e depois tratar oralmente (tratamento). O segundo foi o pré-tratamento: os animais foram primeiramente tratados oralmente e após a indução com CFF foi realizada. Ao final, foram observadas diferenças significativas no peso corporal dos animais e no consumo de água e ração. Além disso, o peso dos órgãos e o peso relativo também foram alterados, todos comparados aos animais não tratados com CFF. Em relação às análises hematológicas e bioquímicas, alterações foram observadas em diferentes parâmetros, indicando supressão no sistema hematopoiético e lesões renais e hepáticas, além de afetar baço e timo. Juntos, esses resultados mostram que a CFF foi capaz de induzir dano nos animais. Tanto o tratamento quanto o pré-tratamento com o EBT não conseguiram restaurar significativamente esses parâmetros alterados pela CFF. A exposição à CFF também

alterou os marcadores do estresse oxidativo (MDA e carbonilação) e reduziu a atividade da SOD. A administração oral com EBT diminuiu os níveis de MDA e carbonilação, enquanto aumentou a atividade da SOD, sendo que o pré-tratamento mostrou os melhores resultados nessas análises. Em princípio, no primeiro experimento os animais apresentaram maiores hepato e nefrotoxicidade, evidenciadas pelas alterações nos pesos do fígado e rim e pelos marcadores de lesão hepática e renal através da análise bioquímica. Já no segundo estudo, foi evidenciado um maior dano ao sistema sanguíneo, evidenciado pelos pesos do timo e baço, além das análises hematológicas. Ademais, a administração com o EBT melhorou o dano oxidativo e a defesa antioxidante enzimática, indicando seu possível mecanismo de ação. Esses efeitos podem estar associados aos compostos fitoquímicos encontrados no extrato, que são conhecidos por sua forte capacidade antioxidante. Essa atividade é associada à neutralização de espécies reativas geradas em excesso, evitando, assim, o dano oxidativo. Por haver poucos estudos *in vivo* sobre as atividades do extrato do fruto de tucumã, pesquisas futuras se fazem necessárias para confirmar os benefícios do consumo da espécie.

8 CONCLUSÃO

Em suma, o extrato de tucumã apresentou um potencial efeito radioprotetor *in vitro*. Entretanto, o modelo de exposição à radiação ionizante proposto neste estudo não foi capaz de gerar os resultados esperados. Por isso, mais estudos são necessários para confirmar o uso do tucumã contra os danos da radiação. As análises fitoquímicas mostraram que o tucumã é um fruto rico em ácidos graxos saturados e insaturados, além de apresentar carotenoides, flavonoides, catequinas e terpenos. O estudo de toxicidade aguda *in vivo* determinou a toxicidade do extrato como sendo maior que 2000 mg/kg, podendo ser classificado na categoria 5 da OECD, na qual apresenta pouca ou nenhuma toxicidade. Por outro lado, a administração repetida e na dose mais alta induziu nefrotoxicidade em ratos machos, evidenciada pela análise histológica. Os outros parâmetros analisados não sofreram alterações, e em fêmeas não foram observados sinais de toxicidade. No geral, os resultados sugerem que a toxicidade apresentada pelo extrato foi dose e gênero dependentes, não sendo indicado seu uso em doses altas e repetidamente, pois pode apresentar toxicidade, especialmente renal. A exposição à ciclofosfamida induziu efeitos tóxicos nos animais, observados através dos parâmetros fisiológicos, análises hematológicas, bioquímicas e marcadores do estresse oxidativo. O pré-tratamento com o extrato mostrou resultados satisfatórios na atenuação do dano oxidativo induzido pela CFF. Esse fato pode ser atribuído à presença de compostos antioxidantes no extrato, capazes de neutralizar as espécies reativas, reduzindo o dano oxidativo. Em geral, os resultados encontrados neste estudo são promissores, indicando que o extrato de tucumã apresenta potenciais atividades farmacológicas. Além disso, pela primeira vez, foram descritas as toxicidades orais aguda e de doses repetidas do extrato em ratos, o que possibilita a escolha de doses seguras para novos estudos *in vivo*. Por fim, torna-se necessária a realização de estudos adicionais sobre as possíveis atividades biológicas do extrato de tucumã para melhor compreender seus mecanismos de ação, especialmente em modelos experimentais *in vivo*.

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ANEXO A – CERTIFICADO DE APROVAÇÃO CEUA (1)

Comissão de Ética no Uso de Animais
da
Universidade Federal de Santa Maria

CERTIFICADO

Certificamos que a proposta intitulada "ESTUDO DA TOXICIDADE AGUDA DO EXTRATO BRUTO DA POLPA DE TUCUMÃ (Astrocaryum aculeatum Meyer) EM MODELO ANIMAL ", protocolada sob o CEUA nº 6572091017, sob a responsabilidade de **Liliane de Freitas Bauermann** *e equipe; Camille Gaube Guex; Amanda Szymansky Heck; Cibele Ferreira Teixeira; Gabriela Buzzati Cassanego* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Universidade Federal de Santa Maria (CEUA/UFSM) na reunião de 09/11/2017.

We certify that the proposal "ACUTE TOXICITY STUDY OF CRUDE EXTRACT OF TUCUMÃ PULP (Astrocaryum aculeatum Meyer) IN RATS ", utilizing 12 Heterogenics rats (12 females), protocol number CEUA 6572091017, under the responsibility of **Liliane de Freitas Bauermann** *and team; Camille Gaube Guex; Amanda Szymansky Heck; Cibele Ferreira Teixeira; Gabriela Buzzati Cassanego* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the Federal University of Santa Maria (CEUA/UFSM) in the meeting of 11/09/2017.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 11/2017 a 04/2018 Área: Farmacologia

Origem:	Biotério Central UFSM						
Espécie:	Ratos heterogênicos	sexo:	Fêmeas	idade:	8 a 12 semanas	N:	12
Linhagem:	Wistar			Peso:	150 a 240 g		

Resumo: Plantas são conhecidas por possuírem compostos bioativos com importantes propriedades farmacológicas e, por serem de fácil acesso à população e possuírem baixo custo, podem ser facilmente implementadas à dieta. Porém, produtos naturais também podem trazer efeitos nocivos à saúde quando não utilizados em condições adequadas. O tucumã (Astrocaryum aculeatum Meyer) é um fruto rico em carotenoides, fibras e proteínas, o que lhe confere importantes atividades biológicas como antioxidante e anti-inflamatória. Porém, estudos sobre sua toxicidade são escassos na literatura, tornando válida a pesquisa acerca da segurança do uso deste fruto. Assim, o objetivo deste trabalho será investigar a possível toxicidade aguda do extrato bruto da polpa de tucumã (TUC). Para isto, serão utilizadas 12 ratas Wistar, nulíparas e não grávidas, divididas em grupos controle e teste. Os animais do grupo teste receberão, via oral (gavagem), uma dose única de 2000 mg/kg (2 mL/kg) do TUC. Ao grupo controle será administrada água destilada (via oral 2 mL/kg). O peso de cada animal será registrado 30 minutos antes da administração e, então, durante todo período de estudo; os animais serão observados durante 14 dias. No 15º dia, os animais serão eutanasiados através de anestesia profunda, seguida de punção cardíaca para coleta do sangue. Análises dos parâmetros bioquímicos e hematológicos serão realizadas; fígado e rins serão removidos para investigação da peroxidação lipídica, atividade de enzimas antioxidantes e análise histopatológica.

Local do experimento: Laboratório de Fisiologia Experimental: prédio 21, sala 5229 - UFSM.

Santa Maria, 09 de novembro de 2017

Prof. Dr. Denis Broock Rosemberg
Coordenador da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

Prof. Dr. Saulo Tadeu Lemos Pinto Filho
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

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Horário de atendimento: das 8:30 ás 12h e 14h ás 17hs : e-mail: ceua.ufsm@gmail.com
CEUA N 6572091017

ANEXO B – CERTIFICADO DE APROVAÇÃO CEUA (2)

Comissão de Ética no Uso de Animais
da
Universidade Federal de Santa Maria

CERTIFICADO

Certificamos que a proposta intitulada "AVALIAÇÃO DE SEGURANÇA DO EXTRATO BRUTO DA POLPA DE TUCUMÃ (Astrocaryum aculeatum) EM RATOS WISTAR: ESTUDO DE TOXICIDADE DE DOSES REPETIDAS ", protocolada sob o CEUA nº 2489120418, sob a responsabilidade de **Liliane de Freitas Bauermann** *e equipe; Camille Gaube Guex; Amanda Szymansky Heck; Gabriela Buzatti Cassanego; Lauren Pappis; Patricia Romualdo de Jesus; Rafaela Castro Dornelles* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Universidade Federal de Santa Maria (CEUA/UFSM) na reunião de 10/05/2018.

We certify that the proposal "SAFETY ASSESSMENT OF CRUDE EXTRACT OF TUCUMÃ PULP (Astrocaryum aculeatum) IN WISTAR RATS: REPEATED DOSE TOXICITY STUDY", utilizing 40 Heterogenics rats (20 males and 20 females), protocol number CEUA 2489120418, under the responsibility of **Liliane de Freitas Bauermann** *and team; Camille Gaube Guex; Amanda Szymansky Heck; Gabriela Buzatti Cassanego; Lauren Pappis; Patricia Romualdo de Jesus; Rafaela Castro Dornelles* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the Federal University of Santa Maria (CEUA/UFSM) in the meeting of 05/10/2018.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 05/2018 a 01/2019 Área: Farmacologia

Origem:	Biotério Central UFSM						
Espécie:	Ratos heterogênicos	sexo:	Machos	idade:	4 a 7 semanas	N:	20
Linhagem:	Wistar			Peso:	70 a 150 g		
Origem:	Biotério Central UFSM						
Espécie:	Ratos heterogênicos	sexo:	Fêmeas	idade:	4 a 7 semanas	N:	20
Linhagem:	Wistar			Peso:	70 a 150 g		

Resumo: O consumo de produtos naturais é uma prática muito comum pela população por serem se fácil acesso, baixo custo e devido a crença de que, por serem naturais, estão livres de efeitos nocivos. Os estudos toxicológicos têm por finalidade os possíveis efeitos adversos decorrentes do uso destes produtos, sendo importantes para a confirmar a segurança e eficácia de seu uso. O tucumã (Astrocaryum aculeatum Meyer) é um fruto nativo do Brasil, fonte de fibras e proteínas. É um fruto que possui confere importantes atividades biológicas como antioxidante, cicatrizante e anti-inflamatória, devido à presente de constituintes como carotenoides e flavonoides. Porém, estudos sobre sua toxicidade são limitados na literatura, tornando válida a pesquisa acerca da segurança do uso deste fruto. Assim, o objetivo deste trabalho será investigar a possível toxicidade subaguda do extrato bruto da polpa de tucumã (EBPT). Para isto, serão utilizados 40 ratos Wistar (20 machos e 20 fêmeas, n=10/grupo), divididos em 4 grupos: I) Controle (animais receberão água destilada 3mL/kg); II, III e IV) Animais tratados com o EBPT nas doses de 200, 400 e 600 mg/kg/dia, respectivamente. O tratamento terá uma duração de 28 dias e a administração do extrato será via oral, com auxílio de gavagem. O peso de cada animal será registrado durante todo período de estudo. No 29º dia, os animais serão eutanasiados através de anestesia profunda, seguida de punção cardíaca para coleta do sangue. O sangue será utilizado para a realização de análises hematológicas e o soro, obtido através de centrifugação, será utilizado para análise de parâmetros bioquímicos. Órgãos (fígado, rins, baço, coração e cérebro) serão removidos para investigação da peroxidação lipídica, atividade de enzimas antioxidantes e análise histopatológica.

Local do experimento: Laboratório de Fisiologia Experimental: prédio 21, sala 5229 - UFSM.

Santa Maria, 10 de maio de 2018

Prof. Dr. Denis Broock Rosemberg
Coordenador da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

Prof. Dr. Saulo Tadeu Lemos Pinto Filho
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

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Horário de atendimento: das 8:30 ás 12h e 14h ás 17hs : e-mail: ceua.ufsm@gmail.com
CEUA N 2489120418

ANEXO C – CERTIFICADO DE APROVAÇÃO CEUA (3)

Comissão de Ética no Uso de Animais
da
Universidade Federal de Santa Maria

CERTIFICADO

Certificamos que a proposta intitulada "EFEITO IMUNOMODULADOR DO EXTRATO BRUTO DA POLPA DE TUCUMÃ (Astrocaryum aculeatum) EM RATOS WISTAR IMUNOSSUPRIMIDOS", protocolada sob o CEUA nº 1039171218, sob a responsabilidade de **Liliane de Freitas Bauermann** *e equipe; Camille Gaube Guex; Gabriela Buzatti Cassanego; Kassia Caroline Figueredo; Lauren Pappis; Patricia Romualdo de Jesus; Rafaela Castro Dornelles* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Universidade Federal de Santa Maria (CEUA/UFSM) na reunião de 26/03/2019.

We certify that the proposal "IMMUNOMODULATOR EFFECT OF CRUDE EXTRACT OF TUCUMÃ PULP (Astrocaryum aculeatum) IN IMMUNOSUPPRESSION INDUCED IN RATS ", utilizing 80 Heterogenics rats (80 males), protocol number CEUA 1039171218, under the responsibility of **Liliane de Freitas Bauermann** *and team; Camille Gaube Guex; Gabriela Buzatti Cassanego; Kassia Caroline Figueredo; Lauren Pappis; Patricia Romualdo de Jesus; Rafaela Castro Dornelles* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the Federal University of Santa Maria (CEUA/UFSM) in the meeting of 03/26/2019.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 05/2019 a 12/2019 Área: Farmacologia

Origem:	Biotério Central UFSM						
Espécie:	Ratos heterogênicos	sexo:	Machos	idade:	6 a 7 semanas	N:	80
Linhagem:	Wistar			Peso:	150 a 200 g		

Resumo: O câncer é uma das principais causas de morte no mundo. Quimioterápicos são frequentemente utilizados para inibir a proliferação de células cancerígenas, no entanto, eles também podem prejudicar as células saudáveis e causar efeitos colaterais, como mielossupressão e imunossupressão, que limitam seu uso no tratamento. Pacientes imunocomprometidos estão mais suscetíveis à invasão e proliferação de microrganismos oportunistas, o que pode levar a sérias complicações e serem letais. As opções farmacológicas do tratamento de desordens imunológicas podem ocasionar a incidência de efeitos adversos severos, limitando seu uso. Assim, produtos naturais estão sendo alvo de pesquisas para que seu uso possa ser seguro e eficiente no tratamento destas desordens. O tucumã (Astrocaryum aculeatum Meyer) é um fruto nativo do Brasil, fonte de fibras e proteínas. É um fruto com importantes atividades biológicas como antioxidante, citoprotetora e antimicrobiana, devido à presença de constituintes como carotenoides e flavonoides. Assim, o objetivo deste trabalho será investigar a potencial atividade imunomoduladora do extrato bruto da polpa de tucumã (EBPT) em ratos Wistar imunossuprimidos. Para a indução da imunossupressão, os animais receberão ciclofosfamida (CFF) na dose de 50 mg/kg durante 3 dias consecutivos, via intraperitoneal, enquanto que os animais sem indução receberão salina 0,9%. Serão utilizados 80 ratos Wistar, divididos em 10 grupos (n=8/grupo): os grupos I, II, III, IV e V receberão salina 0,9% (ip) e serão tratados com água destilada, fármaco padrão ou extrato (200, 400 e 600 mg/kg), via oral, com auxílio de gavagem. Já os grupos VI, VII, VIII, IX e X receberão ciclofosfamida e, então, serão tratados como os grupos acima mencionados. O peso de cada animal será registrado durante todo período de estudo. Ao final do tratamento, os animais serão eutanasiados através de anestesia profunda, seguida de punção cardíaca para coleta do sangue. O sangue será utilizado para a realização de análises hematológicas e o soro, obtido através de centrifugação, será utilizado para análise de parâmetros bioquímicos. Órgãos (cérebro, baço, timo, rins e fígado) serão removidos para investigação da peroxidação lipídica, carbonilação proteica, atividade de enzimas antioxidantes e análise histopatológica.

Local do experimento: Laboratório de Fisiologia Experimental: prédio 21, sala 5229 - UFSM.

Santa Maria, 29 de março de 2019

Prof. Dr. Denis Broock Rosemberg
Coordenador da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

Prof. Dr. Saulo Tadeu Lemos Pinto Filho
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

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ANEXO D – COMPROVANTE DE ACESSO – SisGen (1)

Ministério do Meio Ambiente
CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO
SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

Comprovante de Cadastro de Acesso

Cadastro nº A7539F5

A atividade de acesso ao Patrimônio Genético, nos termos abaixo resumida, foi cadastrada no SisGen, em atendimento ao previsto na Lei nº 13.123/2015 e seus regulamentos.

Número do cadastro:	**A7539F5**
Usuário:	**UFSM**
CPF/CNPJ:	**95.591.764/0001-05**
Objeto do Acesso:	**Patrimônio Genético**
Finalidade do Acesso:	**Pesquisa**

Espécie

Astrocaryum aculeatum

Título da Atividade:	**Estudo do efeito radiomodificador e perfil toxicológico do extrato da polpa de tucumã (Astrocaryum aculeatum)**

Equipe

Liliane de Freitas Bauermann	**UFSM**
Camille Gaube Guex	**UFSM**

Data do Cadastro:	**26/09/2018 20:26:33**
Situação do Cadastro:	**Concluído**

Conselho de Gestão do Patrimônio Genético
Situação cadastral conforme consulta ao SisGen em **20:29** de **26/09/2018**.

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO - **SISGEN**

ANEXO E – COMPROVANTE DE ACESSO– SisGen (2)

Ministério do Meio Ambiente
CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

Comprovante de Cadastro de Acesso

Cadastro nº AADC663

A atividade de acesso ao Patrimônio Genético, nos termos abaixo resumida, foi cadastrada no SisGen, em atendimento ao previsto na Lei nº 13.123/2015 e seus regulamentos.

Número do cadastro:	AADC663
Usuário:	UFSM
CPF/CNPJ:	95.591.764/0001-05
Objeto do Acesso:	Patrimônio Genético
Finalidade do Acesso:	Pesquisa

Espécie

Astrocaryum aculeatum

Título da Atividade:	EFEITO IMUNOMODULADOR DO EXTRATO BRUTO DA POLPA DE TUCUMÃ (Astrocaryum aculeatum) EM RATOS WISTAR IMUNOSSUPRIMIDOS

Equipe

Liliane de Freitas Bauermann	UFSM
Camille Gaube Guex	UFSM

Data do Cadastro:	06/04/2019 15:55:14
Situação do Cadastro:	Concluído

Conselho de Gestão do Patrimônio Genético
Situação cadastral conforme consulta ao SisGen em 16:22 de 06/04/2019.

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO - SISGEN

ANEXO F – COMPROVANTE DE PUBLICAÇÃO (ARTIGO 2)

DRUG AND CHEMICAL TOXICOLOGY
https://doi.org/10.1080/01480545.2020.1777151

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RESEARCH ARTICLE

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Tucumã (*Astrocaryum aculeatum*) extract: phytochemical characterization, acute and subacute oral toxicity studies in *Wistar* rats

Camille Gaube Guex[a], Gabriela Buzatti Cassanego[b], Rafaela Castro Dornelles[a], Rosana Casoti[c,d], Ana Martiele Engelmann[e], Sabrina Somacal[f], Roberto Marinho Maciel[g], Thiago Duarte[a], Warley de Souza Borges[d], Cínthia Melazzo de Andrade[e], Tatiana Emanuelli[f], Cristiane Cademartori Danesi[g], Euler Esteves Ribeiro[h] and Liliane de Freitas Bauermann[a,b]

[a]Programa de Pós-Graduação em Farmacologia, Centro de Ciências da Saúde, Universidade Federal de Santa Maria, Santa Maria, Brasil; [b]Programa de Pós-Graduação em Ciências Farmacêuticas, Centro de Ciências da Saúde, Universidade Federal de Santa Maria, Santa Maria, Brasil; [c]Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, Brasil; [d]Programa de Pós-Graduação em Química, Universidade Federal do Espírito Santo, Vitória, Brasil; [e]Programa de Pós-Graduação em Medicina Veterinária, Centro de Ciências Rurais, Universidade Federal de Santa Maria, Santa Maria, Brasil; [f]Programa de Pós-Graduação em Ciência e Tecnologia dos Alimentos, Centro de Ciências Rurais, Universidade Federal de Santa Maria, Santa Maria, Brasil; [g]Departamento de Patologia, Universidade Federal de Santa Maria, Santa Maria, Brasil; [h]Universidade Aberta da Terceira Idade, Universidade do Estado do Amazonas, Manaus, Brasil

ABSTRACT

Natural products are often used by the population to treat and/or prevent several disorders. Tucumã is an Amazonian fruit widely consumed by local population and no *in vivo* toxicity studies regarding its safety are available in the literature to date. Therefore, the phytochemical characterization, acute and repeated dose 28-day oral toxicities of crude extract of tucumã's pulp (CETP) in *Wistar* rats were evaluated. For the CETP preparation, tucumã pulp was crushed and placed into sealed amber glass jars containing absolute ethanol solution for extraction. CETP phytochemical analyses evidenced the presence of carotenoids, flavonoids, unsaturated and satured fatty acids, and triterpenes. In the acute toxicity, female rats from the test group were treated with CETP at single dose of 2000 mg/kg. For the repeated dose toxicity, CETP was administered to male and female rats at doses of 200, 400 and 600 mg/kg, for 28 days. Body weight was recorded during the experiment and blood, liver and kidney were collected for further analysis. No mortality or toxicity signs were observed during the studies. CETP was classified as safe (category 5, OECD guide), in acute toxicity. In repeated dose study was observed alterations in some biochemical parameters, as well as in oxidative damage and enzymatic activity. Histopathological findings showed renal damage in male rats at higher dose. The data obtained suggest that CETP did not induced toxicity after exposure to a single or repeated doses in female rats. However, in males may be considered safe when given repeatedly in low doses.

ARTICLE HISTORY
Received 10 January 2020
Revised 8 May 2020
Accepted 25 May 2020

KEYWORDS
Carotenoids; tucumã; Arecaceae; toxicity; acute; repeated doses

1. Introduction

Plants are commonly used by human populations and often for medicinal purposes, such as in the cure and/or prevention of diseases. In developed and underdeveloped countries, it is estimated that 70–80% of the population uses complementary or alternative medicine (Araújo *et al.* 2017). Natural products have been targeted by many studies aiming to obtain molecules with therapeutic potential and are known to be important in the development of new drugs (Menegati *et al.* 2016). They represent an alternative treatment that is easily accessible, has low cost and is believed to be safe and without adverse effects because it is 'natural' (Traesel *et al.* 2014). To validate the potential role of natural products in improving the treatment of diseases and use as a functional food, studies on toxicity and safety are necessary.

Brazil has great biodiversity, particularly in its Amazon biome, which is characterized by a dense rainforest with hot and humid climate and heavy rains that are frequent all year round (Matos *et al.* 2019). With such broad diversity, the Amazon offers to local populations several different species of fruit. For example, *Astrocaryum aculeatum* Meyer, popularly known as 'tucumã', 'tucumã-do-amazonas' or 'tucumã-açu', is a palm tree belonging to the Arecaceae family that can be found in degraded environments among secondary vegetation in the Amazonian ecosystem (Maia *et al.* 2014). The fruits of this species are the most consumed part of the palm tree, followed by its roots, seeds, palm heart, leaves, flowers and flower sap (Agostini-Costa 2018).

The tucumã fruits are widely consumed by local population *in natura*, in sandwiches and tapioca, desserts and ice cream (Oliveira *et al.* 2018) and they are traditionally used to treat the respiratory system, infections, infestations and is also associated with digestive system disorders (Macía *et al.* 2011, Agostini-Costa 2018). Many fruits and oleaginous plants

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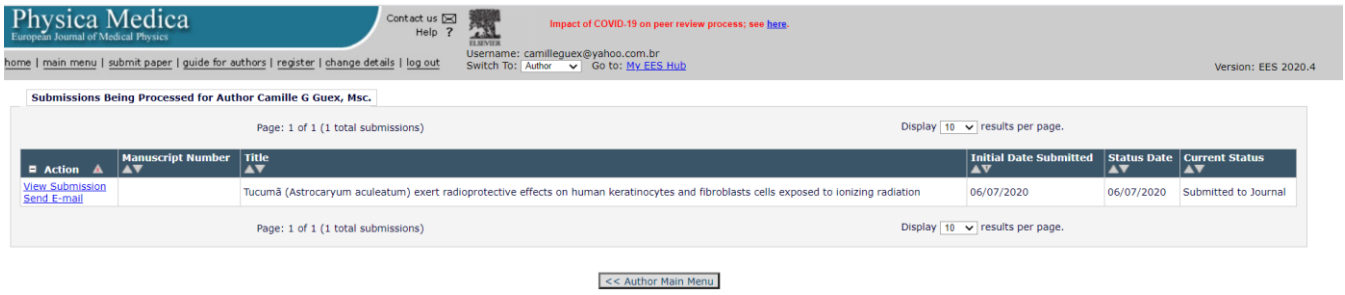
ANEXO G – COMPROVANTE DE SUBMISSÃO (ARTIGO 1)



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Page: 1 of 1 (1 total submissions) Display 10 results per page.

Action	Manuscript Number	Title	Initial Date Submitted	Status Date	Current Status
View Submission Send E-mail		Tucumã (Astrocaryum aculeatum) exert radioprotective effects on human keratinocytes and fibroblasts cells exposed to ionizing radiation	06/07/2020	06/07/2020	Submitted to Journal

Page: 1 of 1 (1 total submissions) Display 10 results per page.

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ANEXO H – COMPROVANTE DE SUBMISSÃO (ARTIGO 3)