Universidade Federal da Paraíba
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Suplementação Alimentar com *Arthrospira* (*Spirulina*) *platensis* Previne os Danos Causados pelo Consumo de Dieta Hipercalórica sobre a Reatividade Contrátil e Estresse Oxidativo no Íleo de Ratos Wistar

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Tese apresentada ao Programa dePós-graduação em Produtos Naturais eSintéticos Bioativos, do Centro de Ciências da Saúde, da Universidade Federal da Paraíba, como parte dos requisitos para obtenção do título de **Doutor em Produtos Naturais e Sintéticos Bioativos**. Área de concentração: **Farmacologia**.

Orientadora: Profa. Dra. Bagnólia Araújo Costa

João Pessoa
2023

PgPNSB

Pós Graduação em Produtos Naturais
e Sintéticos Bioativos

Ata da 365ª (trecentésima sexagésima quinta) Tese de Doutorado do(a) aluno(a) do Programa de Pós-Graduação em Produtos Naturais e Sintéticos Bioativos **Anderson Fellyp Avelino Diniz**, candidato(a) ao Título de "Doutor(a)" em Produtos Naturais e Sintéticos Bioativos na área de concentração Farmacologia.

Às quatorze horas (14h00) do dia vinte e cinco de agosto do ano dois mil e vinte e três (25/08/2023), no Auditório José Ribamar Lemos do Departamento de Ciências Farmacêuticas/CCS/UFPB, reuniram-se em caráter de Solenidade Pública os membros da Comissão designada para examinar o(a) discente **Anderson Fellyp Avelino Diniz**, candidato(a) ao Título de "DOUTOR(A)" em Produtos Naturais e Sintéticos Bioativos na área de concentração Farmacologia. Foram componentes da Banca Examinadora os pesquisadores Alice Valença Araújo, Ph.D em Farmacologia, José Luiz de Brito Alves, Ph.D em Neuropsiquiatria e Ciências do Comportamento, Robson Cavalcante Veras, Ph.D em Farmacologia, Márcia Regina Piuvezam, Ph.D em Microbiologia, e Bagnólia Araújo Costa, Ph.D em Biologia Molecular. Sendo a primeira, integrante do corpo docente da Universidade Federal de Pernambuco; e os demais, integrantes do corpo de pesquisadores da Universidade Federal da Paraíba. Dando início aos trabalhos, o(a) Presidente da Banca, professor(a) Bagnólia Araújo Costa, após declarar os objetivos da reunião, apresentou o(a) candidato(a) **Anderson Fellyp Avelino Diniz,** a quem concedeu a palavra para que dissertasse oral e sucintamente sobre o tema apresentado e intitulado "Suplementação alimentar com *Arthrospira (Spirulina) platensis* previne os danos causados pelo consumo de dieta hipercalórica sobre a reatividade contrátil e estresse oxidativo no íleo de ratos Wistar". Após discorrer sobre o referido tema durante cerca de cinquenta minutos, o(a) candidato(a) foi arguido(a) pelos Examinadores na forma Regimental. Em seguida, passou a comissão, em caráter secreto, a proceder à avaliação e julgamento do trabalho, concluindo por atribuir-lhe o conceito **APROVADO(A)**. Em face da aprovação, declarou o(a) Presidente, achar-se o(a) examinado(a) **Anderson Fellyp Avelino Diniz**, legalmente habilitado(a) a receber o Título de "DOUTOR(A)" em Produtos Naturais e Sintéticos Bioativos, na Área de Concentração Farmacologia, cabendo a Universidade Federal da Paraíba, providências, como de direito, a expedição do Diploma que o(a) mesmo(a) faz jus. Nada mais havendo a tratar, foi lavrada a presente ata que é abaixo assinada pelos membros da Comissão e pelo(a) discente.

Prof.ª Dr.ª Bagnólia Araújo Costa (Orientadora)

Alice Valença Araújo

Prof.ª Dr.ª Alice Valença Araújo (Examinadora)

Prof. Dr. José Luiz de Brito Alves (Examinador)

Prof. Dr. Robson Cavalcante Veras (Examinador)

Prof.ª Dr.ª Márcia Regina Piuvezam (Examinadora)

Anderson Fellyp A-Diniz

Anderson Fellyp Avelino Diniz (Discente)

“Porque dEle, por Ele e para Ele são todas as coisas”

Romanos 11:36

Dedicatórias

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Anderson Fellyp Avelino Diniz

Resumo

RESUMO

A obesidade é uma condição crônica caracterizada pelo acúmulo excessivo de gordura proveniente do consumo de dietas hipercalóricas que podem favorecer o desenvolvimento de doenças e desordens gastrointestinais, associadas ao estresse oxidativo e a secreção e liberação de mediadores inflamatórios. A alga *Arthrospira* (*Spirulina*) *platensis* (AP) é bastante utilizada nas indústrias nutracêutica e farmacêutica, e vem sendo alvo de diversas pesquisas, principalmente por sua rica composição química e nutricional e seus benefícios para a saúde, além de seu potencial antioxidante. No entanto, ainda não se tem estudos envolvendo a suplementação alimentar com AP na prevenção dos efeitos deletérios promovidos pelo consumo de uma dieta hipercalórica sobre o estresse oxidativo e a reatividade intestinal no íleo de ratos, bem como os possíveis mecanismos de ação adjacentes envolvidos. Dessa forma, visando a busca por novas alternativas terapêuticas, avaliou-se os efeitos da AP, em um modelo de obesidade experimental induzida por dieta hipercalórica e os mecanismos pelos quais a alga previne os danos à reatividade contrátil do íleo de rato. Os procedimentos experimentais foram aprovados pela Comissão de Ética no Uso de Animais da UFPB (certidão 2352101019). Os ratos Wistar foram divididos em grupo alimentado com dieta padrão (DP), grupo alimentado com dieta hipercalórica (DHC) e em grupo suplementado com AP na dose de 25 mg/kg (DHC + SP25), durante 8 semanas. Foram analisados os parâmetros nutricionais e histomorfométricos, o estresse oxidativo, os níveis de IL-1β tecidual e os mecanismos de ação funcionais envolvidos nas alterações da reatividade contrátil do íleo, *in vitro*. O consumo da dieta hipercalórica resultou no aumento da massa corporal final, como consequência do ganho de peso pelo consumo calórico, aumento das reservas adiposas epididimal,retroperitoneal e inguinal, e da adiposidade corporal, além disso aumentou o estresse oxidativo e os níveis de IL-1β, reduzindo proteínas antioxidantes (GSSH e SOD) e a capacidade antioxidante (CAT) no íleo. Interessantemente, tais efeitos deletérios promovidos pelo consumo da dieta hipercalórica sobre os parâmetros de obesidade experimental e estresse oxidativo foram prevenidos pela suplementação alimentar com *A. platensis*. Em relação à reatividade contrátil intestinal do íleo, os ratos (DHC) apresentaram redução da eficácia contrátil ao CCh (acoplamento farmacomecânico), que foram prevenidos pela AP. Esses efeitos preventivos da alga foram associados à modulação positiva das vias da Rho cinase (ROCK), do óxido nítrico (NO), dos prostanoides contráteis e do sistema antioxidante através da SOD. A integração de vias que favorecem a contração intestinal pode estar subjacente as alterações preventivas histomorfológicas evidenciadas pela ação da AP no íleo de ratos, bem como a presença do ácido gama- linolênico (GLA), como componente majoritário da alga. Portanto, a suplementação alimentar com *A. platensis* previne os danos associados ao consumo da dieta hipercalórica e desponta como um adjuvante na prevenção da obesidade e de doenças e/ou desordens intestinais agravadas por ela.

Palavras-chave: *Arthospira platensis*; obesidade; antioxidantes; reatividade intestinal; estresse oxidativo; inflamação.

Abstract

ABSTRACT

Obesity is a chronic condition characterized by excessive accumulation of fat from the consumption of hypercaloric diets that may favor the development of gastrintestinal diseases and disorders, associated with oxidative stress and the secretion and release of inflammatory mediators. The seaweed *Arthrospira (Spirulina) platensis* (AP) is widely used in the nutraceutical and pharmaceutical industries, and has been the subject of several studies, mainly due to its rich chemical and nutritional composition and its health benefits, in addition to its antioxidant potential. However, there are still no studies involving food supplementation with AP in preventing the deleterious effects promoted by the consumption of a hypercaloric diet on oxidative stress and intestinal reactivity in the ileum of rats, as well as the possible adjacent mechanisms of action involved. Thus, aiming at the search for new therapeutic alternatives, the effects of AP were evaluated in a model of experimental obesity induced by a hypercaloric diet and the mechanisms by which the seaweed prevents damage to the contractile reactivity of the rat ileum. The experimental procedures were approved by the UFPB Ethics Committee on Animal Use (certificate 2352101019). The Wistar rats were divided into a group fed a standard diet (SD), a group fed a hypercaloric diet (HCD) and a group supplemented with AP at a doseof 25 mg/kg (HCD + AP25), for 8 weeks. Nutritional and histomorphometric parameters, oxidative stress, tissue IL-1β levels and the functional mechanisms of action involved in changes in the contractile reactivity of the ileum, in vitro, were analyzed. Consumption of the hypercaloric diet resulted in an increase in final body mass, as a consequence of weight gain due to caloric consumption, an increase in epididymal, retroperitoneal and inguinal adipose reserves, and in body adiposity, in addition to increasing oxidativestress and IL- 1β, reducing antioxidant proteins and antioxidant capacity (TAC) in the ileum. Interestingly, such deleterious effects promoted by the consumption of a hypercaloric diet on parameters of experimental obesity and oxidative stress were prevented by dietary supplementation with *A. platensis*. Regarding the intestinal contractile reactivity of the ileum, the rats (HCD) showed reduced contractile efficacy to CCh (pharmacomechanical coupling), which were prevented by AP. These preventive effects of the seaweed were associated with the positive modulation of Rho kinase (ROCK), nitric oxide (NO), contractile prostanoids and antioxidant system pathways through SOD. The integration of pathways that favor intestinal contraction may underlie the preventive histomorphological changes evidenced by the action of AP in the ileum of rats, as well as the presence of gamma linolenic acid (GLA), as the major component of the alga. Therefore, food supplementation with *A. platensis* prevents the damage associated with the consumption of a hypercaloric diet and emerges a adjuvant in the prevention of obesity and diseases and/or intestinal disorders aggravated by it.

Keywords: *Arthrospira platensis*; obesity; antioxidants; intestinal reactivity; oxidative stress; inflammation.

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LISTA DE ABREVIATURAS

$[Ca^{2+}]_i$	concentração intracelular de Ca^{2+}
$[K^+]_e$	concentração extracelular de K^+
$4Ca^{2+}$-CaM	complexo cálcio-calmodulina
5-HT	serotonina
AC	ciclase de adenilil
ACh	acetilcolina
AGL	ácido graxo livre
AgRP	peptídio relacionado ao gene agouti
AIN	American Institute of Nutrition
ANG II	angiotensina II
ANOVA	análise de variância
Anvisa	Agência Nacional de Vigilância Sanitária
AP	*Arthrospira (Spirulina) platensis*
ARC	núcleo arqueado
ATP	trifosfato de adenosina
C/EBPα	proteína α estimuladora de ligação a CCAAT
CaM	calmodulina
cAMP	monofosfato cíclico de adenosina
CART	transcrito relacionado à anfetamina e à cocaína
CAT	catalase
Ca_V	canais para cálcio dependentes de voltagem
CCA	coeficiente de conversão alimentar
CCh	carbacol
CCK	colecistocinina
CCS	Centro de Ciências da Saúde
CEA	coeficiente de eficácia alimentar
CEUA	Comissão de Ética no Uso de Animais
cGMP	monofosfato cíclico de guanosina
CGPCC	coeficiente de ganho de peso por consumo calórico
CGRP	peptídio relacionado ao gene da calcitonina
CICR	liberação de Ca^{2+} induzida por Ca^{2+}

CLA	ácido linoleico conjugado
COX	ciclo-oxigenase
CPI-17	proteína inibitória dependente de fosforilação de 17 kDa
CYP	citocromo P450
DA	dopamina
DAG	diacilglicerol
DE	disfunção erétil
DHC	Deita hipercalórica
DPP-4	dipeptidil peptidase 4
e.p.m.	erro padrão da média
EDTA	ácido etilenodiamino tetra-acético
E_{max}	efeito máximo
eNOS	sintase do óxido nítrico endotelial
ERK	cinase reguladas por sinais extracelulares
EROs	espécies reativas de oxigênio
ERNs	espécies reativas de nitrogênio
ET-1	endotelina 1
ETC	cadeia transportadora de elétrons
FASEB	Federação das Sociedades Americanas para Biologia Experimental
FDA	Agência Federal do Departamento de Saúde e Serviços Humanos dos Estados Unidos que faz o controle dos Alimentos e Medicamentos
FSH	hormônio folículo estimulante
$G\alpha_q$	subunidade α da proteína G_q
$G_{12/13}$	proteínas G_{12} e G_{13}
GABA	ácido gama-aminobutírico
GDP	difosfato de guanosina
GLA	ácido gama-linolênico
GLP-1	peptídio semelhante ao glucagon 1
GnRH	hormônio liberador de gonadotrofina
GPCRs	receptores acoplados à proteína G
$G_{q/11}$	proteínas G_q e G_{11}
GSH-PX	glutationa peroxidase

GSH-R	receptor secretagogo de hormônio do crescimento
GSH-Rd	glutationa redutase
GTP	trifosfato de guanosina
HDL-c	fração de lipoproteína de alta densidade
IL	interleucina
IMC	índice de massa corpórea
iNOS	sintase do óxido nítrico induzível
IP_3	1,4,5- trisfosfato de inositol
IP_3R	receptor de IP_3
JAK2	*jannus* cinase 2
LDL-c	fração de lipoproteína de baixa densidade
LH	hormônio luteinizante
LHA	área hipotalâmica lateral
LPL	lipoproteína lipase
MAPK	proteína cinase ativada por mitógeno
MCP-1	proteína quimiotática de monócitos 1
MDA	malondialdeído
MLCK	cinase da cadeia leve da miosina
MLCP	fosfatase da cadeia leve da miosina
MPOA	área pré-óptica medial
MYPT1	subunidade 1 de ligação da MLCP
NA	noradrenalina
NADPH	fosfato de dinucleotídio de adenina e nicotinamida
NANC	não colinérgico não adrenérgico
NCX	trocador Na^+/Ca^{2+}
nNOS	sintase do óxido nítrico neuronal
NO	óxido nítrico
NOX	NADPH oxidase
NPS	nitroprussiato de sódio
NPY	neuropeptídio Y
OMS	Organização Mundial da Saúde
$ONOO^-$	peroxinitrito
OX	xantina oxidase citosólica
PA	ácido fosfatídico

PAI-1	inibidor do ativador de plasminogênio 1
PC	fosfatidilcolina
pCE_{50}	logaritmo negativo, na base 10, da concentração molar de uma substância que produz 50% de seu efeito máximo
PGE	prostaglandina E
$PGF_{2\alpha}$	prostaglandina $F_{2\alpha}$
PGI_2	prostaciclina I_2
PI3K	cinase de fosfatidilinositol- 3
PIP_2	4,5-bisfosfato de fosfatidilinositol
PIP_3	3,4,5-trisfosfato de fosfatidilinositol
PKA	proteína cinase A
PKB	proteína cinase B
PKC	proteína cinase C
PKG	proteína cinase G
$PLC_{\beta1}$	fosfolipase C do tipo β_1
$PLC_{\beta2}$	fosfolipase C do tipo β_2
PLD	fosfolipase D
PMCA	Ca^{2+}-ATPase da membrana plasmática
POMC	pró-opiomelanocortina
PP1c	subunidade catalítica da MLCP
PPARγ	receptor ativador da proliferação de peroxissomos γ
PPgPNSB	Programa de Pós-graduação em Produtos Naturais e Sintéticos Bioativos
PVN	núcleo paraventricular
PYY	peptídio tirosina-tirosina
RDC	Resolução da Diretoria Colegiada
RhoA	pequena proteína G ligante de GTP
RhoGAP	proteína ativadora de GTPase da Rho
RhoGEF	fator de troca de nucleotídio de guanina da Rho
rMLC	cadeia leve regulatória da miosina
ROCK	proteína cinase associada à Rho
RS	retículo sarcoplasmático
RyR	receptor de rianodina
sGC	ciclase de guanilil solúvel

SNC	sistema nervoso central
SOD	superóxido dismutase
STAT3	transdutor de sinal e ativador de transcrição do tipo 3
TAB	tecido adiposo branco
TAM	tecido adiposo marrom
TBA	ácido tiobarbitúrico
TGI	trato gastrintestinal
TNF-α	fator de necrose tumoral α
TxA_2	tromboxano A_2
UCP-1	proteína desacopladora 1
UFPB	Universidade Federal da Paraíba
VET	valor energético total
VIP	peptídio intestinal vasoativo
V_m	potencial de membrana
ZIPK	proteína de interação zíper
α-MSH	hormônio estimulante de melanócitos α

OBS: as abreviaturas e os símbolos utilizados neste trabalho e que não constam nesta relação, encontram- se descritas no texto ou são convenções adotadas universalmente.

SUMÁRIO

1 Introdução

A obesidade é definida como uma condição crônica, complexa e progressiva, resultante do acúmulo excessivo e/ou anormal de tecido adiposo, sendo responsável por acarretar diversos riscos à saúde, estando relacionada com a homeostase energética, a ingestão inadequada de macro e micronutrientes, a não prática exercício físico, a imunidade inata e adaptativa, ao metabolismo celular, microbiota intestinal e ao estresse oxidativo (Afshin *et al.*, 2017; Lancet, 2016; Heymsfield; Wadden, 2022). Portanto, o estilo de vida sedentário e o consumo excessivo de dietas altamente calóricas, são fatores primordiais que favorecem o desenvolvimentoda obesidade e comorbidades associadas (Collado *et al.*, 2008; Erejuwa; Sulaiman; Wahab, 2021).

Nesse contexto, a obesidade é considerada uma síndrome multifatorial, sendo influenciada por interações genéticas, ambientais, comportamentais e pela composição bacteriana do trato gastrintestinal (John; Mullin, 2016; Villanueva- Millán; Pérez-Matute; Oteo, 2018). Essa, portanto, responsável por manter a barreira epitelial intestinal, protegendo contra a colonização de bactérias patogênicas no intestino, pela metabolização de polissacarídeos indigeríveis e pela regulação da absorção de lipídios e armazenamento de energia da dieta (Chakraborti, 2015;Rosenbaum; Knight; Leibel, 2022).

Dessa forma, entre os objetivos desse trabalho, cita-se a compreensão da relação existente entre a obesidade e as desregulações promovidas sobre a reatividade da musculatura lisa do íleo de rato, e como essas alterações poderiam estar envolvidas com o perfil e mediadores inflamatórios, estresse oxidativo, sistema antioxidante, entre outros, correlacionando-os com o efeito preventivo da suplementação alimentar com *Arthrospira* (*Spirulina). Platensis* (AP), colaborando com a descoberta de novas alternativas terapêuticas que possam ser utilizadas para o tratamento/prevenção de tais desordens.

Além disso, já é demonstrado que a obesidade modula a reatividade muscular lisa de vários órgãos e a regulação da contratilidade do músculo liso desempenha um papel fundamental em diversos processos fisiopatológicos, tais como hipertensão arterial, disfunção erétil, asma, cólicas uterinas, constipação intestinal (Felippi, 2014), tornando-se, dessa forma, importante os estudos que envolvam esse tipo de musculatura associada as suas desregulações, a fim de contribuir com a descoberta e o desenvolvimento de novos produtos e alternativas terapêuticas.

Nos últimos anos tem se discutido e demonstrado a *A. platensis* como uma suplementação alimentar alternativa e eficiente para o controle do peso em animais e humanos. A eficácia proveniente da utilização da *Arthrospira* como suplemento alimentar sobre a redução do peso corporal, é dada principalmente devido a sua atuação reduzindo a infiltração de macrófagos no tecido adiposo visceral e prevenindo o acúmulo de lipídios no fígado e o estresse oxidativo (Fujimoto *et al.*, 2012; Mazokopakis *et al.*, 2014). Ademais, já é demonstrado que os antioxidantes presentes na alga, são eficazes no tratamento da obesidade por meio de diferentes efeitos, tais como inibição da lipase, efeito supressor na ingestão de alimentos, entre outros (Hassan; El-Gharib, 2015; Ismail *et al.*, 2017).

Diante do exposto, a suplementação alimentar com AP emerge como fonte promissora para o tratamento de disfunções orgânicas e metabólicas. Dessa forma, nesse estudo foi avaliado o efeito preventivo da suplementação alimentar com AP nas alterações promovidas pelo consumo da dieta hipercalórica sobre os parâmetros nutricionais e morfométricos, estresse oxidativo e perfil inflamatório e investigado as principais vias de sinalização molecular envolvidas com o possível efeito preventivo da AP sobre a reatividade intestinal do íleo de ratos Wistar. Assim, hipotetizamos que a dieta hipercalórica diminui a reatividade contrátil do íleo de ratos obesos e quea suplementação com a alga previne tais danos. A AP por sua potente atividade antioxidante e composição nutricional poderia atuar modulando positivamente e/ou negativamente vias moleculares de sinalização (RhoA/ROCK; óxido nítrico (NO); prostanoides; estresse oxidativo e antioxidantes), e, consequentemente prevenir a obesidade e doenças associadas que acometem o trato gastrintestinal.

2 Fundamentação teórica

2.1 Anatomia e fisiologia do intestino delgado

O trato gastrintestinal (TGI) compreende um órgão tubular altamente especializado que se estende da boca ao ânus (Figura 1). Com uma área superficial de aproximadamente 1000 m^2, o TGI é o maior sistema orgânico e fornece uma interface única entre os ambientes externo e interno do corpo. É a principal portal de entrada de medicamentos, produtos químicos, alimentos e água, e também para partículas inaladas, tendo como principal função a digestao e extração desses componentes que são ingeridos e, eliminar produtos residuais. Esta função é alcançada através da participação de vários órgãos, que incluem as glândulas salivares, o esôfago, o fígado, o pâncreas, a vesícula biliar e os intestinos (Hooser; Earnest, 2018; Miranda-Bautista *et al.*, 2017).

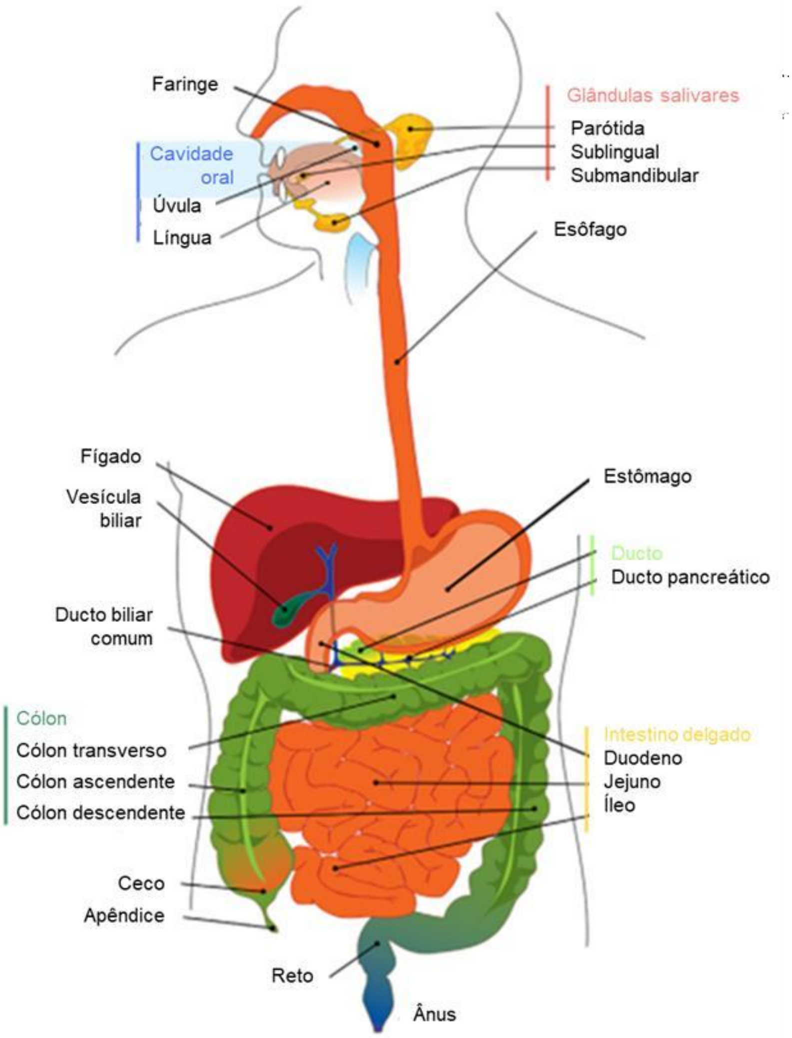
Ademais, o TGI também é composto por uma enorme comunidade microbiana, diversificada e complexa com cerca de 1000 espécies de bactérias, que tem um imenso impacto no metabolismo, fisiologia, nutrição e função imunológica do hospedeiro (Bhattacharyya *et al.*, 2014; Guinane; Cotter, 2013). A fisiologia gastrintestinal descreve as funções normais do trato digestivo, particularmente de seus órgãos ocos. Nutrientes sólidos e fluidos são ingeridos, propelidos, misturados, digeridos e absorvidos enquanto materiais não absorvíveis são armazenados e excretados (Sauer; Merchant, 2018).

O intestino é o principal órgão para a absorção e metabolismo dos alimentos, que também atua como barreira física e imunológica essencial. Suas funções fisiológicas incluem absorção de nutrientes, detecção de patógenos e homeostase intestinal. Este subdivide-se em intestino grosso (cólon) e intestino delgado (duodeno, jejuno e íleo) (Segrist;Cherry, 2020).

O intestino delgado apresenta aproximadamente 6-7 m de comprimento, começando no piloro e terminando na válvula ileocecal (Mowat; Agace, 2014). É dividido em três seções: duodeno, jejuno e íleo. É responsável pela decomposição química final dos alimentos pelas enzimas digestivas e pela absorção de nutrientes, água e eletrólitos, além de apresentar importantes funções secretorias e imunológicas (Amerongen, 2010; Miranda-Bautista; Bañares; Vaquero, 2017) (Figura1). O lúmen do intestino delgado é um complexo arranjo de estruturas que auxiliam na absorção de nutrientes. Cada estrutura é responsável por aumentar a área de superfície para melhorar a digestão e a absorção de nutrientes (Campbell; Berry;

Liangand, 2019).

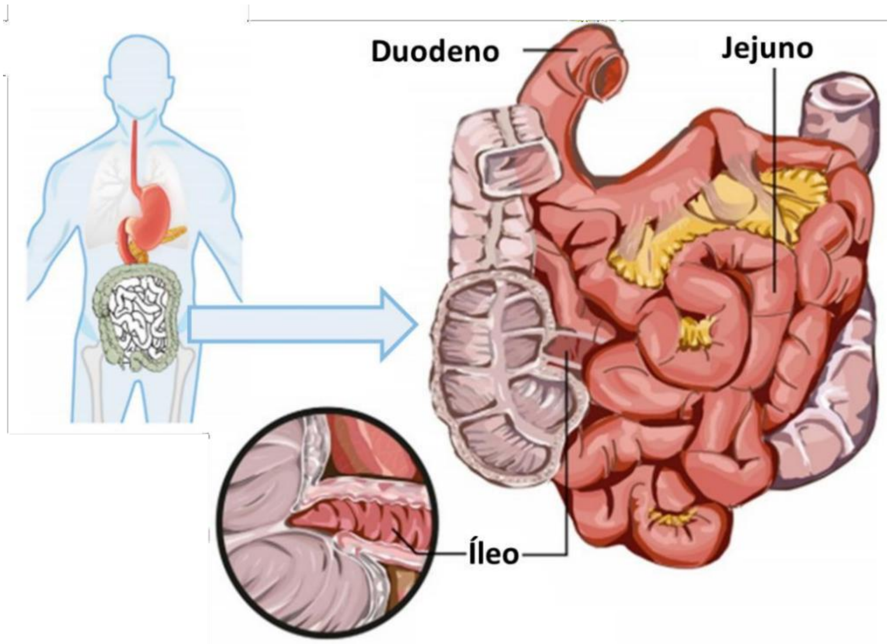
Figura 1 – Anatomia e fisiologia do trato gastrintestinal.



Fonte: Adaptado de Sauer; Merchant, 2018.

O duodeno é a seção mais proximal e mais curta do intestino delgado, medindo aproximadamente entre 20-30 cm, saindo do piloro do estômago e se conecta ao jejuno, em forma de “C” em torno da cabeça do pâncreas (Walthall *et al.*, 2005; Sayeed *et al.*, 2015). O jejuno tem cerca de 2,5 m de comprimento e está localizado na cavidade abdominal superior. O íleo é a última seção do intestino delgado, conectado ao jejuno, apresenta em média 3,5 m de comprimento e está localizado na cavidade abdominal inferior e na pelve (Volk; Lacy, 2017) (Figura 2).

Figura 2 – Anatomia do intestino delgado.



O estômago e partes do cólon removidos: começando com o duodeno, seguido pelo jejuno e o íleo distal se junta ao cólon pela válvula de Bauhin, formando o intestino delgado.

Fonte: Adaptado de Schneider; Feussner, 2017.

A parede do intestino delgado é formada por quatro camadas: mucosa, submucosa, muscular própria e serosa (adventícia). A camada mais interna é a mucosa, composta por três camadas distintas: epitélio, lâmina própria e muscular. A submucosa é a camada de resistência da parede intestinal e é composta de tecido conjuntivo denso, vasos sanguíneos e linfáticos (Höllwarth , 1999; Coffey; O'leary, 2016). O plexo submucoso ou de Meissner, também está localizado na submucosa e é um componente integrante do sistema nervoso entérico (SNE), responsável por regular a motilidade intestinal e o processo de secreção na camada mucosa. O plexo mioentérico, ou plexo de Auerbach, está situado entre essas duas camadas musculares ajuda a controlar a motilidade e a secreção intestinal. A camada serosa é a camada mais externa da parede intestinal e é formada por uma única camada de células mesoteliais (Desesso; Jacobson, 2001; Campbell, 2012; Ma; Lee, 2020).

O intestino delgado é o principal local de absorção de eletrólitos, nutrientes e água. A maior parte da absorção sistêmica ocorre no duodeno e na metade proximal do jejuno. Cerca de 9 L de água por dia (7 L das secreções e 2 L da dieta) entram no intestino delgado (Desesso; Jacobson, 2001). A absorção de vitaminas solúveis em gordura (A, D, E e K) também ocorre no intestino delgado. Alguns minerais como cálcio e ferro e vitaminas como o folato são absorvidos no duodeno e no jejuno superior (Volk; Lacy, 2017).

O jejuno é o principal local de absorção de eletrólitos e água. Aproximadamente 75% do sódio que entra no intestino delgado é absorvido (Campbell, 2012; Guerra *et al.*, 2012).Cerca de 93% dos lipídios da dieta são absorvidos pelos dois terços superiores do jejuno. Quando os lipídios entram no duodeno, as células I e S localizadas na mucosa duodenal secretam colecistocinina (CCK) e secretina, respectivamente. Posteriormente, a CCK e a secretina estimulam a vesícula biliar a se contrair e o pâncreas a secretar as enzimas lipase, colesterol esterase e fosfolipase A_2 (Ma; Lee, 2020; Tucker; Szomstein; Rosenthal, 2007).

Outra importante função do intestino delgado é a motilidade, que permite a mistura e o trânsito das secreções e do conteúdo digerido, mas também se livrar do excesso ou do conteúdo tóxico não absorvido. O SNE é o responsavel por impulsionar a motilidade e é composto por nervos parassimpáticos (nervo vago) e simpáticos (células ganglionares do nervo esplâncnico) (Huizinga *et al.*, 2015; Romanski, 2017; Tharakan *et al.*, 2010).

Adicionalmente, os hormônios intestinais são mensageiros químicos que estão envolvidos na regulação das funções intestinais, incluindo a motilidade, secreção, proliferação celular, digestão e absorção. Quando os alimentos chegamao intestino delgado, desencadeia a secreção de uma série de hormônios para facilitar a absorção de nutrientes. Os hormônios intestinais são secretados pelas células endócrinas localizadas no pâncreas e no trato gastrointestinal (Meek *et al.*, 2016; Sanger *et al.*, 2013; Verkijk *et al.*, 1998) (Quadro 1).

Interessantemente, devido as suas dobras e epitélio complexos, o intestino delgado ainda atua como barreira a diversificada microbiota intestinal. A carga bacteriana da microbiota no intestino delgado é semelhante a do estômago, que se aproxima de 10^4 UFC/mL. Essa colonizaçao de bactérias no intestino delgado aumenta de cerca de 10^2 proximalmente e para 10^8 distalmente. No íleo distal, a concentração de bactérias gram-negativas é maior do que a de bactérias gram-positivas (Okumura; Takeda, 2017; Santaolalla; Abreu, 2012; Simon; Gorbach, 1984).

Simbiontes bacterianos estão envolvidos no metabolismo dos componentes dos alimentos, defesa contra bactérias patogênicas, manutenção da integridade epitelial e desenvolvimento e maturação da mucosa. No entanto, bactérias patogênicas e suas toxinas podem comprometer as respostas imunes corporais, resultando em distúrbios inflamatórios (Furness *et al.*, 2013; Santaolalla; Abreu, 2012).

Quadro 1 – Caracteristicas gerais dos hormônios intestinais do intestino delgado.

Hormônios	Local de produção	Funções
Secretina	Células S da mucosa duodenal	Inibe a secreção de ácido e a motilidade gástrica; envolvido na osmorregulação
GIP	Células K do intestino delgado proximal	Efeito insulinogênico
GLP-1	Células L do jejuno e íleo	Reduz a ingestão de alimentos e inibe a secreção de glucagon
GLP-2	Células L do jejuno e íleo	Inibe a secreção de ácido e o esvaziamento gástrico; aumenta a absorção de nutrientes intestinais
CCK	Células I do duodeno e jejuno	Inibe o esvaziamento gástrico; estimula a secreção de enzimas digestivas pancreáticas
Motilina	Células M do intestino delgado proximal	Induz atividade motora GI
PYY	Células L do jejuno e íleo	Inibe a secreção pancreática e suprime a motilidade intestinal
OXM	Células L do íleo	Saciedade, retardo do esvaziamento gástrico, aumento do gasto energético

GIP = polipeptídio insulinotrópico dependente de glicose; GLP-1 e 2 = peptídio semelhante ao glucagon 1 e 2, respectivamente; CCK = colecistocinina; PYY = peptídio tirosina- tirosina; OXM = oxintomodulina.

Fonte: Adaptado de Ma; Lee, 2020.

A resposta imune inata é iniciada e desencadeada por receptores de reconhecimento de padrões (PRRs), como receptores *toll-like* (TLRs), que atuam como sensores moleculares associadas a patógenos. A sinalização de TLRs está associada à manutenção de junções estreitas e expressão de peptídios antimicrobianos (PAMs), que estão envolvidos na manutenção de uma barreira epitelial saudável (Cornick; Tawiah; Chadee, 2015). No entanto, a maioria dos patógenos de origem alimentar que causam enterocolite são Gram-negativos. Esses patógenos são capazes de bloquear algumas das principais vias de sinalização dos TLRs. Portanto, é muito importante que os PRRs possam reconhecer esses patógenos a fim de protegê-los contra infecções patogênicas (Talbot *et al.*, 2009; Yang; Yan, 2017).

2.2 Aspectos gerais da obesidade

A obesidade é definida como uma doença inflamatória crônica de baixo grau que resulta do acúmulo excessivo de tecido adiposo e classificada atraves do índice de massa corporal (IMC). De acordo com a Organização Mundial de Saúde (OMS), é demarcada como peso em relação à altura (quilograma por metro quadrado), a diretriz é considerada a mais apropriada para descrever obesidade ou sobrepeso. Se o IMC de um indivíduo for superior a 30 kg/m^2, ela é considerada obesa. A obesidade pode ser classificada em três categorias: grupo I (30,0 a 34,9 kg/m^2), grupo II (35,0 a 39,9 kg/m^2) e grupo III (maior que/equivalente a 40 kg/m^2). Quanto maiores os valores de IMC, maiores serão as chances de riscos associados à obesidade (Behera *et al.*, 2022; Brewer; Balen, 2010).

Segundo a OMS, a obesidade é uma epidemia global crescente e um dos problemas de saúde pública mais visíveis, entretanto um dos mais negligenciados. Estima-se que a prevalência global de obesidade ultrapasse 18% nos homens e mais de 21% nas mulheres até o ano de 2025, se as tendências atuais continuarem colocando um fardo significativo nas pessoas, comunidades e nos sistemas de saúde (Blüher, 2019; Ding *et al.*, 2016). De acordo com o *World Obesity Federation* (2023), até 2035, a obesidade (IMC ≥ 30 kg/m^2) terá um aumento de 14 para 24%, afetando, globalmente, quase dois bilhões de pessoas de várias idades. Além disso, adultos com mais de 20 anos terão uma prevalência de obesidade aumentada em 9% tanto nos homens como nas mulheres, entre o período de 2020 a 2035.

Mundialmente, a obesidade ocupa o sexto lugar em relação as taxas de mortalidade e encurta a vida das pessoas em aproximadamente sete anos a partir dos 40 anos de idade (Hruby; Hu, 2015). Representa a segunda causa de mortalidade prematura evitável, depois do tabagismo,afetando mais de 107,7 milhões de crianças e 603,7 milhões de adultos em todo o mundo, o que representa mais de 60% das mortes relacionadas ao alto índice de massa corporal (Afshin *et al.*,2017; Moreno-Franco *et al.*, 2018; WHO, 2017).

As varias doenças ligadas à obesidade, como diabetes tipo 2 (DM2), doenças cardiovasculares, distúrbios musculoesqueléticos , alguns tipos de câncer, doenças hepáticas e intestinais, têm forte impacto sobre saúde humana e reduzem a expectativa de vida (Lumeng; Saltiel, 2011; Proctor *et al.*, 2016; Sheka *et al.*, 2020; Tinahones *et al.*, 2012; Vajro *et al.*, 2013). Os tratamentos para a obesidade até o momento têm sido muito limitados e mal elucidados na abordagem do desafio da epidemia global (Alberti *et al.*, 2009; Boron; Boulpaep, 2017).

Portanto, a obesidade é um problema de saúde pública mundial, sendo considerada a doença nutricional mais prevalente em países desenvolvidos,como os Estados Unidos, mas também em países em desenvolvimento como o Brasil, bem como em outros países da América Latina, consequência da ingestão excessiva de nutrientes e/ou a diminuição do gasto energético (Aitlhadj *et al.*, 2011; Ferreira; Magalhães, 2006; Fussenegger; Pietrobelli; Widhalm, 2008;). Isso, deve-se pricipalmente pela combinação da disfunção do centro da saciedade no nível cerebral, do desequilíbrio de ingestão e uso de energia, e pelas variações genéticas, que se manifestam com um aumento anormal e excessivo ou acúmulo de energia na forma de gordura no tecido adiposo (Cheung; Li, 2012; Cohen, 2016; Tu *et al.*, 2018).

Dessa forma, o ponto chave para o desenvolvimento de novas e eficazes alternativas terapêuticas para a obesidade está em aprofundar a compreensão dos mecanismos envolvidos na regulação do peso corporal e das consequências a nivel sistêmico.

2.3 Papel da inflamação e do estresse oxidativo na obesidade

A obesidade é caracterizada e associada a inflamação sistêmica crônica de baixo grau, como resultado da expansão do tecido adiposo (TA). Essa inflamação sistêmica prejudica o metabolismo da glicose e tem um papel na patogênese de distúrbios metabólicos relacionados à obesidade (Hariharan *et al.*, 2022; Kwaifa *etal.*, 2020). O tecido adiposo serve como um elo importante entre as células imunológicas e a disfunção metabólica no cenário da obesidade. Os adipócitos hipertrofiados secretam citocinas e quimiocinas inflamatórias (Wei *et al.*, 2020),como a MCP-1 (CCL2). Isso desencadeia o acúmulo de várias células imunes pró- inflamatórias (macrófagos M1, CD8 $^{+}$células T e células Th1) e uma diminuição concomitante em células imunes anti-inflamatórias (macrófagos M2, células Th2, células invariantes *natural killer* T (Inkt). Além do TA, outros órgãos, incluindo fígado, músculo, pâncreas, coração, cérebro e intestino delgado e grosso, exibem inflamação de baixo grau que pode contribuir para a resistência à insulina induzida pela obesidade(Man *et al.*, 2022; Park; Shastri, 2022).

Como a inflamação é comumente associada à obesidade, ela desempenha um papel importante no comprometimento da saúde humana pela flutuação do metabolismo da glicose e lipídios no músculo esquelético, fígado e tecido adiposo (Man; Kallies; Vasanthakumar, 2022). O desenvolvimento da inflamação de baixo grau na obesidade é referido como inflamação metabólica (Wu; Ballantyne, 2020). Uma das principais características da inflamação induzida pela obesidade é a infiltração do tecido adiposo, fígado e pâncreas devido a células imunes, como macrófagos M1, células T e interleucinas (IL) (Park, 2022). Além disso, várias vias de sinalização são conhecidas por atuar como um mediador para desenvolver a inflamação e a resistência à insulina na obesidade, incluindo NF-κB, proteínas SOCS(Suppressors of Cytokine Signalling), TLR, JNK (Cinase c-Jun N-terminal) e ROCK (Land *et al.*, 2021).

Fisiologicamente, o tecido adiposo é formado principalmente por pré- adipócitos e adipócitos, com a presença de poucos leucócitos. No entanto, durante aobesidade, a composição e as funções do tecido adiposo são alteradas. Uma vez que a capacidade de armazenamento do tecido adiposo está sobrecarregada com gordura, essa podendo ser armazenada intra ou extravascularmente em outrosórgãos vitais, incluindo músculo esquelético, coração, rins, fígado e intestinos (Hernández, *et al.*, 2013; Saltiel; Olefsky, 2017).

O aumento do aporte calórico faz com que os adipócitos sofram hipertrofia (aumento do volume), o que pode resultar em complicações, como hipóxia, necrose dos adipócitos, secreção de quimiocinas e fluxo irregular de ácidos graxos. A hipertrofia dos adipócitos perturba o equilíbrio dascitocinas e adipocinas derivadas do tecido adiposo. Isso também está associado ao aumento da secreção de citocinas pró-inflamatórias e proteína quimioatrativa de monócitos 1 (MCP-1), causando uma infiltração típica de macrófagos queexacerbam ainda mais o processo inflamatório. O nível de concentração de leptina circulante é elevado com a consequente redução da concentração de adiponectina (Mongraw-Chaffin, 2017; Segovia *et al.*, 2019). A superexpressão de citocinas pró- inflamatórias durante a obesidade é considerada o maior elo entre obesidade e inflamação (Ellulu; Patimah, 2017).

A inflamação funciona como um mecanismo de defesa para as respostas dos tecidos à lesão ou dano tecidual por meio da remodelação, redução ou mesmo destruição dos agentes contribuintes ou remoção de todo o tecido, no processo conhecido como apoptose. A inflamação induzida pela obesidade é uma condição multifacetada que afeta muitos órgãos, incluindo músculo esquelético, tecido adiposo, fígado, cérebro, coração, pâncreas e intestino. A disfunção endotelial resulta de um desequilíbrio na produção de agentes vasodilatadores, como NO, fator hiperpolarizante derivado do endotélio (EDHF), prostaciclina (PGI_2) e agentes vasoconstritores, incluindo prostaglandina H_2 (PGH_2), endotelina-1 (ET-1) e angiotensina II (Ang-II) (Sena *et al.*, 2018; Mussbacher *et al.*, 2019).

Na fisiologia normal, ocorre liberação equilibrada de fatores contráteis e relaxantes derivados do endotélio. Alterações neste equilíbrio predispõem o endotélio vascular a estados pró-trombóticos e pró-aterogênicos, resultando em ativação plaquetária, adesão leucocitária, vasoconstrição, pró-oxidação, mitogênese, inflamação vascular, coagulação prejudicada, aterosclerose e trombose com subsequentes doenças cardiovasculares. Durante a obesidade, esse delicado equilíbrio geralmente é interrompido, promovendo o desenvolvimento e posterior progressão para disfunção endotelial vascular com subsequente dano a algunsórgãos vitais (Carnevale *et al.*, 2017; Dhananjayan *et al.*, 2016;). (Figura 3).

O aporte nutricional irregular proveniente do consumo de dietas hipercalóricas e hiperlipídicas, é responsável por comprometer a capacidade fisiológica do tecido adiposo levando a lipotoxicidade, glicotoxicidade sistêmica e aumento da produção e liberação de adipocinas pró-inflamatórias, tais como o fator de necrose tumoral α (TNF-α), a MCP-1, leptina, IL-1β, IL-6 e IL-8 e o ligante indutor de apoptose relacionado ao TNF (TRAIL), que caracterizam o quadro de obesidade (Rakotoarivelo *et al.*, 2018; Sartipy; Loskutoff, 2003; Zoller *et al.*, 2017).

O desequilíbrio na secreção de adipocinas pró-inflamatórias em detrimento das anti-inflamatórias está diretamente associado ao aumento de processos oxidativos, principalmente em reposta a exacerbada infiltração de macrófagos no tecido adiposo que se dá pela elevação dos níveis do fator nuclear kappa B (NF-κB),favorecendo a formação de um ciclo vicioso de inflamação (Hill *et al.*, 2015; Kim *et al.*, 2019). Tais fatores são responsáveis por aumentar os substratos energéticos para vias metabólicas oxidativas, envolvidas no metabolismo da glicose e lipídios, que por sua vez levarão a um aumento dos níveis circulantes de ácidos graxos livres(AGL) e a uma superprodução de espécies reativas de nitrogênio (ERNs) e espéciesreativas de oxigênio (EROs) (Marchev *et al.*, 2023; McMurray; Patten; Harper, 2020).

Figura 3 – Mecanismo de disfunção endotelial associados à inflamação durante aobesidade.

O processo de desenvolvimento da obesidade é caracterizado por uma inflamação sistêmica crônica que leva a expansão do tecido adiposo corporal favorecendo e contribuindo para disfunção das céluas endoteliais que irá culminar em disfunção do metabolismo do ácido araquidônico. A disfunção do AA leva ao desequilíbrio entre as propriedades pró-inflamatórias bem como na produção de prostanoides relaxantes e contratéis a partir das celulas mulsculares lisas, contribuindo assim para a perturbação do sistema hemostático de relaxamento e contração do músculo liso de diversos orgãos. prostaciclina (PGI_2); prostaglandina (PGE_2); prostaglandina F2α ($PGF_{2\alpha}$) tromboxano (TxA_2);

Fonte: Autor, 2023

As EROs, em níveis fisiológicos, são moléculas que desempenham um papel fundamental na sinalização celular sendo consideradas sinalizadores em condições fisiológicas normais e que estão envolvidas em vários processos, como manutenção do tônus muscular liso, apoptose, cicatrização e na indução da diferenciação celular, entretanto, seu acúmulo e altos níveis danificam proteínas, lipídios e DNA, levando por consequência ao comprometimento da função celular (Finkel; Holbrook, 2000; Gutteridge, 2015; Halliwell; Bhattacharyya *et al.*, 2014; McMurray; Patten; Harper, 2016; Piché *et al.*, 2018), a esse fenômeno denomina-se estresse oxidativo. Adicionalmente, o estresse oxidativo caracteriza-se por um estado de desequilíbrio entre o processo de produção e eliminação das EROs (Hussain *et al.*, 2021), moléculas instáveis e ativas que contêm elétrons desemparelhados na camada de valência (Lushchak, 2020) (Figura 4).

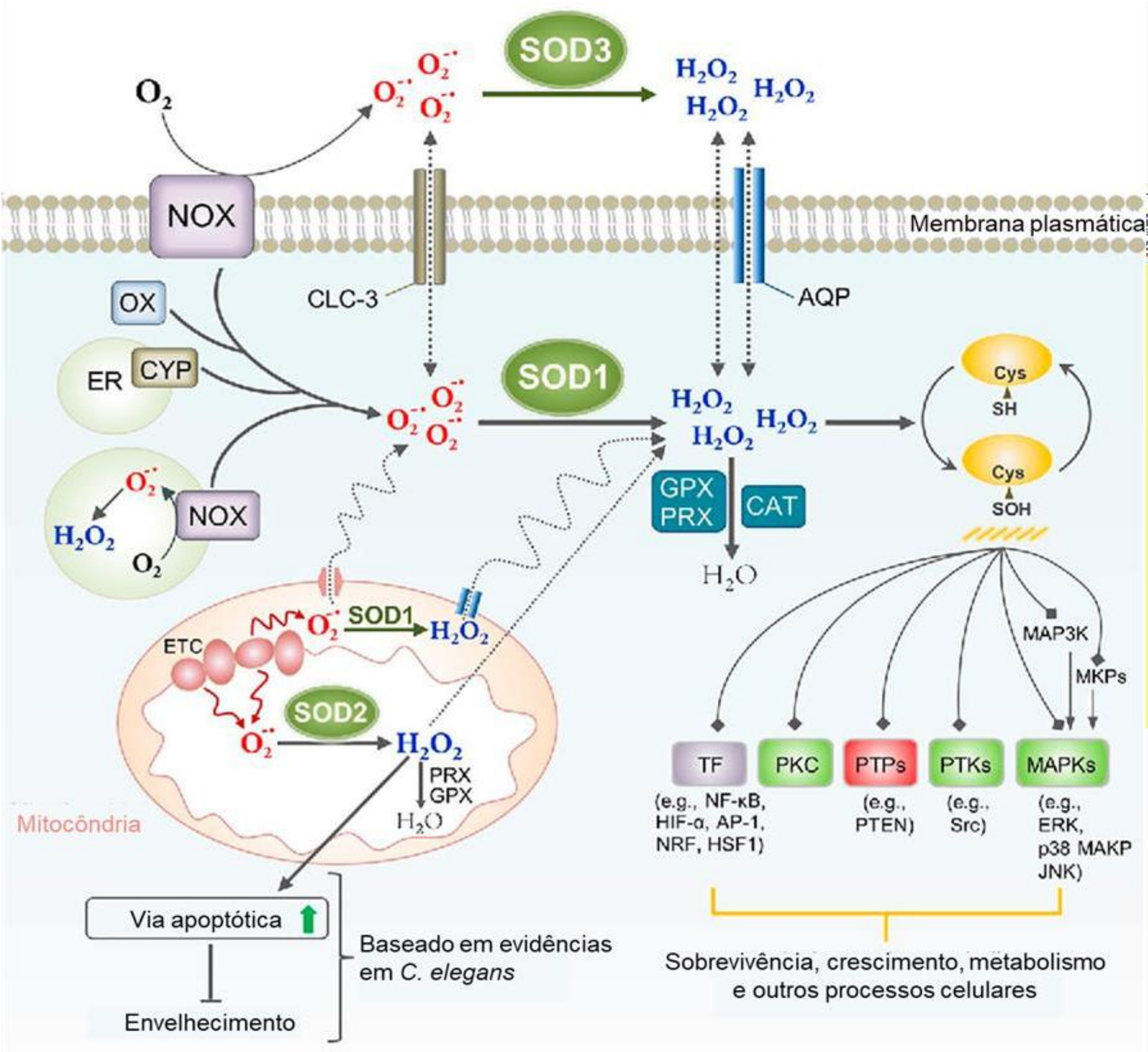
Em organismos aeróbicos, muitos processos produzem o ânion superóxido ($O^{2\bullet-}$), incluindo a xantina oxidase citosólica (OX), as mono-oxigenases do citocromo P450 (CYP) no retículo endoplasmático (RE), a cadeia transportadora de elétrons

(ETC) mitocondrial e a nicotinamida adenina dinucleotídeo fosfato (NADPH) oxidase (NOX). NOX é um complexo enzimático ligado à membrana que pode ser encontrado na membrana plasmática, bem como dentro de estruturas de membrana intracelular ou vesículas (Meitzler *et al.*, 2014).

O $O^{2\bullet-}$ produzido pelo NOX ligado à membrana plasmática (por exemplo, NOX2) pode atuar intra e extracelularmente. O peróxido de hidrogênio (H_2O_2) produzido por superóxido dismutase tipo 3 (SOD3) pode atravessar para o interior dacélula através de canais de aquaporina para iniciar a sinalização intracelular, enquanto $O^{2\bullet-}$ através do canal de cloreto-3 (Fisher, 2009). Os complexos NOX intracelulares produzem EROs no lúmen de um compartimento vesicular, onde as EROs atuam localmente ou entram no citosol (Brown; Griendling, 2009).

O H_2O_2 tem sido implicado na sinalização de EROs por meio da modificação oxidativa de cisteínas sensíveis a redox críticas em proteínas de sinalização. Os alvos relativamente bem reconhecidos da sinalização de EROs incluem proteínas fosfatases (PTPs), tirosina cinases não receptoras (PTKs), proteína cinase C (PKC)e ativadas por mitógenos (MAPKs) e fatores de transcrição (TFs). Foi demonstrado que as EROs mitocondriais atuam sinalizando tais como as proteínas citadas (Ristow, 2018) (Figura 4).

Figura 4 – Sinalização mediada por espécies reativas de oxigênio e superóxido dismutase.

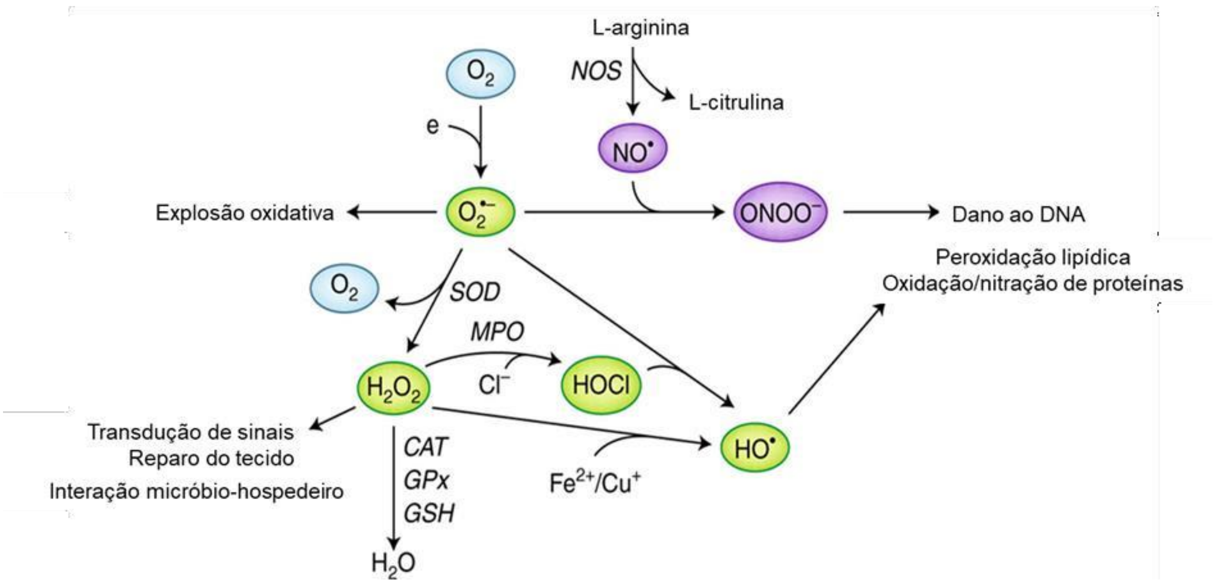


Em organismos aeróbicos, muitos processos produzem $O_2^{•-}$, incluindo a xantina oxidase citosólica (OX), as monooxigenases do citocromo P450 (CYP) no RE, a ETC mitocondrial e a NAD PH oxidase (NOX). NOX é um complexo enzimático ligado à membrana que pode ser encontrado na membrana plasmática, bem como dentro de estruturas de membrana intracelular ou vesículas (Meitzler et al., 2014). $O_2^{•-}$ produzido pelo NOX ligado à membrana plasmática (por exemplo, NOX2) pode atuar intra e extracelularmente. H_2O_2 produzido por SOD3 fora da célula pode atravessar para o interior da célula em parte através de canais de aquaporina para iniciar a sinalização intracelular, enquanto O2•- poderia influir através do canal de cloreto-3 (Fisher, 2009). Os complexos NOX intracelulares produzem EROs no lúmen de um compartimento vesicular, onde o ROS atua localmente ou a partir do qual entra no citosol (Brown e Griendling, 2009). O H_2O_2 tem sido implicado na sinalização de EROs por meio da modificação oxidativa de cisteínas sensíveis a redox críticas em proteínas de sinalização. Os alvos relativamente bem reconhecidos da sinalização EROs incluem proteínas fosfatases (PTPs), proteínas tirosina quinases não receptoras (PTKs), proteína quinase C (PKC), proteínas quinases ativadas por mitógenos (MAPKs) e fatores de transcrição (TFs). A função de sinalização do O2•- ainda é amplamente não caracterizada.

Fonte: Adaptado Wang *et al.*, 2018.

As EROs podem ser divididas em radicais livres e não radicais, que dependem de os elétrons estarem livres. Os radicais livres incluem $O^{2•–}$, radical hidroxila (•OH), radical peroxila (ROO•) e radical alcoxila (RO•). Os compostos não radicais consistem em oxigênio (O_2), ozônio (O_3), peróxido de hidrogênio (H_2O_2), ácido hipocloroso (HOCl), ácido hipobromoso (HOBr), cloraminas (RNHCl) e hidroperóxidos orgânicos (ROOH) (Bedard; Krause, 2007; Sies; Jones, 2020). Os •OH são as principais substâncias de sinal redox, incluindo NADPH oxidase e cadeia de transporte de elétrons mitocondrial. A variedade de EROs determinam suas propriedades, incluindo instável, ativa e extensa. Ademais, existem alguns compostos altamente ativos semelhantes às EROs denominados espécies reativas de nitrogênio (ERNs), incluindo óxido nítrico (•NO), dióxido de nitrogênio (•NO_2), peroxinitrito ($ONOO^-$) e trióxido de dinitrogênio (N_2O_3) (Figura 5).

Figura 5 – Produção e eliminação de espécies reativas de oxigênio/nitrogênio.



As enzimas SOD catalisam a dismutação do superóxido ($O_2^{\bullet-}$), gerando peróxido de hidrogênio (H_2O_2). A catalase (CAT), glutationa peroxidases (GPXs) e PRXs convertem H_2O_2 em água. H_2O_2 pode reagir com metais redox-ativos (por exemplo, ferro) para gerar o radical hidroxi (OH•) através da reação de Fenton/Haber-Weiss. A reação entre $O_2^{\bullet-}$ e óxido nítrico ($NO^\bullet$) produz ONOO-, cuja decomposição, por sua vez, dá origem a alguns intermediários altamente oxidantes, incluindo $NO_2^\bullet$ OH• e $CO_3^{\bullet-}$, bem como, finalmente, NO_3^- estável. Portanto, níveis elevados de O2•- também podem diminuir a biodisponibilidade de NO• e gerar toxicidade de $ONOO^-$. O $_2^{\bullet-}$ por si só pode reduzir o ferro férrico (Fe^{3+}) a ferro ferroso (Fe^{2+}) nos centros ferro-enxofre das proteínas, levando à inativação enzimática e perda concomitante de Fe^{2+} das enzimas, que por sua vez alimenta a química de Fenton. A protonação do $O_2^{\bullet-}$ pode formar o radical hidroperoxila mais reativo ($HO_2^\bullet$).

Fonte: Adaptado de Aviello; Knaus, 2018.

O O_2 é reduzido a $O^{2\bullet-}$, que pode ser reduzido a H_2O_2 pela superóxido dismutase (SOD) ou convertido a $HO^\bullet$. O H_2O_2 pode sofrer a reação de Fenton e é transformado em $HO^{\bullet}$ ou é reduzido a água (H_2O) pela catalase (CAT) ou pelo sistema glutationa peroxidase (GSH-GPx). Na presença de íons cloreto (Cl^{-}) H_2O_2 pode ser convertido em ácido hipocloroso (HOCl) pela mieloperoxidase neutrofílica (MPO) e essa reação pode gerar ainda mais $HO^{\bullet}$. A sintase do óxido nítrico (NOS) catalisa a oxidação da L-arginina a L-citrulina, liberando radicais óxido nítrico ($NO^{\bullet}$) que formam o potente oxidante $ONOO^{-}$ após reação com $O^{2\bullet-}$ (Figura 5).

Em contrapartida, as células são protegidas por enzimas antioxidantes tais como a SOD, as glutationa peroxidase (GSH-PX) e redutase (GSH-Rd) e a catalase, além disso temos também os sistemas antioxidantes não enzimáticos que podem ser glutationa, ubiquinona e do ácido úrico, compostos produzidos *in vivo*, e também representados por compostos obtidos da dieta alimentar como o α-tocoferol (vitamina E), o β-caroteno, o ácido ascórbico (vitamina C) e compostos fenólicos, com a finalidade de barrar os efeitos deletérios prevenindo os processos oxidativos resultantes da ação dos radicais livres (Broinizi *et al.*, 2008; Niemann *et al.*, 2021; Pietta, 2000; Pisoschi; Pop, 2015; Sies, 1997) (Figura 5).

A capacidade limitada do sistema homeostático de controlar o desbalanço resultante do excesso de EROs e da ineficiencia do sistema antioxidante, resultantes do excesso de tecido adiposo, levam a desregulações da sinalização da leptina e da insulina no tecido adiposo, muscular e hepático. Consequentemente, no tecido adiposo, essas desordens induzem a secreção de adipocinas pró-inflamatórias, ativação do sistema imune e, portanto, exacerbando ainda mais o ambiente inflamatório favorecendo o desenvolvimento de inflamação crônica (Keuper *et al.*, 2017; Sharma *et al.*, 2018; Vasileva; Marchev; Georgiev, 2020). Dessa forma, o estresse oxidativo causado pelo aumento desproporcional da produção de EROs contribui diretamente para o desenvolvimento de doenças cardiovasculares, neurodegenerativas e intestinais, estando associado a expectativa de vida humana (Finkel; Holbrook, 2000; KIM; Patel *et al.*, 2016; Sieburth, 2022).

2.4 Estresse oxidativo e sistema de defesa antioxidante intestinal

O estresse oxidativo é um estado de desequilíbrio entre o processo de produção e eliminação de EROs, incluindo radicais livres e não radicais, são moléculas centradas em oxigênio instáveis e ativas que contêm elétrons de camada de valência desemparelhados. Como importantes moléculas de sinalização, as

EROs atuam em funções fisiológicas normais no processo de metabolismo humano e no sistema imunológico (Hussain *et al.*, 2016; Lushchak, 2014). Em situação normal, os organismos possuem capacidades efetivas de geração e eliminação de EROs. No entanto, independentemente das fontes endógenas, poluentes, radiação, dieta e estilo de vida podem contribuir para a elevação das EROs. O aumento de EROs intracelulares não apenas leva a reações frequentes com componentes celulares, como proteínas, gorduras e DNA, mas também desencadeia as vias de sinalização relacionadas (Bhattacharyya *et al.*, 2014; Finkel; Holbrook, 2021). O estresse oxidativo causado pela produção desproporcional de EROs contribui para muitas doenças intestinais, cardiovasculares, neurodegenerativas e até mesmo associado ao tempo de vida humano (Kim; Sieburth, 2018; Patel *et al.*, 2018).

O intestino é o órgão primário para a digestão, absorção e metabolismo dos alimentos, que também atua como barreiras físicas e imunológicas essenciais. Suas funções fisiológicas incluem absorção de nutrientes, detecção de patógenos e homeostase intestinal (Segrist; Cherry, 2020). O estresse oxidativo intestinal desempenha um papel importante no estágio inicial da lesão intestinal. Ele atua como o fator ativador da disfunção da barreira intestinal, desencadeando assim o desequilíbrio imunológico e a inflamação (Han *et al.*, 2016; Yang *et al.*, 2019). Muitas doenças intestinais são iniciadas e agravadas pelo estresse oxidativo, como doenças inflamatórias intestinais (DII), infecções entéricas, lesão intestinal isquêmicae câncer colorretal (Bhattacharyya *et al.*, 2014).

O dano do estresse oxidativo ocorre quando o sistema de defesa antioxidante não consegue remover o excesso de EROs do corpo humano. Manifestando-se principalmente como danos a proteínas, lipídios e DNA (Han; Huang; Wang, 2017). Como componentes básicos de tecidos e órgãos que realizam a função fisiológica vital nos organismos, as proteínas são importantes moléculas-alvo para o ataquedas EROs. Uma vez que a forte afinidade entre EROs e ácidos graxos insaturados na bicamada fosfolipídica, a peroxidação lipídica da biomembrana promove danos que levam a alterações na estrutura, permeabilidade e fluidez do biofilme e destruição das funções celulares normais (Michel *et al.*, 2008; Sargis; Subbaiah, 2006).

O malondialdeído (MDA) é o metabólito dos radicais livres e da peroxidação lipídica do biofilme. Seu conteúdo pode refletir diretamente o grau de peroxidação tecidual e indiretamente refletir o grau de dano celular causado pelos radicais livres de oxigênio. O estresse oxidativo foi confirmado significativamente relacionado a danos no DNA e pode causar quebras na cadeia de DNA e modificações nas bases nitrogenadas. O radical hidroxila, o peróxido de hidrogênio e o ânion superóxido estão envolvidos na maioria dos danos oxidativos ao DNA (Moller; Loft, 2010; Van Remmen *et al.*, 2021). No intestino, potenciais substâncias estranhas que causam estresse oxidativo podem facilmente destruir proteínas, lipídios e DNA em nível molecular, levando a doenças intestinais macroscópicas.

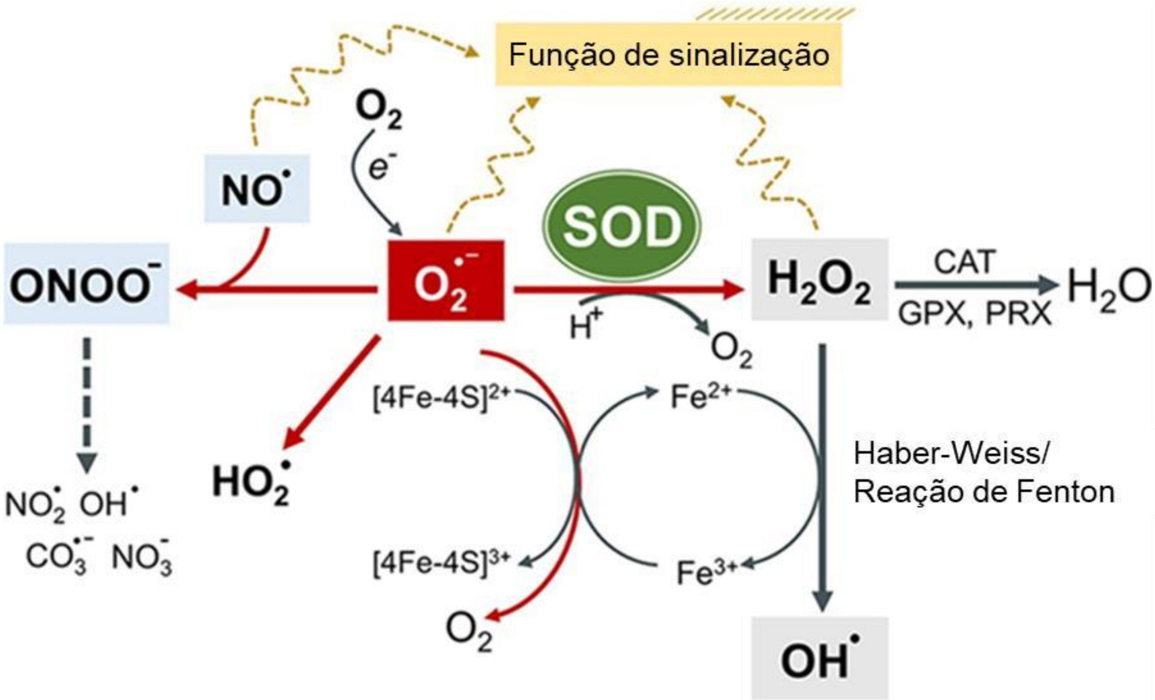
As células epiteliais intestinais consistem em células indiferenciadas (células-tronco) e células imunológicas diferenciadas (células B, células T, macrófagos), incluindo células absortivas (enterócitos) e células secretoras (células de Paneth, células caliciformes e células enteroendócrinas), são candidatos cruciais para as barreiras intestinais (Circu; Aw, 2019).

Sob condições de estresse oxidativo, o status redox da glutationa e dissulfeto de glutationa afeta o ciclo de crescimento das células epiteliais intestinais. Proliferação anormal, estagnação do crescimento, diferenciação e apoptose causam dano intestinal às células e lesão da barreira intestinal, levando a doenças inflamatórias intestinais graves e câncer de cólon (Aw, 2015).

As EROs são uma das causas da resposta inflamatória, envolvendo a participação de macrófagos e neutrófilos que se infiltram no intestino e produzem EROs, levando ao agravamento do estresse oxidativo e inflamação. Esta é a razão para o feedback positivo dos macrófagos e a principal razão para a dificuldade em aliviar a inflamação intestinal (Coant *et al.*, 2020). Em células normais, as células caliciformes e as microvilosidades são reduzidas ou até mesmo desaparecem em ratos com estresse oxidativo induzido (Vardi *et al.*, 2018).

Para manter o equilíbrio de EROs, o corpo humano é equipado com um sistema básico de defesa antioxidante contra o desequilíbrio de ROS, que consiste em antioxidantes enzimáticos endógenos e antioxidantes não enzimáticos endógenos (Wang *et al.*, 2018). As enzimas SOD catalisam a dismutação do superóxido ($O^{2\bullet-}$), gerando H_2O_2. A catalase (CAT), glutationa peroxidases (GPXs) e PRXs convertem H_2O_2 em água. O H_2O_2 pode reagir com metais redox-ativos (por exemplo, o ferro) para gerar o radical hidroxi (OH•) através da reação de Fenton/Haber-Weiss. A reação entre $O^{2\bullet-}$ e NO• produz o peroxinitrito ($ONOO^-$), cujadecomposição, por sua vez, dá origem a alguns intermediários altamente oxidantes, incluindo $NO_2\bullet$, OH• e $CO_3^{\bullet-}$ bem como, em última análise, NO_3^-. Portanto, níveis elevados de $O^{2\bullet-}$ também podem diminuir a biodisponibilidade de NO• e potencializar a toxicidade por meio da formação de $ONOO^-$. $O^{2\bullet-}$ por si só pode reduzir o ferro férrico (Fe^{3+}) a ferro ferroso (Fe^{2+}) nos centros ferro-enxofre das proteínas, levando à inativação enzimática e perda concomitante de Fe^{2+} das enzimas (Figura 6).

Figura 6 – Reações do ânion superóxido e atuação do sistema antioxidante.



O oxigênio (O_2) é reduzido a superóxido ($O_2^{\bullet-}$), que pode ser reduzido a peróxido de hidrogênio (H_2O_2) pela superóxido dismutase (SOD) ou convertido em radical hidroxila ($HO^\bullet$). O H_2O_2 pode sofrer a reação de Fenton e é transformado em HO• ou reduzido a água (H_2O) pela catalase (CAT) ou pelo sistema glutationa (GSH)/glutationa peroxidase (GPx). Na presença de íons cloreto (Cl^-), H_2O_2 pode ser convertido em ácido hipocloroso (HOCl) pela mieloperoxidase neutrofílica (MPO) e essa reação pode gerar ainda mais $HO^\bullet$. A sintase do óxido nítrico (NOS) catalisa a oxidação de l-arginina a l-citrulina, liberando radicais de óxido nítrico ($NO^\bullet$) que formam o potente oxidante peroxinitrito ($ONOO^-$) após reação com $O2^{\bullet-}$.

Fonte: Adaptado de Wang *et al.,* 2018.

Algumas enzimas ou componentes do corpo humano são alterados quando no estresse oxidativo intestinal, entre elas a SOD. Por isso, comumente pode ser utilizada como um indicador para avaliar a capacidade de resposta ao estresse oxidativo (Li *et al.*, 2019). A glutationa peroxidase (GSH-Px) está envolvida na transformação entre glutationa (GSH) e dissulfeto de glutationa (GSSG), durante a qual o H_2O_2 é reduzido a H_2O e os hidroperóxidos lipídicos são reduzidos a álcoois (Moine *et al.*, 2018). Por outro lado, a glutationa redutase (GSR) pode reduzir GSSG a GSH, mantendo o equilíbrio da diferenciação celular, crescimento e apoptose

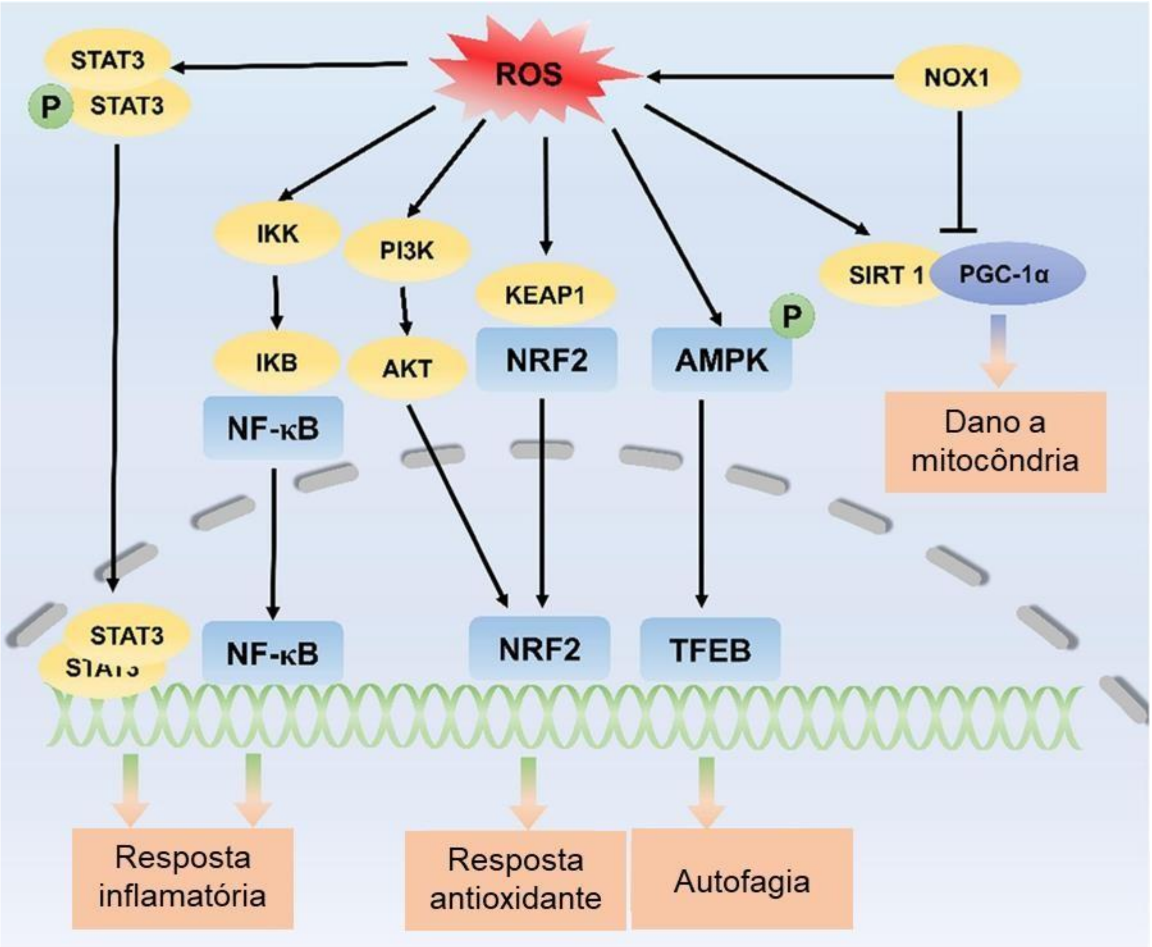
(Diaz-Vivancos *et al.*, 2015; Liu *et al.*, 2019). A GSH-Px, GSR e glutationa S-transferases (GST) constituem o sistema glutationa, servindo como uma barreira contra o estresse oxidativo. Embora a heme oxigenase não possa atuar como uma enzima antioxidante tradicional, ela é sensível ao estresse oxidativo e crucial ao ciclo celular, apoptose no câncer de cólon (Oates; West, 2016).

A tiorredoxina (Trx), tioredoxina redutase (TrxR) e forma reduzida de fosfato de dinucleotídio de adenina e nicotinamida (NADPH) constituem o sistema Trx, que possui a função de eliminar espécies reativas de oxigênio e reduzir pontes dissulfeto e mantendo a homeostase redox celular. Os antioxidantes enzimáticos endógenos não são enzimas específicas para o intestino, portanto, servem como uma barreira ao estresse oxidativo em todo o corpo (Watson, 2014).

Outro elemento importante para o sistema de defesa antioxidante são os Nrf2, um fator de transcrição e centro regulador na resposta ao estresse oxidativo. A via de sinalização da proteína 1 relacionada à epicloridrina semelhante a Kelch (Keap1)/Nrf2-elemento de resposta antioxidante (ARE) é a via de sinalização antioxidante mais comum (Gan *et al.*, 2016). Em condições normais, o Nrf2 pode se ligar ao Keap1, resultando na ubiquitinação e degradação do proteassoma do Nrf2 e mantendo o Nrf2 em um nível baixo (Shukla *et al.*, 2020) (Figura 7).

Quando ocorre o estresse oxidativo, a ubiquitinação e a degradação do Nrf2 são diminuídas, o que contribui para o acúmulo de Nrf2 no núcleo e ativação do elemento de resposta antioxidante (ARE). Esta via regula principalmente a expressão de suas enzimas antioxidantes a jusante, como SOD, GSH-PX, CAT, etc. (Erlank, 2011). A via de sinalização do AMPK-TFEB (fator de transcrição EB) tem sido considerada a razão que causa a indução da mitofagia, estresse antioxidante, funções de proteção mitocondrial e intestinal pela curcumina (Cao *et al.*, 2020). O inibidor de NOX1 pode aliviar o dano mitocondrial induzido pelo estresse oxidativo e a lesão da mucosa intestinal por meio da modulação da geração de EROs e da via de sinalização sirtuína 1 (SIRT1)/receptor ativado por proliferador de peroxissoma-γcoativador-1α (PGC-1α) (Li *et al.*, 2020). As vias de sinalização alvo mediadas por EROs também incluem NFκB, MAPK e transdutor de sinal e ativador da transcrição
3 (STAT3). Esses mecanismos desempenham um papel importante no desenvolvimento e agravamento de doenças bem como na elucidação novas drogas com efeitos preventivos contra o estresse oxidativo (Figura 7).

Figura 7 – Vias de sinalização envolvidas no estresse oxidativo intestinal.



Nrf2 é um importante fator de transcrição na resposta ao estresse oxidativo, e também é o centro regulador da resposta ao estresse oxidativo. A via de sinalização da proteína 1 relacionada à epicloridrina semelhante a Kelch (Keap1)/Nrf2-elemento de resposta antioxidante (ARE) é a via de sinalização antioxidante mais comum (Gan et al., 2016). Em condições normais, o Nrf2 pode se ligar ao Keap1, resultando na ubiquitinação e degradação do proteassoma do Nrf2 e mantendo o Nrf2 em um nível baixo. Quando ocorre o estresse oxidativo, a ubiquitinação e a degradação do Nrf2 são diminuídas, o que contribui para o acúmulo de Nrf2 no núcleo e ativação do elemento de resposta antioxidante (ARE). Esta via regula principalmente a expressão de suas enzimas antioxidantes a jusante, como SOD, GSH-PX, CAT, etc. (Erlank, Elmann, Kohen, & Kanner, 2011). Um estudo recente mostrou que o ácido lisofosfatídico e os análogos da radioproteína-1 podem reverter a oxidação do tiol proteico induzida por irradiação parcial do corpo, reduzir a expressão de Nrf2 e ativar as enzimas antioxidantes associadas ao receptor do ácido lisofosfatídico 2 (LPAR2), que são provavelmente o mecanismo de prevenir e atenuar a ruptura da junção apertada induzida por radiação, disfunção da barreira e endotoxemia (Shukla *et al.,* 2020). A via de sinalização do AMPK- TFEB (fator de transcrição EB) tem sido considerada a razão que causa a indução da mitofagia, estresse antioxidante, funções de proteção mitocondrial e intestinal pela curcumina (Cao et al., 2020). O inibidor de NOX1 pode aliviar o dano mitocondrial induzido pelo estresse oxidativo e a lesão da mucosa intestinal por meio da modulação da geração de ROS e da via de sinalização SIRT1 (sirtuína 1)/PGC-1α (receptor ativado por proliferador de peroxissoma-γ coativador-1α) (Li et al. , 2020). As vias de sinal alvo mediadas por ROS também incluem NFκB, MAPK (proteína quinase ativada por mitógeno), STAT3 (transdutor de sinal e ativador da transcrição 3) e assim por diante. Esses mecanismos desempenham um papel importante no desenvolvimento de novos alimentos ou drogas com efeitos protetores do estresse oxidativo.

Fonte: Adaptado de WANG *et al.*, 2020.

2.5 O papel das vias de sinalizaçao molecular na funcionalidade do intestino

2.5.1 RhoA/ROCK

A integração funcional do sistema nervoso entérico, sistema nervoso central e músculo liso, é responsável pela motilidade intestinal envolvendo contração e relaxamento sistemáticos do músculo liso. No entanto, alguns processos nos tônus do músculo liso podem ocorrer independentemente dessa inervação sendo de natureza miogênica (Cook; Brookes, 2010).

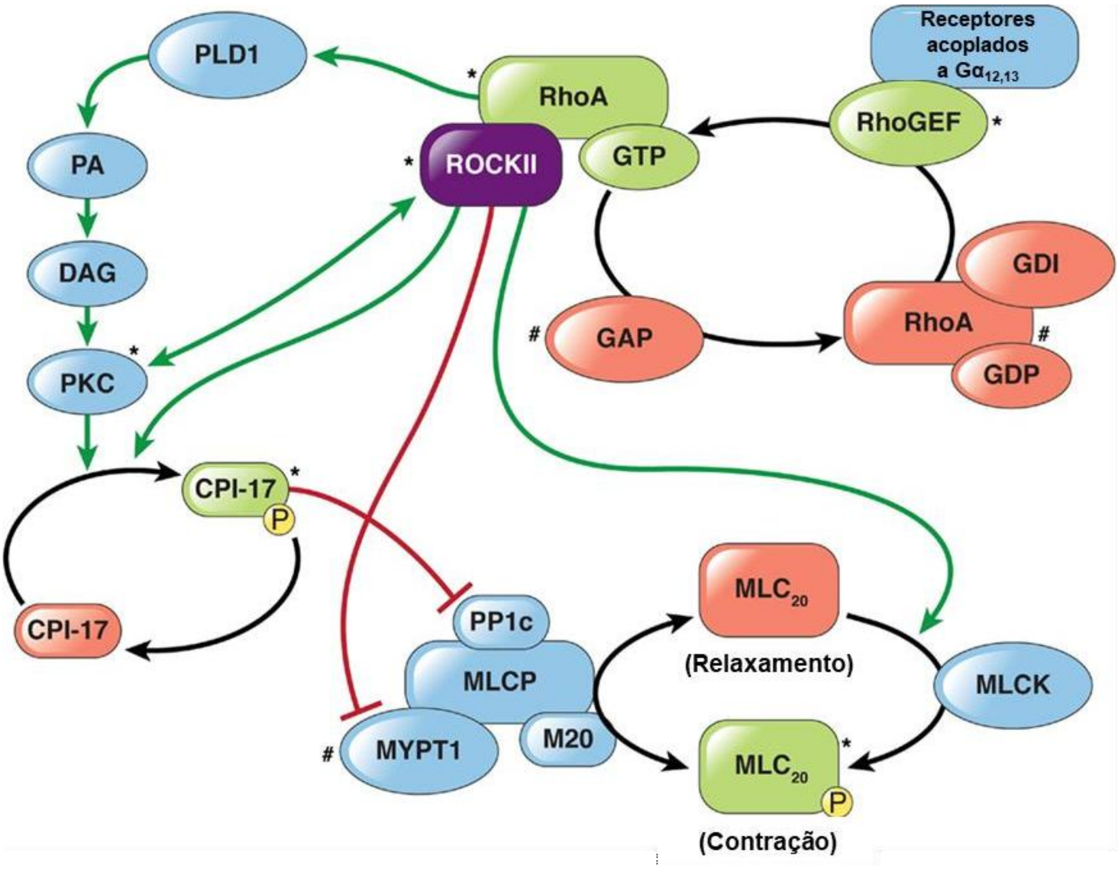
Algumas vias de sinalização molecular estão envolvidas diretamente na funcionalidade intestinal, alterando a contratilidade do músculo liso, dentre elas a via Ca^{2+}/calmodulina/cinase da cadeia leve da miosina (MLCK), PKC/proteína inibitória dependente de fosforilação (CPI-17) e RhoA/Rho-cinase (ROCK)/fosfatase da cadeia leve da miosina (MLCP) (Murthy, 2006). A via da RhoA/ROCK funciona como importante mediador da responsividade contrátil e disfunção do músculo liso intestinal (Gerthoffer, 2005; Somlyo; Somlyo 2000).

O mecanismo de transdução de sinal em células musculares lisas envolve 3 tipos de proteínas ligadas à membrana: um receptor de membrana, uma proteína de ligação ao GTP e enzimas efetoras capazes de gerar vias regulatórias intracelulares. Os ligantes no músculo liso intestinal são oriundos de várias fontes, como de células adventícias e via circulação. Os ligantes incluem angiotensina II, eicosanoides, ácido lisofosfatídico (LPA) e endocanabinoides (Casselbrant *et al.*, 2007; Cong *et al.*, 2008; Godoy; Rattan; Rattan, 2009; Rattan, 2005; Rattan; Phillips; Maxwell IV, 2010). Uma vasta gama de receptores reside na superfície do trato intestinal de células musculares lisas que ativam proteínas $G_{12/13}$ resultando na ativação de RhoA/ROCK (Figura 8).

A RhoA é uma pequena e importante GTPase que medeia múltiplos processos celulares, incluindo a polimerização, proliferação e migração da actina (Cáceres *et al.*, 2005, Jaffe; Hall, 2005). As ROCKs são os efetores mais importantesa jusante de RhoA e contribuem para o controle de tensão entre as células epiteliais intestinais (Li *et al.*, 2018). Estudos demonstram que a cinase dependente de Ca^{2+}, aMLCK modula a organização actina-miosina (Cunningham; Turner, 2012), a ativação da da via Rho/ROCK e MLCK fosforila sinergicamente a cadeia leve de miosina, quepor sua vez promove a contração perijuncional e a ruptura da junções intestinais

(Arnold *et al.*, 2017; Campos *et al.*, 2009; Cunningham; Turner, 2012), além disso, é relatado ainda que citocinas pró-inflamatórias resultaram na fosforilação de MLC2 por meio da super ativação de RhoA, contribuindo para o aumento da inflamação da mucosa intestinal (Capaldo; Nusrat, 2009). Por outro lado, a inibição da sinalização Rho/ROCK ou MLCK melhora a a integridade da barreira, a permeabilidade e motilidade intestinal (Schwarz *et al.*, 2007).

Figura 8 – Representação esquemática das principais vias envolvidas no acoplamento excitação-contração e relaxamento do músculo liso intestinal.



Setas verdes e substratos indicam estimulação; linhas vermelhas e substratos indicam inibição. GDP = difosfato de guanosina; GTP = trifosfato de guanosina; GAP = proteína ativadora de GTPase; RhoA = pequena proteína ligante de GTP; GDI = inibidor de dissociação de troca de guanina; RhoGEF = fator de troca GDP-GTP; ROCK = proteína cinase associada à Rho; PLD = fosfolipase D; PA = ácido fosfatídico; DAG = diacilglicerol; PKC = proteína cinase C; CPI- 17 = proteína inibitória dependente de fosforilação de 17 kDa; MLC = cadeia leve da miosina; MLCK = cinase da MLC;MLCP = fosfatase da MLC; MYPT1 = subunidade 1 de ligação da MLCP; PP1c = subunidade catalítica da MLCP; MLCK.

Fonte: Adaptado de Rattan; Phillips; Maxwell IV, 2010.

A ROCK (cinase específica de serina/treonina citoplasmática) se transloca para a membrana plasmática onde se liga à RhoA ligada a GTP, levando à autofosforilação e ativação (Figura 4) (Chen *et al.*, 2002; Leung *et al.*, 2007). ROCK possui 2 isoformas, ROCKI (ROKβ) e ROCKII (ROKα), expressas na musculatura lisa de vários órgãos. A ROCKII é a isoforma mais comum envolvida na contração do músculo liso intestinal (Wang *et al.*, 2009). A via de consenso para a manutenção do tônus do músculo liso gastrintestinal envolve a estimulação do receptor causando a ativação de RhoA/ROCK para inibir o MLCP, resultando na contração muscular sustentada (Gerthoffer, 2005). Além de seu papel na contração sustentada, vários estudos mostraram que o relaxamento ativo do músculo liso intestinal é mediadopela inibição das vias de transdução de sinal RhoA/ROCK (Chitaley; Webb, 2002; Ridick; Ohtani; Surks, 2008).

2.5.2 Óxido Nítrico

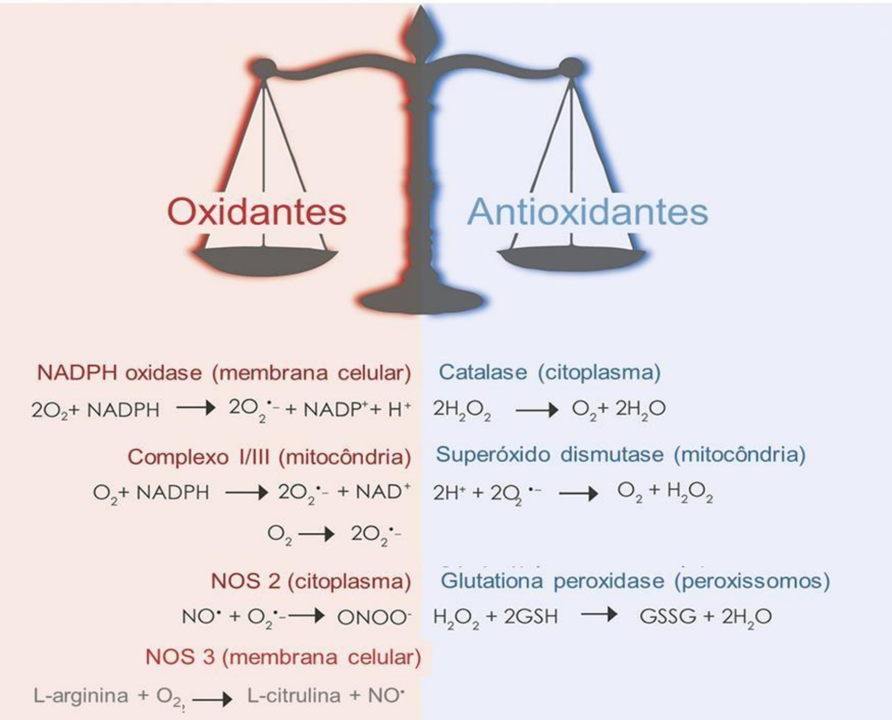
O NO, é um radical livre produzido endogenamente, membro do grupo de moléculas sinalizadoras gasosas referidas como neurotransmissores gasosos (Brenman; Bredt, 1997). Participa de várias funções fisiologias, tais como autócrinas e parácrinas, desde a regulação da função imunológica e neurológica até a preservação da homeostase cardiovascular (Mustafa; Gadalla, 2019). O NO afeta diretamente seus alvos intracelulares porque pode permear passivamente a membrana celular, em contraste com as vias tradicionais de sinalização de neurotransmissores que precisam de interação com o receptor correspondente. Funciona como uma molécula de sinalização comum, estando envolvido em uma ampla gama potencial de processos, como por exemplo na função celular, sinalização retrógrada, fluxo sanguíneo, neuroinflamação e na saúde intestinal (Garthwaite, 2008; Picón-Pagès *et al.*, 2019; Subedi *et al.*, 2021).

A síntese endógena de NO é regulada por mecanismos enzimáticos e não enzimáticos. A NO sintase (NOS) catalisa a síntese enzimática de NO através de uma série de processos redox, incluindo a quebra de L-arginina em L-citrulina e presença de NO de oxigênio e NADPH. NOS neuronal (nNOS ou NOS1), NOS induzível (iNOS ou NOS2) e NOS endotelial (eNOS ou NOS3) são os três tipos de NOS. Embora NOS2 seja independente do transiente de Ca^{2+}, NOS1 e NOS3 são enzimas constitutivas dependentes de Ca^{2+}/calmodulina (Lanzetti *et al.*, 2012). Cada subunidade da enzima homodimérica contém N- e C-terminal domínios de oxigenasee redutase. A cNOS consiste em eNOS e nNOS, sendo a última predominante no intestino. Mais de 90% do NO fisiológico é produzido pela nNOS. Já a iNOS só é expressa e liberada em grandes quantidades em resposta a citocinas e fatores de crescimento envolvidos em vários processos fisiopatológicos (Qu *et al.*, 1999. Li *et al.*, 2013).

As espécies derivadas de NO têm atividades químicas específicas que podem levar à oxidação, nitrosação ou nitração de lipídios, proteínas e outras moléculas. Essas moléculas modificadas podem participar de uma ampla gama de reações químicas com consequências positivas, neutras ou indesejadas para a fisiologia celular (Radi, 2018). A superprodução de NO foi associada a muitas patologias associadas à inflamação. Sob essas condições indesejáveis, pode haver uma mudança para o estresse oxidativo que é o responsável final pelos efeitos prejudiciais (Kolios *et al.*, 2004; Lanzetti *et al.*, 2012) (Figura 9).

A atividade da NOS é regulada pela disponibilidade de substratos e cofatores, inibidores e uma complexa orquestração de processos transcricionais e pós-traducionais, incluindo: i) fosforilação multissítio; ii) interações proteína-proteína;e iii) localização intracelular (Mount; Power, 2006). Além disso, o nitrogênio para formar NO é fornecido pela arginina, que pode ser sintetizada em mamíferos ou ingerida nos alimentos. No último caso, deve-se observar que o impacto da arginina dietética na biodisponibilidade de NO ainda não está claro (Lundberg; Weitzberg, 2022; Aviello; Knaus, 2017).

Figura 9 – Principais fontes de EROs/ERNs e sistemas de defesa antioxidante no TGI



Fonte: Adaptado de Aviello; Knaus, 2017.

2.5.3 Complexo NADPH oxidase

A obesidade, juntamente com outras síndromes metabólicas, tais como incluindo diabetes tipo 2 e hipertensão, tornou-se um desafio importante na saúde pública (Ezenwaka *et al.*, 2014; Hossain; Kawar; El Nahas, 2007). Componentes das paredes celulares de bactérias gram-negativas, incluindo lipopolissacarídeos, estimulam a liberação de mediadores inflamatórios de macrófagos e neutrófilos, como resultado, ocorre inflamação sistêmica, que favorecem o desenvolvimento da obesidade e diversos disturbios associados (Hoogland *et al.*, 2015; Michels *et al.*, 2019). Além disso, descobriu-se que o LPS induz respostas inflamatórias sistêmicas como resultado de múltiplos danos no epitélio intestinal , incluindo, aumento da apoptose, disfunção da barreira e produção de citocinas (Wu *et al.*, 2019; Wu *et al.*, 2020).

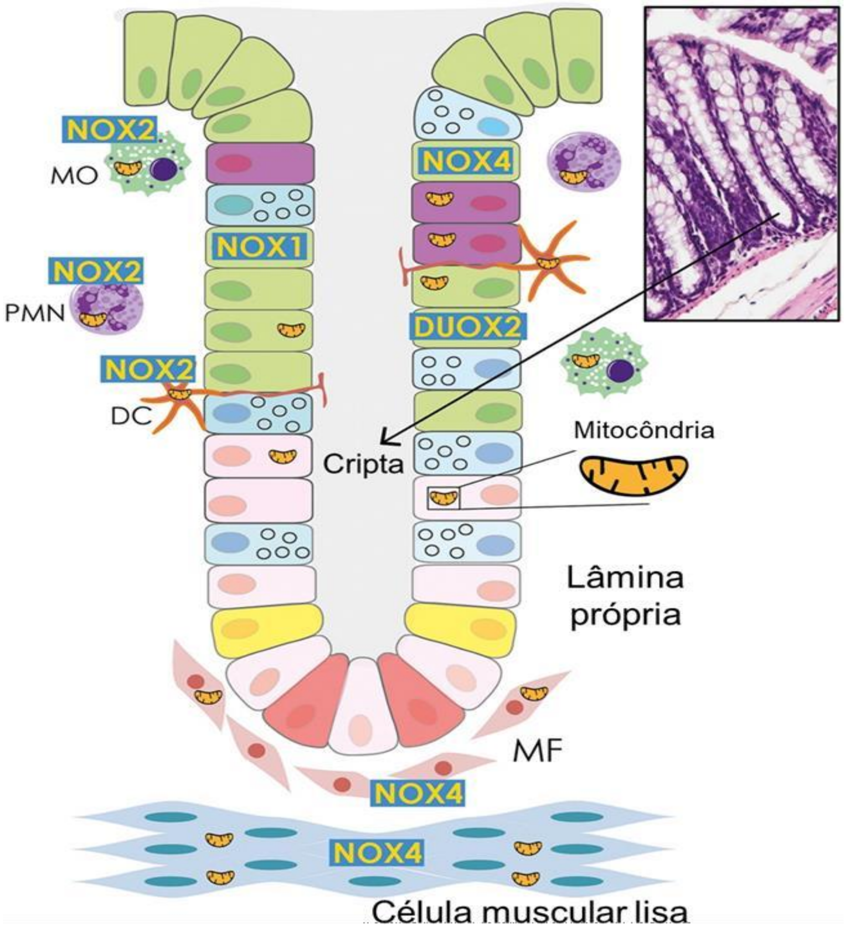
A NADPH oxidases (NOXs) são as principais fontes de EROs, que desempenham um papel essencial nas doenças intestinais induzidas pela obesidade. NOX2 e NOX4 são as isoformas Nox predominantes no trato gastrintestinal, nomeadamente NOX2 em células imunes inatas da lâmina própria e NOX4 expressa em fibroblastos intestinais e endotélio (Aviello; Knaus, 2018). As NOXs regulam a integridade da barreira intestinal via ROS, modulando assim as respostas a doenças infecciosas e inflamação intestinal (Makhezer *et al.*, 2019; Pérez, 2017).

A família das oxidases de mamíferos compreende cinco membros NOX (NOX1-5) e duas oxidases duplas (DUOX1-2). Com exceção do NOX5 que não é expressa em roedores, tanto humanos quanto camundongos expressam todas as isoformas da oxidase. Os homólogos NOX e DUOX diferem em suas estruturas, mecanismos de ativação e padrões de expressão tecidual. As principais oxidases expressas ao longo do TGI são NOX1 e DUOX2 encontradas no epitélio (NOX1 no íleo, ceco e cólon; DUOX2 em todos os segmentos do intestino), NOX2 expressa porfagócitos e células dendríticas, e NOX4 presente no epitélio, fibroblastos e células musculares lisas (Fonte: Aviello; Knaus, 2017; Bedard; Krause, 2007) (Figura 10).

As NADPH oxidases formam complexos multiméricos que geram superóxido ou H_2O_2 de maneira rigorosamente controlada pela transferência de elétrons do NADPH via FAD e através de dois hemes não equivalentes ao oxigênio molecular, o aceptor de elétrons. O transporte de elétrons completo está contido no domínio NOX de seis transmembranas, que é parte integrante de todos os sete membros da família NADPH oxidase (NOX1-5, DUOX1-2) (Figura11)

NOX1 é um complexo multissubunidade com um núcleo catalítico composto por duas proteínas transmembrana: p22^{phox} e NOX1. A fim de converter oxigênio molecular em superóxido, NOX1 heterodimeriza com p22^{phox}, duas subunidades citosólicas denominadas NOXA1 e NOXO1 e RAC1 GTPase ativada. O heterodímero NOX2 é composto por p22^{phox} e gp91^{phox}. O complexo NOX2 é tipicamente inativo em células em repouso, mas quando estimulado, as subunidades citosólicas p40^{phox}, p47^{phox} e p67^{phox} e RAC ativado se unem com NOX2-p22^{phox} para reduzir o oxigênio a superóxido por transporte de elétrons. DUOX2 é uma proteína contendo o domínio de ligação com a peroxidase que forma um heterodímero ligado à membrana com seu fator de maturação DUOXA2 e produz H_2O_2 de maneira dependente de Ca^{2+} (Aviello; Knaus, 2018) A NOX1 é expressa, principalmente, em células epiteliais do cólon e reto, mas também pode ser encontrado em outros tipos celulares, como como células musculares lisas e células endoteliais (Figura 11).

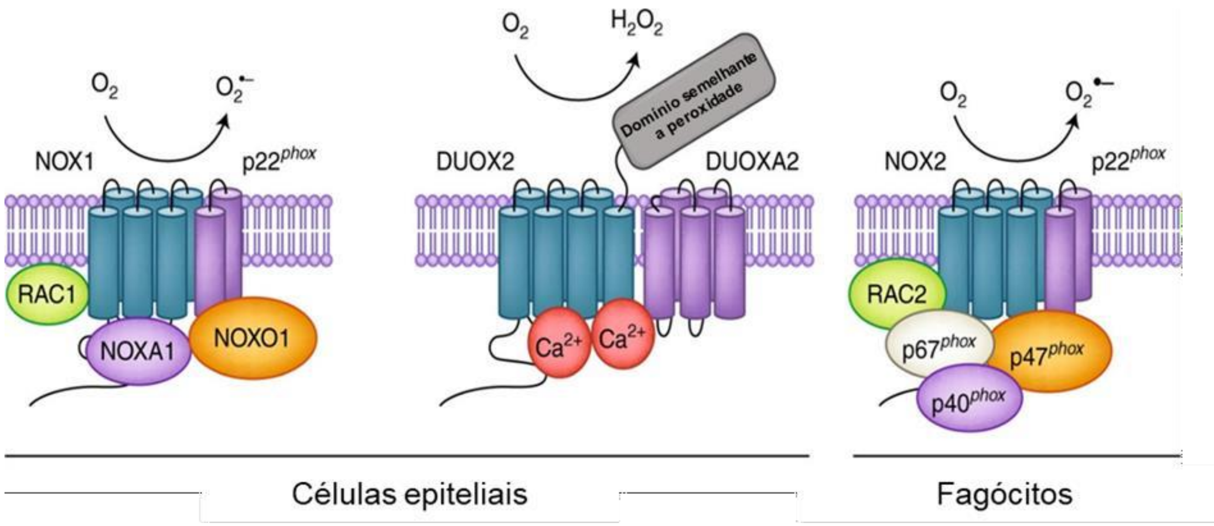
Figura 10 – Localização das isoformas da NADPH oxidase na mucosa gastrintestinal.



NOX = NADPH oxidase; DUOX = oxidase dual; MO = macrófago; PMN = célula polimorfonuclear; MF = miofibroblasto; SMC = célula muscular lisa; DC = células dendriticas. Código de cores para células: verde: célula absortiva de enterócitos; roxa: célula enteroendócrina; rosa: célula progenitora; azul: célula caliciforme.

Fonte: Adaptado de Aviello; Knaus, 2017.

Figura 11 – Representação esquemática das NADPH oxidases presentes no intestino.



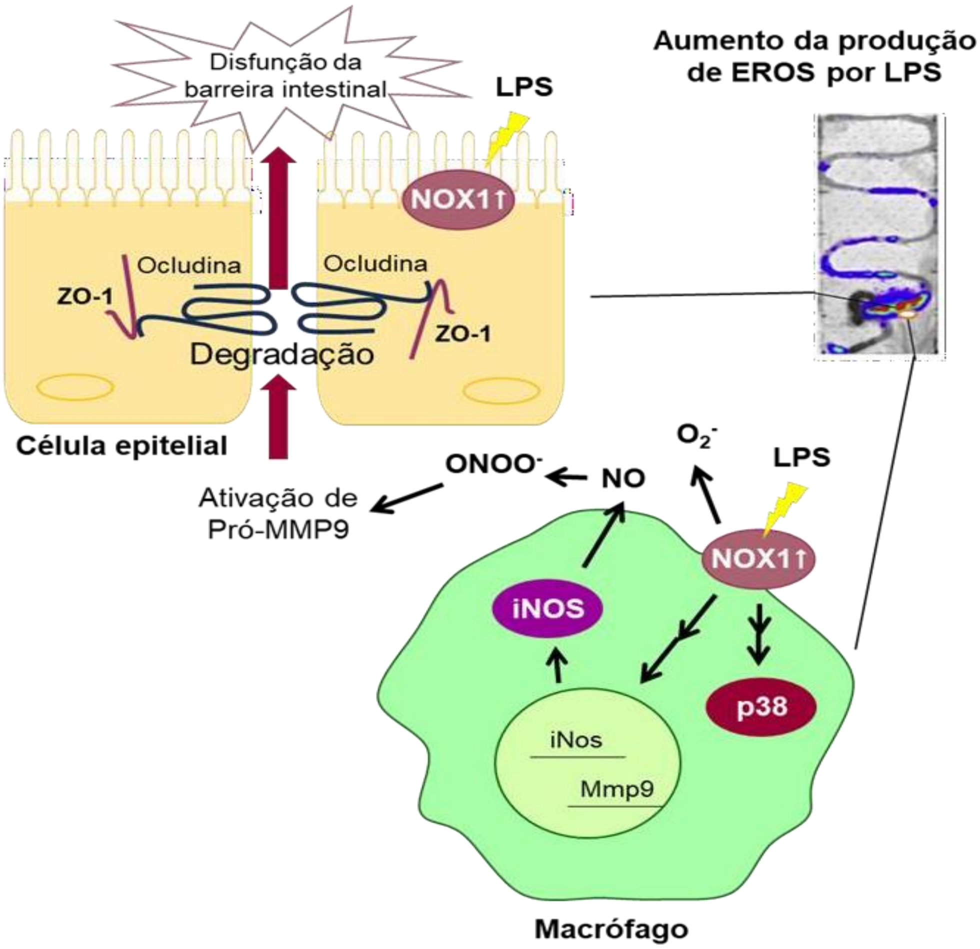
Fonte: Adaptado de Aviello; Knaus, 2018.

As NADPH oxidases estão intimamente envolvidas no *crosstalk* com a microbiota e vice-versa. Quando as NADPH oxidases são parcialmente ou completamente inativadas, mudanças no gradiente de H_2O_2 atingindo bactérias comensais na camada de muco frouxa modificarão a estrutura da comunidade bacteriana alterando a transcrição gênica e a sinalização intrabacteriana, o que também afetará a comunicação interbacteriana. No epitélio, a redução de superóxido (NOX1) e/ou H_2O_2 (NOX1, DUOX2) interromperá as vias intracelulares, levando a alterações na secreção de citocinas ou outros mediadores imunológicos, o que modificará ainda mais a composição da microbiota intestinal (Larsson *et al.*, 2022; Pérez, 2017).

Os complexos NOX produzem EROs usando NADPH e O_2 como substratos.A regulação das isoformas NOX é diversa, incluindo a simples ativação de NOX5 dependente de Ca^{2+} e a modulação complexa das atividades de NOX-1/2 via associação com várias proteínas efetoras, como a proteína G Rac, NOX A1, $p47^{phox}$ e $p67^{phox}$, que, por sua vez, são regulados por várias vias de sinalização celular (Panday *et al.*, 2020). A regulação positiva ou negativa da expressão da subunidade NOX pode aumentar ou diminuir, respectivamente, a atividade enzimática e, consequentemente, aumentar ou atenuar a produção vascular de EROs (Montezano; Touyz, 2022).

Foi demonstrado em um estudo recente que o tratamento com LPS aumentou a imunocoloração de NOX1 no lado apical do epitélio ileal, mas não na lâmina própria. A hiper permeabilidade intestinal induzida por LPS foi abolida em camundongos deficientes em NOX1 ou iNOS. O aumento da atividade e expressão de MMP9 foi acompanhado pela ativação de p38 MAPK, e suprimido em macrófagos deficientes em NOX1. Consequentemente, o eixo NOX1/iNOS nas células derivadas da medula óssea, pode perturbar a integridade da barreira intestinal durante a endotoxemia pela ativação de MMP9. Dessa forma, na endotoxemia, um processo comum durante a disbiose intestinal causada pelo consumo de dietas hipercalóricas, a regulação positiva de NOX1 em macrófagos promove a expressão de iNOS e a geração de peroxinitrito que ativa diretamente a MMP9 para provocar a disfunção da barreira intestinal. A ativação da MAPK p38 também pode fazer parte desse processo (Liu *et al.*, 2020) (Figura 12).

Figura 12 – Mecanismo de disfunção da barreira intestinal mediada por NOX1.



NO = óxido nítrico; iNOS = sintase do NO induzível; LPS = lipopolissacarídio S; MMP = metaloproteinase de matriz; $ONOO^-$ = peroxinitrito; NOX1 = NADPH oxidase 1;ZO- 1 = proteína de zona de oclusão 1.

Fonte: Adaptado de Liu *et al.*, 2020.

2.5.4 Prostanoides

A via do ácido araquidônico (AA) é responsável pela geração de uma grande variedade de metabólitos bioativos. Esses metabólitos, também conhecidos como eicosanoides, demonstraram estar envolvidos em diferentes e variadas doenças, incluindo câncer, inflamação e obesidade (Greene *et al.*, 2011). O ácido araquidônico pode ser metabolizado em eicosanoides biologicamente ativos por meio da ação de três grupos separados de enzimas: ciclo-oxigenases (COX), lipoxigenases (LOX) e epoxigenases (ciclocromo P450). As enzimas COX catalisam o primeiro passo na síntese de prostanoides a partir do AA. A COX foi inicialmente descoberta como duas isoformas distintas no início de 1990, incluindo a COX-1 expressa constitutivamente e a forma induzível de COX-2, associada à inflamação (Wang; Dubois, 2010; Warner; Mitchell, 2019).

Uma terceira isoforma de COX também foi identificada, conhecida como COX-3 (Chandrasekharan et al, 2002). No entanto, estudos subsequentes mostraram que não tem atividade de COX, ou seja, é improvável sua atividade na produção de prostaglandinas em tecidos humanos (Civelek; Ozen, 2022; Snipes, 2015).

Os produtos derivados das COXs, prostaglandinas e tromboxanos, denominados de prostanoides, são mediadores lipídicos biologicamente ativos envolvidos em uma ampla gama de processos fisiológicos, como modulação do tônus vascular, resposta inflamatória e citoproteção gástrica. Os prostanoides também foram implicados em vários estados de doença, como artrite, doença cardíaca, hipertensão pulmonar e obesidade (Smyth *et al.*, 2009; Vianello *et al.*, 2020).

A COX-1 é expressa constitutivamente na maioria dos tipos de tecidos. Os prostanoides produzidos por esta isoforma geralmente medeiam funções de "manutenção", como citoproteção da mucosa gástrica, regulação do fluxo sanguíneo renal e agregação plaquetária. No entanto, a COX-1 também demonstrou ser induzível, particularmente em locais de inflamação (Dirig; Isakson; Yaksh, 1998; Kirkby *et al.*, 2013; Morteau *et al.*, 2000). Muitos estudos demonstraram que a expressão de COX-1 pode ser induzida em células endoteliais em resposta a uma variedade de estímulos. A COX-1 é a única isoforma expressa nas plaquetas e é um importante mediador da agregação plaquetária (Hu *et al.*, 2016; Murphy; Fitzgerald, 2001; Schwab *et al.*, 2002).

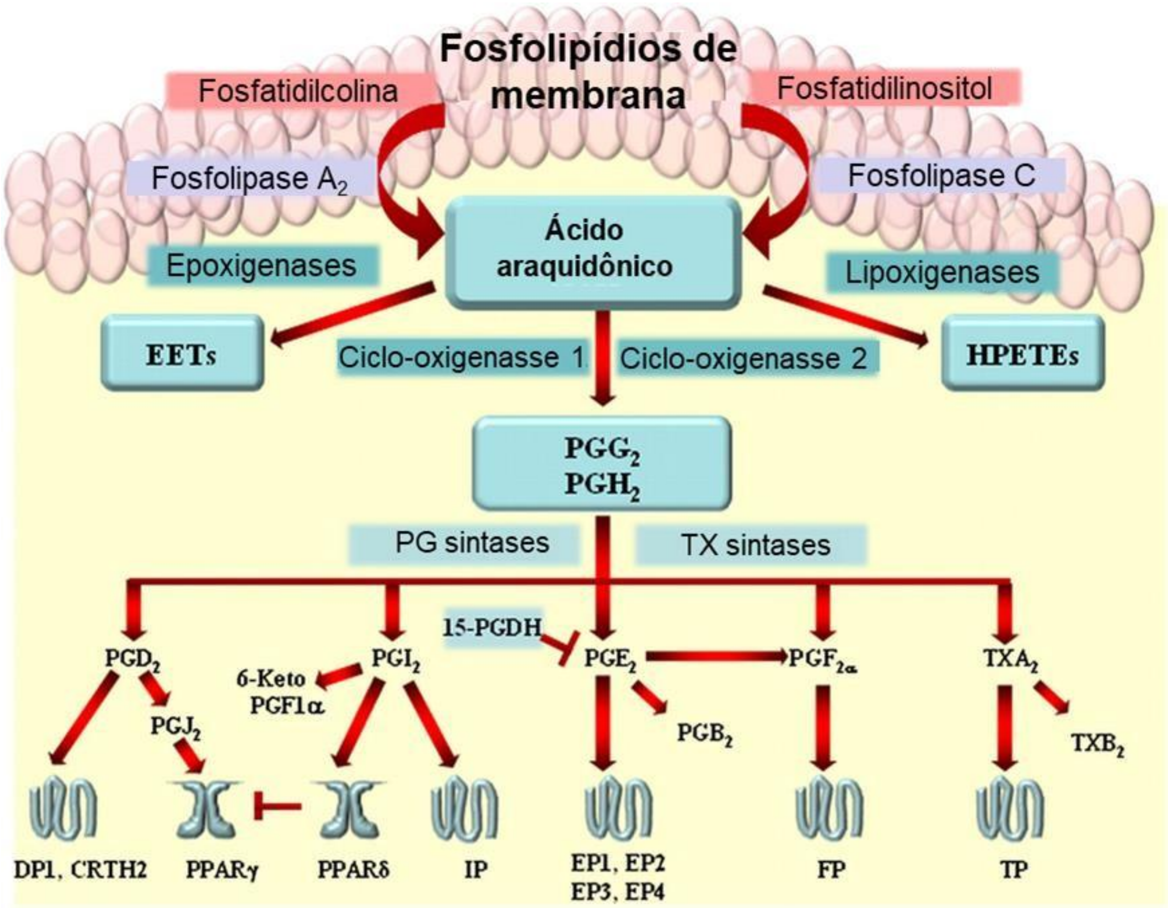
A COX-2, por outro lado, é uma enzima altamente induzível em condições fisiológicas, embora seja expressa constitutivamente em alguns tecidos, como cérebro, medula espinhal, rins e trato gastrintestinal (Harries *et al.*, 1994; Lukiw *et al.*,2003; Norel *et al.*, 2020). Embora a expressão de COX-2 seja altamente restrita em condições basais, sua expressão é dramaticamente aumentada em locais de inflamação em resposta a citocinas como interferon γ, TNFα e IL-1β, hormônios, fatores de crescimento e hipóxia (Asano *et al.*, 2017; Pichiule; Chavez; Lamanna, 2004). A expressão de COX-2 também foi observada em células neoplásicas e endoteliais em muitos tumores diferentes (Masferrer *et al.*, 2020).

A etapa regulatória chave na via de sinalização da COX é a conversão enzimática do ácido araquidônico em PGH_2, que é então reduzido a um intermediário endoperóxido instável, PGH_2. A PGH_2 é então convertida cataliticamente nos vários prostanoides por meio de redução, rearranjo ou isomerização pelas enzimas terminais da sintase (sintase de PGE, sintase de PGD, sintase de PGF, sintase de prostaciclina e sintase de tromboxano). Os produtos prostanoides resultantes (prostaglandinas e tromboxanos) são compostos instáveis e,portanto, são rapidamente metabolizados *in vivo* (Wang; Dubois, 2017).

Os prostanoides derivados de COX são facilmente gerados por vários tipos de células. Plaquetas, mastócitos e monócitos/macrófagos sintetizam TXA_2, PGD_2, PGE_2 e $PGF_{2\alpha}$, enquanto o endotélio é a principal fonte de PGI_2 (Bogatcheva *et al.*, 2015). Os prostanoides derivados do ácido araquidônico são mediadores lipídicos biologicamente ativos envolvidos em uma ampla gama de processos fisiológicos, como modulação do tônus vascular, resposta inflamatória e citoproteção gástrica. No entanto, eles também foram implicados em vários estados de doença, como artrite, doença cardíaca e hipertensão pulmonar (Hata; Breyer, 2014; Needleman; Isakson, 1998).

Os prostanoides exercem suas funções celulares ligando-se a receptores de superfície celular pertencentes a uma família de sete receptores acoplados à proteína G de domínio transmembranar. A nomenclatura do receptor prostanoide é atribuída com base no ligante ligado por oposição a relações genéticas ou funcionais. TP_α/TP_β liga-se a TXA_2, DP liga-se a PGD_2, $EP_{1\text{-}4}$ liga-se a PGE_2, FP liga- se a $PGF_{2\alpha}$, enquanto IP liga-se a PGI_2. Em alguns casos, os prostanoides e seus metabólitos se ligam a seus receptores nucleares, como os receptores ativados por proliferadores de peroxissoma (PPARs) (Alfranca *et al.*, 2016; Lopategi *et al.*, 2016). Três isotipos distintos de PPARs foram identificados até o momento e podem ser geralmente designados como PPARα, PPARβ/δ e PPARγ (Norel *et al.*, 2020; Willson *et al.*, 2020) (Figura 13).

Figura 13 – Geração de prostanoides derivados da COX via metabolismo do ácido araquidônico.

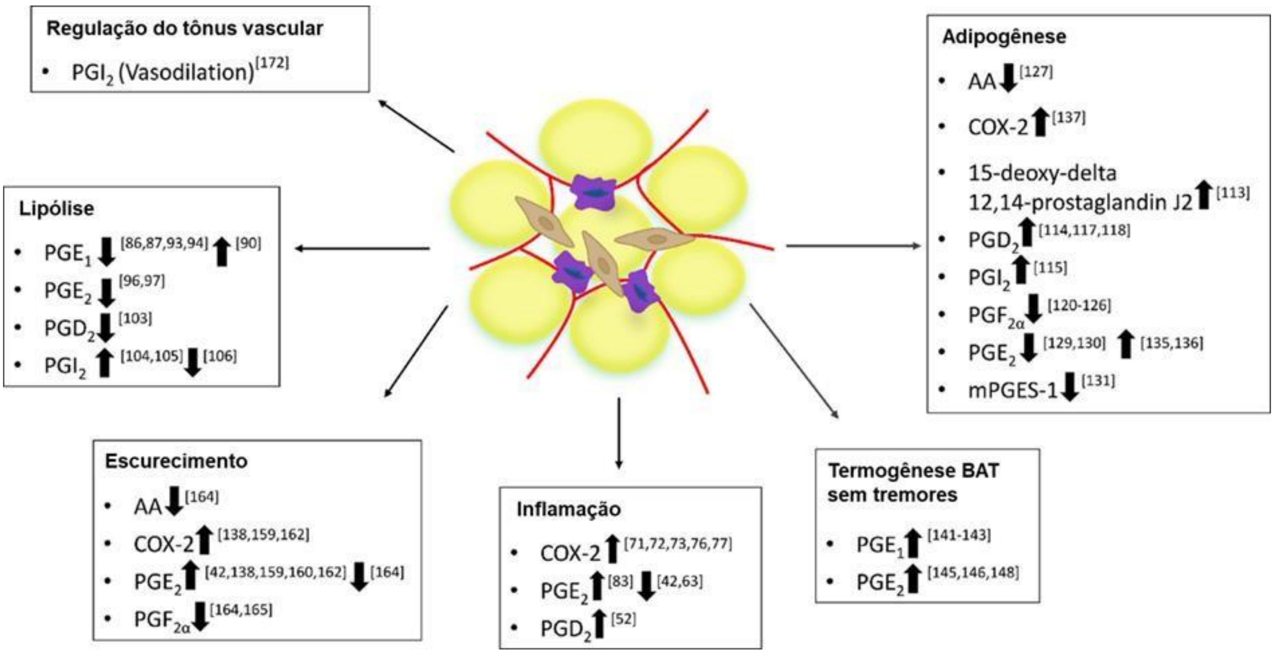


Fonte: Adaptado de Cathcart *et al.*, 2022

Além de seus papéis fisiológicos, os prostanoides derivados da COX estão envolvidos em uma variedade de processos patológicos, incluindo inflamação e câncer. Nos últimos anos, numerosos estudos examinaram o papel de prostanoides individuais no processo de carcinogênese e as características do câncer. Desses prostanoides derivados de COX, a PGE_2 é a mais amplamente investigada em cânceres gastrointestinais, com super expressão frequentemente relatada em tumores do trato gastrointestinal (Gonçalves *et al.*, 2021; Rigas; Goldman; Levine, 1993).

Sabe-se que a obesidade está associada à inflamação do tecido adiposo e à desregulação na secreção de adipocinas, que são denominadas coletivamente como disfunção do tecido adiposo (Longo *et al.*, 2019). Os prostanoides podem modular a função do tecido adiposo através da regulação da secreção de adipocinas (Fain *et al.*, 2001; Peeraully *et al.*, 2006). Assim, os prostanoides, principalmente a PGE_2, têm efeitos importantes na lipólise, inflamação do tecido adiposo, adipogênese, termogênese do tecido adiposo branco, escurecimento do tecido adiposo e regulação do tônus vascular (Figura 14).

Figura 14 – Resumo dos efeitos dos prostanoides na regulação do tônus vascular, da lipólise, do escurecimento do tecido adiposo, da inflamação, da termogênese e da adipogênese.



Fonte: Adaptado de Civelek; Ozen, 2022.

Estudos posteriores confirmaram a produção de prostanoides a partir dotecido adiposo de ratos (Richelsen, 1992). PGE_2 e PGI_2 foram os prostanoides mais liberados do tecido adiposo de ratos e humanos. Em consonância com esses estudos, Katz, Rudick e Knittle (1988) relataram que a produção *in vitro* de prostanoides a partir do tecido adiposo subcutâneo humano tinha a seguinte ordem de quantidade: PGE_2>6-keto- $PGF_{1\alpha}$ (um metabólito estável de PGI_2) > TxB_2 (um metabólito estável de TxA_2) (Timur *et al.*, 2018). Em outro estudo, o tecido adiposo branco de ratos mostrou produzir níveis 2-3 vezes maiores de $PGF_{2\alpha}$ em comparação com PGE_2. Por outro lado, foi demonstrado que o PGE_2 era o prostanoide mais abundante, consistente com os estudos anteriores, outros prostanoides como o PGI_2, PGD_2 ou $PGF_{2\alpha}$ não foram detectados em concentrações altas o suficiente para ligar seus receptores em camundongos, sugerindo que dependendo da espécie e do tipo de tecido adiposo, a quantidade de produção de prostanoides pode ser modulada (Jaworski *et al.*, 2009; Katz; Rudick; Knittle, 1988).

2.6 Arthrospira (*Spirulina*) *platensis*

Historicamente, os produtos naturais serviram como importantes drogas contra uma ampla variedade de doenças humanas. Desde a década de oitenta entretodos os agentes terapêuticos de pequenas moléculas aprovados, os medicamentos derivados a partir de produtos naturais correspondem aproximadamente 65% dos medicamentos comercializados, os quais são usados inalterados ou desenvolvidos com base nas estruturas que ocorrem naturalmente (Newman; Cragg, 2020). O interesse por produtos naturais de origem marinha se intensificou, devido principalmente à conscientização da enorme biodiversidade existente no ambiente marinho, bem como aos inúmeros avanços emambas as técnicas de pesquisa e estratégias para abordar as principais questões que dificultam a descoberta e o desenvolvimento de medicamentos provenientes de produtos naturais marinhos (Gerwick; Moore, 2012; Montaser; Luesch, 2011).

As pesquisas com produtos naturais marinhos no Brasil iniciaram na década de 60 no Centro de Pesquisas de Produtos Naturais na Faculdade de Farmácia da UFRJ (Kelecom, 1997). No entanto, ainda são poucas as pesquisas documentadas em artigos científicos, sobre substâncias isoladas e a atividade biológica de produtos naturais de organismos marinhos coletados ao longo do litoral brasileiro. As poucas informações existentes sobre a química desses organismos, muitos dos quais são espécies endêmicas, indicam um grande potencial de pesquisa para a área no Brasil (Chagas-Paula, 2021).

O ambiente marinho fornece uma valiosa plataforma para a descoberta de novos compostos biologicamente ativos. Mais de 4000 produtos naturais marinhos bioativos foram relatados desde 1985 (Blunt *et al.*, 2015; Harvey; Edrada-Ebel; Quinn, 2015; Hu *et al.*, 2015; Sakai; Swanson, 2014). Eles foram descobertos em uma ampla variedade de organismos marinhos, como invertebrados (Fisher *et al.*, 2012; Hegazy *et al.*, 2015; Leal *et al.*, 2013), microrganismos derivados de sedimentos (Aksoy; Uzel; Bedir, 2016; Fukuda *et al.*, 2015) além de algas marinhas e microrganismos associados a estas (Abdelmohsen; Bayer; Hentschel, 2014; Andrade, 2016; Barbosa; Valentao; El-Hossary *et al.*, 2017). Leal *et al.*, 2013; Saleem *et al.*, 2007).

Entre o grande número de funcionalidades biológicas dos produtos naturais provenientes das algas marinhas, vários efeitos já foram comprovados, tais como efeitos antivirais (Cheng *et al.*, 2016; Moghadamtousi *et al.*, 2015), antituberculosos (Sieniawska, 2015; Sun *et al.*, 2014) antidiabéticos (Ko; Jeon, 2013; Zheng *et al.*, 2015), imunomoduladores (Shrestha; Clair; O'neill, 2015; Tabares *et al.*, 2011), anti-inflamatórios (Abad; Bedoya; Bermejo, 2018; Gonzalez *et al.*, 2015;), antioxidantes (Grkovic *et al.*, 2014; Wada; Sakamoto; Matsugo, 2018), antiprotozoários (Bu; Yang; Hu, 2021; Jones *et al.*, 2013) e antimicrobianos (Aksoy; Uzel; Bedir, 2022; Hensler *et al.*, 2014; Singh; Abraham, 2014).

Dentre as algas marinhas, as pertencentes ao filo das cianobactérias e da família Spirulinaceae, representam os procariontes quimiossintéticos mais encontrados nos ecossistemas aquáticos. Certas espécies, incluindo *Aphanizomenon flos-aquae, Spirulina maxima, Spirulina fusiformis, Spirulina sp, Nostoc* e *Spirulina platensis*, têmsido consumidos pelos seres humanos há séculos (Torres-Duran; Juarez- Oropeza, 2010; Rasmussen *et al.*, 2010).

A *Spirulina platensis*, também conhecida como *Arthrospira*, é uma alga verde-azulada, multicelular e filamentosa, pertencente ao filo das cianobactérias,que vem ganhando considerável popularidade na indústria de alimentos, sendoconsiderada como um suplemento alimentar para dietas de humanos, gado, aves e aquicultura. Durante séculos tem sido utilizada especificamente como suplemento dietético por populações que habitam próximo de lagos alcalinos, onde crescem naturalmente, incluindo, o Lago Chade, na África, e o Lago Texcoco, no México. Além disso, a *Spirulina* pode ser encontrada em oceanos, rios e lagos de água doce, sendo facilmente colhida e produzida (Ciferri; Tiboni, 1985; Sotiroudis; Sotiroudis, 2013; Wan; Wu; Kuca, 2016).

Semelhantemente a maioria das cianobactérias, a *Spirulina* é reduz o dióxido de carbono do ambiente e absorve principalmente os nitratos, sendo o glicogênio o principal produto de assimilação da fotossíntese. Demonstra ótimo crescimento entre 35 e 37 °C em condições laboratoriais, altamente resistente aos raios ultravioleta, no entanto, esta alga é sensível à baixas temperaturas (< 15 °C) (Wan; Wu; Kuca, 2022).

Possui teores muito elevados de macro e micronutrientes, como proteínas, aminoácidos, ácidos graxos insaturados, minerais, vitaminas, antioxidantes carotenoides, tocoferóis e compostos fenólicos. Sua composição consiste de 55- 70% de proteína, 15-25% de polissacarídeos, 5-6% de lipídios totais, 6-13% de ácidos nucléicos e 2,2-4,8% de minerais (Hosseini; Khosravi-Darani; Mozafari, 2016). Os ácidos graxos essenciais insaturados constituem 1,5 a 2,0% do conteúdo lipídico total dessas algas, incluindo o ácido γ-linoleico (GLA; 30-35% do total), ácido estearidônico (SDA), ácido eicosapentaenoico (EPA), ácido docosahexaenoico (DHA) e ácido araquidônico (AA). Além disso, possui baixos níveis de colesterol (32,5 mg/100 g), sendo também de fácil digestão (com até 90% de digestibilidade absoluta) devido à ausência de celulose em suas paredes celulares (Belay, 2002; El-Sheekh; Hamad; Gomaa, 2014; Habib *et al.*, 2008; Reboleira *et al.*, 2019; Sasson, 1997).

Composta majoritariamente por proteínas, que constituem a maioria do seu peso seco, a *Spirulina* é a fonte mais rica deste macronutriente, quando comparada a carnes, ovos, leite, grãos e soja, sendo, portanto, representada como um alimento nutracêutico. Contém todos os aminoácidos essenciais, com concentrações particularmente elevadas de leucina, valina e isoleucina, agregando ainda mais valor ao seu uso como fonte proteica alternativa (Belay, 2008; Hosseini; Khosravi-Darani; Karkos *et al.*, 2011; Mozafari, 2016).

Os pigmentos naturais presentes na *Spirulina* são bastante importantes para o metabolismo fotossintético da alga. As ficobiliproteínas, como a ficocianina-C e a aloficocianina, estão presentes em quantidades significativas dentro da fração proteica da *Spirulina*, representando cerca de 20 a 25% do peso seco total da alga e constituem os principais responsáveis por diversas atividades biológicas e farmacológicas benéficas e já descritas, tais como antioxidante, anticarcinogênico, anti- inflamatório, antiangiogênica, neuroprotetora e antiobesidade (Romay *et al.*, 2003; Serban *et al.*, 2016; Shokri; Khosravi; Taghavi, 2014; Reboleira *et al.*, 2019). Adicionalmente, a ficocianobilina presente na *Spirulina* funciona como um potente inibidor de NOX, sendo responsável pela eliminação de radicais livres com a capacidade de reduzir não apenas os radicais derivados das EROs, mas também os radicais hidroxila, peroxila, hipoclorito e peroxinitrito (Liwa *et al.*, 2017; Mccarty, 2007;Romay *et al.*, 2013).

Nos últimos anos tem se discutido e demonstrado a *Spiruilina platensis* como uma suplementação alimentar alternativa e eficiente para o controle do peso em animais e humanos. Estudos clinicos demosntraram que a suplementação com essa alga durante o periodo de 12 semanas (1 g/dia) resultou na redução do peso e do Indice de Massa Corporal (IMC), outro estudo relatou que após três meses de suplementação na dose de (2 g/dia), a *Spirulina* também promoveu uma redução significativa no peso, IMC e na circunferência abdominal de pacientes hipertensos e obesos (Miczke *et al.*, 2016; Szulinska *et al.*, 2017; Zeinalian *et al.*, 2017).

A eficácia proveniente da utilização da *Spirulina* como suplemento alimentar sobre a redução do peso corporal, é dada principalmente devido a sua atuação reduzindo a infiltração de macrófagos no tecido adiposo visceral e prevenindo o acúmulo de lipídios no fígado e o estresse oxidativo. Outra caracteristica importante para tal propriedade, é a presença de fenilalanina em sua composição, um potente liberador de colecistocinina, hormônio que afeta diretamente o centro do apetite no cérebro, agindo portanto, como um inibidor do peso corporal (Fujimoto *et al.*, 2012; Mazokopakis *et al.*, 2014). Ademais, já é demonstrado que os antioxidantes presentes na alga, são eficazes no tratamento da obesidade por meio de diferentes efeitos, tais como inibição da lipase, efeito supressor na ingestão de alimentos, efeito inibitório sobre a diferenciação dos adipócitos, efeitos estimulatórios no gasto energético e efeito regulatório sobre o metabolismo lipídico (Hassan; El-Gharib, 2015;Ismail *et al.*, 2020).

2.6.1 Arthrospira (Spirulina) platensis e musculatura lisa

Estudos pré-clínicos e clínicos demonstram os benefícios que a ingestão de *A. platensis* pode promover na diminuição dos triglicerídeos séricos e lipoproteínas de baixa densidade, no controle glicêmico, na redução da rinite alérgica, no crescimento da microflora intestinal saudável, efeitos anticâncer, anti-inflamatório, anti-hipertensivo, antioxidante e na redução da disfunção endotelial (Pan *et al.,* 2014; Morais *et al.,* 2016). Diante disso, há 9 anos, aproximadamente, nosso grupo de pesquisa vem se debruçando em estudos sobre essa alga e seus mais variados efeitos na musculatura lisa, em diferentes condições fisiológicas e patológicas, evidenciando propriedades, atividades biológicas e farmacológicas a cerca da suplementação alimentar com *Arthrospira (Spirulina) platensis* em diferentes modelos experimentais.

Entre esses estudos, já foi demosntrado e evidenciado que na reatividade da aorta, ocorre participação da via do NO. No tecido aórtico na presença de L-NAME, a suplementação crônica com S*. platensis* (150/500 mg/kg) causou diminuição da resposta contrátil e aumento dos níveis de relaxamento e dos níveis de nitrito, indicando maior produção de NO, associado à diminuição do estresse oxidativo e aumento da atividade antioxidante a partir da alga (Brito et al., 2019).

Posteriormente, demostramos que ratos alimentados com uma dieta hipercalórica, a suplementação dietética com *S. platensis* aumentou efetivamente o número de ereções, enquanto diminuiu a latência para iniciar uma ereção peniana. Além disso, a *S. platensis* aumenta a biodisponibilidade de NO, reduz a inflamação reduzindo a liberação de prostanóides contráteis em tecido cavernoso, aumenta o efeito de relaxamento promovido pela acetilcolina (ACh), restaura o dano a reatividade contrátil e ao relaxamento cavernoso, reduz as espécies reativas de oxigênio e aumenta a capacidade antioxidante cavernosa. Evidenciando, portanto, o papel da suplementação alimentar com *S. platensis* na restauraçao da função erétil de ratos obesos e aumento da biodisponibilidade de NO (Diniz *el al*., 2020).

S. platensis reduziu a reatividade do íleo de rato ao carbacol e ao KCl,enquanto o treinamento de força reduziu apenas a eficácia do CCh. Além disso, a associação potencializou a redução da reatividade contrátil. A suplementação reduziu o estresse oxidativo e aumentou a inibição da oxidação. Portanto, este estudo demonstrou que tanto a suplementação quanto sua associação com o treinamento de força promovem efeitos benéficos em relação à reatividade contrátil intestinal e ao estresse oxidativo, fornecendo novos *insights* para o manejo dedistúrbios intestinais (Araujo et al., 2020).

Similarmente, o treinamento de força aumenta a reatividade contrátil e diminui o componente farmacomecânico da reatividade relaxante no útero de ratas. Entretando, a suplementação com AP preveniu esse efeito e potencializou o aumento da capacidade antioxidante uterina. Assim, *S. platensis* é um meio alternativo de prevenir disfunções uterinas causadas pelo exercício de força (Ferreira *et al*., 2021).

A dieta hipercalórica foi eficaz em promover obesidade em ratos, bem como diminuir a potência e a eficácias relaxante e contrátil do íleo. Em contraste, a suplementação dietética com SP foi capaz de prevenir alguns dos parâmetros da obesidade experimental. Além disso, SP impediu a redução da reatividade contratil intestinal, possivelmente devido a uma modulação positiva dos canais de cálcio dependentes de voltagem (Ca_V) e regulação negativa dos receptores muscarínicos (M3). Assim, a suplementação alimentar com AP torna-se uma alternativapromissora na prevenção de doenças gastrointestinais induzidas e/ou agravadas pela obesidade (Diniz *et al.,* 2021).

Foi observado uma diminuição na peroxidação lipídica no plasma e aumento na inibição da oxidação para a traquéia e pulmão em SG150 e SG500, sugerindo aumento da atividade antioxidante proveniente da alga. Bem como, a *S. platensis* (150/500 mg/kg) diminuiu a resposta contrátil e aumentou o relaxamento por aumentar a atividade antioxidante e os níveis de nitrito, modulando a respostainflamatória. Sugerindo assim, uma maior atividade antioxidante da alga (Brito *et al.*, 2022). Ademais, os efeitos deletérios causados pelo consumo de dieta hipercalórica por 8 semanas, sobre a função erétil e reatividades contrátil e relaxante no corpo cavernoso de rato, foram evitados pela suplementação com SP nas doses de 25, 50 e/ou 100 mg/kg. Dessa forma, a suplementação com *S. platensis* previne os danos associados ao consumo de uma dieta hipercalórica e surge como adjuvante na prevenção da disfunçao eretil (Souza *et al.,* 2022).

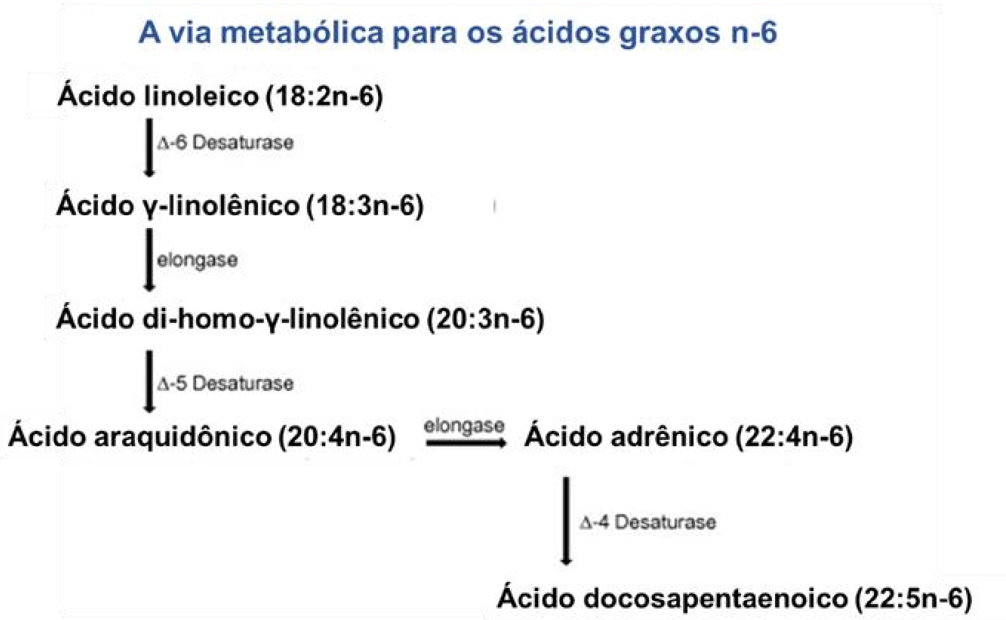
2.6.2 Arthrospira (Spirulina) platensis e ácido gama linolênico (GLA)

Os ácidos graxos poli-insaturados de cadeia longa (PUFAs) são uma classe de nutrientes de grande interesse, pois estão envolvidos no desenvolvimento do cérebro, cognição e em muitas doenças cardiovasculares, câncer, diabetes (Bazinet; Layé, 2014; Kris-Etherton *et al.*, 2003; Soyuğur, 2021). São eficazes em melhorar a sensibilidade à insulina, restaurar a ovulação, regular a menstruação e reduzir a inflamação, tais propriedades estão relacionadas a redução dos distúrbios associados a infertilidade, diabetes, condições cardiometabólicas e obesidade (Prabhu; Abilash, 2021).

Os PUFAs são constituídos por dezoito a vinte e dois átomos de carbono e mais de duas ligações duplas (2 a 6) (Fischer, 2022) e contribuem para a regulação de vários processos fisiológicos em humanos (Calder, 2015). O ácido γ-linolênico (GLA) é um PUFA bioativo que no organismo é metabolizado em ácido di-homo-γ- linolênico (DGLA) e posteriormente em AA. A partir destes dois últimos PUFAs, ocorre a produção de eicosanoides de diferentes séries (Guil-Guerrero, 2007). OGLA está envolvido em diversas funções fisiológicas, tais como, expressão genicade funções imunes e apoptose (Kapoor; Huang, 2006) e inibição do ciclo celular tumoral (Xu; Qian, 2014). Além disso, a suplementação dietética com GLA melhora operfil lipídico do sangue e da pele bem como na prevenção de síndromes e doenças inflamatórias (Kawamura *et al.*, 2011; Tso *et al.*, 2012; Tasset-Cuevas *et al.*, 2013).

O ácido all-cis-6,9,12-octadecatrienoico (GLA) e ácido estearidônico (SDA, ácido all-cis-6,9,12,15-octadecatetraenóico) são PUFAs pertencentes às séries n-6 e n-3, respectivamente. Ambos os ácidos graxos são produzidos no corpo a partir de seus precursores metabólicos ácido linoleico (LA, ácido all- cis- 9,12-octadecadienoico) e ácido α-linolênico (ALA, ácido all-cis-9,12,15-octadecatrienoico), respectivamente, pela ação da enzima Δ6-dessaturase. O GLA é posteriormente metabolizado em ácido di- homo-γ-linolênico (DGLA, ácido all-cis-8,11,14-eicosatrienoico), que sofre metabolismo oxidativo por ciclo-oxigenases e lipoxigenases para produzir eicosanoides anti-inflamatórios (prostaglandinas e leucotrienos) que são compostos bioativos semelhantes a hormônios envolvidos na regulação de vários mecanismos fisiológicos e patológicos em animais e humanos (Guil-Guerrero, 2007; Horrobin, 1992; Meesapyodsuk; Qiu, 2022) (Figura 15).

Figura 15 – A via metabólica para o ácido all-cis-6,9,12-octadecatrienoico.



Fonte: Adaptado de Colquhoun, 2020.

Além disso, o GLA e seus metabólitos também afetam a expressão de vários genes, tendo um papel significativo nas funções imunes e no apoptose (Kapoor; Huang, 2006), e vários estudos indicam que o GLA possui propriedades anticancerígenas, incluindo inibição da proliferação celular e indução de apoptose (Menéndez *et al.*, 2001; Xu; Qian, 2015). Além disso, trabalhos recentes têm atribuído benefícios proeminentes à saúde à suplementação alimentar com GLA, como melhora do perfil lipídico do sangue e da transpiração cutânea, mostrando efeitos promissores no tratamento de dermatites, hiper proliferação cutânea e osteoporose, entre outras síndromes (Kawamura *et al.*, 2011; Tasset-Cuevas *et al.*, 2013; Tso *et al.*, 2012). Consequentemente, devido às suas ações fisiológicas benéficas amplamente divulgadas, o GLA é cada vez mais utilizado na indústria cosmética (Greenery; Bruheim, 2013) e indústrias alimentícias (Flider, 2015).

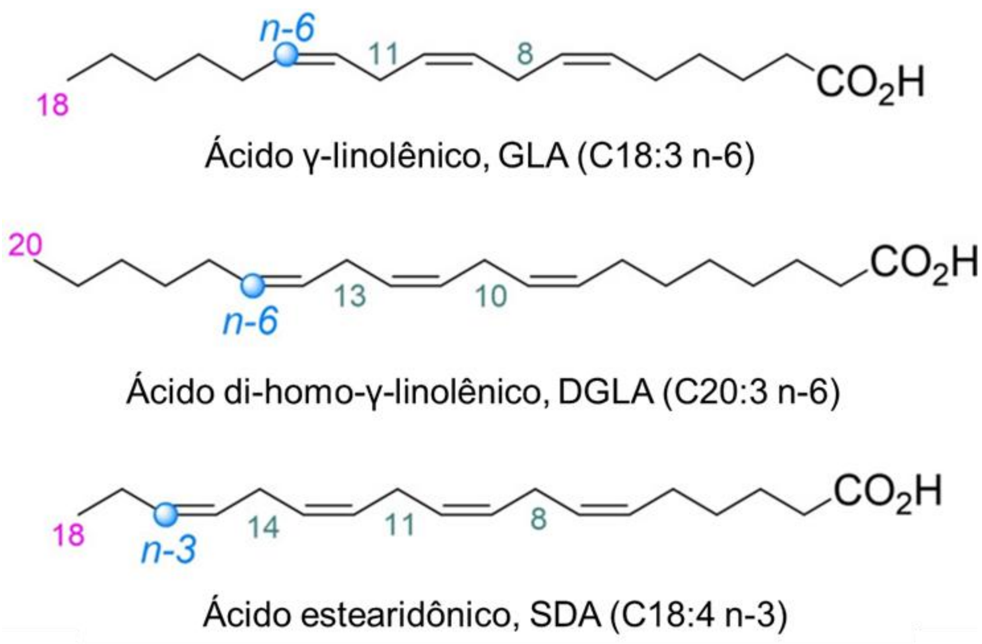
O GLA possui múltiplas funções nutracêuticas e farmacêuticas. A pesquisa envolvendo o GLA centrou-se em seu papel terapêutico no alívio de doenças crônicas, como doenças inflamatórias (obesidade, artrite reumatoide e dermatite), neuropatia diabética, síndrome pré-menstrual e fatores de risco para doenças cardiovasculares (Fan; Chapkin, 1998).

As fontes primárias de GDGs-GLA na natureza são derivadas dos lipídios da membrana fotossintética de plantas e algas. É abundante em algumas plantas superiores (as sementes de plantas de borragem e prímula) e algas verde-azuladas (*Spirulina platensis* e Nostoccommune). Vários estudos demonstraram que os PUFAs podem reduzir o estresse oxidativo e as respostas inflamatórias ao inibir as vias de sinalização de citocinas inflamatórias (Novak *et al.*, 2003) e confirmou os efeitos terapêuticos dos PUFAs na artrite, asma e outras doenças relacionadas (O'donovan; Fernandes, 2000). Numerosos estudos clínicos mostraram que o GLA é um suplemento dietético de alto valor em todo o mundo e os compostos contendo GLA são eficazes na terapia adjuvante, no tratamento e prevenção de câncer,diabetes, infecção viral e obesidade (Zhang *et al.*, 2013; Zhou *et al.*, 2021). Yang e colaboradores demonstraram que o GDGs-GLA extraído da *Spirulina platensis,* possui uma gama de atividades funcionais, incluindo propriedades anti-inflamatórias, antioxidantes e antialérgicas que promovem a regeneração de feridas em peixe- zebra (Cho *et al.*, 2018; Thompson *et al.*, 2019).

Os ácidos γ-linolênico (GLA, C18: 3 n-6) e estearidônico (SDA, C18: 4 n-3) são fontes sustentáveis de ácidos graxos ômega-6 e ômega-3. O GLA é encontrado na gordura do leite de vaca ou humano, alguns vegetais verdes folhosos ou algas, prímula (*Oetothera beinnis e lamarckiana*), borragem (*Borage officinalis*) ou óleos de groselha negra (*Ribes nigrum),* bem como em microalgas. SDA por sua vez, pode ser encontrado em óleos de sementes de echium (*Echium plantagineum*), maconha (*Cannabis sativa L.*) e groselha negra, Buglossoides (*Buglossoides arvensis)* (Lee *et al.*, 2016).

O GLA e SDA são mais estáveis, devido ao seu menor índice de insaturação (Figura 19). A presença de insaturações é uma grande preocupação, pois os PUFAs contêm posições bisalílicas que são altamente propensas à abstração de hidrogênio por radicais livres liberados na membrana. Essas espécies reativas sofrem peroxidação lipídica levando à formação de metabólitos de PUFA agrupados sob o nome de oxilipinas (*Fischer et al.*, 2022). Esses metabólitos são formados sem a intervenção de enzimas, são chamados de oxilipinas não enzimáticas. As oxilipinas não enzimáticas foram descobertas pela primeira vez por Morrow *et al.* (1990), do ácido araquidônico (AA, C20: 4 n-6), e desde 1990 foram detectados metabólitos não enzimáticos *in vivo* de outros PUFAs (AA, ALA, DHA, EPA, AdA) (Jahn; Galano, 2008). Portanto, pode-se presumir indubitavelmente que GLA, DGLA (ácidodi- homo- γ-linolênico) e SDA também serão peroxidados (Fischer *et al.*, 2022) (Figura 16).

Figura 16 – Estruturas dos ácidos γ-linolênico, di-homo-γ-linolênico e estearidônico.



Fonte: Adaptado de Fischer *et al.*, 2022.

Os PUFAS, incluindo o GLA, têm sido relatados como exercendo efeitos antitumorais em vários tipos de tumor (Newell, et a., 2019). O GLA altera o metabolismo da ciclo-oxigenase, podendo estimular e/ou inibir espécies reativas de oxigênio, envolvendo a ativação de caspases, liberação de citocromo C e apoptose (Colquhoun; Schumacher, 2001). Além disso, o GLA atua no metabolismo energético C6 (Ramos; Colquhoun, 2003)., na angiogênese e no ciclo celular (Miyake; Benadiba; Colquhoun, 2009). Células que absorvem quantidades aumentadas de GLA, incorporam este ácido graxo na membrana fosfolipídica, posteriormente este ácido graxo será metabolizado pela PLA_2, que transforma o ácido graxo em eicosanoides pela enzima ciclooxigenase-2 (COX-2). A COX-2 forma prostaglandinas (PGs), leucotrienos e prostaciclinas. A COX-2 metaboliza o AA e produz eicosanoides da série 2 (por exemplo, PGE_2). O AA livre é metabolizado em prostaglandinas, ácidos hidroperoxieicosatetraenoicos e ácidos epóxieicosatrienoicos por meio da ação de COX, LOX e CYP 450 epoxigenases, respectivamente (Pang et al, 2021).

Os PUFAs competem com os AA e quando a COX-2 metaboliza os PUFAs produzindo eicosanoides como a PGE_3, que tem efeitos menores que a PGE_2, diminuindo a ação da PLA_2 e da COX-2. Devido a esses efeitos, o GLA pode modificar a produção dos eicosanoides na célula (Ensign *et al.*, 2013) e esses lipídios têm características inflamatórias e menos agressiva do que a PGE_2 (Colquhoun, 2020; Colquhoun; Miyake; Benadiba, 2009; Hawcroft *et al.*, 2012; Miyake; Gomes).

Diante de tais premissas, destaca-se o papel promissor da *Arthrospira (Spirulina) platensis* como potente agente antioxidante na melhora da reatividade muscular lisa vascular, da disfunção endotelial, do estresse oxidativo, de problemas relacionados a contratilidade uterina e da disfunção erétil. Entretanto, ainda não há evidências da aplicabilidade da utilização da alga na prevenção dos danos causados sobre a contratilidade intestinal do íleo, sendo, portanto, necessários estudos para evidenciar o potencial efeito protetor da *A. platensis*, bem como os mecanismos de ação subjacentes envolvidos nas alterações de adiposidade corporal e na reatividade muscular lisa intestinal de ratos alimentados com dieta hipercalórica.

3 Objetivos

3.1 Objetivo geral

Avaliar o possível efeito preventivo da suplementação alimentar com *Arthrospira* (*Spirulina*) *platensis* nas alterações promovidas pelo consumo de uma dieta hipercalórica sobre os parâmetros nutricionais e morfométricos, a reatividade do músculo liso intestinal, o estresse oxidativo e o perfil inflamatório, em ratos Wistar.

3.2 Objetivos específicos

- Avaliar o possível efeito preventivo da *A. platensis* sobre os parâmetros murinométricos e de obesidade experimental;

- Investigar os efeitos da obesidade e da suplementação alimentar com *A. platensis* sobre:
  - Histomorfometria do íleo;
  - Perfil inflamatório do íleo através da técnica de imuno- histoquímica;
  - Estresse oxidativo;
  - Defesas antioxidantes.

- Investigar e caracterizar, em nível funcional, o mecanismo de ação da *A. platensis* na prevenção das alterações sobre a reatividade contrátil, envolvendo as vias do(a)s:
  - RhoA/ROCK;
  - Oxido nítrico;
  - Prostanoides;
  - Antioxidantes;
  - Estresse oxidativo.

4 Material e métodos

4.1 Material

4.1.1 Produto-teste

A *Arthrospira (Spirulina) platensis* foi adquirida do laboratório INFINITY Pharma® (HONG KONG, China) na forma de pó. Uma amostra foi analisada e certificada pela Farmácia de Manipulaçao Roval (João Pessoa, Paraíba, Brasil).

4.1.2 Animais

Eram utilizados ratos Wistar (*Rattus norvegicus*) com 8 semanas de idade, pesando entre 170-190 g, provenientes da Unidade de Produção Animal (UPA) do Instituto de Pesquisa em Fármacos e Medicamentos (IPeFarM)/UFPB e do Bioterio Professor Eduardo Barbosa Beserra da Universidade Estadual da Paraiba (UEPB). Os animais foram mantidos sob rigoroso controle alimentar com uma dieta balanceada a base de ração tipo *pellets* (Presence®), com acesso a água *ad libitum*, ventilação, umidade e temperatura (21 ± 1 °C) controladas e constantes, submetidosdiariamente a um ciclo claro escuro de 12 h, sendo o período claro das 6-18 h. Os experimentos eram realizados no período das 8-22 h e seguirão os princípios de cuidados com animais, de acordo com o *"Guidelines for the ethical use of animals in applied ethology studies"* (Sherwin *et al.*, 2003) e com o Guia Brasileiro de Produção,Manutenção ou Utilização de Animais em Atividades de Ensino ou Pesquisa Científica, do Conselho Nacional de Controle de Experimentação Animal (CONCEA) (Brasil, 2016). Os procedimentos experimentais foram aprovados pela Comissão de Ética no Uso de Animais da UFPB (protocolo nº 2352101019) (Anexo 1).

4.1.3 Substâncias e reagentes

Os cloretos de cálcio di-hidratado ($CaCl_2.2H_2O$), de sódio (NaCl) e de potássio (KCl), a glicose ($C_6H_{12}O_6$), o bicarbonato de sódio ($NaHCO_3$), os fosfatos de potássio monobásico anidro (KH_2PO_4) e de sódio dibásico (Na_2HPO_4), o sulfato de magnésio hepta hidratado ($MgSO_4.7H_2O$), o hidróxido de sódio (NaOH) e o ácido clorídrico (HCl) foram adquiridos da Êxodo Científica (Brasil). O fosfato de sódio monobásico hidratado ($NaH_2PO_4.H_2O$) e o álcool etílico absoluto foram adquiridos da FMaia

(Brasil). O $CaCl_2.2H_2O$, o KCl, o KH_2PO_4 e o $MgSO_4.7H_2O$ foram dissolvidos e diluídos em água destilada para obtenção de cada solução estoque que foram mantidas sob refrigeração. As demais substâncias foram mantidas em temperatura ambiente e diluídas em água destilada ou ultrapura (Milli-Q®) de acordo com cada protocolo experimental. Estas substâncias, exceto a glicose, o bicarbonato de sódio e o cloreto de sódio foram dissolvidas e diluídas em água destilada para obtenção de cada solução-estoque que era mantida sob refrigeração. O formaldeído era utilizado para conservar as amostras de tecidos utilizadas para cortes histológicos. A mistura carbogênica (95% de O_2 e 5% de CO_2) foi adquirida da White Martins (Brasil).

As substâncias que foram utilizadas nos protocolos em nível funcional, bem como aquelas das metodologias em nível molecular, foram adquiridas da Merck® (Brasil). Todas eram dissolvidas segundo as recomendações do fornecedor e eram diluídas em água destilada purificada para concentrações apropriadas ao protocolo experimental adotado.

4.1.4 Solução nutritiva

A solução nutritiva utilizada era a de Krebs Henseleit em mM: NaCl 118; KCl 1,2; KH_2PO_4 1,18; $MgSO_4.7H_2O$ 1,18; $NaHCO_3$ 25; $CaCl_2.2H_2O$ 2,5; glicose 1,1 (Henriques, 2011), tinha o pH ajustado com HCl ou NaCl (1 N) até atingir um valor de 7,4, era aerada com mistura carbogênica em todas as etapas dos experimentos funcionais, e mantida à temperatura de 37 °C.

4.1.5 Deitas alimentares

Os animais dos grupos DP (dieta padrão) receberão uma dieta balanceada à base de ração tipo pellets (Presence®) contendo por peso 23% de proteína, 63% de carboidrato e 4% de lipídios, totalizando o valor energético total (VET) em 380 kcal/100g. Os grupos alimentados com a dieta hipercalórica (DHC) receberão uma dieta composta por uma mistura de ração padrão tipo pellets (Presence®), chocolate, amendoim *in natura* torrado e biscoito de maizena na proporção de 3:2:2:1, apresentando VET de 417 kcal/100 g (Souza *et al.,* 2017). A dieta era preparada semanalmente e ofertada aos animais na forma de pellets (Figura 17).

Figura 17 – Composição e preparação da dieta hipercalórica.

Ração padrão tipo *pellets* (Presence®) (1), biscoito de maizena (2), amendoim *in natura* torrado (3), chocolate ao leite (4), ração padrão tipo *pellets* (Presence®) moída e triturada (5), biscoito demaizena triturada (6), amendoim *in natura* torrado moído (7), chocolate ao leite derretido (8), materiaissecos e líquidos misturados (9), formação e moldagem dieta (10), dieta na estufa (11) e dieta pronta para consumo dos animais (12).

Fonte: O autor (2023).

4.1.6 Grupos experimentais

Os animais foram distribuidos randomicamente em 3 grupos experimentais: grupos alimentados com dieta padrão que receberam solução salina (NaCl 0,9%) (DP), grupos alimentados com dieta hipercalórica que receberamsolução salina (NaCl 0,9%) (DHC), ou que foram suplementados simultaneamente com *S. platensis* na dose de 25 mg/kg (DHC + SP25).Cada grupo experimental foi alimentado por um período de 8 semanas (El-Desoky *et al.*, 2013; Ferreira, 2017; Souza, 2018) (Figura 18).

Figura 18 – Desenho dos grupos experimentais.

Os ratos foram divididos em três grupos experimentais, alimentados com suas respectivas dietas e suplementados com *Arthrospira* (*Spirulina*) *platensis* durante 8 semanas. DP + salina (grupo alimentado com dieta padrão e suplementado com solução salina); DHC + salina (grupo alimentado com dieta hipercalórica e suplementado com solução salina); DHC + SP25 (grupo alimentado com dieta hipercalórica e suplementado, simultaneamente com *Arthrospira* (*Spirulina*) *platensis* na dose de25 mg/kg).

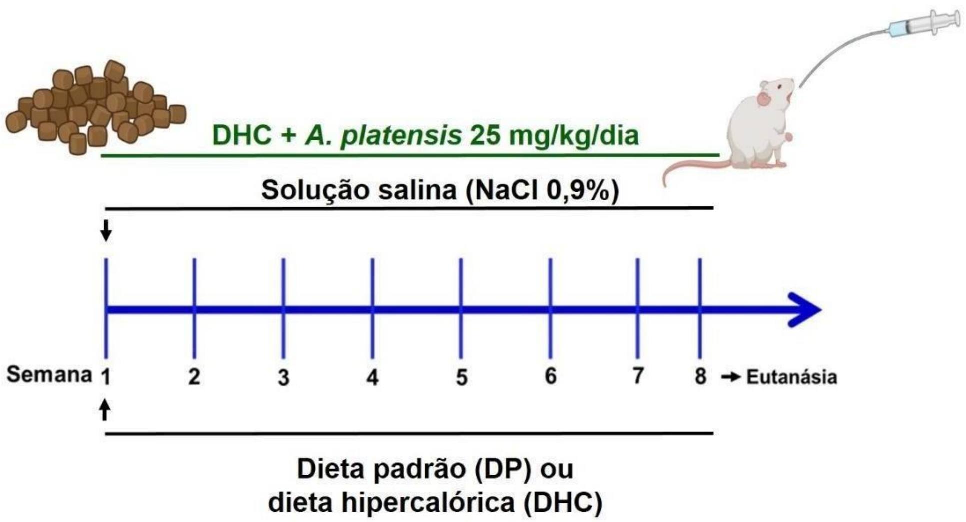
Fonte: O autor (2023).

4.1.7 Preparo e administração da A. platensis

O pó de *A. platensis* era diariamente dissolvido em solução salina (NaCl 0,9%) para o preparo das doses utilizadas no estudo, que foram administradas aos ratos ao fim da preparação. A suplementação na dose de 25 mg/kg era realizada uma vez ao dia, por um período de 8 semanas, (El- Desoky *et al.*, 2013; Ferreira, 2017; Adaptado de Juárez-Oropeza *et al.*, 2009; Souza, 2018). Todos os grupos iniciaram a suplementação (salina ou *A. platensis*) no mesmo dia do início da dieta e continuaram até o dia da eutanásia (Figura 20). A dose de 25 mg/kg foi escolhida após estudos/experimentos de triagem farmacológica realizados com 3 doses (25, 50 e 100 mg/kg) em que a dose de 25 mg/kg foi a menor dose com o melhor efeito.

As administrações foram realizadas por via oral no período das 12 às 14 h, uma vez ao dia durante todos os dias da semana (8 semanas), com o auxílio de agulhas de aço inoxidável para gavagem (BD-12, Insight, Ribeirão Preto, São Paulo, Brasil) e seringas descartáveis de 10 mL com precisão de 0,2 mL (BD, Higilab, João Pessoa, Paraíba, Brasil) (Figura 20).

Figura 8 – Delineamento experimental.



Todos os grupos experimentais foram alimentados e suplementados por 8 semanas, após esse período os animais ficaram de jejum por 12 horas, e posteriormente foram eutanasiados.

Fonte: O autor (2023).

4.1.8 Isolamento do íleo

Os animais foram eutanasiados por guilhotina e o íleo foi removido, limpo de tecido conjuntivo e gordura, imerso em solução fisiológica à temperatura ambiente e colocados em cubas de banho para orgãos isolados com mistura de carbogênio. Para o registro das contrações isotônicas, segmentos do íleo (2-3 cm) foram suspensos individualmente em banhos de órgãos (5 mL) por fio de algodão e acoplados a um sistema de aquisição digital.

4.1.9 Reatividade relaxamente

Após o período de estabilização, foi induzida contração com KCl 25 mM para verificar a funcionalidade do órgão. Em seguida, o preparado foi lavado e nova contração foi induzida com KCl 25 mM, e sob o componente tônico desta contração foi adicionado verapamil (10^{-16} a 3 x 10^{-7} M), um bloqueador dos canais de cálcio dependentes de voltagem, cumulativamente à cuba, em todas as preparações. Os resultados foram avaliados comparando as respostas dos grupos controle suplementados com solução salina, obesos suplementados com solução salina e obesos suplementados com *A. platensis* (25 mg/kg) e os valores de Emax e pCE_{50} foram avaliados como parâmetros de eficácia e potência, respectivamente . Os valores de E_{max} e pCE_{50} foram obtidos por regressão não linear.

4.1.10 Reatividade contratil

Após o período de estabilização, o íleo foi contraído com KCl 25 mM para verificar a integridade do órgão, em seguida o órgão foi lavado e após retornar à linha de base, atropina foi pré-incubada (10^{-6} e 10^{-5}), um antagonista não seletivo de receptores muscarínicos. Posteriormente, foi realizada uma curva de concentração acumulada reabastecida para carbacol, um análogo da acetilcolina. Os resultados foram avaliados comparando as respostas dos grupos controle suplementados com solução salina (SD e HCD) e suplementados com S. platensis (HCD + AP25) e os valores de E_{max} e pCE_{50} foram avaliados como parâmetros de eficácia e potência, respectivamente.

4.2 *Análise imuno-histoquímica dos níveis de interleucina 1 beta (IL-1β) no íleo*

Fragmentos de íleo (3 µm) foram obtidos em micrótomo e transferidos para lâminas silanizadas (Dako, Glostrup, Dinamarca) e submetidos aos processos de desparafinização e hidratação. As lâminas foram então lavadas com Triton X-100 a 0,3% em tampão fosfato, tratadas com peróxido de hidrogênio a 3% e incubadas durante a noite a 4 °C com o anticorpo primário anti-interleucina 1β (IL-1β) (Santa Cruz Biotechnology, Interprise, Brasil). Após lavagem com tampão fosfato, as lâminas foram incubadas com anticorpo secundário conjugado com estreptavidina-HRP (Biocare medical, Concord, CA, EUA) por 30 min e a imunorreatividade para IL-1β foi realizada por meio de teste colorimétrico. baseado em um kit de detecção, seguindo o protocolo fornecido pelo fabricante (rótulo Trek Avidin-HRP + Medical Biocare Kit, Dako, EUA). As amostras foram visualizadas em microscópio óptico (Leica DM750, Suíça) com sistema Qwin acoplado a uma câmera (Leica ICC50 HD) com objetiva de 400x. Em cada imagem, todos os pixels marrom-acastanhados (coloração imuno-histoquímica positiva) foram utilizados para criar uma imagem binária por processamento digital.

5 Resultados e Discussões

5.1 Capítulo I

Artigo 1

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Research Article

Spirulina platensis Consumption Prevents Obesity and Improves the Deleterious Effects on Intestinal Reactivity in Rats Fed a Hypercaloric Diet

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The consumption of hypercaloric diets is related to the development of obesity, favoring the etiology of gastrintestinal disorders. In this context, *Spirulina platensis* (SP), some blue-green algae with antioxidant action, appears as a potential therapeutic alternative to prevent obesity and associated intestinal disorders. Thus, the present study is aimed at evaluating the deleterious effects of the hypercaloric diet on the contractile and relaxing reactivity of the ileum of rats, as well as the possible preventive mechanisms of dietary supplementation with SP. Wistar rats were divided into three groups: fed a standard diet (SD), a hypercaloric diet (HCD), and/or supplemented with 25 mg/kg SP (HCD + SP25) for 8 weeks. The hypercaloric diet was effective in promoting obesity in rats, as well as decreasing potency and ileal relaxing and contractile efficacy. In contrast, dietary supplementation with SP was able to prevent some of the parameters of experimental obesity. In addition, SP prevented the reduction of intestinal reactivity, possibly due to a positive modulation of voltage-gated calcium channels (Ca_V) and negative regulation of muscarinic receptors (M_3). Thus, food supplementation with Spirulina platensis becomes a promising alternative in the prevention ofgastrintestinal diseases induced and/or aggravated by obesity.

1. Introduction

Defined as the abnormal and/or excessive deposition of body fat, which directly interferes with the individual's health, obesity is a chronic noncommunicable disease caused by the energy imbalance between consumption and caloric expenditure, representing an important risk factor for development of cardiovascular diseases, type 2 diabetes mellitus, musculoskeletal disorders, and some types of cancer [1–4]. The causes can be influenced by genetic, hormonal, and environmental factors, especially those related to poor eating habits, determined by the consumption of hypercaloric diets leading to increased rates of obesity and overweight and consequently to the development of various gastrintestinal disorders [5, 6].

Thus, with the growing global obesity epidemic, researchers have turned their attention to studies that demonstrate the relationship between obesity and the main metabolic and endocrine disorders that affect the gastrintestinal system (gastroesophageal reflux disease, dyspepsia, constipation, bowel syndrome irritable, and diarrhea) that contribute to the existence of similar proinflammatory mechanisms, linking both diseases [7–9]. In addition, preclinical studies report that obesity impairs inhibitory neuromuscular transmission andrelaxation of enteric smooth muscle, in addition to changes in intestinal motility [10].

In this context, the marine environment represents a rich therapeutic arsenal composed of several organisms that function as sources of bioactive metabolites, with great potential for the discovery of new drugs [11]. Among these organisms, in recent years as previous marine algae gaining prominence, being increasingly inserted in human food, demonstrating antiobesity effects, mainly to its nutritional composition of bioactive compounds, photosynthetic pigments, sterols, polyunsaturated fatty acids, vitamins, minerals, fiber, and proteins, used as nutraceuticals and/or food supplements. In addition, the interest in inserting algae as one of the additives present in animal feed has been growing, mainly because theyare a natural source of biomass and easily cultivated, enhancing performance and animal health [12–15].

Among the algae, *Spirulina platensis* (*Arthrospira platensis*) stands out, a blue-green, unicellular alga with nutraceutical, probiotic, antioxidant, anti-inflammatory, hypolipidemic, hypoglycemic, antihypertensive, and immunomodulatory properties [16–18]. Spirulina has been shown to be an efficient dietary supplement for weight control in animals and humans, due to its excellent nutritional profile and the large amount of protein elements, phycocyanin, carotenoids, and all important amino acids for the body's balance [19–22].

In recent years, our research group has studied the various effects of food supplementation with *S. platensis*, showing that the algae were able to decrease lipid peroxidation and inhibit oxidation in the aorta [23], cavernous body [24], and ileum [25] of rats, as well as the concentration of reactive oxygen species and the inflammation induced by the exercise of force [26]. In addition to reducing adipose reserves and restoring intestinal contractile reactivity in Wistar rats fed a high-fat diet for 16 weeks [27].

Therefore, it is important to evaluate the functional changes and the deleterious effects caused by the consumption of the high calorie diet on parameters related to experimental obesity and intestinal reactivity and the possible therapeutic role of food supplementation with *S. platensis* in preventing changes in reactivity relaxing and contractile ileum of Wistar rats fed for eight weeks on a calorie-rich diet, investigating the possible mechanisms of action involved in such effects.

2. Materials and Methods

2.1. Chemicals. Potassium chloride (KCl), calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$), sodium chloride (NaCl), and formaldehyde were purchased from Vetec Química Fina Ltda. (João Pessoa, Brazil). Sodium bicarbonate ($NaHCO_3$) and glucose ($C_6H_{12}O_6$) were purchased from Dinâmica (Brazil). Sodium monobasic phosphate (NaH_2PO_4), sodium hydroxide (NaOH), and hydrochloric acid (HCl) were purchased from Nuclear (Brazil). These substances, except glucose, NaCl, and $NaHCO_3$, were diluted in distilled water to obtain each solution, which were maintained under refrigeration.

Carbamylcholine hydrochloride (CCh) was purchased from Merck (USA). Cremophor®, thiobarbituric acid, tetramethoxypropane, perchloric acid, Mayer's hematoxylin, and eosin were acquired from Sigma-Aldrich (Brazil). All substances were diluted in distilled water as needed for each experimental protocol. The carbogen mixture (95% O_2 and 5% CO_2) was obtained from White Martins (Brazil).

2.2. Animals. Wistar male rats (*Rattus norvegicus*), 2 months old (approximately 170 g), were obtained from the Animal Production Unit (UPA) of the Research Institute for Drugs and Medicines, João Pessoa, Brazil (IpeFarM/UFPB). The animals were maintained under controlled ventilation and temperature (21 ± 1 °C) with water ad libitum in a 12 h light-dark cycle (light on from 6 to 18 h). The experimental procedures were performed following the principles of guidelines for the ethical use of animals in applied etiology studies [28] and from the Conselho Nacional de Controle de Experimentação Animal of Brazil [29] and were previously approved by the Ethics Committee on Animal Use of UFPB with certificatenumber 6061090318.

The animals were randomly divided into three groups (8 rats/group): rats given a standard diet (SD), rats fed a hypercaloric diet (HCD), and fed a hypercaloric diet and simultaneously supplemented with 25 mg/kg Spirulina platensis for 8 weeks (HCD + SP25). The experimental groups were fed for 8 weeks. After this period, the animals were anesthetized with thiopental sodium (100 mg/kg body weight) mixed with lidocaine (10 mg/mL), then euthanized by decapitated by guillotine.

2.3. Preparation and Supplementation with Spirulina platensis. Spirulina platensis (*Arthrospira platensis*) was obtained from the INFINITY Pharma laboratory (HONG KONG, China) (lot No. 20130320), in powder form, and a sample was analyzed, fractionated, and distributed by the Roval Manipulation Pharmacy (João Pessoa, Paraíba, Brazil) (lot No. 405894) to certify that S. platensis lyophilized powder has been obtained.

The *S. platensis* powder was dissolved in saline solution (NaCl 0.9%) to prepare the dose of 25 mg/kg. The groups supplemented with 25 mg/kg received the supplementation for a period of 8 weeks [30]. Oral administration was done daily through stainless steel needles for gavage and 5 mL syringes with a precision of 0.2 mL, between 12 and 14 hours.

2.4. Food Diets. The standard diet (Presence®) contains 23% protein, 63% carbohydrate, and 4% lipids with 3.8 kcal energy density/g; the hypercaloric diet consisted of standard diet (Presence®), milk chocolate, peanuts, and sweet biscuits at a ratio of 3:2:2:1 [31]. The hypercaloric diet contains 23% protein, 45% carbohydrate, and 16% lipids with energy density 4.17 kcal/100 g by weight; this diet was prepared weekly and fed to the animals as granules [32, 33] (Table 1). To prepare the hypercaloric diet, the feed, peanuts, and biscuits were ground and mixed, and the chocolate melted in a water bath and added to the mixture to forma homogeneous material that was molded and then dried in an oven (70 °C) for 24 h andstored at room temperature (Table 1). The diet was prepared weekly and offered to rats in the form of pellets [30].

2.5. Ileum Isolation. All animals were fasted for 12 hours before being euthanized. After this period, the rats were anesthetized with ketamine 100 mg/kg (i.p.) and xylazine 10 mg/kg (i.p.), and the euthanasia process was completed by decapitation in a guillotine. Then, an abdominal incision was made, and the ileum segment was removed and placed in a Petri dish containing the Krebs Henseleit solution at 37 °C under aeration with carbogen.

Record isometric contractions, the adjacent connective and adipose tissues of the ileum were removed, and ileum segments (2-3 cm) were suspended in bath tubs for isolated organs (6 mL) containing Krebs Henseleit solution aerated with carbogen at 37 °C and remained at rest under tension of 1 g for 30 minutes, the time necessary for the stabilization ofthe organ. During the stabilization period, the nutrient solution was changed every 10 minutes to avoid the interference of metabolites [34].

The physiological solution of Tyrode was used and has the composition as follows (in mM): NaCl (150.0), KCl (2.7), $CaCl_2$ (1.8), $MgCl_2$ (2.0), $NaHCO_3$ (12.0), NaH_2PO_4 (0.4), and D-glucose (5.5). The pH was adjusted to 7.4, and the ileum was stabilized for 1 h under a resting tension of 1 g at 37 °C and bubbled with a carbogen mixture [33, 34].

TABLE 1: Macronutrients and caloric value of standard and hypercaloric diets.

Diets	Carbohydrates (%)	Proteins (%)	Lipids (%)	Total Energy Value (kcal/100g)
Standard	63	23	4	3800
Hypercaloric	45,53	22,76	16	4170

Souza et al. [32].

2.6. Parameters of experimental obesity

2.6.1. Murinometric Parameters. On the day of euthanasia, the rats were weighed, and the nasoanal length (cm) was used, which was used to calculate the Lee index, from the ratio between the cube root of body mass (g) and the nasoanal length (cm) of the animal [35]. The body mass index (BMI) was calculated from the ratio between body mass (g) and the square of body length (cm^2) [36]. The abdominal circumference, located in the anterior part of the animal's rear paw, and the thoracic circumference, located in the posterior portion of the front paw, were measured using an anthropometric body measuring tape [36].

2.6.2. Mass of Adipose Tissue Deposits. Twenty-four hours after the last exposure of the diet and supplementation, the rats were euthanized by guillotine, and after careful dissection, the epididymal, retroperitoneal, and inguinal adipose tissues were weighed, which represent the main components of central adiposity in rats [37]. The abdominal fat located in the lower abdomen connected to the epididymis represents the epididymal fat. The fat connected to the posterior abdominal wall around the kidneys and the abdominal part of the ureter represents the retroperitoneal fat. The subcutaneous fat that is located between the lower portion of the rib cage and the median portion of the thigh represents the inguinal fat [38, 39].

2.6.3. Adiposity Index. The adiposity index was calculated from the sum of the individual masses of the epididymal, retroperitoneal, and inguinal fat layers, using the following formula: (epididymal fat + retroperitoneal fat + inguinal fat) × 100/final body mass [40, 41].

2.7. Relaxation Reactivity Measurement. After the stabilization period, a contraction was induced with 25 mM KCl to check the organ's functionality. Then, the preparation was washed, and a new contraction was induced with 25 mM KCl, and under the tonic component of this contraction was added verapamil (10^{-16} to 3×10^{-7} M), a blocker of calcium channels dependent on voltage, cumulatively to the vat, in all preparations [42]. The results were evaluated by comparing the responses of the control groups supplemented with saline, obese supplemented with saline, and obese supplemented with S. platensis (25 mg/kg), and the values of E_{max} and pCE_{50} were evaluated as parameters of efficacy and potency, respectively. The values of E_{max} and pCE_{50} were obtained by nonlinear regression.

2.8. Contraction Reactivity Measurement. After the stabilization period, the ileum was contracted with 25 mM KCl to check the integrity of the organ; then, the organ was washed, and after returning to baseline, atropine was preincubated (10^{-6} and 10^{-5}) [43, 44], a nonselective antagonist of muscarinic receptors. Subsequently, a cumulative replenished concentration curve for carbacol, an analogue of acetylcholine, was performed. The results were evaluated by comparing the responses of the control groups supplemented with saline (SD and HCD) and supplemented with *S. platensis* (HCD + SP25), and the values of E_{max} and pCE_{50} were evaluated as parameters of efficacy and potency, respectively.

2.9. Ileal Histological and Morphometric Analysis. For a general analysis of the histoarchitecture of the analyzed tissues, the samples were placed in an automatic tissue processor, embedded in paraffin and then cut in a 4 µm microtome, placed on histological slides and deparaffinized in xylene for 30 minutes, hydrated in alcohols in decreasing concentrations for 25 minutes and washed in running water for 5 minutes, and then washed in distilled water. The samples were then treated with Harris hematoxylin for 1 minute, washed again in distilled water for 5 minutes and again stained with eosin for 3 minutes, and later washed in running water for another 30 seconds. Finally, the slides were dehydrated in increasing concentrations of alcohols, cleared in xylene, and mounted with Entellan®.

3. Statistical analysis

The results were expressed as mean and standard error of the mean, being analyzed statistically using the *t*-test (unpaired) or the analysis of variance (ANOVA) one way followedby the Tukey posttest. The null hypothesis was rejected when p value < 0.05. As a power parameter, pCE_{50} values were used; calculated by nonlinear regression and as an efficiency parameter, E*max* was used [45]. All data were analyzed using the GraphPad Prism® program version 6.01 (GraphPad Software Inc., San Diego, CA, USA).

4. Results

4.1. Effect of Hypercaloric Diet and Supplementation with S. platensis on Final Body Mass and Murinometric Parameters. In rats fed the hypercaloric diet, an increase in final body mass (470.1 ± 8.2 g) was observed when compared to rats fed the standard diet (345.7 ± 4.7g). The rats fed the hypercaloric diet and supplemented with S. platensis at a dose of 25 mg/kg(376.7 ± 5.9) showed an increase in body mass when compared to the SD group and a decrease in relation to the HCD group (Figure 1(a)).

When analyzing murinometric parameters, the DHC group had both nasoanal (27.67 ± 0.3 cm) and abdominal (22.0 ± 0.6 cm) and thoracic (19.14 ± 0.4 cm) length difference in relation to the SD group (25.0 ± 0.5 cm, 16.71 ± 0.3 cm, and 14.29 ± 0.2 cm, respectively). Analyzing the HCD + SP25 group, it had nasoanal length (24.17 ± 0.2 cm), lower abdominal (16.00 ± 0.1 cm), and thoracic (5 cm) circumferences when compared to the group HCD and with no difference with the SD group (Figures 1(b)–1(d)).

Regarding the Lee index, the HCD group (0.817 ± 0.003 g/cm) was higher than the SD (0.72 ± 0.01 g/cm) (Figure 1(e)). In the body mass index (BMI), the HCD group (0.66 ± 0.010) showed an increase in relation to the SD group (0.52 ± 0.01), but the HCD + SP25 group (0.64 ± 0.01) did not differ from the HCD group, showing a difference only when compared to the SD control group (Figure 1(f)). As for the adiposity index, both the HCD group

(9.74 ± 0.33) and the HCD + SP25 group (5.61 ± 0.11) showed an increase in relation to the SD group (4.47 ± 0.15). The HCD + SP25 group, when compared to the obese group, had a lower adiposity index (5.61 ± 0.11) (Figure 1(g)).

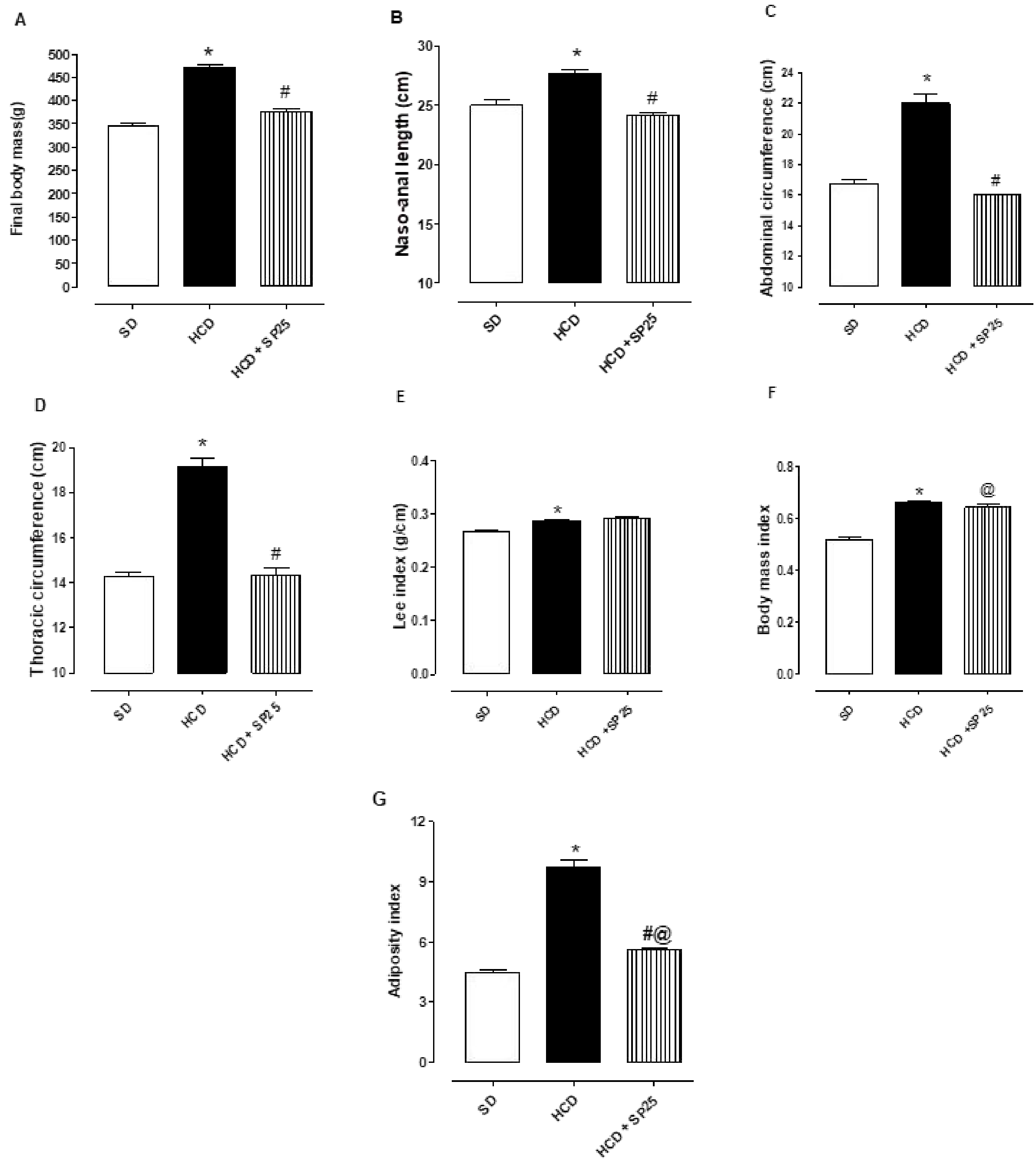


FIGURE 1: Values of final body mass (a), nasoanal length (b), waist circumference (c), chest circumference (d), Lee index (e), body mass index (f), and adiposity index (g) in rats from group DS, HCD, and HCD + SP25. Columns and vertical bars represent the mean and S.E.M., respectively (n = 5). ANOVA was one-way followed by Tukey's posttest. *p < 0.05 (SD vs. HCD); #p < 0.05 (HCD vs. HCD + SP25); @p < 0.05 (SD vs. HCD + SP25). SD: standard diet group supplemented with saline; HCD: hypercaloric diet group supplemented with saline; HCD + SP25: hypercaloric diet group and supplemented with *S. platensis* at a dose of 25 mg/kg.

4.2. Effect of Consumption of the Hypercaloric Diet and Food Supplementation with S. platensis on the Mass of Adipose Tissue Deposits. Analyzing the mass of adipose tissue deposits, it was possible to observe that the HCD group rats showed an increase in retroperitoneal (23.16 ± 1.0 g), epididymal (12.76 ± 0.4 g), and inguinal (10.4 ± 1.1 g) when compared to the SD group (6.05 ± 0.4 g, 4.94 ± 0.4 g, and 3.92 ± 0.3 g, respectively) (Figures 2(a)–2(c)). However, the group fed a hypercaloric diet and supplemented with 25 mg/kg *S. platensis* observed in the retroperitoneal adipose tissues (10.32 ± 0.7 g), epididymal (6.22 ± 0.3 g), and inguinal (13.76 ± 0.2 g) relative decrease in fat deposition when compared to the obese HCD group, with no significant difference in SD (Figures 2(a)–2(c)).

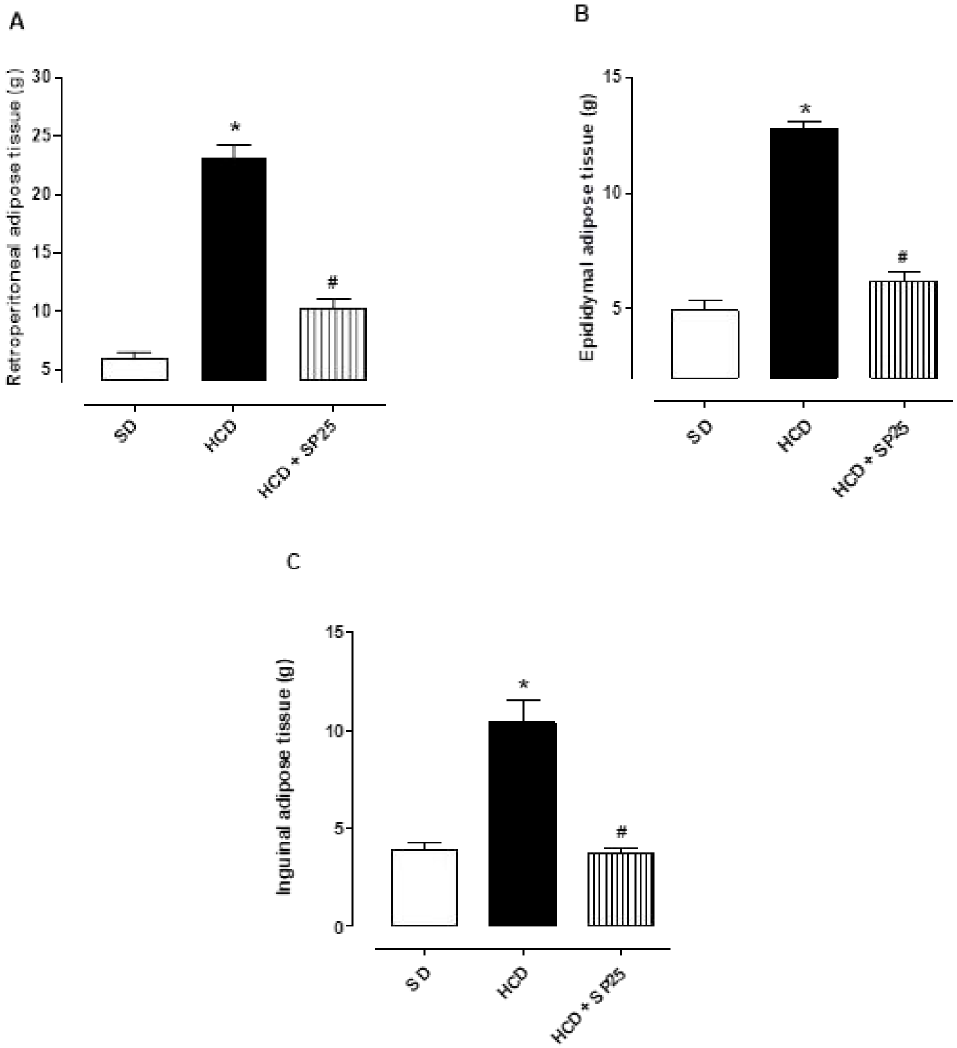


FIGURE 2: Values of the masses of adipose tissue deposits: retroperitoneal (a), epididymal (b), and inguinal (c) in rats from groups SD, HCD, and HCD + SP25. Columns and vertical bars represent the mean and S.E.M., respectively (n = 5). ANOVA was one-way followed by Tukey's posttest. *$p < 0.05$ (SD vs. HCD); #$p < 0.05$ (HCD vs. HCD + SP25); @$p < 0.05$ (SD vs. HCD + SP25), (n = 5). SD: standard diet group supplemented with saline; HCD: hypercaloric diet group supplemented with saline; HCD + SP25: hypercaloric diet group and supplemented with *S. platensis* at a dose of 25 mg/kg.

4.3. Effect of Consumption of the Hypercaloric Diet and Food Supplementation with S. platensis on the Relaxing Reactivity of Isolated Rat Ileum. In the HCD group, a decreased KCl contractile power was observed in the presence of verapamil (pCE_{50} = 6.09 ± 0.21) when compared to the group that consumed only the standard diet (pCE_{50} = 10.90 ± 0.52). In addition, it can be observed that there were no changes regarding the relaxing potency of verapamil between the

HCD + SP25 group (pCE_{50} = 10.55 ± 0.33) and SD; however, it is possible to verify an increase in power when compared to the HCD group (Figure 3).

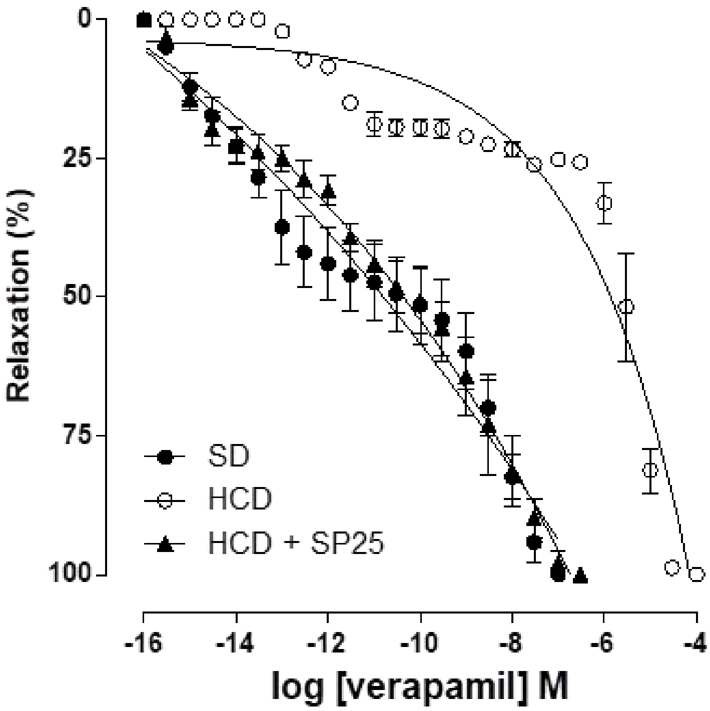


FIGURE 3: Cumulative concentration-response curve for verapamil in rat ileum in the SD (●), HCD (○), and HCD + SP25 (▲) groups. The symbols and vertical bars represent the mean and S.E.M., respectively (n = 5). ANOVA was one-way followed by Tukey's posttest. *p < 0.05 (SD vs. HCD), #p < 0.05 (HCD vs. HCD + SP25),and @p < 0.05 (SD vs. DHC + SP25). SD: group fed a standard diet; HCD: group fed a hypercaloric diet; HCD + SP25: groups fed a hypercaloric diet and supplemented with S. platensis 25 mg/kg, respectively.

4.4. Effect of Consumption of the Hypercaloric Diet and Food Supplementation with S. platensis on the Cumulative Curve of Contraction Induced by Carbachol in the Absence and Presence of Atropine in the Groups Fed the Standard Diet. In the presence of 10^{-6} M atropine, it was observed that the cumulative contraction curve for CCh shifted to the right with an increase in pCE_{50} (3.5 ± 0.2) when compared to the contraction curve in the absence of the antagonist (6.3 ± 0.04) without changing E_{max} (100%). Similar results were observed in the presence of 10^{-5} M atropine, in which the CCh contraction curve was shifted to the right, with an increase in pCE_{50} (2.3 ± 0.01) without changing E_{max} (100%).

4.5. Effect of consumption of the hypercaloric diet and food supplementation with S. platensis on the cumulative curve of contraction induced by carbachol in the absence and presence of atropine in the groups fed the hypercaloric diet. In the group fed during the eight weeks with the hypercaloric diet, a decrease in the maximum effect was observed (32.0 ± 2.9) when compared to the contraction curve of the group of animals that were fed a standard diet (6.3 ± 0.04) without changing the pCE_{50} (6.6 ± 0.01 and 6.3 ± 0.04 respectively) (Figure 4).

In the HCD group in the presence of 10^{-6} M atropine, it was observed that the cumulative contraction curve in CCh was shifted to the right with an increase in pCE_{50} (3.9 ± 0.08) when compared to the contraction curve in the absence of the antagonist in the group HCD (6.6 ± 0.01) with a change in E_{max} (32.0 ± 2.9 and 44.6 ± 2.0% respectively). Similar results were observed in the presence of 10^{-5} M atropine, in which the CCh contraction curve was shifted to the right, with an increase in pCE50 (2.9 ± 0.03) with an E_{max} change (30.2 ± 0.9%) (Figure 4).

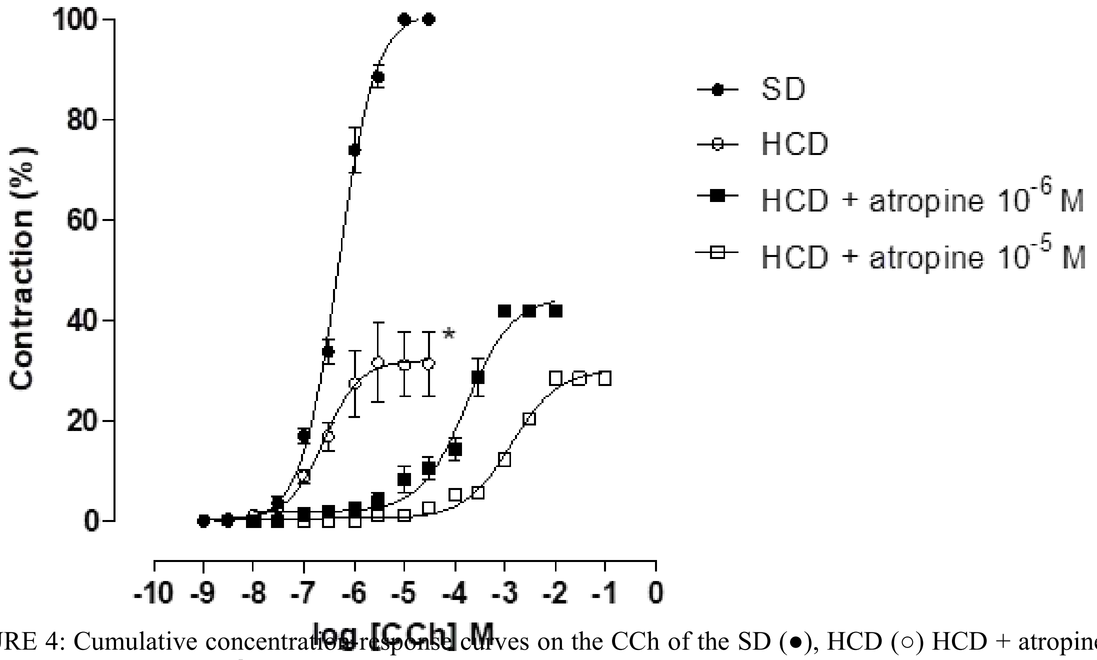


FIGURE 4: Cumulative concentration-response curves on the CCh of the SD (●), HCD (○) HCD + atropine 10^{-6} M (■), HCD + Atropine 10^{-5} M (□) groups in isolated rat ileum. The symbols and vertical bars represent the mean and S.E.M., respectively (n = 5). ANOVA was one-way followed by Tukey's post-test. *p < 0.05 (SD vs. HCD), #p < 0.05 (HCD vs. HCD + atropine 10^{-6} M and 10^{-5} M) and @p < 0,05 (SD vs. HCD + atropine 10^{-6} M and 10^{-5} M). SD = group fed a standard diet; HCD = group fed a hypercaloric diet; HCD + SP25 = groups fed a hypercaloric diet and supplemented with *S. platensis* 25 mg/kg, respectively.

4.6. Effect of consumption of the hypercaloric diet and food supplementation with S. platensis on the cumulative curve of contraction induced by carbachol in the absence and presence of atropine in the groups fed a hypercaloric diet and supplemented simultaneously with S. platensis at a dose of 25 mg/kg. In the group submitted to a hypercaloric diet and supplemented with *Spirulina platensis* at a dose of 25 mg/kg (HCD + SP 25) in the presenceof 10^{-6} M atropine, it was observed that the cumulative contraction curve shifted to the right with decreased potency (pCE_{50} = 3.4 ± 0.4) when compared to the contraction curve in the absence of the antagonist in the SD group (pCE_{50} = 6.3 ± 0.04) with a change in (E_{max} = 59.89 ± 1.2 and 100% respectively), similar results occurred when compared to the contraction curve in the absence of the antagonist in the HCD group (pCE_{50} = 6.6 ± 0.1) with change in the maximum effect (E_{max} = 59.89 ± 1.2 and 32.0 ± 2.9% respectively) and when compared to the contraction curve in the absence of the antagonist in the HCD + SP 25 group (pCE_{50} = 6.2 ± 0.1) with change in the maximum effect (E_{max} = 59.89 ± 1.2 and 63.87 ± 1.0% respectively) (Figure 5).

Similar results were observed in the group submitted to a hypercaloric diet and supplemented with *Spirulina platensis* at a dose of 25 mg/kg (HCD + SP 25) in the presence of 10^{-5} M atropine, in which the CCh contraction curve was shifted to right with decreased contractile force (pCE_{50} = 2.8 ± 0.3) with a change in the maximum effect (E_{max} = 47.93 ± 4.8%) (Figure 5).

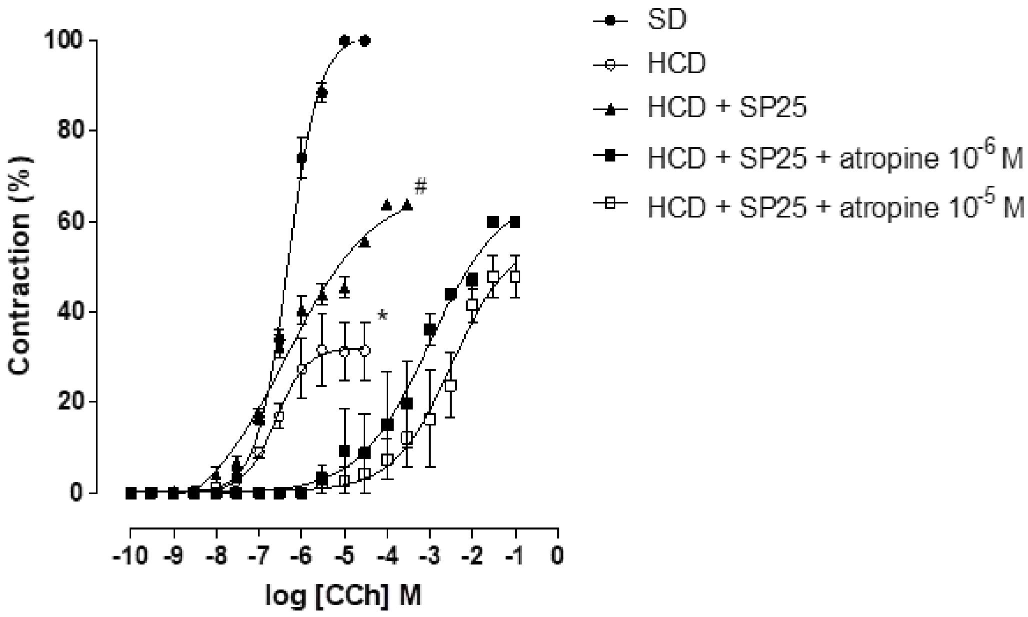


FIGURE 5: Cumulative concentration-response curves on the CCh of the SD (●), HCD (○), HCD + SP25, (▲), HCD + SP25 + atropine 10^{-6} M (■), HCD + SP25 + atropine 10^{-5} M (□) groups in isolated rat ileum. The symbols and vertical bars represent the mean and S.E.M., respectively (n = 5). ANOVA was one-way followed by Tukey's post-test. *p < 0.05 (SD vs. HCD), $^{\#}$p < 0.05 (HCD vs. HCD + SP25) and $^{@}$p < 0.05 (HCD + SP25 vs. HCD + SP25 + atropine 10^{-6} M and 10^{-5} M). SD = group fed a standard diet; HCD = group fed a hypercaloric diet; HCD + SP25 = groups fed a hypercaloric diet and supplemented with *S. platensis* 25 mg/kg, respectively.

4.7. Effect of hypercaloric diet and Spirulina platensis supplementation on histology and ileal morphometry of rats. In animals in the SD group, it is observed that the mucosa has some mononuclear inflammatory cells, such as macrophages and few plasmocytes, with preservation of goblet cells and enterocytes (Figures 6(a) and 6(d)). In animals in the HCD group, it is observed that the inflammatory infiltrate in the mucosa is extensive and multifocal (***), rich in mononuclear cells, such as macrophages and many plasmocytes (Figures 6(b) and 6(e)). In animals from the HCD-SP25 group, a slight increase in the number of mononuclear cells (*), with the same phenotype, macrophages and lymphocytes, is observedin the mucosa (Figures 6(c) and 6(f)).

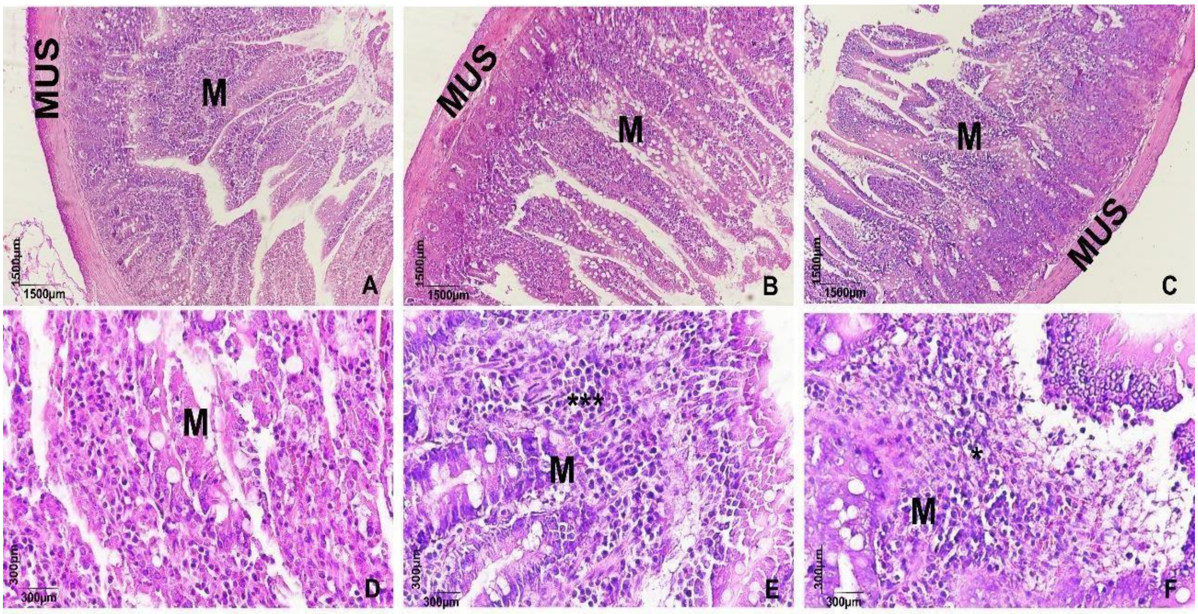


FIGURE 6: Histological section of rat ileum stained in hematoxylin and eosin showing parts of the organ, highlighting the mucosa (M) and the external muscle (MUS). Panoramic view (A, B and C) and higher magnification (D, E and F) showing histological images of the ileum of rats from SD, HCD and HCD + SP25 groups, respectively.

In the ileal morphometric analysis of the mucosal area, it was observed that the animals in the HCD group (149.9 ± 1.6) had a decrease in the length of the intestinal villi when compared to the SD control group (166.3 ± 0.8). The HCD+SP25 group (164.4 ± 0.8) showed no difference compared to the SD group (Figure 7(a)). Similarly, when analyzing the width ofthe intestinal villi of the ileum of rats in the HCD group (125.6 ± 1.2), a decrease was observed in relation to the SD group (145 ± 1.0). The group supplemented with *Spirulina* at a dose of 25 mg/kg (143.0 ± 2.0) also showed no difference when compared to the control group (145.0 ± 1.0) (Figure 7(b)).

Likewise, in the morphometric analysis of the outer muscle layer, it was observed that the group fed the high-calorie diet HCD (81.7 ± 0.9) presented a decrease in the muscle layer compared to the SD group (98.5 ± 0.4). Interestingly, the ileal muscle layer of the HCD+SP25 group (90.5 ± 1.5) presented differences in relation to both the HCD (81.7 ± 0.9) and SD (98.5 ± 0.4) groups (Figure 7(c)).

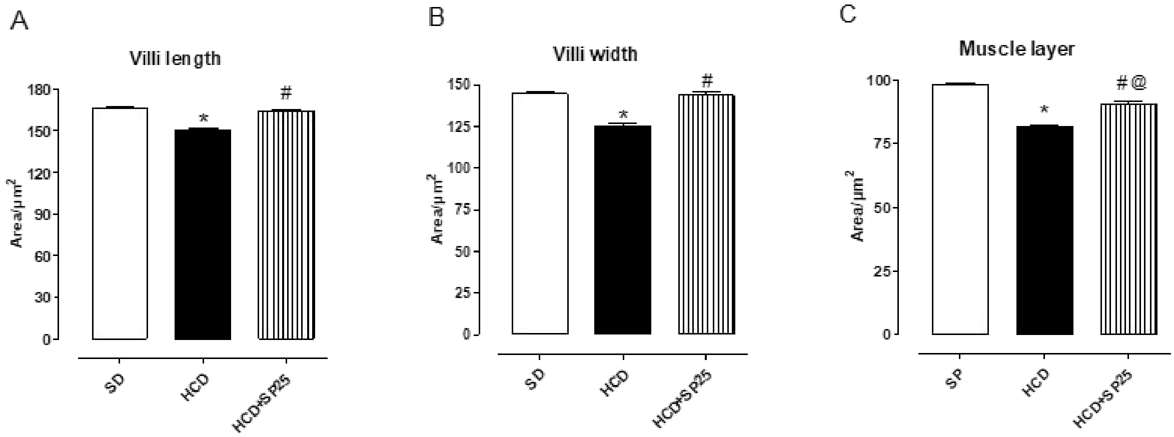


FIGURE 7: Ileum morphometry showing villi length (A), villi width (B) and muscle layer (C) of groups the SD, HCD and HCD + SP25. Columns and vertical bars represent the mean and S.E.M., respectively (n = 5). ANOVA was one-way followed by Tukey's post-test. *$p < 0.05$ (SD vs. HCD), #$p < 0.05$ (HCD vs. HCD + SP25) and @p < 0,05 (HCD + SP25 vs. HCD + SP25. SD = group fed a standard diet; HCD = group fed a hypercaloric diet; HCD + SP25 = groups fed a hypercaloric diet and supplemented with *S. platensis* 25 mg/kg, respectively.

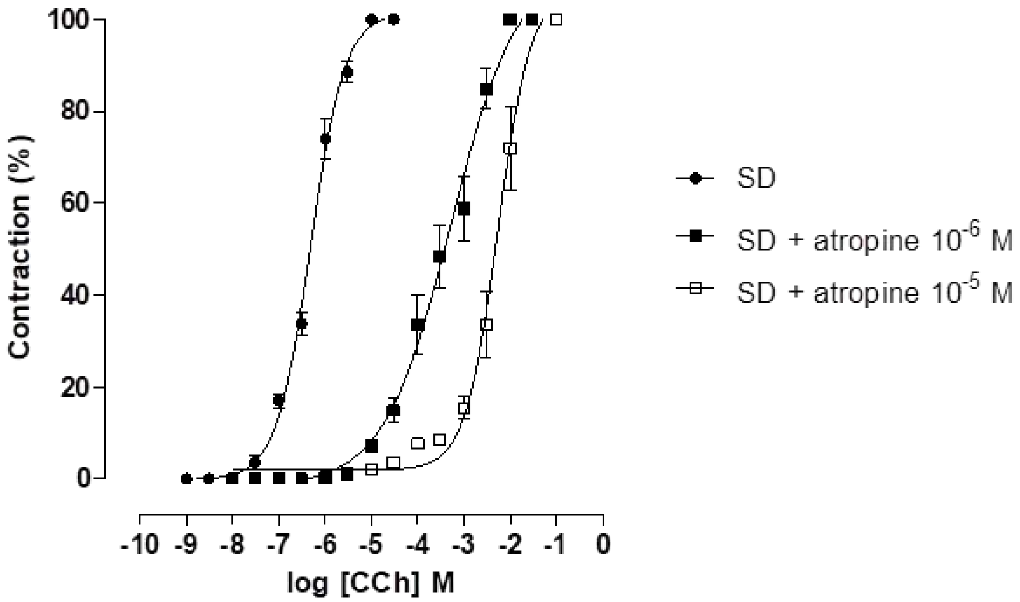


FIGURE 8: Cumulative concentration-response curves for the CCh of the SD (●), SD + atropine 10^{-6} M (■), SD + atropine 10^{-5} M (□) groups in isolated rat ileum. The symbols and vertical bars represent the mean and S.E.M., respectively (n = 5). ANOVA was one-way followed by Tukey's post- test. *$p < 0.05$ (SD vs. HCD), #$p < 0.05$ (HCD vs. HCD + SP25) and @p < 0,05 (SD vs. HCD + SP25). SD = group fed a standard diet;HCD = group fed a hypercaloric diet; HCD + SP25 = groups fed a hypercaloric diet and supplemented with *S. platensis* 25 mg/kg, respectively.

5. Discussion

In the present study, the experimental obesity model was induced by consuming a hypercaloric diet in Wistar rats for 8 weeks, which resulted in increased final body mass, murinometric parameters, and body adiposity index, as well as promoting a reduction in relaxing and ileal contractile reactivity. Interestingly, the deleterious effects promoted by the consumption of the hypercaloric diet were prevented by supplementing food with *S. platensis*.

Obesity for being able to cause comorbidities in individuals is already considered a serious public health problem [46]. Thus, to better understand the effects and mechanisms that the disease brings to, the individual, hypercaloric diets are used to induce a state of experimental obesity in rats, mimicking the consequences that they would bring to humans [47]. Wistar rats were fed a standard and/or hypercaloric diet for eight weeks, and simultaneously received saline solution and/or supplementation with *S. platensis* at a dose of 25 mg/kg/day, based on the principle that in previous studies, carried out by our research group, this was the dose that had the best effects [48].

When analyzing the data related to experimental obesity, it is possible to observe that the rats fed with the hypercaloric diet had an increase in the final body mass and that the supplementation with *Spirulina platensis* at a dose of 25 mg/kg totally prevented this increase, not having a significant difference when compared to the control group fed a standard diet (Figure 1(a)). Such results suggest that the consumption of the hypercaloric diet was effective in increasing the final body mass, consequently favoring the onset of obesity in these animals. In addition, the protective effect attributed to kelp can be related to its composition and properties, characterized by a high content of proteins (approximately 70%), bioactive compounds, and antioxidants, which contribute to the thermogenesis process and decrease lipogenesis, preventing therefore, the accumulation of lipid and the deposition of fat from consumption of the hypercaloric diet [49, 50].

To verify the effectiveness of the hypercaloric diet in the development of obesity as well as the possible protective role of supplementation with *S. platensis*, parameters related to experimental obesity were evaluated (Lee index, BMI, adiposity, nasoanal length, measured abdominal and thoracic circumference and mass of fat reserves). From these results, it is possible to infer the evidence of the onset of obesity from the hypercaloric diet used in this study. Additionally, dietary supplementation with algae at a dose of 25 mg/kg was effective in partially and/or totally preventing some of the analyzed parameters (Figures 1(b)-1(d) and 1(g)), thus reaffirming its anti-obesity effects. However, no differences were observed between the Lee index and the BMI of the experimental groups (Figures 1(e) and 1(f)), similar to that observed in different studies with animal obesity induced by excessive intake of sucrose (300 g/L) or lipids (42.9% lipids per kcal) [51, 52].

In animals, mainly rats, fat deposition occurs predominantly in three distinct regions, these reserves are located in the retroperitoneal, epididymal, and inguinal adipose tissues. The assessment of the mass of these regions helps to determine the physiological dysfunctions resulting from the ingestion of hyperlipidic and/or hypercaloric diets [53-55]. Thus, it was observed that the obese rats showed an increase in adipose, retroperitoneal (Figure 2(a)), epididymal (Figure 2(b)), and inguinal (Figure 2(c)) deposits and that food supplementation with *S. platensis* was able to prevent this increase in all of them. Furthermore, based on these results, a possible modulating activity of *S. platensis* in preventing and reducing adipose deposits of rats fed a hypercaloric diet is demonstrated.

Once the development of obesity and the physiological changes caused by the consumption of the hypercaloric diet were confirmed, and that supplementation with *S.platensis* prevented such dysfunction, the investigation of the impact of diet and supplementation with algae on relaxing reactivity was continued intestinal, since the increase in body adiposity is associated with decreased relaxing reactivity in ileum of Wistar rats, and food supplementation with *S. platensis* at a dose of 50 mg/kg was able to reverse these changes [27].

In this study, it was demonstrated that the hypercaloric diet decreased the ileal relaxing reactivity, confirmed by the decrease in the relaxing efficacy of verapamil (Figure 8), making this relaxation difficult. In addition, supplementation with *S. platensis* completely prevented the decrease in both the potency and the relaxing effectiveness of the electromechanical component, bringing them back to what was observed in the control group, confirmed by the overlapping curves. Likewise, the hypercaloric diet reduced the contractile effectiveness of KCl, proving that it reduces the contractions caused by electromechanical coupling [33]. In contrast, Ferreira [27] did not observe any difference between animals fed a standard diet supplemented with *S. platensis* in relation to the obese group, and it is not possible to observe a reversal of such effects. However, our study demonstrated that food supplementation with *S.platensis* at a dose of 25 mg/kg prevented changes caused by the high calorie diet, thus inferring that algae may be a possible alternative for the prevention of intestinal diseases in obese people.

Studies have shown that obesity increases vasoconstriction in the aorta of obese rats, in which possibly the excessive accumulation of adipose tissue is related to increasing the expression of proteins in the calcium sensitization pathway [56] and therefore decreasing the potency of ileal relaxants, and it may be suggested that this effect is associated with a decrease in the expression of voltage-gated calcium channels (Ca_V) [57, 58]. Consequently, *S. platensis* may be preventing the decrease in calcium influx, improving changes in intestinal reactivity. Thus, to validate this hypothesis, there is a need for investigative studies on the participation of these channels in the ileum of obese rats supplemented with *S. platensis*.

Once all these deleterious damages resulting from the consumption of the hypercaloric diet were verified, and as in previous studies, it was observed that the consumption of this diet is directly related to the increase in contractile reactivity and reduction in the relaxing response of various organs in rats [24, 59, 60], it was decided to investigate the mechanism of action by which the ingestion of the hypercaloric diet promotes a reduction in the contractile reactivity of the ileum [48], effects that were prevented in rats fed the hypercaloric diet by supplemental feeding with *S. platensis*.

The intestinal contractile reactivity was evaluated using as a premise the property that the intestinal smooth muscle has to undergo actions mediated by acetylcholine (ACh), which when it binds to its muscarinic receptor type M_3, highly expressed in this musculature, triggers contractile responses [61]. Thus, to mimic the pharmacological action of this hormone, carbachol, an agonist of ACh muscarinic receptors, resistant to degradation by acetylcholinesterase, was used to assess the pharmacomechanical coupling of ileum contraction in Wistar rats [62].

Recently, Souza et al. [33] found that Wistar rats fed for 8 weeks on a hypercaloric diet showed reduced intestinal contractile efficacy compared to KCl and CCh. Thus, once the onset of obesity was confirmed and a reduction in ileal contractile reactivity was verified, the hypothesis was raised that the consumption of the hypercaloric diet could decrease the contractile response to CCh by negatively modulating the muscarinic receptors present in the intestinal smooth muscle and that by its antioxidant role, supplementation could prevent this modulation by possibly acting on calcium influx.

For this, in the group fed a standard diet, atropine (10^{-6} and 10^{-5} M), an antagonist of muscarinic receptors, was incubated, showing that in both concentrations the cumulative contraction curve to CCh was shifted to the right with decreased force and no change in the maximum effect (Figure 8). These results were already expected, since atropine is a competitive antagonist of muscarinic receptors, requiring increasing concentrations of carbachol to displace it from the M_3 receptor in the ileum to obtain the same contractile effect [63]. However, this protocol is of fundamental importance for the analysis and comparison of the effects and potencies of the maximum amplitudes of the HCD and HCD + SP25 mg/kg groups with those of the control group, fed only with a standard diet. It is also possible to observe that the presence of atropine in both concentrations continued to shift the contraction curve to the right without changing the maximum effect, suggesting, therefore, that obesity and/or the hypercaloric diet did not alter the expression of muscarinic receptors, per se.

In this study, it was demonstrated that the consumption of a hypercaloric diet significantly decreased the ileal contractile response to CCh. In the HCD group in the presence of 10^{-6} and 10^{-5} M atropine, it was observed that the cumulative contraction curves of the CCh shifted to the right with decreased contractile force, with a change in the maximum effect (Figure 4). This decrease in ileal contractile reactivity does not involve the participation of muscarinic receptors, possibly possibly being associated with modulation of the Ca_V and/or related to mechanisms involving the antioxidant system, mainly due to the increased expression of malondialdehyde (MDA), the main product of lipid peroxidation, corroborating the findings of previous studies by our research group [27, 33].

Previous studies carried out by our research group showed that food supplementation with the alga *S. platensis* was able to improve contractile reactivity in the aorta, avoiding oxidative stress [23], restoring erectile function in obese rats [24], as well as is related to increased contractile reactivity in the ileum of rats fed a hypercaloric diet [25] by promoting increased bioavailability of nitric oxide.

Additionally, excessive consumption of fats and calories are associated with metabolic and gastrintestinal disorders. Furthermore, animal research has shown that long-term consumption of high-calorie diets is particularly related to damage to the gastrintestinalmucosa [64, 65]. Thus, intestinal histological and morphometric parameters are very important and used as scientific tools to evidence such findings and particularly the effects of the hypercaloric diet as well as *Spirulina platensis* on the histomorphology of the ileum ofrats have not yet been elucidated.

The intestinal mucosal barrier plays essential roles in preserving intestinal health [66]. Among the components of the intestinal epithelial mucus layer is mucin, synthesized and secreted by goblet cells, this mucus layer is responsible for separating part of the intestinal content from the intestinal epithelial cells, protecting the cells against the invasion of harmful substances and has an active role in regulating mucosal immunity [67-69]. Our results showed that the consumption of the hypercaloric diet considerably reduced the number of goblet cells and enterocytes (Figures 6(b) and 6(e)) important for digestion and absorption of nutrients from the diet, which is associated with changes in the intestinal permeability of the intestinal epithelial cell barrier, inducing oxidative stress and apoptosis. Furthermore, the high-calorie diet considerably increased the inflammatory profile of the ileum (Figures 6(b) and 6(e)) observed in the images due to the rich presence of mononuclear cells, specifically macrophages and plasma cells, characteristic of the body's adipose tissue accumulation process (D7, D8). Morphometric analyzes of the intestinal mucosa of the ileum confirm the histological results, since it is possible to observe that the length (Figure 7(a)) and width (Figure 7(b)) of the ileum intestinal villi as well as the muscle layer (Figure 7(c)) presented a decrease in relation to the control group.

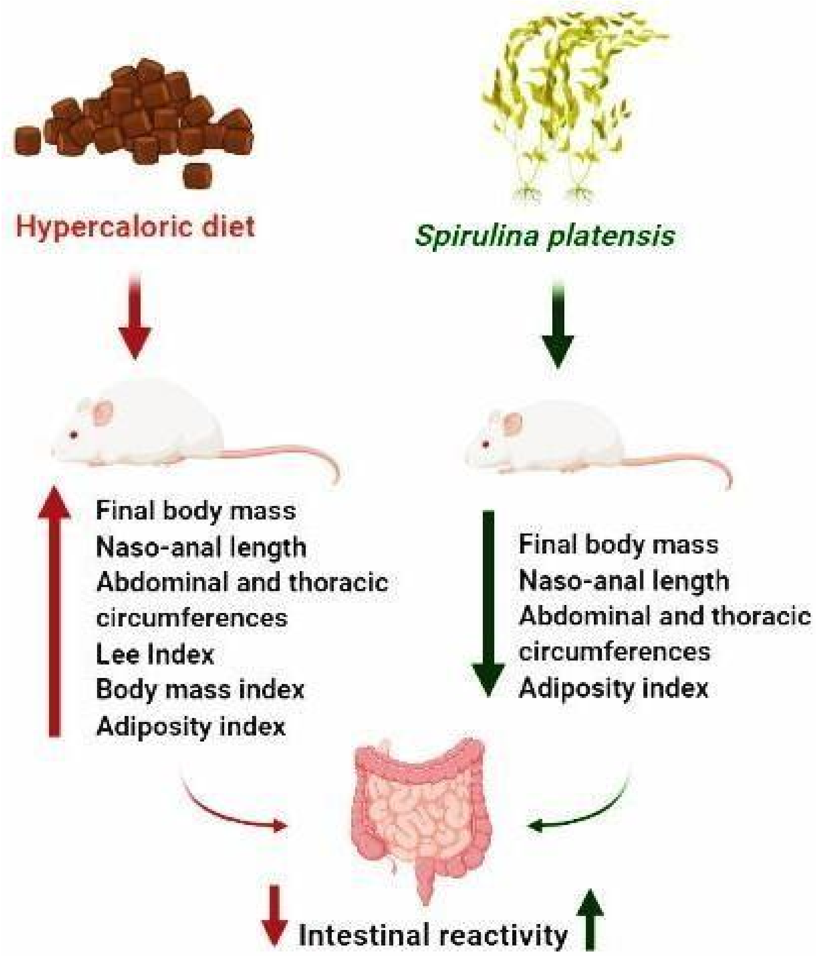
On the other hand, *Spirulina platensis* was able to prevent the harmful effects on the ileum mucous layer caused by the consumption of the high-calorie diet, approaching the histological findings of the control group, characterized by a slight increase in the number of mononuclear cells of the same phenotype, number of reduced macrophages and lymphocytes (Figures 6(c) and 6(f)). This effect may be associated with the nutritional composition of the alga, composed of high levels of antioxidants, carotenoids, phycocyanins and vitamins, in addition to the pharmacological and biological activities already evidenced, which are related to gastropopotetic activities, anti-inflammatory and antioxidant effects, in addition to increased antioxidant capacity of various organs (17, 68-70), which was confirmed by the morphometric analysis of the ileal mucosa in which all parameters evaluated were preserved (Figure 7) and did not distinguish from the healthy control group shown in Figure 7.

In view of these results, it is possible to evidence the effectiveness of the experimental obesity model in promoting damage on the intestinal reactivity of the ileum of Wistar rats, and that the supplementation with *Spirulina platensis* at a dose of 25 mg/kg was potentially capable of preventing the deleterious effects induced by the consumption of hypercaloric diet during the 8-weeks period. In addition, this research demonstrated the beneficial potential of dietary supplementation with *S. platensis* in obesity, as well as in the prevention of intestinal disorders associated with reactivity of intestinal smooth muscle, such as diarrhea, constipation, and poor digestion, which may be associated with improvement and prevention of intestinal dysbiosis, very associated with obesity and intake of irregular diets. In view of the popularity of *Spirulina platensis* consumption as a functional food, and its safety already proven, the results of this research and previous works in the literature justify the execution of clinical trials to investigate the possible protective role of this blue-green algae in diseases and intestinal disorders associated with obesity and other inflammatory pathologies.

6. Conclusion

Food supplementation with the algae *Spirulina platensis*, the research star of this study, points to a possible preventive anti-obesity role and a protector of deleterious effects and preserving the intestinal histomorphological environment on intestinal reactivity (Figure 9). In addition, the hypercaloric diet decreases contractile reactivity in rat ileum by mechanisms that are related to a downregulation of muscarinic receptors, and dietary supplementation with algae prevents the reduction of intestinal reactivity induced by the consumption of the hypercaloric diet, possibly by modulating the expression positively of the Ca_V. These data suggest the beneficial effects of algae as a potential therapeutic arsenal in the prevention of obesity and associated intestinal diseases, diarrhea, irritable bowel disease, and some types of gastrintestinal cancer.

FIGURE 9: The consumption of the hypercaloric diet induces the onset of obesity in rats by increasing all



parameters evaluated in this study and consequently leads to a decrease in ileal reactivity. Food supplementation with *Spirulina platensis* prevents the development of obesity and the reduction of contractile reactives and intestinal relaxants.

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Data Availability Statement

The hypothesis and review data used to support the findings of this study are included within the article.

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Conflict of Interest

The authors declare no conflict of interest.

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5.2 Capítulo II

Artigo 2

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Arthrospira platensis prevents oxidative stress and suppresses IL-1β expression in the ileum of rats fed a hypercaloric diet

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ABSTRACT

Obesity is characterized by an energy imbalance caused by caloric intake and expenditure, being a risk factor associated with a wide range of pathophysiological conditions including gastrintestinal diseases. In recent years, it has been shown that overexpression of oxidative stress and pro-inflammatory cytokines is a mechanistic link between obesity and cellular functions in animals and humans. Some natural products are reported to be effective in counteracting the adverse effects of oxidative stress. Thus, *Arthorpira platensis (AP)* stands out, an alga with anti-inflammatory and antioxidant activities. Thus, the objective is to evaluate the preventive effects of AP supplementation on oxidative stress and interleukin-1β (IL-1β) levels in the ileum of rats fed a hypercaloric diet. The rats were divided into a group fed a standard diet (SD), a hypercaloric diet (HCD) and/or fed a hypercaloric diet andsupplemented simultaneously with AP 25 mg/Kg (HCD + AP25), after 8 weeks of treatment the ileum was collected for the analyses. It was observed that in the DHC group there was underproduction of antioxidant enzymes as well as overexpression of oxidative stress in the ileum of rats, interestingly this damage was prevented by the alga. Furthermore, the DHC group showed high levels of IL-1β (940.6 ± 34.5) such an increase was prevented in the HCD + AP25 group (597.2 ± 33.3). It is evident, therefore, that AP prevents the increase in oxidative stress and the inflammatory profile in the ileum of obese rats, making it a promisingtherapeutic alternative in the treatment of inflammatory bowel diseases aggravated by obesity.

Keywords: Obesity; *Arthrospira platensis*; Oxidative stress; Gut microbiota.

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1. Introduction

Obesity is a disease characterized by the abnormal accumulation of fat, which brings several health damages (Kopelman, 2000; Iantorno et al., 2014). Excessive increase in body adipose tissue favors the chronic inflammatory state and oxidative stress that results in metabolic dysfunctions and cellular damage (Lopategi et al., 2016; Gil-Cardoso et al., 2017; Bonfim et al., 2020). This characteristic makes obesity a crucial point for the development and worsening of comorbidities such as diabetes, cardiovascular and renal diseases, dyslipidemias, hepatic steatosis, neurological damage and compromised intestinal health (Gil-Cardoso et al., 2017; Kovesdy et al., 2017; O'Brien et al., 2017; Gasparin et al., 2018; Vekic et al., 2019). Fig. 1.

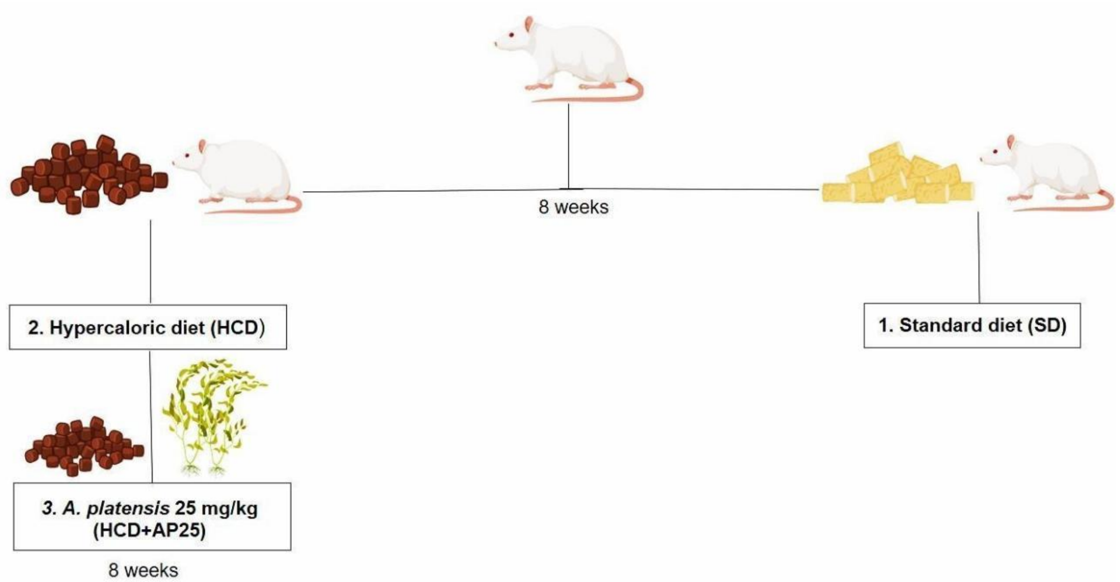


Fig. 1. Design of experimental groups. Rats were divided into three experimental groups: 1. Fed for 8 weeks with standard diet only (SD); 2. Fed for 8 weeks only on the hypercaloric diet (HCD) and 3. Fed on the hypercaloric diet and supplemented ously with *Arthrospira platensis* at a dose of 25 mg/kg (HCD + AP25), this group received both the diet and supplementation together for 8 weeks.

Among the main factors associated with the etiology of the obesity epidemic, genetic and behavioral factors stand out, especially those related to the individual's lifestyle, including sedentary lifestyle and inadequate eating habits that result in an imbalance in the energy homeostasis of adipose tissue. tissue (Frota et al., 2015; Kadouh et al., 2017; Mundo, 2021). In addition, it has been shown that environmental factors also contribute to determining body weight and obesity maintenance, among these factors changes in bacterial phyla, have an impactful role in the development of obesity (Abenavoli et al., 2019; Castaner; Goday, 2018; Kobyliak; Virchenko; Falalyeyeva, 2016). Adipose tissue in turn, in addition to fat storage, represents the main function, from the production of numerous regulatory proteins, called adipokines, such as leptin, adiponectin and interleukins (IL) in circulation (Hotamisligil et al., 1995; Dianarello, 1996; Bruun et al., 2002; Febbraio, 2014; Rodríguez, et al., 2015). However, in obesity this cross-talk is disturbed, resulting in increased secretion of pro-inflammatory cytokines and macrophages from adipose tissue. This process leads to the infiltration of immune cells and induces the increase of the inflammatory response mainly through the interleukin-1 (IL-1) system that includes one of the most powerful inflammatory molecules, as well as the excessive production of reactive oxygen species (ROS) (Kintscher et al., 2008; Sell and Eckel, 2012; Sokhanvar et al., 2011; Piya et al., 2013; Ballak et al., 2015).

In view of these facts, in recent years there has been an increase in the growing search for therapeutic alternatives that aim to prevent or control diseases associated with obesity, mainly for natural compounds that can be used in food diets that improve growth performance, increase immunity, reduce oxidative stress and improve intestinal histology and digestibility in humans and animals (Ibrahim et al., 2020; Amer et al., 2021). In this sense, studies have focused on the potential nutritional and antioxidant value of natural foods of mineral, animal and plant origin, such as algae and microalgae.

Arthrospira platensis (Cyanobacteria) (AP) is a blue-green filamentous, planktonic photosynthetic algae used as a source of active biomolecules, such as amino acids, vitamins, carotenoids, minerals, phenolic compounds and phycobiliprotein pigments 70% of protein (Mariey; Samak; Ibrahem, 2012; Yang et al., 2020; Freitas et al., 2021; Omar et al., 2022). It has been widely used as a functional resource and in food supplementation in humans and animals (Kay, 1991; Lupatini et al., 2017; Yuhong Yang, Du, Hosokawa; Miyashita, 2020; Nakata et al., 2021). AP is also composed of a high content of antioxidant compounds which makes it an important supplement in the reduction of blood triglycerides and cholesterol, oxidative stress and inflammation, mainly through the elimination of free radicals and unstable molecules and ROS inhibition (Maddaly, 2010; Bolonho et al., 2014; Kumar, Agam et al., 2022). In addition, potential *A. platesis* plays an important role in intestinal tissue homeostasis, stimulating the growth of probiotics that facilitate digestion and protect the intestinal mucosa against toxic and pathogenic substances that can alter the intestinal microbiota (Simpore et al., 2006; Asmaz; Nilay, 2022; Seyidoglu; Aydin, 2020).

In a previous study carried out by our research group, it was evidenced that the consumption of *A. platensis* prevents obesity and the deleterious effects on intestinal reactivity induced by a hypercaloric diet (Diniz et al., 2021) this context, there is a general paucity of research investigating the actions of AP in the management of obesity, associated with diet, inflammatory diseases, oxidative stress and underlying mechanisms in the intestinal tract. Furthermore, it is known that the effects of dietary supplementation with AP have never been investigated on inflammation and oxidative stress of the ileum induced by a hypercaloric diet. Therefore, this study examined the effects of *A. platensis* supplementation on reducing IL-1β expression and preventing oxidative stress in a murine model of experimental obesity induced by a hypercaloric diet.

2. Materials and methods

2.1. Test product

Arthrospira platensis used in this study was purchased from the INFINITY Pharma laboratory (HONG KONG, China) (Lot N◦. 20130320). In powder form. A sample was analyzed, fractionated and certified by the Roval Manipulation Pharmacy (João Pessoa, Paraíba, Brazil) (Lot N◦. 405894).

The *A. platensis* powder was dissolved in saline solution (NaCl 0.9%) at a dose of 25 mg/kg. Food supplementation was performed for 8 weeks simultaneously with the hypercaloric diet (Diniz et al., 2021). Oral administration was daily between 12 and 2 pm, using stainless steel gavage needles (BD-12, Insight, Ribeirão Preto, SP) and 5 mL syringes with a precision of 0.2 mL (BD, HIGILAB, João Pessoa, PB).

2.2. Animals

To carry out the experimental models, 8-week-old Wistar rats (*Rattus norvegicus*) were used, weighing approximately 170–190 g, from the Animal Production Unit (UPA) of the Institute for Research in Drugs and Medicines (IPeFarM)/UFPB. The animals were kept under food control with a diet based on pellets (Presence®), with access to water ad libitum, ventilation, humidity and temperature (21 ± 1 °C) controlled and constant, submitted daily to a light cycle 12 h dark.

The animals were fasted for 12 h before the start of the experimental protocols. The animals were euthanized by a complementary method after prior anesthesia in accordance with Resolution n◦. 1000/2012 of the Federal Council of Veterinary Medicine with the recommendations of the American Veterinary Medicine Association (Avma, 2013). All experimental protocols followed the international principles for the study with laboratory animals (Zimmernam, 1983) and were approved by the Ethics Committee in the Use of Animals of the UFPB under registration n◦ 2352101019.

2.3. Experimental groups

Rats were randomly divided into 3 experimental groups: fed a standard diet and received saline solution (NaCl 0.9%) (SD); fed with a hypercaloric diet and receiving saline solution (NaCl 0.9%) (HCD) and those fed with a hypercaloric diet and supplemented simultaneously with *A. platensis* at a dose of 25 mg/kg (HCD + AP25). Each experimental group was fed and/or supplemented for a period of 8 weeks (El-Desoky et al., 2013; Diniz et al., 2021).

2.4. Diets

The study consisted of two types of diets that were fed to the rats for 8 weeksaccording to their specific group. The first diet was a balanced diet based on pellets (Presence®) containing 23% protein, 63% carbohydrates and 4% lipids by weight, totaling the total energy value (TEV) of 380 kcal/100 g and was fed to the rats of the SD group. The second diet was used to induce obesity in the rats and consisted of a mixture of standard pelletfood (Presence®), milk chocolate, fresh roasted peanuts and cornstarch in a 3:2:2:1 ratio, respectively, presenting a TEV of 417 kcal/100 g (Table 1) (Souza et al., 2017). To prepare the hypercaloric diet, the food, peanuts and cookies were ground and mixed, the chocolate was melted in a bain-marie and added to the mixture until a homogeneous mass was formed, and then dried in an oven (70 °C) for 24 h. and stored at room temperature. The diet was prepared weekly and offered to the rats in the form of pellets (Estadella et al., 2004).

Table 1. Centesimal composition of the hypercaloric diet.

Parameters	Mean ± e.p.m.
Moisture (%)	10.5 ± 0.003
Ashes (%)	4.6 ± 0.05
Carbohydrates (%)	45.5 ± 0.02
Proteins (%)	22.8 ± 0.01
Lipids (%)	16.0 ± 0.02
VET (kcal/100 g)	417.0 ± 0.001

TEV = total energy value.

2.5. Preparation and administration of A. Platensis

The *A. platensis* powder was dissolved daily in saline solution (NaCl 0.9%) for the preparation of a dose of 25 mg/kg, being administered to the rats at the end of the preparation. Seaweed supplementation was performed for a period of 8 weeks only for the HCD + AP25 group (adapted from Juárez-Oropeza et al., 2009; El-Desoky et al., 2013; Diniz et al., 2021). All groups started saline and/or *A. platensis* on the same day as the beginning of the diets and continued until the day of euthanasia. The administrations (saline and *A. platensis*) were performed orally between 12 pm and 2 pm with the aid of stainless steel needles for gastric gavage (Diniz et al., 2021).

2.6. Antioxidant activity and oxidative stress parameters

2.6.1. Determination of non-protein sulfhydryl group (GSH) levels

GSH levels were determined according to the protocol of Faure and Lafond (1995). Through the reaction with 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) the ileum samples were suspended in 0.02 M EDTA 1:10 (v/ v) and perforated with scissors for 15 sec on a plate in contact with ice. The resulting suspension was homogenized for 2 min. Then, 400 μL of the homogenate were removed and 320 μL of distilled water and 80 μL of 50% trichloroacetic acid were added, which were centrifuged at 3000 rpm at 4 °C for 15 min. After this period, 100 μL of the resulting supernatant was pipetted into a 96-well microplate, and 200 μL of Trisand 25 μL of DTNB were added. This microplate was incubated at room temperature and after 15 min the reading was performed in a spectrophotometer at a wavelength of 412 nm. The calibration curve was made with reduced L-glutathione. The GSH values of the samples were calculated by interpolating the values with the standard curve and expressed in mg NPHS (non- protein thiol groups)/g tissue.

2.6.2. Determination of the antioxidant activity of superoxide dismutase (SOD)

The amount of SOD enzyme was measured through the ability to inhibit the photochemical reduction of nitro-tetrazolium blue (NBT). Photochemically reduced riboflavin generates O_2^- which reduces NBT and produces formazan which absorbs at the wavelength of 546–630 nm. In the presence of SOD, the reduction of NBT is inhibited. Results were expressed in units of enzyme per gram of protein. For this, the ileum samples were homogenized in phosphate buffer (0.4 M, pH 7.0) and centrifuged for 15 min at 10,000 rpm at 4 °C. The supernatant was removed and used in the assay. The plates containing the reaction medium (10 mM phosphate buffer), L-methionine (1.79 mg/mL, pH 7.8), riboflavin (0.2 mg/mL, pH 7.8), NBT (1, 5 mg/mL, pH 7.8) and 10 µL of the sample supernatant were exposed to a fluorescent lamp (15 W) for 10 min. After this period, the material was taken to the 630 nm spectrophotometer.

2.6.3. Determination of the total antioxidant capacity (TAC)

The quantification of the total antioxidant capacity was based on the DPPH method described by Brand-Williams et al. (1995). In centrifuge tubes, protected from light, 50 µL of the ileum homogenate and 2 mL of the DPPH solution dissolved in absolute ethanol (0.012 g/L) were added; then, the tubes were vortexed for 10 sec and kept at rest for 30 min. Then, the samples were centrifuged at 7,489 × g for 15 min at 179 20 °C. The supernatant was read in a spectrophotometer at 515 nm.

2.6.4. Determination of malondialdehyde (MDA) levels

The measurement of malondealdehyde (MDA) production was performed following the methodology described by Ohkawa et al. (1970). After obtaining the ileum homogenate, 250 µL aliquots were incubated at 37 ◦C in a water bath for 60 min. Then, the samples were precipitated with 400 µL of 35% perchloric acid and centrifuged at 16,851 × g for 20 min at 4 °C. The supernatant was transferred to Eppendorf® tubes and 400 µL of 0.6% thiobarbituric acid was added to the samples and incubated at 95–100 °C for 1 h. After this period, the cooled samples were read in a spectrophotometer at 532 nm.

2.6.5. Determination of myeloperoxidase (MPO) activity

MPO activity was quantified using the methodology described by Krawisz et al.(1984). The ileum homogenates were homogenized in the hexadecyltrimethylammonium bromide (HTAB) buffer, which has a detergent function, lysing the neutrophil granules that contain the myeloperoxidase released in the proportion of 400 µL for 15 mg of tissue. The material was centrifuged at 4500 rpm for 12 min at 4 °C and the sample was subjected to a triple process of freezing and thawing to facilitate the disruption of cell structures and consequently the release of the enzyme. 150 µL of reaction buffer (o-dianisidinehydrochloride, 50 mM phosphate buffer and 33% H_2O_2) was added. The reading was performed using a 460 nm spectrophotometer, at times of 0, 30 and 78 sec and 5 min. The curve was obtained with the results at the mentioned times and the time of 1 min was selected as the time that best represented the event. Results were expressed as myeloperoxidase units per gram of tissue.

2.7. Immunohistochemical analysis of interleukin 1 beta (IL-1β) levels in ileum

Ileum fragments (3 µm) were obtained in a microtome and transferred to silanized slides (Dako, Glostrup, Den-mark) and submitted to the deparaffinization and hydrationprocesses. The slides were then washed with 0.3% Triton X-100 in phosphate buffer, treated with 3% hydrogen peroxide, and incubated overnight at 4 °C with the primary antibody anti- interleukin 1β (IL-1β) (Santa Cruz Biotechnology, Interprise, Brazil). After washing with phosphate buffer, the slides were incubated with a secondary antibody conjugated with streptavidin-HRP (Biocare medical, Concord, CA, USA) for 30 min and immunoreactivity forIL-1β was performed using a colorimetric test. based on a detection kit, following the protocolprovided by the manufacturer (Trek Avidin-HRP label + Medical Biocare Kit, Dako, USA). The samples were visualized in an optical microscope (Leica DM750, Switzerland) with a Qwin system coupled to a camera (Leica ICC50 HD) with a 400x objective. In each image, allbrownish-brown pixels (positive immunohistochemical staining) were used to create a binary image by digital processing.

2.8. Statistical analysis

Results were expressed as mean and standard error of the mean (e.p. m.) and statistically analyzed using ANOVA followed by Tukey's post-test. The null hypothesis was rejected when $p < 0.05$. The results were analyzed using GraphPad Prism® 6.0 software, San Diego, CA, USA.

3. Results

3.1. Determination of non-protein sulfhydryl group (GSH) levels

According to the results obtained, it was observed that in the obese HCD group (31.56 ± 2.0 nmol of GSH/mg of proteins) there was a reduction in the levels of GSH when compared to the healthy control group SD (66.52 ± 2, 8 nmol GSH/mg protein). However, supplementation with AP at a dose of 25 mg/kg administered orally increased GSH levels to 49.50 ± 1.8 GSH/mg protein, partially preventing its reduction when compared to the obese HCD group (31, 56 ± 2.0 nmol GSH/mg protein) (Fig. 2A, n = 5).

3.2. Determination of superoxide dismutase (SOD) activity

Obese rats in the HCD group demonstrated a reduction in SOD enzyme activity(4.7 ± 0.22 U of SOD/mg of protein) when compared to the control SD group (9.0 ± 0.31 U of SOD/mg of protein). However, when the rats were fed the hypercaloric diet and simultaneously supplemented with AP (25 mg/kg) the SOD activity was increased to 8.54 ± 0.32 when compared to the HCD group (4.7 ± 0.22 U of SOD/mg of protein) (Fig. 2B, n = 5).

3.3. Determination of the total antioxidant capacity (TAC)

In the isolated rat ileum, a decrease in total antioxidant capacity was observed between rats fed the hypercaloric diet (73.0 ± 2.7%) and those fed the standard diet (89.4 ± 2.2%). Interestingly, in rats fed a hypercaloric diet and supplemented with *A. platensis* at a dose of 25 mg/kg (85.4 ± 2.8%), there was an increase in the total antioxidant capacity of the organ in relation to the HCD group, however not was a significant difference between the HCD + AP25 (85.4 ± 2.8%) and SD (89.4 ± 2.2%) groups (Fig. 2C, n = 5).

3.4. Determination of malondialdehyde (MDA) levels

In rats fed a hypercaloric diet from the HCD group (500.7 ± 20.14 nmol of MDA/g of tissue) it was observed that the levels of MDA increased when compared to rats fed only with the standard diet of the SD group (195.4 ± 11.7 nmol MDA/g tissue). However, *S. platensis* significantly reduced MDA levels to 135.0 ± 8.3 nmol MDA/g tissue, when compared to the HCD group (500.7 ± 20.14 nmol MDA/g tissue). Similarly, supplementation with AP (135.0 ± 8.3 nmol MDA/g tissue) further reduced MDA levels when compared to the SD control group (195.4 ± 11.7 nmol MDA/g tissue) (Fig. 2D, n = 5).

3.5. Determination of myeloperoxidase (MPO) levels

The results showed that in the ileum of obese rats from the HCD group (46.45 ± 1.7 MPO units/g of tissue) the levels of MPO increased when compared to the healthy SD group (4.68 ± 0.87 MPO units/g of tissue). Interestingly, when these mice were supplemented with AP, MPO levels were dramatically reduced to 7.42 ± 0.26 MPO units/g tissue, when compared to the HCD group (46.45 ± 1.7 MPO units/g tissue) (Fig. 2E, n = 5).

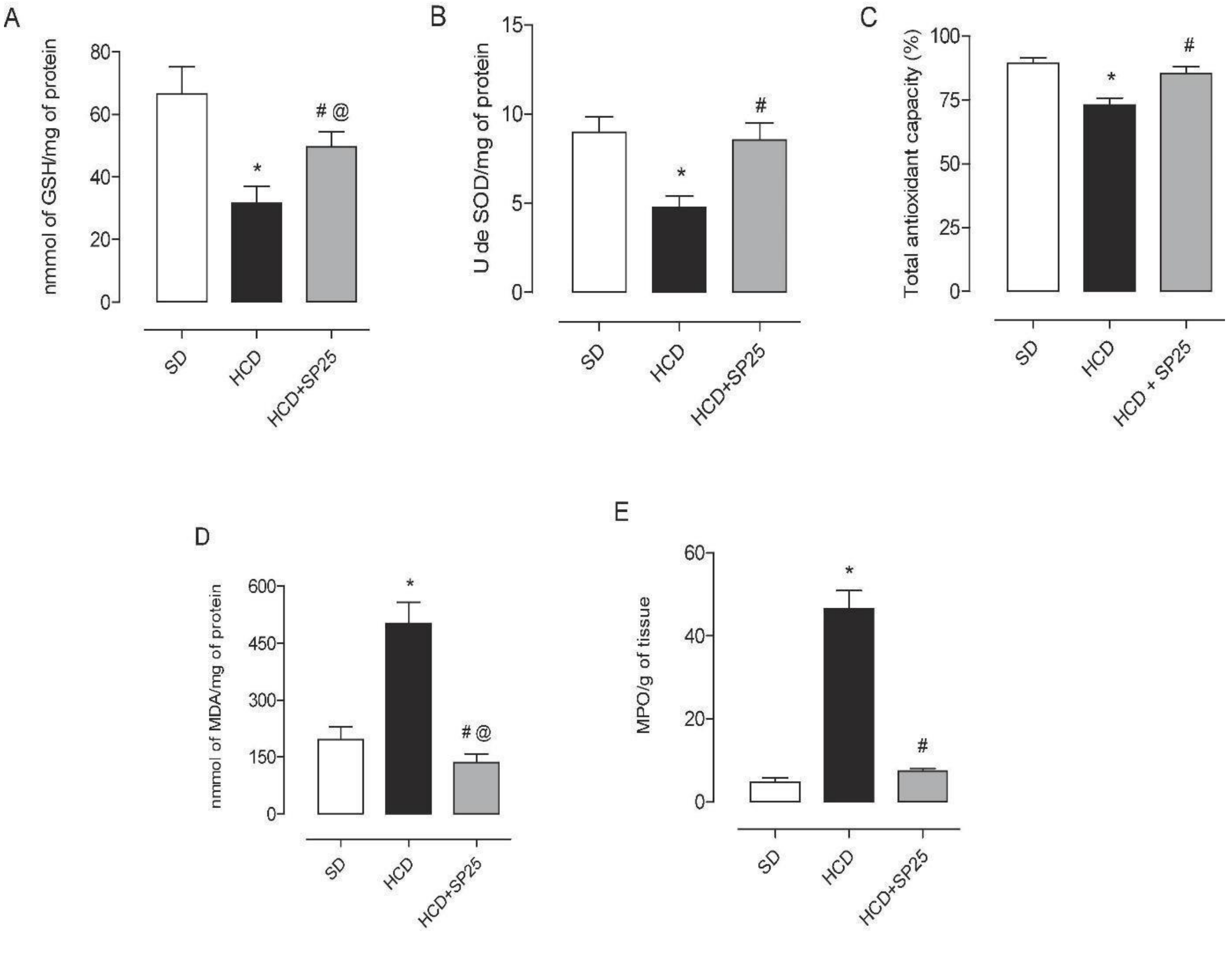
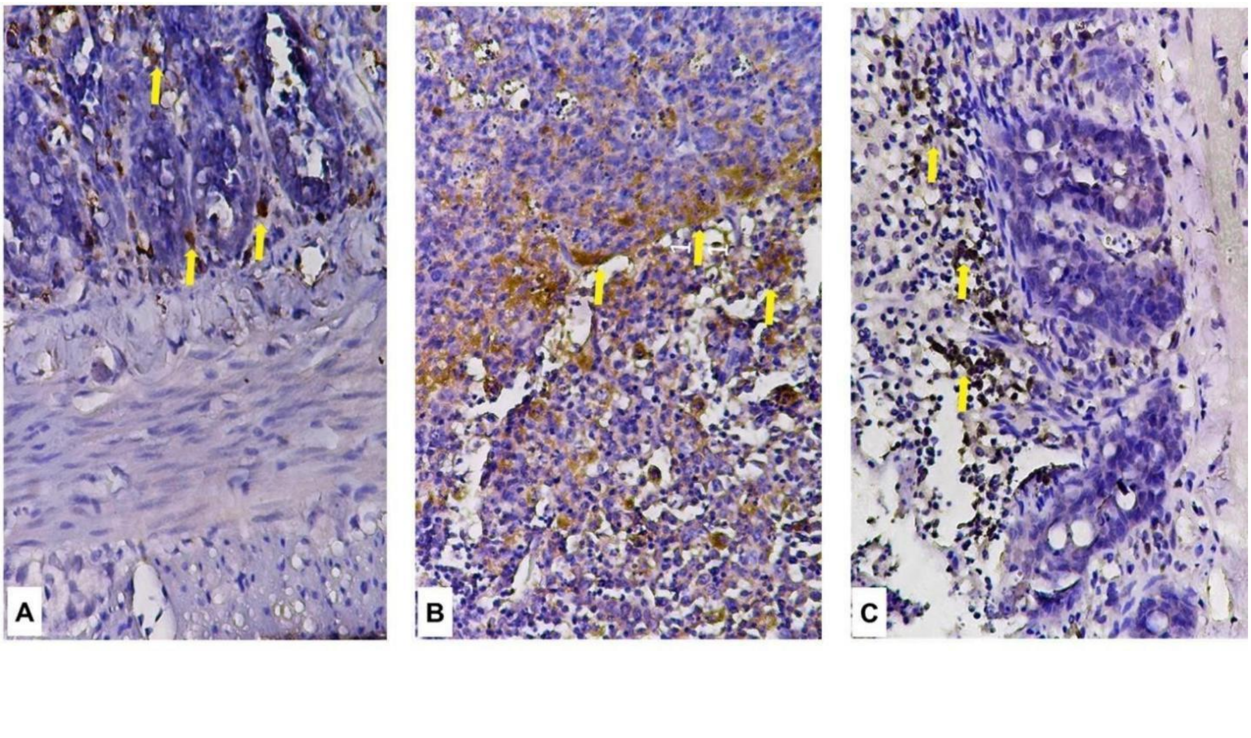


Fig. 2. Effect of dietary supplementation with *Arthrospira platensis* at a dose of 25 mg/kg on GSH levels (A), SOD (B), TAC (C), MDA (D) and MPO (E) in ileum of rats fed a hypercaloric diet. Columns and vertical bars represent the mean and S.E.M., respectively (n = 5). ANOVA one-way followed by Tukey's post- test. *p < 0.05 (SD vs. HCD); #p < 0.05 (HCD vs. HCD + AP25); @p < 0.05 (SD vs. HCD + AP25). SD = standard diet group supplemented with saline; HCD = hypercaloric diet group supplemented with saline; HCD + AP25 = hypercaloric diet group and supplemented with *A. platensis* at a dose of 25 mg/kg.

3.6. Immunohistochemical analysis of interleukin 1 beta (IL-1β) levels in ileum

In the evaluation of the immunostaining of the ileum of obese rats from the HCDgroup, an increase in the presence of IL-1B-labeled enterocytes, dispersed throughout the intestinal lamina propria, was observed, as evidenced by the brownish-brown coloration (Fig. 3B), when compared to the SD control group (Fig. 3A). However, dietary supplementation with AP reduced immunostaining with IL-1B (Fig. 3C) when compared to the expression of this cytokine in the HCD group (Fig. 3B), similarly to that observed in the SD group (Fig. 3A).According to the results obtained for IL-1β levels, the obese HCD group fed a hypercaloric diet showed an increase in IL-1β levels (940.6 ± 34.5 pg of IL-1β/mL) when compared to the control SD group fed only with standard diet (403.4 ± 26.23 pg IL1β/mL). Dietary supplementation with *A. platensis* from the HCD + AP25 group reduced the levels of this pro- inflammatory interleukin to 597.2 ± 33.3 IL-1β/mL, when compared to the HCD group (940.6 ± 34.5 pg IL- 1β/mL) (Fig. 3D).



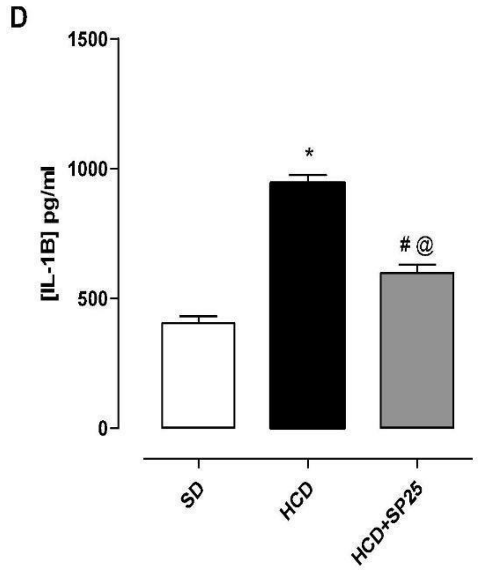


Fig. 3. Photomicrographs with immunohistochemical staining for interleukin 1 beta (IL-1β) in ileum samples from Wistar rats. 3A - SD Group; 3B- HCD Group; 3C - HCD + AP25 group (25 mg/kg). Scale: 50 µm. 400x. 3D - Quantification of IL-1β cytokine levels in ileum of rats in SD, HCD and HCD + AP25 groups.

4. Discussion

It was recently evidenced by our research group that *Arthrospira platensis* prevents obesity and improves the deleterious effects on intestinal reactivity of rats fed a hypercaloric diet (Diniz et al., 2021). In this context, the present study investigated the potential benefit of dietary supplementation with *A. platensis* (25 mg/kg), which contains a rich and vast nutritional composition with high levels of proteins and bioactive compounds, in modulating inflammation and oxidative stress of obese rats fed a hypercaloric diet. Thus, it was demonstrated with this research that *Arthrospira platensis* suppresses the production and release of the pro-inflammatory cytokine IL-1β and improves the antioxidant activity of the ileum by reducing oxidative stress.

Changes in eating habits and greater accessibility to high-calorie foods have made obesity one of the biggest and most serious health problems in the world. The consumption of high-calorie foods has influenced the composition of the intestinal microflora, which is a key point in the pathophysiology of many extraintestinal diseases. In recent years, several studies have confirmed a mechanistic association of the intestinal microbiota in the pathophysiology of obesity (Sanmiguel et al., 2015).

The gastrintestinal tract has the greatest potential for producing circulating inflammatory cytokines. High-fat diets promote negative modulation, increasing inflammation and intestinal permeability (Lim et al 2016; Guadagnini et al., 2019). In addition, hypercaloric diets are capable of leading to a process of intestinal dysbiosis, thus influencing the development of obesity through inflammatory mechanisms mediated by lipopolysaccharides (LPS), increasing oxidative stress and exacerbated secretion of pro-inflammatory cytokines (Cortez-Cooper et al., 2013; Park et al., 2013; Agagündüz, et al., 2023) (Fig. 4). Therefore, for the induction of experimental obesity, Wistar rats were fed with an HCD. Regarding the treatment with the seaweed, the rats concomitantly received HCD and *A. platensis* at a dose of 25 mg/kg for 8 weeks (preventive model), in order to verify the possibility of delaying the progression of inflammation and/or oxidative stress in the ileum.

Knowing that in obesity there is a greater secretion of adipokines from adipose tissue, favoring the pro-inflammatory state, which both influences the composition and physiology of the intestinal microbiota and also increases oxidative reactions, generating an increase in reactive oxygen species, culminating in the oxidative stress and, consequently, cell and/or tissue damage (Yang et al., 2017; Crawford et al., 2019). In turn, obesity is characterized as a chronic condition of oxidative stress. In this case, high levels of glucose and circulating lipids result in the excessive supply of energy substrates for metabolic pathways, which favors increased ROS production (McMurray et al., 2016). In view of this, in this study we initially investigated how obesity induced by a hypercaloric diet would be participating in oxidative stress, as well as the antioxidant mechanism of *A. platensis* in preventing such damage through the quantification of markers, such as glutathione redutase (GSH), total antioxidant capacity (TAC), superoxide dismutase (SOD) and malondialdehyde (MDA) levels, in additionto the evaluation of possible anti-inflammatory modulation through a marker of neutrophilic infiltration, myeloperoxidase (MPO) in the ileum of rats.

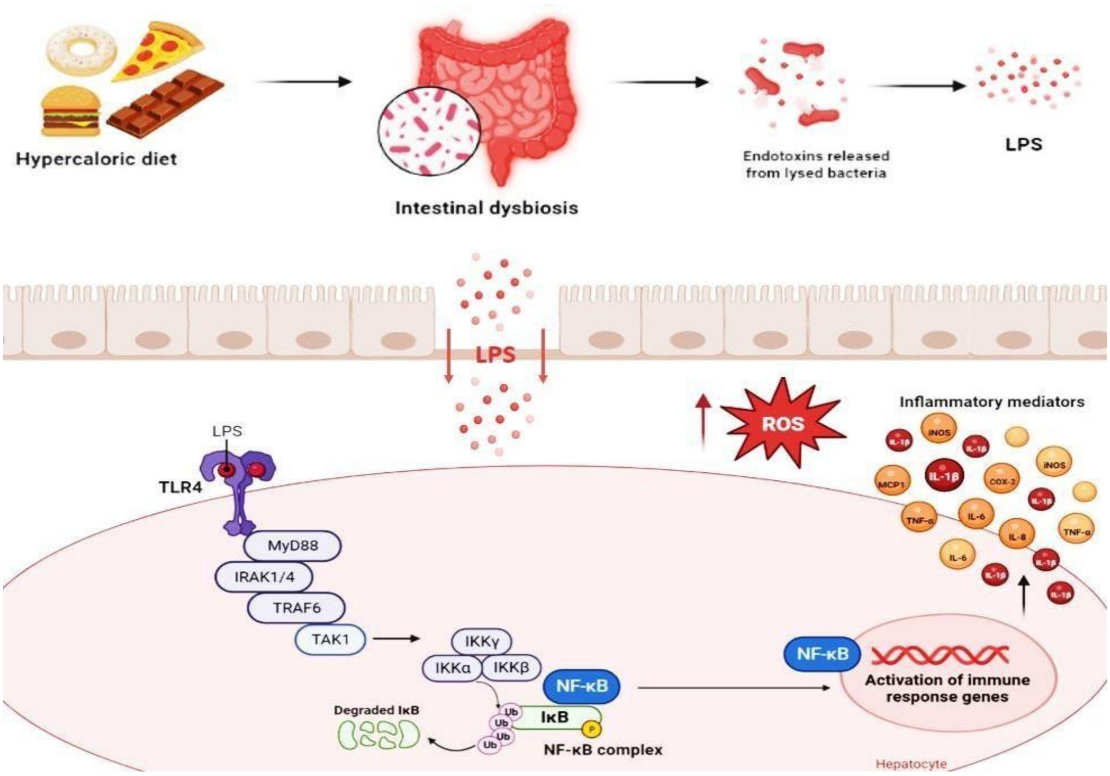


Fig. 4. Interaction between hypercaloric diet, intestinal microbiota and host in the development of obesity.

The antioxidant system helps in the prevention of some diseases, including intestinal disorders, much of its production occurs physiologically and can combat oxidative stress. Among them are enzymatic antioxidants such as SOD and GPx, as well as non-enzymatic antioxidant systems such as reduced glutathione (GSH) (Calcabrini et al., 2017; Kivrak et al., 2017; Sarangarajan et al., 2017). GSH is a tripeptide that acts as a protective agent for the intestinal mucosa by regulating intracellular redox homeostasis, in addition to directly channeling and neutralizing ROS (Kivrak et al., 2017; Jaeschke; Ramachandran, 2018). Thus, our results showed that the rats that consumed the hypercaloric diet, in the HCD group, the levels of GSH in the ileum were reduced to those quantified in the SD control group, in contrast, the dietary supplementation with AP prevented the reduction of GSH levels in the ileum. Ileus from obese rats, a mechanism attributed to the antioxidant effect of the seaweed, enhancing the non-enzymatic tissue antioxidant system.

Physiologically, gastrintestinal tract cells are protected against oxidative stress by a set of free radical scavengers, such as SOD (Garcia- Irigoyen et al., 2022). SODs are a set of enzymes whose main function is to catalyze the dismutation of reactive superoxide anions into hydrogen peroxide (H_2O_2), a more stable and easily diffusible molecule, which can be easily converted into water by other antioxidants, thus representing the main cellular defense against superoxide radicals (Halliwell, 1989; Zelko et al., 2002; Miler, 2013). Our results show that obese rats achieved a decrease in SOD levels when compared to control group SOD levels. However, when supplemented with AP, the rats increased the levels of this antioxidant, thus showing a protective effect of the seaweed in preventing the reduction of SOD caused by the hypercaloric diet. These results corroborate what was observed by Garcia-Irigoyen (2022) in which SOD depletion in the intestinal epithelium leads to inflammation and obesity through activation of phospholipase A_2 (PLA_2) and increased release of arachidonic acid in mice. On the other hand, the preventive effect associated with AP is attributed to its rich nutritional composition, since it contains natural pigments, such as phycocyanin, carotenoids and xanthophylls, molecules responsible for its antioxidant activity, elimination of free radicals and inflammatory modulation (Miranda et al., 1998; Gad et al., 2011; Kumar et al., 2022).

Another important parameter in the evaluation of antioxidant activity is the total antioxidant capacity (TAC), a biomarker of oxidative stress, which represents the organ's ability to scavenge free radicals through redox systems (Crews et al., 2000; Brighenti et al., 2005). Thus, analyzing the rats fed the hypercaloric diet, in the HCD group, the TAC was lower than in the SD control group. Well-established studies demonstrate that the loss of the homeostatic balance between ROS and antioxidant defenses, with a reduction in antioxidant capacity, consequently leads to an increase in the expression of oxidative stress, mainly given by the increase in NADPH concentrations, resulting in an increased production of electron donors that reduce molecular oxygen to superoxide (Brownlee, 2001).

In rats fed a hypercaloric diet and supplemented with *A. platensis* at a dose of 25 mg/kg, an increase in tissue TAC was observed when compared to the HCD obese group. These data indicate that the increase in tissue antioxidant defenses caused by seaweed consumption is correlated with the removal of free radicals, thus reducing the deleterious effects of ROS in the ileum. In vitro and in vivo studies show that treatment with *Arthrospira* significantly reduces oxidative stress, preventing lipid peroxidation and DNA damage, eliminating free radicals and enhancing the activity of the enzymatic antioxidant system, effects that are mediated by the chemical composition and nutritional status of the algae (Upasani; Balaraman, 2003; Abdel-Daim et al., 2013; Abdelkhalek et al. 2015; Wu et al., 2016).

The gastrintestinal (GI) tract is an important element in maintaining redox homeostasis, as it represents the interface between the organism and the environment. Furthermore, evidence shows that oxidative stress is a determining factor in the pathogenicity of gastrintestinal diseases, such as gastrintestinal cancer, peptic ulcers and inflammatory bowel diseases (Bhattacharyya et al., 2014). In this sense, studies involving oxidative stress, MDA is the end product of lipid peroxidation, representing the most frequently quantified systemic and tissue biomarker (Yoshikawa et al., 2002; Del Rio; Stewart; Pellegrini, 2005; Uddin et al., 2017).

According to our results, MDA levels in obese rats were higher than levels observed in healthy rats in the control group. These results corroborate studies that demonstrate that MDA concentrations are increased and antioxidant defense markers are reduced in obese individuals (Pihl et al., 2006; Chrysohoou et al., 2007; Hartwich et al. al., 2007; Adnan et al., 2019). Therefore, it can be inferred that the increase in MDA levels may be related to the increase in body adiposity, a consequence of the high production of pro-inflammatory cytokines, such as TNF-α and IL-1β, stimulated by the infiltration of macrophages in the adipose tissue. (Morrow, 2003; Fonseca-Alaniz et al., 2007).

In contrast, dietary supplementation with AP prevented the increase in MDA levels in rats fed a hypercaloric diet. Such observed effect can be attributed to antioxidant compounds such as β-carotene, phycocyanins, vitamins and minerals present in *A. platensis*. Studies also show that the protective action of the seaweed may be associated with the presence of phycocyanin, a protein responsible for scavenging free radicals and for modulating cyclooxygenase-2 (COX-2), a catalytic enzyme that mediates inflammatory processes and oxidative stress (Karadeniz et al., 2009; Alirezaei et al., 2011; Abdel-Daim et al., 2013).

The enzyme myeloperoxidase (MPO) is secreted mainly by neutrophils and to a lesser extent by monocytes and macrophages. One of the main effects of MPO is the formation of reactive species, such as chloramines, involved in the production of inflammatory cytokines and activation of kinases (Jones et al., 2016; Selders et al., 2017). All these processes favor the increase of intestinal damage. It was observed in this study that the rats in the HCD group had significantly higher levels of MPO than the rats in the SD group. On the other hand, the levels of this enzyme were reduced in rats supplemented with AP when compared to the obesegroup. The phycocyanin present in AP was responsible for reducing MPO activity, which was increased in an experimental model of colitis in rats (González et al., 1999). Thus, we evidence the preventive antioxidant action of the seaweed in protecting the ileum against damage caused by the exacerbated increase in MPO levels induced by obesity.

Food exerts a great influence on the health of humans and animals. Irregular eating habits are important precursors that favor the development of obesity (Botelho et al., 2018). Thus, the consumption of hypercaloric and hyperlipidic diets are associated with the accumulation of fat in the adipose tissue, where the commitment of adipocytes to store excess triglycerides leads to hypertrophy and hyperplasia of these cells, deregulating the positive energy balance and altering the pattern of production and secretion of adipokines (Reynés et al., 2017). During obesity we have a decrease in anti-inflammatory chemokines such as adiponectin and an increase in macrophage infiltration as well as activation of the intestinal immune system resulting in the production of pro-inflammatory cytokines such as tumor necrosis factor (TNF-α), prostaglandins (PG), leukotrienes (LT), IL-6 and IL-1β (Krimsky et al., 2003; Berg, Anders, & Scherer, 2005; Shoelson et al., 2006).

Our results showed that in rats fed a hypercaloric diet, IL-1β expression was higher when compared to the group of animals fed a standard diet. These findings can be visualized through the increase in brownish-brown markings dispersed throughout the lamina propria, in addition, we also have an increase in the infiltration of macrophages and immune cells (Fig. 3B; 3D). However, in mice from the HCD + AP25 group, dietary supplementation with AP suppressed IL-1β expression when compared to obese mice from the HCD group (Fig. 3C; 3D). Abu-Taweel and colleagues showed that spirulina exerts anti-inflammatory effects in rats exposed to the writhing response induced by acetic acid, associated with reduced levels of IL- 6, tumor necrosis factor (TNF), IL- 1β, nitric oxide (NO) and suppression of COX-2 and iNOS activities (Abu-Taweel et al., 2019). Inferring that the seaweed was able to prevent the increase in IL-1β levels in the ileum, caused by the hypercaloric diet, reducing the intestinal inflammatory profile, possibly due to its immunomodulatory, antioxidant and anti- inflammatoryactivity.

The consumption of hypercaloric diets can affect intestinal permeability through the secretion of inflammatory mediators from the process of intestinal dysbiosis, which will cause an imbalance in the production of pathogenic bacterial phyla, promoting the release of endotoxins called lipopolysaccharides (LPS) that will break the intestinal integrity, increasing permeability and allowing the translocation of LPS to the circulation. Elevated concentrations of LPS in the blood are primarily responsible for increased fat deposition in various organs and act as an antigen to stimulate the host's immune response. An increase in LPS levels activates the toll-like receptor 4 (TLR-4), present on hepatocytes, which binds to LPS and induces low- grade inflammation. Once activated, these transmembrane receptors recruit a series of protein families MyD88 (myeloid differentiation factor), IRAK-1 (interleukin-1 receptor- associated kinases), TRAF6 (tumor necrosis receptor-associated factor 6), TAK1 β growth associated with kinase 1, which in turn promotes activation of the IKK complex (formed by three subunits IKKα, IKKβ and IKKγ). The IKK complex phosphorylates the IkB present in NF-kB (nuclear kappa B factor) which will undergo ubiquitination and degradation. NF-kB will then be translocated to the nucleus and induce the expression of adhesion molecules and inflammatory mediators, including tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-1β, IL-8, cyclooxygenase-2 (COX-2) and chemokines, such as monocyte chemotactic protein 1 (MCP1) and inducible nitric oxide synthase (iNOS) (CARTWRIGHTet al., 2016; LAPPAS et al., 2005). dysregulation of adipokines and increased inflammatory infiltrates in adipose tissue result in chronic inflammation and oxidative stress, which contribute to increased body adiposity and the development of obesity.

5. Conclusions

The study concludes that dietary supplementation with *Arthrospira platensis* prevents the increase of oxidative stress by negatively modulating the expression of MDA and MPO, in addition to potentiating the enzymatic antioxidant system through the positive modulation of SOD and GSH activities, improving the total antioxidant capacity from the ileum of obese rats fed a hypercaloric diet, thus demonstrating the potential preventive effect of seaweed in protecting against oxidative stress. AP was also responsible for preventing the increase in IL- 1β levels in the ileum of obese rats fed a hypercaloric diet, possibly due to its anti-inflammatory and immunomodulatory activity, negatively regulating the percentage of the interleukin immunostaining index, by inhibiting the obesity-induced inflammatory responses (Fig. 5). Although studies in humans are limited, this set of results corroborates the histomorphological findings evidenced by Diniz et al. (2017). Altogether, we demonstrated that supplementation with *Arthrospira platensis* exerts preventive effects on damage to the intestinal tract by modulating signaling pathways of oxidative stress and inflammation in a model of experimental obesity induced by a hypercaloric diet, becoming a natural anti-inflammatory strategy and promising adjuvant for the treatment of different intestinal diseasescaused by obesity, and a possible candidate for phytotherapy.

6. Institutional review board statement

All work protocols were approved by the Ethics Committee on the Use of Animals of UFPB n◦ 2352101019) and followed the norms for the ethical use of animals of the National Council for Experimental Control Animals (Brazil).

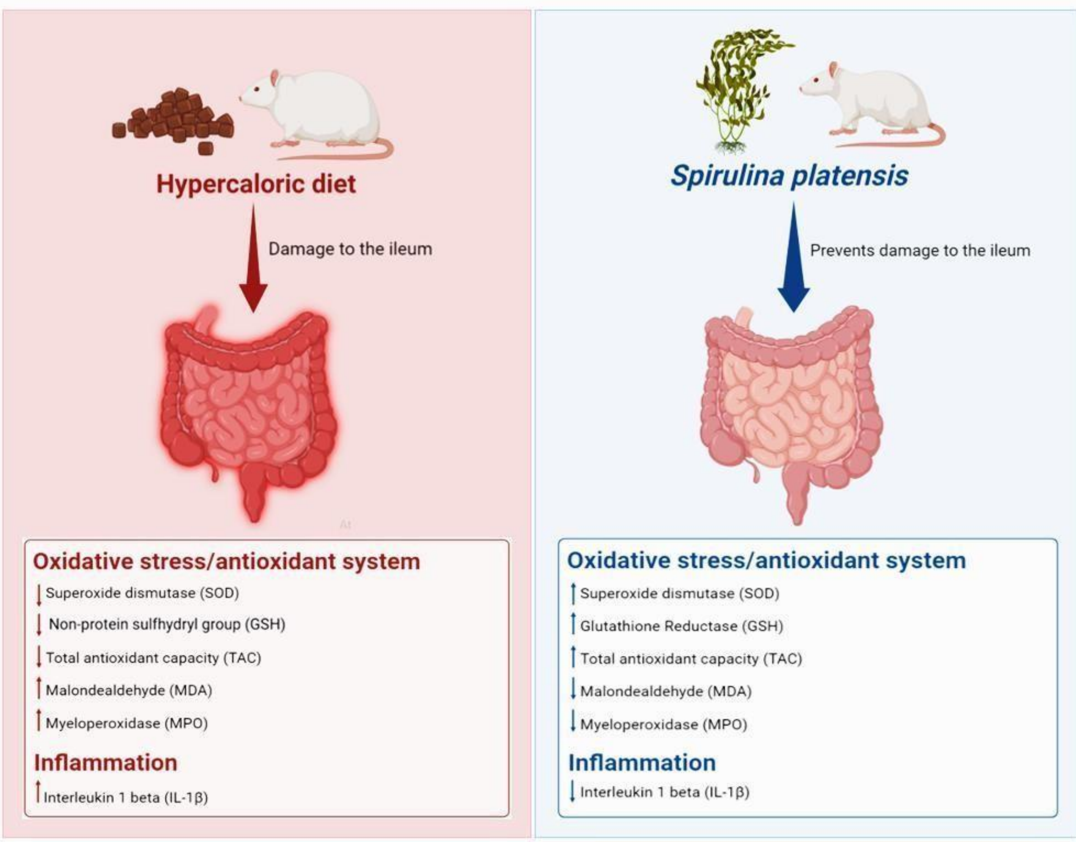


Fig. 5. Conceptual map. Consumption of the hypercaloric diet for 8 weeks caused damage to the ileum of rats, evidenced by increased oxidative stress (MDA and MPO) and reduced SOD activity and GSH levels, as well as TAC. Furthermore, it was able to increase the levels of the inflammatory cytokine IL-1β. In contrast, dietary supplementation with *A. platensis* prevented such damage to the oxidative/antioxidant stress system and the inflammatory profile by suppressing IL-1β levels in the ileum of obese rats.

Author contributions

A.F.A.D. and B.A.d.S. developed the hypothesis and experimental design. A.F.A.D., P.B.F. and B.C.B. analyzed the data and wrote the manuscript. A.F.A.D., B.F.O.C., D.M.C.F. and E.B.A.J. performed the experimental work. A.F.A.D, R.R.A., A.F.A. and L.M.B. contributed to the in vivo work. All authors have read and agreed to the published version of the manuscript.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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5.3 Capítulo III

Artigo 3

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Arthrospira (Spirulina) platensis supplementation prevents contractile reactivity damage in obese rats fed a hypercaloric diet by positive modulating the Rho-A/Rho-kinase pathway, inflammation and oxidativestress

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ABSTRACT

Obesity is characterized by excessive accumulation of fat that favors the development of diseases, associated with changes in intestinal reactivity. The alga *Arthrospira (Spirulina) platensis* (AP) has been the subject of several studies. However, there are still no studies involving its use in theprevention of deleterious effects promoted by the consumption of a hypercaloric diet on ileal reactivityand the possible mechanisms of action involved. Thus, the effects of PA and the mechanisms by whichalgae prevent damage to the contractile reactivity of the ileum were evaluated. The experimental procedures were approved by the UFPB Ethics Committee on Animal Use (certificate 2352101019). The rats were divided into standard diet (SD), hypercaloric diet (HCD) and supplemented with AP at adose of 25 mg/kg (HCD + SP25). Rats (HCD) showed reduced contractile efficacy to CCh (pharmacomechanical coupling), which was prevented by PA. These preventive effects of the seaweedwere associated with the positive modulation of Rho kinase (ROCK), nitric oxide (NO), contractile prostanoids, superoxide dismutase (SOD) and NADPH oxidase complex pathways, which may be associated with the presence of acid gamma linolenic acid (GLA), as a major component of the seaweed. Therefore, AP emerges as an adjuvant in the prevention of obesity and intestinal diseases.

Keywords: 1. *Arthrospira (Spirulina) platensis;* 2. Obesity; 3. Oxidative stress; 4. Inflammation;

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1. Introduction

Defined as a chronic, complex and progressive condition, resulting from excessive and abnormal accumulation of body adipose tissue, obesity is responsible for causing several health risks, being related to energy homeostasis, inadequate intake of macro and micronutrients, non-practice physical exercise, innate and adaptive immunity, cellular metabolism and intestinal microbiota (LANCET, 2016; AFSHIN et al., 2017; ABARCA- GOMEZ et al., 2017).

In this context, obesity is considered a multifactorial syndrome, being influenced by genetic, environmental, behavioral interactions and by the composition of the intestinal microbiota (VILLANUEVA-MILLÁN; PÉREZ-MATUTE; OTEO, 2015; JOHN; MULLIN, 2016). In addition, it has already been demonstrated that obesity modulates the smoothmuscle reactivity of various organs and the regulation of smooth muscle contractility plays a key role in several pathophysiological processes, such as arterial hypertension, erectile dysfunction, asthma, uterine cramps, intestinal constipation (FELIPPI, 2014), thus making it important for studies involving this type of musculature associated with its dysregulations, in order to contribute to the discovery and development of new products and therapeutic alternatives.

The inclusion of functional foods that include phenolic compounds, bioactives and fatty acids in the daily dietary routine has shown beneficial effects on lipid metabolism and consequently anti-obesity (BATISTA et al., 2018, FONTE-FARIA et al., 2019). Thus, in recent years, the role of *Arthrospira platensis* as an effective alternative food supplement for weight control in animals and humans has been discussed. The effectiveness of seaweed consumption as a dietary supplement on reducing body weight has been attributed mainly to its action by reducing the infiltration of macrophages in the visceral adipose tissue and preventing the accumulation of lipids in the liver and oxidative stress (FUJIMOTO et al., 2012; MAZOKOPAKIS et al., 2014). Furthermore, it has already been demonstrated that the antioxidants present in seaweed are effective in the treatment of obesity through different effects, such as lipase inhibition and suppressive effect on food intake (HASSAN; EL-GHARIB, 2015; ISMAIL et al., 2017).

Arthrospira platensis is already widely used as a functional resource and food supplement, presenting several benefits for human and animal intestinal health, but fewstudies have revealed the preventive effects of the seaweed on obesity and the intestinal tract, in addition to the mechanisms of action by which the seaweed exerts its effects on intestinal reactivity remain unknown and must be characterized. A recent study carried out by our research group showed that dietary supplementation with 25 mg/kg of *Arthrospira platensis* has an anti-obesity effect, as well as preventing damage to the intestinal reactivity of rats fed a hypercaloric diet (DINIZ et al., 2021). We hypothesize that *Arthrospira platensis* may prevent damage to contractile reactivity in the ileum through negative modulation of oxidative stress and inflammation. The potential modulatory preventive mechanisms of AP on intestinal reactivity of the ileum have not been elucidated. In this study, we aimed to investigate the influence of preventive treatment with *A. platensis* on intestinal health and clarify the possible underlying mechanisms of action involved in ileal reactivity in obese rats.

2. Materials and methods

2.1. Obtaining and preparing Arthrospira (Spirulina)platensis

Arthrospira platensis (*Spirulina platensis*) was acquired from the laboratory INFINITY Pharma (HONG KONG, China) in the form of lyophilized powder (lot nº. 17J11- B004-020541). A sample of the powder was certified and fractionated by Roval Manipulation Pharmacy (João Pessoa), Paraiba, Brazil).

2.2. Animals and experiments groups

Eight-week-old healthy male Wistar rats (*Rattus norvegicus*), weighing between 170-190 g, purchased from the Animal Production Unit (APU) of the Institute for Research in Pharmaceuticals and Medicines (IPeFarM)/UFPB (João Pessoa, Brazil). Rats were housed in an experimental room for animals with ad libitum access to water, under a light-dark cycle of 12 h:12 h at 21 °C ± 1 °C, relative humidity of 50 ± 10%. After the acclimatization period, the rats were randomly divided into three groups (n = 5), namely: SD group, which was fed a standard diet (Presence® pellets); HCD group, fed a hypercaloric diet; and HCD + AP25 group, which was fed with HCD and simultaneously supplemented with *Arthrospira platensis*(25 mg/kg), which was administered with saline solution (NaCl 0.9%) by oral gavage once a day for 8 weeks (DINIZ et al., 2021). The experimental procedures were approved by the UFPB Ethics Committee on Animal Use (protocol nº. 2352101019).

Rats in the SD group received a balanced diet based on pellets containing by weight 23% protein, 63% carbohydrate and 4% lipids, totaling the total energy value (TEV) at

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380 kcal/100 g. The groups fed the hypercaloric diet (HCD and HCD+AP25) received a diet composed of a mixture of standard chow, milk chocolate, roasted in natura peanuts and cornstarch biscuits in a ratio of 3:2:2:1, whose VET is 417 kcal/100 g. The HCD was prepared weekly and offered to the animals in the form of pellets.

2.3. Substances and reagents

Dihydrate calcium chloride ($CaCl_2.2H_2O$), hepta-hydrated magnesium sulfate ($MgSO_4.7H_2O$) and glucose ($C_6H_{12}O_6$) were purchased from VETEC (Brazil). Sodium bicarbonate ($NaHCO_3$) was purchased from Fmaia (Brazil). Sodium chloride (NaCl) and potassium chloride (KCl) were acquired from modern chemistry (Brazil). Monobasic potassium phosphate (KH_2PO_4), sodium hydroxide (NaOH) and hydrochloric acid (HCl) werepurchased from nuclear (Brazil). These substances, except glucose, sodium bicarbonate and sodium chloride were dissolved and diluted in distilled water to obtain each solution that werekept under refrigeration. Carbamylcholine hydrochloride (CCh) and Verapamil were purchased from Sigma-Aldrich (Brazil). These substances were dissolved and diluted indistilled water to obtain the stock solution (10^{-2} M) and maintained at 0 °C in refrigeration. These solutions were diluted in distilled water to obtain appropriate concentrations for experimental protocol.

Nω-nitro-L-arginine methyl ester (L-NAME), indomethacin, tempol, apocynin, (R-(+)- trans-4-(1-aminoethyl)-N-(4 pyridyl) cyclohexanecarboxamide (Y27632) was purchased from Cayman Chemical (Brazil). The substances used in the functional experiments were dissolved and diluted in distilled water, except for indomethacin, apocynin and Y27632 which were dissolved in absolute alcohol (96 °GL) to obtain each stock solution, which were kept at 4 or -20 °C. The pH of the nutrient solution was adjusted to 7.4 with HCl or NaOH 1 N. The Krebs Henseleit solution (mM) was composed of: NaCl (118.0); KCl (1.2); $CaCl_2$ (2.5); KH_2PO_4 (1.18); $MgSO_4$ (1.18); $C_6H_{12}O_6$ (1,1); $NaHCO_3$ (25.0) (HENRIQUES, 2011). The carbogenic mixture (95% O_2 and 5% CO_2) was purchased from White Martins (Brazil).

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3. Methods

3.1. Analysis of the chemical profile of Arthrospira (Spirulina) platensis

3.1.1. Extraction

100 mg of the green powder was subjected to extraction with 100 mL of chloroform for 15 min in an ultrasound bath and after this procedure a filtration was performed. The solution was concentrated under reduced pressure. The extract was analyzed by NMR.

3.1.2. Nuclear Magnetic Resonance (NMR)

The ^{1}H NMR and ^{13}C NMR were recorded on Bruker AVANCE NEO (500 MHz FT-NMR) and AVANCE HD (400 MHz FT-NMR) using deuterated chloroform ($CDCl_3$) as solvents and TMS as internal standard.

3.2. Effect of food supplementation with A. platensis on contractile reactivity of isolated rat ileum

3.2.1. Obtaining and preparing rat ileum segments

All rats were fasted for 12 hours (only receiving water ad libitum during this period). After this period, the animals were euthanized by guillotine. An abdominal incision was made and the segment of ileum approximately 15 cm long was removed and placed in a Petri dish containing Krebs Henseleit nutrient solution at 37 °C under carbogen aeration (RADENKOVIC et al., 2006). The segment of the ileum was sectioned into fragments of 2 to 3 cm in length and destined for reactivity. The connective and adipose tissues adjacent to the ileum segments were removed, which were individually suspended in bath tubs for isolated organ (6 mL) containing Krebs Henseleit solution aerated with carbogen at 37 °C and kept at rest under tension of 1 g for 30 minutes, time necessary for the stabilization of the organ. During the stabilization period, the nutrient solution was changed every 15 minutes to prevent metabolite interference (RADENKOVIC et al., 2006).

3.2.2. Effect of supplementation with A. platensis on cumulative CCh in the absence and presence of Y27632

The ileum was assembled as described in item *3.1.1.* After the stabilization period, cumulative concentration-response curves to CCh (10^{-9}-3 x 10^{-4} M) were obtained in the

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absence and presence of Y27632 (10^{-6} M), a selective ROCK inhibitor, incubated for 20 minutes (MILLS et al., 2001).

The contractile response of the ileum in the presence of the inhibitor was calculated based on the mean amplitude of the curve obtained in the control group. Contractile reactivity was evaluated based on CCh E_{max} and pEC_{50} values, in the absence and presence of Y27632, and the effect of the inhibitor on the cumulative concentration-response curve to CCh was compared between SD and HCD or HCD + AP25.

3.2.3. Effect of supplementation with A. platensis on the CCh cumulative curve in the absence and presence of apocynin and tempol

After the ileum stabilization period, apocynin (10^{-4} M), an NADPH oxidase inhibitor (CÔCO et al., 2016), tempol (10^{-3} M), a SOD mimetic (PEIXOTO et al., 2009), in different preparations, and then cumulative concentration-response curves to CCh were obtained.

The contractile response of the ileum to CCh in the presence of apocynin or tempol was calculated based on the average amplitude of the curve obtained in the control group, and the contractile reactivity was evaluated according to the values of E_{max} and pEC_{50} of CCh, in the absence and in the presence of inhibitors, and compared between SD and HCD or HCD + AP25.

3.2.4. Effect of supplementation with A. platensis on the cumulative CCh curve in the absence and presence of L-NAME or indomethacin

After the ileum stabilization period, L-NAME (10^{-4} M), a non-selective NOS inhibitor, was incubated for 30 minutes (VIGNOZZI et al., 2006), and indomethacin (10^{-5} M), anon-selective COX (CARTLEDGE; EARDLEY; MORRISON, 2000), in different preparations, and then cumulative concentration-response curves to CCh were obtained. The contractile response of the ileum in the presence of the inhibitor was calculated based on the mean amplitude of the curve obtained in the control group. Contractile reactivity was evaluated based on CCh E_{max} and pEC_{50} values, in the absence and presence of L-NAME and indomethacin, and the effect of the inhibitors on the cumulative concentration-response curve to CCh was compared between SD and HCD or HCD + AP25.

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3.3. Statistical analysis

The results were expressed as the mean and standard error of the mean (S.E.M.) and statistically analyzed using the *t* test (unpaired), for comparison between two experimental groups, the analysis of variance (ANOVA) one way followed by Tukey's post-test, for multiple comparison between experimental groups. The null hypothesis was rejected when $p < 0.05$.

The pEC_{50} was used as a potency parameter, calculated by non-linear regression, and the E_{max} as an efficacy parameter (NEUBIG et al., 2003). Data were analyzed using the GraphPad Prism® program version 6.01 (GraphPad Software Inc., San Diego CA, U.S.A.).

4. Results

4.1. NMR analysis of chloroform extract

In the [1]H NMR spectrum (Fig. 1) was possible observed signals in δ_H 5.3 (m, olefinics C6, C7, C9, C10, C12, C13), 3.3 (s, CH_3 ester C19), 2.7 (m, CH_2 between double bonds C8, C11), 2.2 (m, CH_2 adjacent to C = O, C17), 2.1 (m, CH_2 adjacent to double bond C5, C14), 1.7 (m, C3, C4, C15 and C16) 1.3 (m, CH_2 next to CH_3 C2), 0.9 (t, CH_3 terminal C1). The signals observed in regions of [13]C NMR spectrum (Fig. 2) δ_C 14.4- C1, 22.9- 34.7 (C2, C3, C4,C5, C8, C11, C14, C15, C16, C17) 50.9- C19, 127.56 – C7, C12, 129.55 – C9, C10, 130.41 – C6, C13, 174.82 – C18.

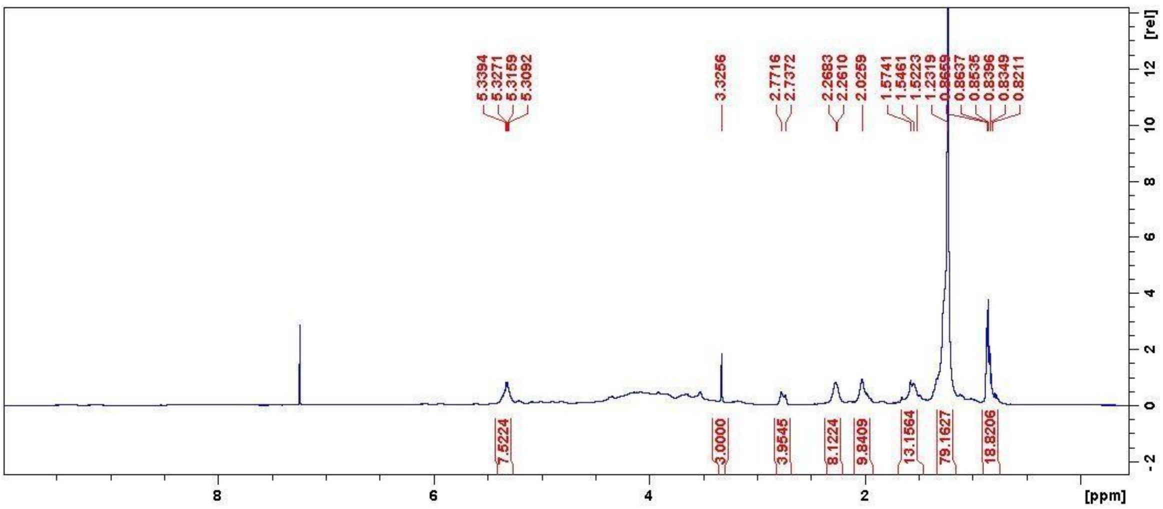

Fig. 1. [1]H NMR of chloroform extract from *A. platensis*.

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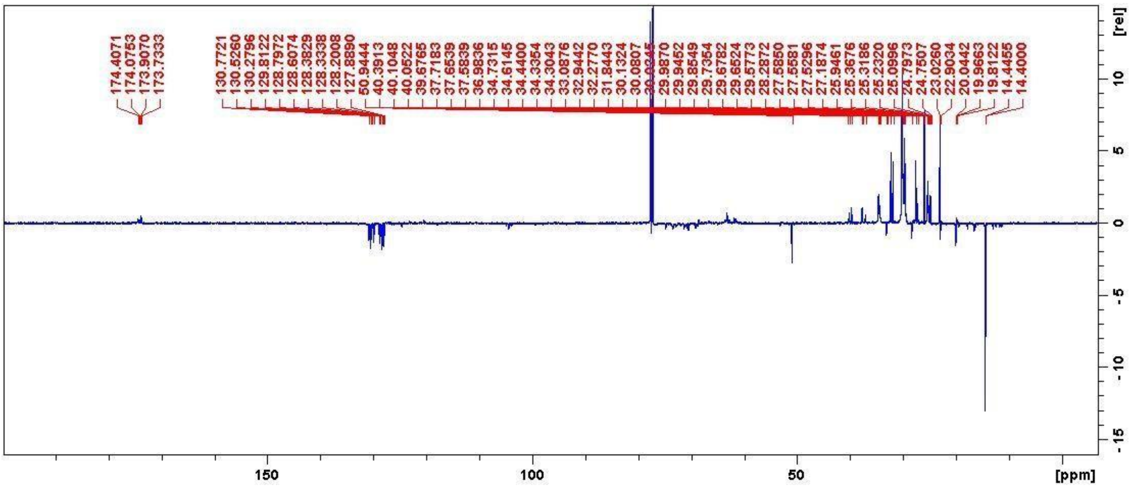

Fig. 2. ^{13}C NMR of chloroform extract from *A. platensis.*

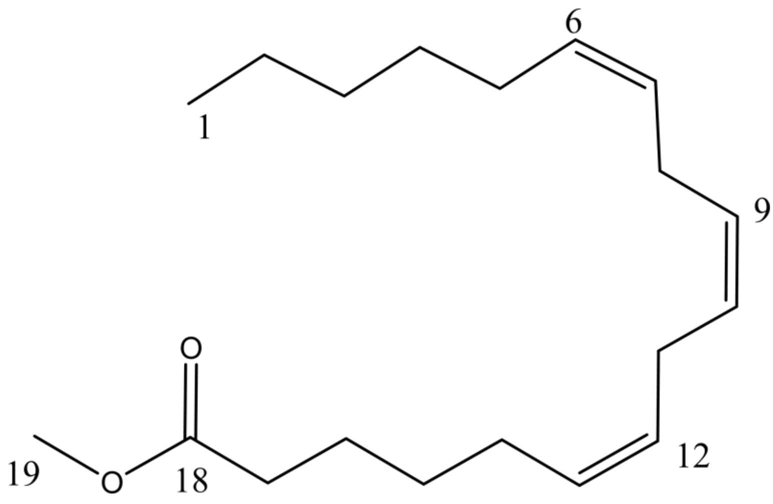

Fig. 3. Structure of gamma linolenic acid (GLA).

4.2. Effect of supplementation with Arthrospira platensis on the cumulative CCh curve in the absence and presence of Y27632

In rats fed only the standard diet, the contractile efficacy of CCh was reduced in the presence of Y27632 (E_{max} = 64.4 ± 2.1%), a ROCK inhibitor, when compared to its absence in the SD group (E_{max} = 100 %). Furthermore, there was no change in agonist contractile potency in the absence (6.3 ± 0.05) and in the presence of Y27632 in this experimental group (6.0 ± 0.05) (Table 1, Fig. 4A and B n = 5).

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With the consumption of the hypercaloric diet, in the HCD group, the cumulative curve for CCh did not change the maximum effect, but the contractile potency was reduced in the presence of the blocker (E_{max} = 23.2 ± 0.9%; pEC_{50} = 6.1 ± 0.2), when compared to yours (E_{max} = 32.7 ± 7.5%; pEC_{50} = 6.6 ± 0.1) (Table 1, Fig. 4B, n = 5).

In rats that consumed the hypercaloric diet and were supplemented with 25 mg/kg of seaweed, the contractile efficacy of CCh was reduced 1.3 times in the presence of Y27632 (E_{max} = 49.1 ± 3.1%) when compared to its absence. inhibitor (E_{max} = 63.9 ± 0.9%). Similarly, the contractile potency of the agonist in the presence of Y27632 (pEC_{50} = 5.6 ± 0.1) was also altered when compared to its absence (pEC_{50} = 6.2 ± 0.1) (Table 1, Fig. 4B, n = 5).

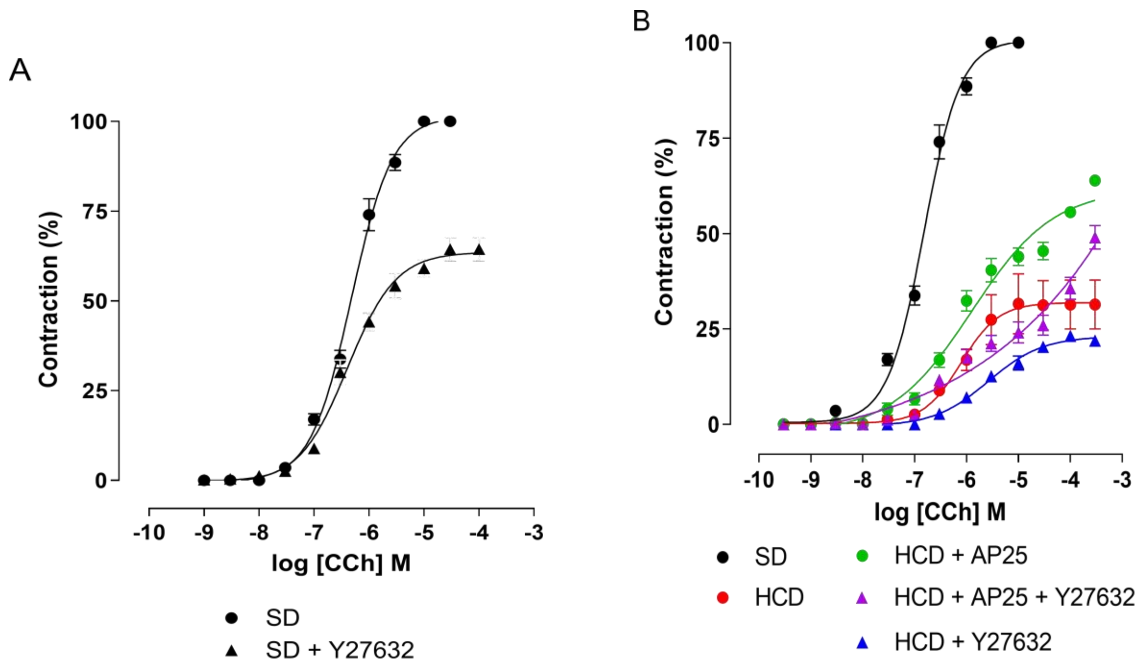

Fig. 4. Cumulative concentration-response curves to CCh in the absence (●) and in the presence of Y27632 (▲) in isolated rat ileum from SD (A); SD, HCD, HCD + Y27632, HCD + AP25 and HCD + AP25 + Y27632 groups (B). Symbols and vertical bars represent mean and S.E.M., respectively (n = 5). One-way ANOVA followed by Tukey's post-test. *$p < 0.05$, $^{\#}p < 0.05$ and $^{\&}p < 0.05$ (absence vs. Y27632) on SD, HCD and HCD + AP25, respectively. CCh = carbachol; SD = standard diet group; HCD = fed a hypercaloric diet group; HCD + AP25 = HCD group simultaneously supplemented with *Arthrospira platensis*; Y27632 = (R- (+) -trans-4-(1-aminoethyl)-N-(4-pyridyl) cyclo-hexanecarboxamide dihydrochloride.

	SD		**HCD**		**HCD + AP25**	
	Absence	**Y27632**	**Absence**	**Y27632**	**Absence**	**Y27632**
E_{max} (%)	100	64.4 ± 2.1*	32.7 ± 7.5	23.2 ± 0.9	63.9 ± 0.9	49.1 ± 3.1$^{\&}$
pCE_{50}	6.3 ± 0.05	6.0 ± 0.05	6.6 ± 0.1	6.1 ± 0.2$^{\#}$	6.2 ± 0.1	5.6 ± 0.1$^{\&}$

Table 1. E_{max} and pEC_{50} values to CCh in the absence and in the presence of Y27632 in isolated rat ileum. One-way ANOVA followed by Tukey's post-test. *$p < 0.05$, $^{\#}p < 0.05$ and $^{\&}p < 0.05$ (absence vs. Y27632) on

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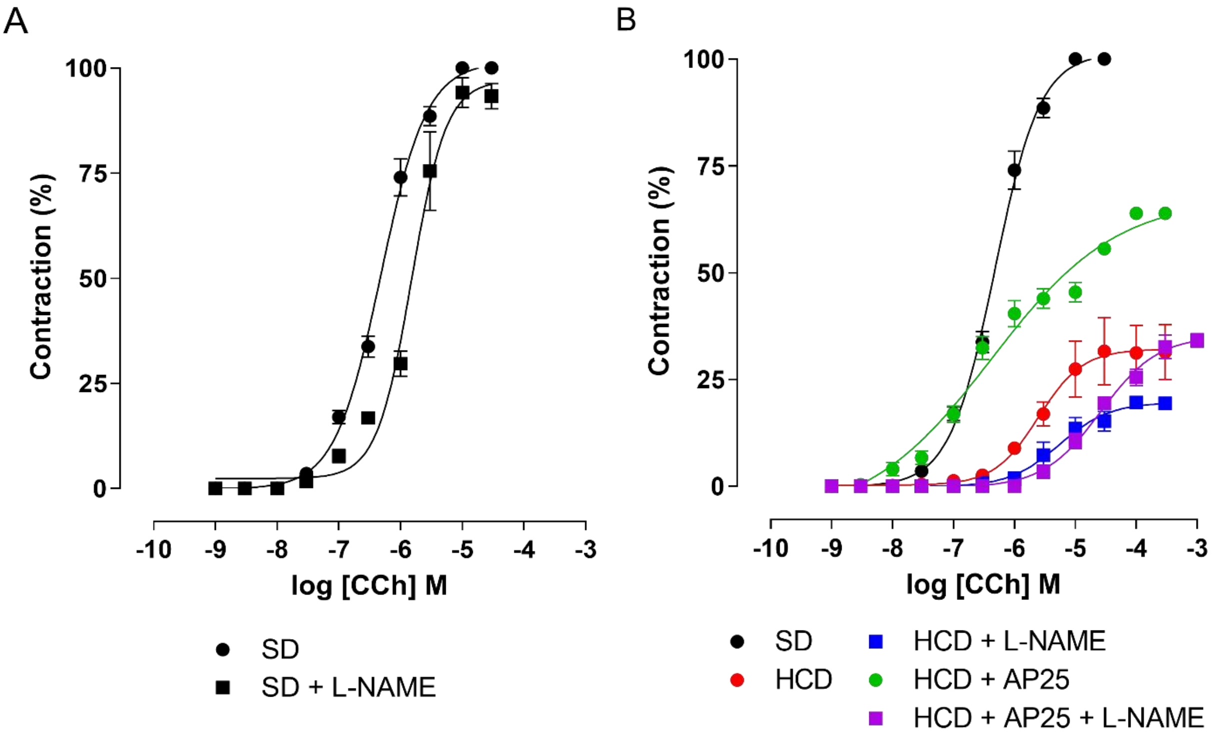
SD, HCD and HCD + AP25, respectively (n = 5). SD = standard diet group; HCD = fed a hypercaloric diet group; HCD + AP25 = HCD group simultaneously supplemented with *Arthrospira platensis*.

4.3. Effect of Arthrospira platensis supplementation on the CCh cumulative curve in the absence and presence of L-NAME

When analyzing the CCh curve in the presence of L-NAME, a non-selective NOS inhibitor, in rats fed a standard diet, it was observed that there was no change in the maximum effect (E_{max} = 94.1 ± 2.7%; pEC_{50} = 5.9 ± 0.1) when compared to the absence of this inhibitor (E_{max} = 100%; pEC_{50} = 6.3 ± 0.05) (Table 2, Fig. 5A, n = 5).

When evaluating the CCh curve in the presence of L-NAME in rats that consumed the hypercaloric diet of the HCD group, the contractile efficacy of CCh was reduced by approximately 1.7 times in the presence of L-NAME (E_{max} = 19.4 ± 1 .1%), when compared



to the absence of this inhibitor (E_{max} = 32.7 ± 7.5%). Furthermore, the contractile potency of the agonist in the presence of L-NAME was also reduced (pEC_{50} = 5.2 ± 0.2) when compared to its absence (pEC_{50} = 6.6 ± 0.1) (Table 2, Fig. 5B, n = 5).

Fig. 5. Cumulative concentration-response curves to CCh in the absence (●) and in the presence of L- NAME (▪) in isolated rat ileum from SD (A); SD, HCD, HCD + L-NAME, HCD + AP25 and HCD + AP25 + L-NAME groups (B). Symbols and vertical bars represent mean and S.E.M., respectively (n = 5). One-way ANOVA followed by Tukey's post-test. #$p < 0.05$ and &$p < 0.05$ (absence vs. L-NAME) on SD, HCD and HCD + AP25, respectively. CCh = carbachol; SD = standard diet group; HCD = fed a hypercaloric diet group; HCD + AP25 = HCD group simultaneously supplemented with *Arthrospira platensis*; L-NAME = Nω- nitro L- arginine methyl ester.

	SD		HCD		HCD + AP25	
	Absence	**L-NAME**	**Absence**	**L-NAME**	**Absence**	**L-NAME**
E_{max} (%)	100	94.1 ± 2.7	32.7 ± 7.5	19.4 ± 1.1$^{\#}$	63.9 ± 0.9	34.1 ± 1.6$^{\&}$
pCE_{50}	6.3 ± 0.05	5.9 ± 0.1	6.6 ± 0.1	5.2 ± 0.2$^{\#}$	6.2 ± 0.1	4.6 ± 0.06$^{\&}$

Table 2. E_{max} and pEC_{50} values to CCh in the absence and in the presence of L-NAME in isolated rat ileum. One-way ANOVA followed by Tukey's post-test. $^{\#}p < 0.05$ and $^{\&}p < 0.05$ (absence vs. L-NAME) on HCD and HCD + AP25, respectively (n = 5). SD = standard diet group; HCD = fed a hypercaloric diet group; HCD + AP25 = HCD group simultaneously supplemented with *Arthrospira platensis*.

It was verified that the contractile efficiency of CCh was reduced 1.8 times in the presence of L-NAME (E_{max} = 34.1 ± 1.6%) in rats fed a hypercaloric diet and supplemented with 25 mg/kg of *A. platensis* when compared to the absence of this blocker (E_{max} = 63.9 ± 0.9%). As well as reduced CCh contractile potency in the presence of the inhibitor (pEC_{50} = 4.6 ± 0.06) when compared to its absence (pEC_{50} = 6.2 ± 0.1) (Table 2, Fig. 5B, n = 5).

4.4. Effect of Arthrospira platensis supplementation on the CCh cumulative curve in the absence and presence of indomethacin

In the evaluation of the contraction induced by CCh in the presence of indomethacin, a non-selective COX blocker, in rats fed the standard diet of the SD group, it was shown that there was a reduction in the maximum effect of the cumulative curve of the agonist in the presence of the inhibitor (E_{max} = 71.4 ± 3.4%; pEC_{50} = 6.0 ± 0.01) compared to its absence (E_{max} = 100%; pEC_{50} = 6.3 ± 0.05) (Table 3, Fig. 6A, n = 5).

When analyzing the CCh curve in the presence of indomethacin in rats that consumed the hypercaloric diet of the HCD group, the contractile efficacy of CCh was not altered (E_{max} = 21.6 ± 2.2%) when compared to the absence of this inhibitor (E_{max} = 32 7 ± 7.5%), but with a change in the contractile potency of the agonist in the presence of indomethacin (pEC_{50} = 6.2 ± 0.1) (Table 3, Fig. 6B, n = 5).

In rats fed a hypercaloric diet and supplemented with 25 mg/kg of *A. platensis* in the HCD+AP25 group, the presence of indomethacin (E_{max} = 54.4 ± 2.1%) did not change the maximum effect of CCh when compared to its absence (E_{max} = 63.9 ± 0.9%). Interestingly, the contractile potency of CCh was reduced in the presence of the COX inhibitor (pEC_{50} = 7.2 ± 0.1; pEC_{50} = 5.2 ± 0.04) (Table 3, Fig. 6B, n = 5).

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4.5. Effect of Arthrospira platensis supplementation on the CCh cumulative curve in the absence and presence of apocynin

In the evaluation of the CCh curve in the presence of apocynin, an NADPH oxidase inhibitor, in rats fed the standard diet, there was a reduction both in the maximal effect and in the contractile potency of the agonist in the presence of the NOX inhibitor (E_{max} = 76 ± 2.1%; pEC_{50} = 5.9 ± 0.05) when compared to the curve in the absence of apocynin (E_{max} = 100%; pEC_{50} = 6.3 ± 0.05) (Table 4, Fig. 7A, n = 5).

When analyzing the CCh curve in the presence of apocynin in rats from the HCD group, both contractile efficiency and potency were reduced in the presence of the inhibitor (E_{max} = 16.7 ± 1.4%; pEC_{50} = 5.1 ± 0.2) when compared to the absence (E_{max} = 32.7 ± 7.5%; pEC_{50} = 6.6 ± 0.1) (Table 4, Fig. 7B, n = 5).

In rats fed a hypercaloric diet and supplemented with 25 mg/kg of *A. platensis* in the HCD+AP25 group, the presence of apocynin (E_{max} =40.9 ± 2.5%) reduced the maximum effect of CCh by 1.6 times when compared to its absence (E_{max} = 63.9 ± 0.9%). Similarly, the contractile potency of CCh was also reduced in the presence of the NOX inhibitor (pEC_{50} = 6.2 ± 0.1; pEC_{50} = 4.8 ± 0.1) (Table 4, Fig. 7B, n = 5).

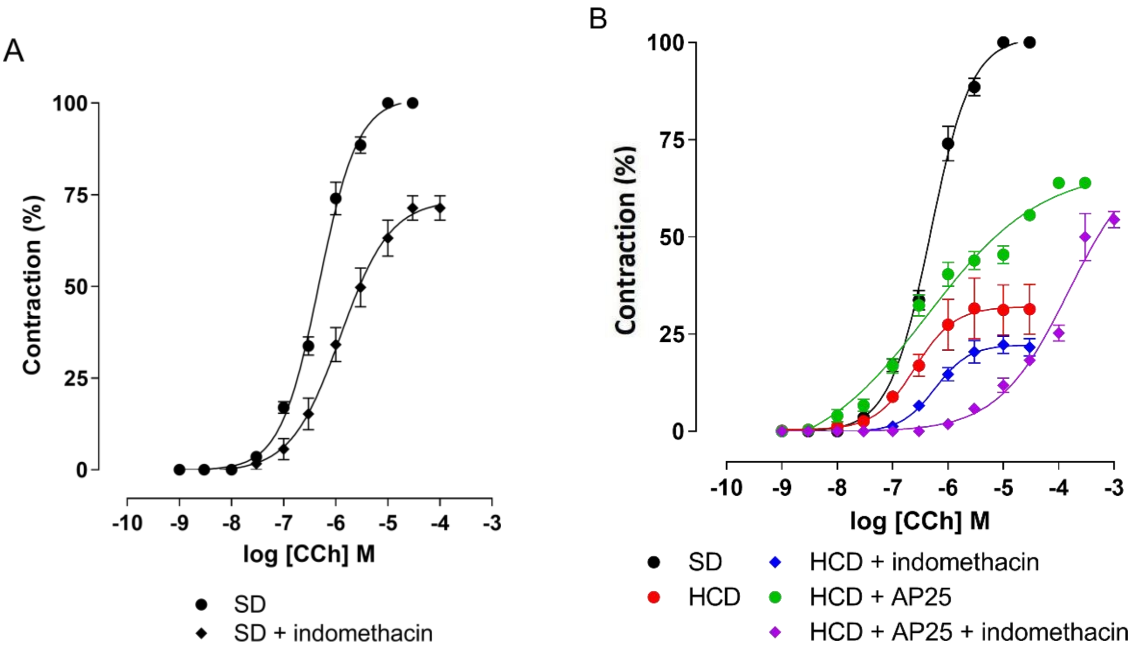

Fig. 6. Cumulative concentration-response curves to CCh in the absence (●) and in the presence of indomethacin (✦) in isolated rat ileum from SD (A); SD, HCD, HCD + indomethacin, HCD + AP25 and HCD + AP25 + indomethacin groups (B). Symbols and vertical bars represent mean and S.E.M., respectively (n = 5). One-way ANOVA followed by Tukey's post-test. *p < 0.05, #p < 0.05 and &p < 0.05 (absence vs. indomethacin) on SD, HCD and HCD + AP25, respectively. CCh = carbachol; SD = standard diet group;

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HCD = fed a hypercaloric diet group; HCD + AP25 = HCD group simultaneously supplemented with *Arthrospira platensis*.

	SD		HCD		HCD + AP25	
	Absence	**Indomethacin**	**Absence**	**Indomethacin**	**Absence**	**Indomethacin**
E_{max} (%)	100	71.4 ± 3.4*	32.7 ± 7.5	21.6 ± 2.2	63.9 ± 0.9	54.4 ± 2.1
pCE_{50}	6.3 ± 0.05	6.0 ± 0.01	6.6 ± 0.1	$6.2 \pm 0.1^{\#}$	6.2 ± 0.1	$5.2 \pm 0.04^{\&}$

Table 3. E_{max} and pEC_{50} values to CCh in the absence and in the presence of indomethacin in isolated rat ileum. One-way ANOVA followed by Tukey's post-test. *p < 0.05, $^{\#}p < 0.05$ and $^{\&}p < 0.05$ (absence vs. indomethacin) on SD, HCD and HCD + AP25, respectively (n = 5). SD = standard diet group; HCD = fed a hypercaloric diet group; HCD + AP25 = HCD group simultaneously supplemented with *Arthrospira platensis*.

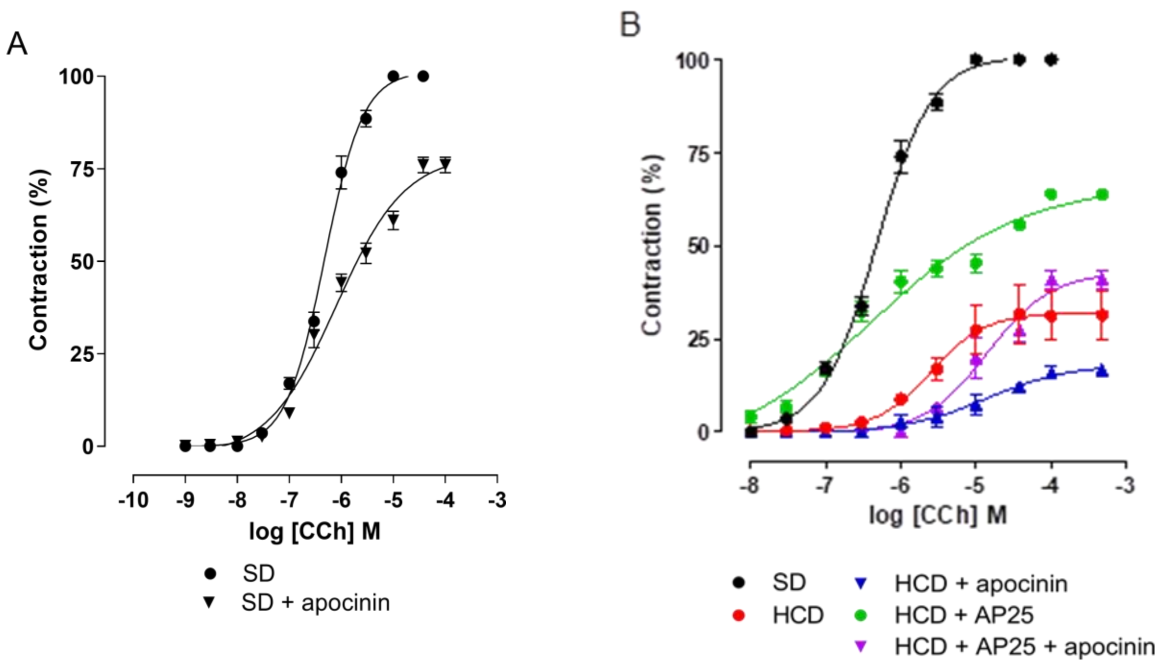

Fig. 7. Cumulative concentration-response curves to CCh in the absence (●) and in the presence of apocynin (▼) in isolated rat ileum from SD (A); SD, HCD, HCD + apocynin, HCD + AP25 and HCD + AP25 + apocynin groups (B). Symbols and vertical bars represent mean and S.E.M., respectively (n = 5). One- way ANOVA followed by Tukey's post-test. *p < 0.05, $^{\#}p < 0.05$ and $^{\&}p < 0.05$ (absence vs. apocynin) on SD, HCD and HCD + AP25, respectively. CCh = carbachol; SD = standard diet group; HCD = fed a hypercaloric diet group; HCD + AP25 = HCD group simultaneously supplemented with *Arthrospira platensis*.

	SD		HCD		HCD + AP25	
	Absence	**Apocynin**	**Absence**	**Apocynin**	**Absence**	**Apocynin**
E_{max} (%)	100	76.0 ± 2.1*	32.7 ± 7.5	$16.7 \pm 1.4^{\#}$	63.9 ± 0.9	$40.9 \pm 2.5^{\&}$
pCE_{50}	6.3 ± 0.05	5.9 ± 0.05	6.6 ± 0.1	$5.1 \pm 0.2^{\#}$	6.2 ± 0.1	$4.8 \pm 0.1^{\&}$

Table 4. E_{max} and pEC_{50} values to CCh in the absence and in the presence of apocynin in isolated rat ileum. One-way ANOVA followed by Tukey's post-test. *p < 0.05, $^{\#}p < 0.05$ and $^{\&}p < 0.05$ (absence vs. apocynin) on SD, HCD and HCD + AP25, respectively (n = 5). SD = standard diet group; HCD = fed a hypercaloric diet group; HCD + AP25 = HCD group simultaneously supplemented with *Arthrospira platensis*.

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4.6. Effect of Arthrospira platensis supplementation on the CCh cumulative curve in the absence and presence of tempol

In the evaluation of the CCh curve in the presence of tempol, a SOD minetic, in rats fed a standard diet, the effectiveness and contractile potency of CCh was not altered (E_{max} = 86.1 ± 3.2%; pEC_{50} = 6.1 ± 0.05) when compared to the curve in the absence of this mimetic (E_{max} = 100%; pEC_{50} = 6.3 ± 0.05) (Table 5, Fig. 8A, n = 5).

Additionally, the evaluation of the CCh contraction curve in the presence of tempol, in the HCD group rats, showed that there was a reduction in the contractile efficacy of this agonist (E_{max} = 11.2 ± 0.7%), with an increase in potency (pEC_{50} = 7. 2 ± 0.1) when compared with the curve in the absence of this substance (E_{max} = 32.7 ± 7.5%; pEC_{50} = 6.6 ± 0.1) (Table 5, Fig. 8B, n = 5).

Supplementation with 25 mg/kg *A. platensis* in rats fed a hypercaloric diet increased the contractile efficacy of CCh by 1.3 times (E_{max} = 84.9 ± 2.1%; pEC_{50} = 6.3 ± 0.1) when compared to the absence of this inhibitor no change in potency (E_{max} = 63.9 ± 0.9%; pEC_{50} = 6.2 ± 0.1) (Table 5, Fig. 8B, n = 5).

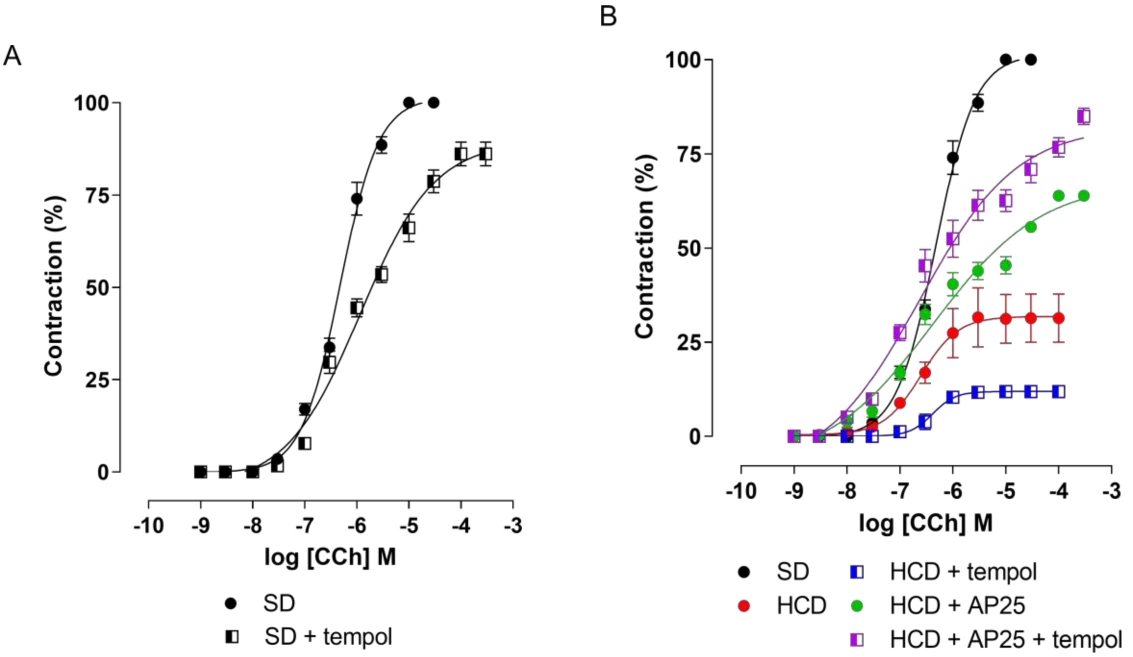

Fig. 8. Cumulative concentration-response curves to CCh in the absence (●) and in the presence of tempol (◧) in isolated rat ileum from SD (A); SD, HCD, HCD + tempol, HCD + AP25 and HCD + AP25 + tempol groups (B). Symbols and vertical bars represent mean and S.E.M., respectively (n = 5). One-way ANOVA followed by Tukey's post-test. $^{\#}p < 0.05$ and $^{\&}p < 0.05$ (absence vs. tempol) on HCD and HCD + AP25, respectively. CCh = carbachol; SD = standard diet group; HCD = fed a hypercaloric diet group; HCD + AP25 = HCD group simultaneously supplemented with *Arthrospira platensis*.

	SD		HCD		HCD + AP25	
	Absence	**Tempol**	**Absence**	**Tempol**	**Absence**	**Tempol**
E_{max} (%)	100	86.1 ± 3.2	32.7 ± 7.5	$11.2 \pm 0.7^{\#}$	63.9 ± 0.9	$84.9 \pm 2.1^{\&}$
pCE_{50}	6.3 ± 0.05	6.1 ± 0.05	6.6 ± 0.1	$7.2 \pm 0.1^{\#}$	6.2 ± 0.1	6.3 ± 0.1

Table 5. E_{max} and pEC_{50} values to CCh in the absence and in the presence of tempol in isolated rat ileum. One-way ANOVA followed by Tukey's post-test. $^{\#}p < 0.05$ and $^{\&}p < 0.05$ (absence vs. tempol) on HCD and HCD + AP25, respectively (n = 5). SD = standard diet group; HCD = fed a hypercaloric diet group; HCD + AP25 = HCD group simultaneously supplemented with *Arthrospira platensis*.

5. Discussion

The present study aimed to investigate the mechanisms of action underlying the preventive effect of supplementation with Arthrospira (Spirulina) platensis on the decrease in contractile reactivity of the ileum to carbachol induced by the consumption of a hypercaloric diet in Wistar rats. The main finding of this work was the demonstration, for the first time, that the mechanism of action by which A. platensis produces such preventive effects involves the modulation of the RhoA/ROCK pathway, nitric oxide, prostanoids, oxidative stress and activation of antioxidant signaling pathways.

Arthrospira (Spirulina) platensis is a blue-green microalgae traditionally used as a food supplement, characterized by its varied chemical and nutritional composition that determine its pharmacological activities in health promotion, such as anticancer, antioxidant, immunomodulatory, hypolipidemic and hypoglycemic, anti- inflammatory, antiviral, regulation of the intestinal microbiota and oxidative stress (FERREIRA et al., 2021; KUMAR et al., 2022; WANG et al., 2022; WANG et al., 2023; XIAOPENG, et al., 2023). Thus, it is extremely important to analyze the chemical profile of the seaweed to proceed with the elucidation of the mechanisms of action that are involved with the preventive effects on intestinal reactivity.

The determination of the chemical constituents of AP was performed using the Nuclear Magnetic Resonance (NMR) technique. The chemical profile of the AP chloroform extract shown in the spectra of figures 1 and 2 showed the predominance of gamma linolenic acid (GLA) and its derivatives in the sample. These signals compared with literature data were possible attribution to GLA (Figure 3) with main compound (JUBIE, et al. 2015). AP isa rich source of proteins, nutrients, vitamins and polyunsaturated fatty acids (PUFAs) such as GLA, a metabolite of linoleic acid (LA), which functions as a potent nutraceutical effective in

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adjuvant therapy, in the treatment and prevention of cancer, diabetes, viral infections and obesity (Zhang et al., 2013; ZHOU et al., 2021; ROUGHAN, 1989; SAJILATA et al., 2008; XIAO et al., 2011; XIONG et al., 2018; XIONG et al., 2023). Furthermore, GLA extracted from A. platensis has anti-allergic, antioxidant and anti-inflammatory activities (YANG et al., 2019). Given these assumptions and given the importance of the chemical characterization of AP evidenced by the major chemical component GLA, it is suggested the participation of this functional glycolipid in the mechanisms of action evidenced in this study.

We recently showed that the consumption of AP for 8 weeks prevents obesity and the deleterious effects caused by the hypercaloric diet on the reactivity of the ileum in rats. We suggest that the consumption of a hypercaloric diet decreases the contractile and relaxant reactivity in the ileum possibly by mechanisms of downregulation of muscarinic receptors and that supplementation with seaweed prevents the reduction of the relaxing reactivity of the ileum possibly by the positive modulation of the expression of voltage-dependent calcium channels (Ca_V) (DINIZ et al, 2021). Knowing the impact that the consumption of a hypercaloric diet promoted on the contractile reactivity of the ileum and that supplementation with 25 mg/kg of AP was potentially effective in preventing such damage, we decided to investigate which mechanisms of action could be involved.

In this context, the hypothesis was raised that the consumption of a hypercaloric diet decreases the contractile reactivity of the ileum of rats, by a decrease in CaV expression and/or negative modulation of the RhoA/ROCK pathway, and, consequently, favors intestinal disorders such as the constipation. The RhoA/ROCK molecular signaling pathway is responsible for affecting gastrointestinal smooth muscle contractility, emerging as an important mediator of intestinal endothelial contraction and dysfunction (MURTHY, 2006).In addition, a range of receptors are expressed on the surface of intestinal cells, which can activate G proteins12,13, resulting in the activation of RhoA/ROCK. Furthermore, in guinea pigs, the tonic component of intestinal smooth muscle contraction in response to CCh, LPA and ACh proved to be dependent on ROCK (SOMLYO; SOMLYO, 2000; SATISH RATTAN et al., 2010).

Therefore, to investigate the participation of the RhoA/ROCK pathway, Y27632, a ROCK inhibitor (MILLS et al., 2001), was incubated in the preparations and then cumulative contractions induced with carbachol (CCh). In the SD group, a reduction in contractileefficacy was observed (Figure 4A), demonstrating the influence of the Ca2+ sensitization pathway on intestinal smooth muscle contraction, as previously described (MURTHY, 2006). Analyzing the HCD group, in the presence of Y27632, the maximum effect and the contractile potency of CCh were not altered (Figure 4B), suggesting a possible inhibition of ROCK activity, justifying the lesser effectiveness of Y27632 in increasing the contractile reactivity of the ileum of rats fed a hypercaloric diet. Similar to what was observed in the SD group, AP supplementation in the HCD+AP25 group (Figure 4B) promoted a reduction in the contractile efficiency of CCh, in the presence of the blocker, confirming the participation of the ROCK pathway. It is inferred that PA partially attenuates the reduction in contractile efficiency by positively modulating the RhoA/ROCK pathway, activating it and/or increasing its expression.

Knowing that the attenuation of the preventive effect of the seaweed on the contractile reactivity of the ileum of rats was partially influenced by the participation of ROCK and that obesity is a determining complicating factor in the development of gastrointestinal diseases, it is necessary to investigate the involvement of other pathways of signaling, such as NO, prostanoids and oxidative stress.

In this sense, the small intestine functions as an important site of digestion, absorption and energy entry point, with nitric oxide synthase (NOS) playing an important role in these processes (ZANI; BOHLEN, 2005; COON et al., 2015). All three isoforms, constitutive and inducible, of the NOS family of enzymes are present in the small intestine, with physiological and pathophysiological roles in gastrointestinal injury and inflammation by acting as a non-adrenergic non-cholinergic inhibitory neurotransmitter (NANC) (POTOKA; NADLER; UPPERMAN, 2002; VALLANCE et al., 2004; YAN et al., 2012).

Therefore, L-NAME, a non-selective NOS inhibitor, was incubated (VIGNOZZI et al., 2006), and it was shown that there was no change in the maximum effect and contractile potency of CCh, in the presence of this inhibitor, in the SD group (Figure 5A), inferring that NOX does not participate in an effective and totalitarian way in the normal physiology of ileum contraction. In the obese group, HCD, the contractile efficacy of CCh was also not altered in the presence of L-NAME (Figure 5B). Studies that have shown that the intake of diets rich in fat and calories is associated with increased production and overexpression mainly of the iNOS protein, free radicals and ROS in the small intestine (OU et al., 2012; KOLIOS et al., 1995; SILVA; CERCHIARO; HONORIO, 2011;).

Interestingly, when analyzing the HCD+AP25 group, it is observed that in the presence of the NOS inhibitor, the contractile efficacy of CCh is reduced by approximately 2 times compared to the absence of L-NAME, confirming the participation of the NO pathway in the mechanism of preventive action of the AP. Furthermore, when comparing the contraction curve of the HCD group with HCD + AP25 + L-NAME, it can be seen that the

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preventive effect of AP is abolished (Figure 5B). Inferring that AP would be increasing the expression of endothelial NOS, this then reacts with ROS from the hypercaloric diet, leading to the formation of contractile oxidative radicals such as peroxynitrite (ONOO-), whichfavored the contractile effect of CCh (JOHNSON et al.., 2011; XIONG et al., 2023). These results show the preventive effect of AP through the positive modulation of NOS, an effect directly related to the potent protective role of AP in the processes of nutrient absorption, motility, inflammation and intestinal permeability.

Endothelial dysfunction during the development and worsening of obesity is characterized by alterations in arachidonic acid metabolism that lead to an imbalance between the production of contractile and relaxing prostanoids, by both COX-1 and COX-2, underlying the abnormal functioning of muscle reactivity smooth cells of rats and mice (GOODWILL; JAMES; FRISBEE, 2008; XIANG et al., 2008; LOBATO et al., 2011).

The modulation of the prostanoid pathway on contractility in the ileum was evaluated using indomethacin, a non-selective COX inhibitor (CARTLEDGE; EARDLEY;MORRISON, 2000). In SD group rats, the CCh contraction curve was reduced in the presenceof the inhibitor (Figure 6A), suggesting that the contractile reactivity of the rat ileum depends on contractile prostanoids. However, the consumption of a hypercaloric diet, in the HCD group, did not alter the contractile efficacy of CCh in the presence of indomethacin (Figure 6B), suggesting a negative modulation of the diet on the prostanoid pathway.

In rats supplemented with *A. platensis* (HCD + AP25), the presence of indomethacin (Figure 6B) reduced the effectiveness and contractile potency of CCh when compared to its absence, confirming the involvement of the COX pathway in AP-mediated contraction. Analyzing these results, it is demonstrated that the same concentration of the inhibitor was less effective in reducing the contraction induced by CCh. Inferring that the seaweed promotes an increase in the synthesis of contractile prostanoids. In this context, *A. platensis* prevents alterations through the arachidonic acid pathway by producing contractile prostanoids, preventing the reduction of contractile reactivity caused by the hypercaloric diet. This result can be explained by the nutritional composition of the seaweed, rich source of GLA, precursor of prostaglandins E_1 and E_2, $PGF_{2\alpha}$, TxA_2 (ZIBOH, 1989), as well as several bioactive signaling molecules with pro-inflammatory and anti-inflammatory characteristics, in addition to is involved in the NF-Kb inflammatory signaling pathway, through cyclooxygenase and lipoxygenase reactions (INNES; CALDER, 2018; YANG at al., 2019). GLA alters the metabolism of COX and ROS and involves activation of caspases (COLQUHOUN; SCHUMACHER, 2001).

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Considering the influence of oxidative stress and the importance of antioxidant systems in the contractile responsiveness of the rat ileum. In obesity, intestinal dysfunction plays an important role in mediating the systemic inflammatory response syndrome, mainly through the exacerbated release of ROS (ANDERSEN et al., 2017). Nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOXs) are the only enzymes exclusively dedicated to the production of ROS, mainly the superoxide anion (O2 • −), in vascular tissues,which play an essential role in intestinal functions (BRANDES; KREUZER, 2005; KONIOR et al., 2014; AVIELLO; KNAUS, 2018).

For this, apocynin, an inhibitor of NADPH oxidase was used (SOVARI; MORITA; KARAGUEUZIAN, 2008). In SD group rats, the contractile efficacy of CCh, in the presence of this agonist, was reduced (Figure 7A), demonstrating the influence of O_2^-, produced by the NADPH oxidase complex, on rat ileum contraction. During physiological steady-state conditions in the gut, NADPH oxidase in epithelial cells are the primary sources of ROS/RNS, producing superoxide ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) (LETO; GEISZT, 2006; BEDARD; KRAUSE, 2007; FARIA; FORTUNATO, 2020; WU et al., 2020). Additionally, the HCD group rats, which consumed the hypercaloric diet, in the presence of apocynin, the contraction curve was reduced by approximately 2 times in relation to the absence of this enzymatic inhibitor. Confirming, therefore, the production of superoxide anion from the hypercaloric diet and that by blocking the NADPH oxidase complex pathway, the finalresulting contraction will be even more difficult (Figure 7B). Previously, such results corroborate our studies in which the deleterious effect of high-calorie diet consumption on oxidative stress parameters (MDA, MPO, SOD, CAT, GSSH) was evidenced (DINIZ et al., 2023).

In the HCD + AP25 + apocynin group, the effect and contractile power of CCh were reduced in relation to the absence of this inhibitor, inferring that due to the decrease in O_2^- production, due to the presence of the blocker, the formation of $ONOO^-$ was reduced, and consequently hindered intestinal contraction. Confirming the participation of the NADPH oxidase complex on the preventive effects of AP in the ileum of obese rats, suggesting a positive modulation of AP on this enzymatic complex. HATANAKA et al. (2006), demonstrated the systematic effect of ROS production induced by GLA in human and rat neutrophils.

The consumption of a hypercaloric diet favored the increase in ROS, previously evidenced by the increase in O2- production from the enzymatic complex NADPH oxidase. Furthermore, we previously demonstrated that the hypercaloric diet impaired the production of superoxide dismutase (SOD) (DINIZ et al., 2023). Given these assumptions, the possible participation of SOD in the preventive effects of PA on intestinal contraction in obese rats was investigated. For this, tempol was used, a SOD mimetic (PEIXOTO et al., 2009). In rats fed the standard diet, SD group, the contractile efficacy was not altered in the presence of SOD (Figure 8A), confirming that in the basal physiology of contraction of the ileum, healthy rats, the production of O2- is lower. The presence of tempol reduced the maximum contraction effect of CCh in the obese rats of the HCD group (Figure 8B). Therefore, suggesting that the hypercaloric diet is exacerbating the superoxide anion production pattern, and that the antioxidant action of the enzymatic presence of SOD in the medium is being effective in combating this ROS. Furthermore, we have previously demonstrated that the hypercaloric diet through the production of O_2^- is responsible for promoting apoptosis of the muscle layer and loss of functionality in the ileum of obese rats (DINIZ et al., 2021).

In the AP-supplemented group of the HCD+AP25 group, the maximum contraction effect was increased in the presence of tempol. Confirming the participation of SOD in the preventive effect of PA (Figure 8B). It was previously shown that PA prevents the decrease in SOD caused by a hypercaloric diet, suggesting its role as a donor and activator of this enzymatic antioxidant (DINIZ et al., 2023). SOD, by neutralizing the actions of O_2^- from the hypercaloric diet, promotes the production of H_2O_2, which leads to the oxidation of thiol groups (RSH) of cysteines (FINKEL, 2001) and consequent direct redox activation of signaling pathways that favor intestinal contractility (MAPK/PKC) (NAKASHIMA et al., 2002; CORCORAN; COTTER, 2013; WANG et al., 2018). In view of these results, it is suggested that *A. platensis* acts by preventing the harmful effects caused by the hypercaloric diet on contractile reactivity in the ileum of obese rats, with the effective participation of all molecular pathways investigated in this study, positively modulating them. Emerging as a potential phytotherapeutic candidate in the prevention and treatment of intestinal diseases and disorders, associated with intestinal reactivity, permeability, absorption and motility.

5. Conclusions

In summary, consumption of a hypercaloric diet for 8 weeks further aggravated the damage to contractile reactivity in the ileum of rats. However, with this study, we showed the potential preventive role of food supplementation with *A. platensis* at a dose of 25 mg/kg, on intestinal damage induced by HCD, through the positive modulation of molecular signaling pathways: ROCK; prostanoids; nitric oxide; superoxide dismutase; NADPH oxidase complex. It was concluded that the integration of pathways that favor intestinal contraction in the ileum

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may underlie the nutritional and chemical composition of the PA, mostly composed of GLA (Figure 9). Thus, *A. platensis* emerges as a candidate for herbal medicine, adjuvant in the prevention of obesity and diseases and/or intestinal disorders aggravated by it.

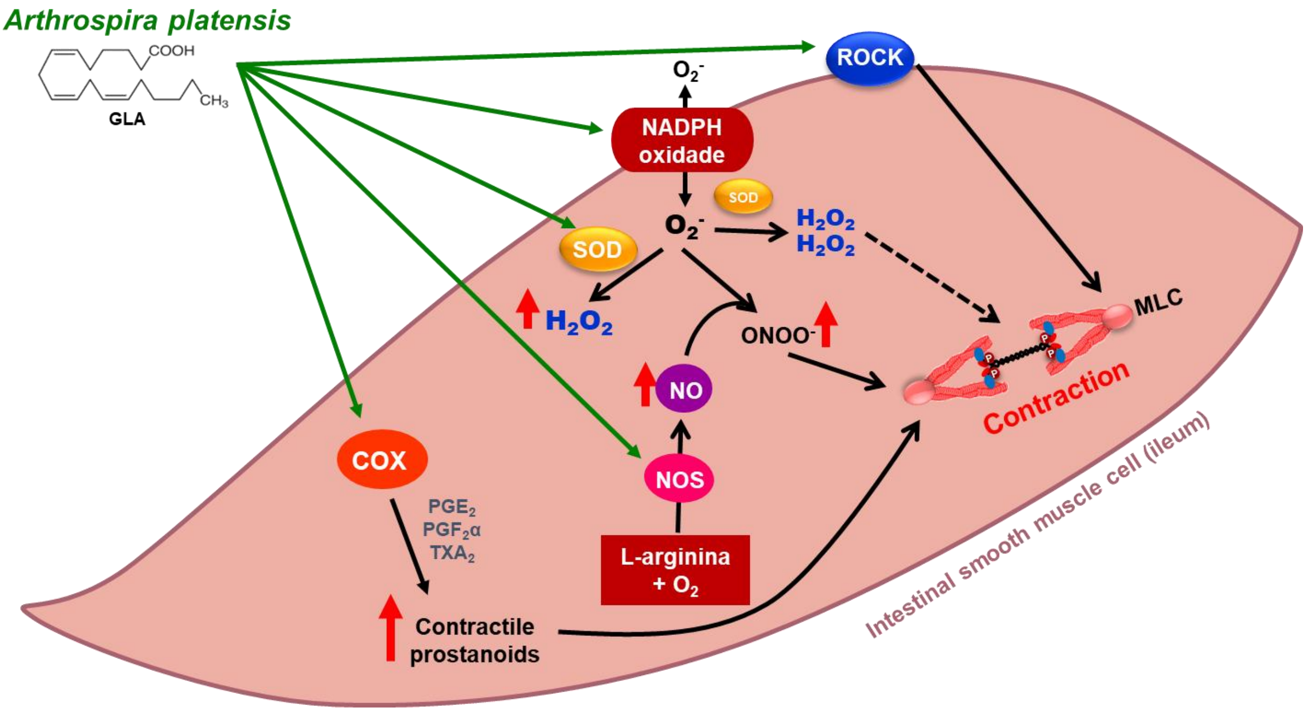

Fig. 9. Conceptual map. *Arthrospira (Spirulina) platensis* prevents the damage caused by the consumption of a hypercaloric diet on the intestinal contractile reactivity of the ileum of rats through the positive modulation of molecular signaling pathways: 1. prostanoid pathway (COX); 2. nitric oxide (NO) pathway; 3. superoxide dismutase (SOD); 4. NADPH oxidase complex (NOX); 5. via da ROCK. Activation of these pathways will have the final effect of increasing intestinal contractile reactivity, resulting in increased contraction that had been reduced by the hypercaloric diet.

6. Institutional review board statement

All work protocols were approved by the Ethics Committee on the Use of Animals of UFPB nº 2352101019) and followed the norms for the ethical use of animals of the National Council for Experimental Control Animals (Brazil).

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Author contributions

A.F.A.D. and B.A.d.S. developed the hypothesis and experimental design. A.F.A.D., P.B.F. and B.C.B. analyzed the data and wrote the manuscript. A.F.A.D., B.F.O.C., D.M.C.F., J.M.A.d.S., M.K.d.N.M. and F.F.L.J. performed the experimental work. L.S.A., Y.M.N. and J.F.T. performed the RMN work. M.d.C.C.S. contributed to the conceptual map design and corrections. All authors have read and agreed to the published version of the manuscript.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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6 Conclusões

Na avaliação da suplementação alimentar com a *Arthrospira* (*Spirulina) platensis* em ratos Wistar que consumiram a dieta hipercalórica, conclui-se que os efeitos danosos provenientes do consumo desta dieta são prevenidos, uma vez que a alga promove:

- - Melhora dos parâmetros de obesidade experimental;
- - Aumento da eficácia e potência relaxante do íleo;
- - Aumento da eficácia contrátil intestinal (*downregulation* dos receptores muscarínicos);
- - Redução do estresse oxidativo ileal;
- - Melhora das defesas antioxidantes;
- - Redução do perfil inflamatório;
- - Preservação do ambiente histomorfológico intestinal

O mecanismo de ação plelo qual a *A. platensis* previne os efeitos deletérios causados pela dieta hipercalórica envolve a modulação positiva das vias de sinalização do(a)s:

- sensibilização ao cálcio (RhoA-ROCK).
- óxido nítrico (NO);
- prostanóides;
- Superóxido dismutase (SOD);
- Complexo NADPH oxidase (NOX);

Tomados em conjunto, esses resultados reforçam a hipótese de que a *Arthrospira* (*Spirulina*) *platensis* é uma forte candidata a um medicamentofitoterápico para tratar, preventivamente, a obesidade e as doenças intestinais associadas.

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Anexos

ANEXO A – CERTIFICADO DE ANÁLISE DA *Arthrospira* (*Spirulina*) *platensis*.

1

CERTIFICADO DE ANÁLISE

Insumo:	Espirulina 60%	**Data de Análise:**	27-10-2017
Lote Interno:	17J11-B004-025041	**Lote Fabricante:**	SP17132
Data de Fabricação:	19-06-2017	**Data de Validade:**	18-06-2020
Origem:	China	**Procedência:**	Hong Kong
Condições de Armazenamento:	Temperatura Ambiente	**Ordem de Fracionamento:**	025041

DCB:	-		**DCI:**	-	
CAS:	-		**Peso Molecular:**	-	
Fórmula Molecular:	-				
Observações:	**Parte Utilizada:**	Toda a planta	**Nome Científico:**	Spirulina platensis	

Testes	Especificações	Resultados	Unidade	Referências
Descrição *	Po fino verde, com leve odor de algas	Conforme		Fabricante
Sabor	Leve de algas	Conforme		Fabricante
Perda por Dessecação *	<= 8,0% (2g/ 105ºC/ Peso constante)	4,44	%	Fabricante
Proteina	>= 60	65,3	%	Fabricante
Cinzas totais *	<= 8,0	6,83	%	Fabricante
Total Carotenoides *	>= 0,3	0,30	g/100g	Fabricante
Arsênio *	<= 1,0	0,081	ppm	Fabricante
Chumbo *	<= 2,0	Não detectado	ppm	Fabricante
Cádmio *	<= 0,1	0,012	ppm	Fabricante
Mercúrio	<= 0,1	Conforme	ppm	Fabricante
Granulometria	100 passa malha 80	Conforme	%	Fabricante
Densidade Aparente *	+ ou - 0,5	0,47	g/mL	Fabricante
Testes Microbiológicos				
Contagem total de bactérias *	<= 100000	< 1000	UFC/g	Fabricante
Fungos e Leveduras *	<= 300	< 10	UFC/g	Fabricante
Salmonella *	Negativo	Negativo		Fabricante
Echerichia coli *	Negativo	Negativo		Fabricante
Coliformes	<= 10	< 10	UFC/g	Fabricante
Staphylococcus *	Negativo	Negativo		Fabricante

(Continua)

(Continuação)

infinity pharma

2

CERTIFICADO DE ANÁLISE

Insumo:	Espirulina 60%	**Data de Análise:**	27-10-2017
Lote Interno:	17J11-B004-025041	**Lote Fabricante:**	SP17132
Data de Fabricação:	19-06-2017	**Data de Validade:**	18-06-2020
Origem:	China	**Procedência:**	Hong Kong
Condições de Armazenamento:	Temperatura Ambiente	**Ordem de Fracionamento:**	025041

DCB:	-		**DCI:**		-
CAS:	-		**Peso Molecular:**		-
Fórmula Molecular:	-				
Observações:	**Parte Utilizada:**	Toda a planta	**Nome Científico:**	Spirulina platensis	

* Resultados obtidos em análises realizadas no Laboratório de Controle de Qualidade SM EMPREENDIMENTOS FARMACÊUTICOS LTDA. E os demais foram transcritos conforme certificado de análise do fabricante.

Conclusão:

Aprovado (X)

Reprovado ( )

Responsável Técnico
João Paulo Sartin Mendes
CRF-GO: Nº 7355

Responsável Técnico Substituto
Olívia Neiva Mesquita Mendes
CRF-GO: Nº 5227

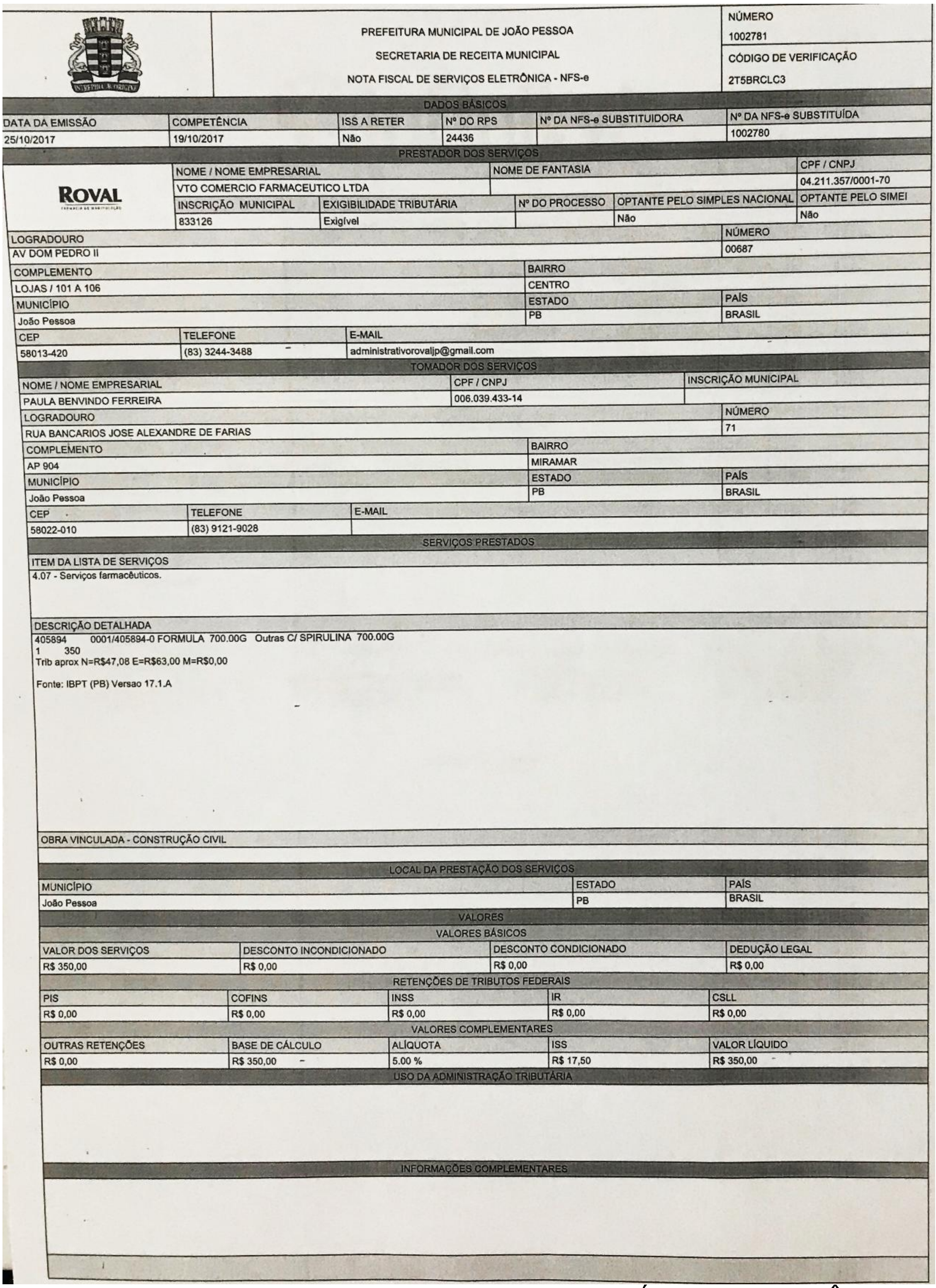
Responsável Técnico
Ana Ligia Boer Barbosa
CRF-SP: 72.656

Responsável Técnico
Eliene Ribeiro de Lima
CRF-GO: Nº 11.361

Fim do Documento

Fonte: Laboratório de Controle de Qualidade SM EMPREENDIMENTOS FARMACÊUTICOSLTDA, 2017.

ANEXO B – CÓPIA DO COMPROVANTE DE AQUISIÇÃO DO PÓ DA *Arthrospira* (*Spirulina*) *platensis*.



PREFEITURA MUNICIPAL DE JOÃO PESSOA
SECRETARIA DE RECEITA MUNICIPAL
NOTA FISCAL DE SERVIÇOS ELETRÔNICA - NFS-e

NÚMERO: 1002781
CÓDIGO DE VERIFICAÇÃO: 2T5BRCLC3

DADOS BÁSICOS

DATA DA EMISSÃO	COMPETÊNCIA	ISS A RETER	Nº DO RPS	Nº DA NFS-e SUBSTITUIDORA	Nº DA NFS-e SUBSTITUÍDA
25/10/2017	19/10/2017	Não	24436		1002780

PRESTADOR DOS SERVIÇOS

ROVAL

NOME / NOME EMPRESARIAL	NOME DE FANTASIA	CPF / CNPJ
VTO COMERCIO FARMACEUTICO LTDA		04.211.357/0001-70

INSCRIÇÃO MUNICIPAL	EXIGIBILIDADE TRIBUTÁRIA	Nº DO PROCESSO	OPTANTE PELO SIMPLES NACIONAL	OPTANTE PELO SIMEI
833126	Exigível		Não	Não

LOGRADOURO	NÚMERO
AV DOM PEDRO II	00687

COMPLEMENTO	BAIRRO
LOJAS / 101 A 106	CENTRO

MUNICÍPIO	ESTADO	PAÍS
João Pessoa	PB	BRASIL

CEP	TELEFONE	E-MAIL
58013-420	(83) 3244-3488	administrativorovaljp@gmail.com

TOMADOR DOS SERVIÇOS

NOME / NOME EMPRESARIAL	CPF / CNPJ	INSCRIÇÃO MUNICIPAL
PAULA BENVINDO FERREIRA	006.039.433-14	

LOGRADOURO	NÚMERO
RUA BANCARIOS JOSE ALEXANDRE DE FARIAS	71

COMPLEMENTO	BAIRRO
AP 904	MIRAMAR

MUNICÍPIO	ESTADO	PAÍS
João Pessoa	PB	BRASIL

CEP	TELEFONE	E-MAIL
58022-010	(83) 9121-9028	

SERVIÇOS PRESTADOS

ITEM DA LISTA DE SERVIÇOS
4.07 - Serviços farmacêuticos.

DESCRIÇÃO DETALHADA
405894 0001/405894-0 FORMULA 700.00G Outras C/ SPIRULINA 700.00G
1 350
Trib aprox N=R$47,08 E=R$63,00 M=R$0,00

Fonte: IBPT (PB) Versao 17.1.A

OBRA VINCULADA - CONSTRUÇÃO CIVIL

LOCAL DA PRESTAÇÃO DOS SERVIÇOS

MUNICÍPIO	ESTADO	PAÍS
João Pessoa	PB	BRASIL

VALORES

VALORES BÁSICOS

VALOR DOS SERVIÇOS	DESCONTO INCONDICIONADO	DESCONTO CONDICIONADO	DEDUÇÃO LEGAL
R$ 350,00	R$ 0,00	R$ 0,00	R$ 0,00

RETENÇÕES DE TRIBUTOS FEDERAIS

PIS	COFINS	INSS	IR	CSLL
R$ 0,00	R$ 0,00	R$ 0,00	R$ 0,00	R$ 0,00

VALORES COMPLEMENTARES

OUTRAS RETENÇÕES	BASE DE CÁLCULO	ALÍQUOTA	ISS	VALOR LÍQUIDO
R$ 0,00	R$ 350,00	5.00 %	R$ 17,50	R$ 350,00

USO DA ADMINISTRAÇÃO TRIBUTÁRIA

INFORMAÇÕES COMPLEMENTARES

Fonte: Laboratório de Controle de Qualidade VTO COMÉRCIO FARMACÊUTICO LTDA, 2017.

ANEXO C – CÓPIA DA CERTIDÃO DE APROVAÇÃO DO PROJETO JUNTO À CEUA.

Universidade Federal da Paraíba

Comissão de Ética no Uso de Animais
Reitoria

CERTIFICADO

Certificamos que a proposta intitulada "Avaliação do mecanismo de ação da Spirulina platensis na reatividade contrátil e relaxante do íleo de ratos Wistar submetidos à dieta hipercalórica ", protocolada sob o CEUA nº 6061090318 (ID 000247), sob a responsabilidade de **Bagnólia Araújo Costa** *e equipe; Anderson Fellyp Avelino Diniz* - 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 da Paraíba (CEUA/UFPB) na reunião de 06/07/2018.

We certify that the proposal "Evaluation of the mechanism of action of Spirulina platensis on the contractile and relaxing reactivity of the ileum of Wistar rats submitted to the hypercaloric diet", utilizing 128 Heterogenics rats (128 males), protocol number CEUA 6061090318 (ID 000247), under the responsibility of **Bagnólia Araújo Costa** *and team; Anderson Fellyp Avelino Diniz* - 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 Paraíba (CEUA/UFPB) in the meeting of 07/06/2018.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 04/2018 a 02/2020 Área: Ciências Farmacêuticas

Origem:	Unidade de Produção Animal - IPeFarM						
Espécie:	Ratos heterogênicos	sexo:	Machos	idade:	8 a 8 semanas	N:	128
Linhagem:	Rattus Norvegicus - Wistar			Peso:	150 a 160 g		

Local do experimento: O citado projeto será realizado no Laboratório de Farmacologia Funcional (LFF) e na Unidade de Produção Animal, localizados no Instituto de Pesquisa em Fármacos e Medicamentos (IPeFarM) onde funciona o Programa de Pós-Graduação em Produtos Naturais e Sintéticos Bioativos, do Centro de Ciências da Saúde (CCS/UFPB), todos da Universidade Federal da Paraíba (UFPB), sob responsabilidade da orientadora.

João Pessoa, 06 de julho de 2018

Islania Gisela A. Gonçalves

Profa. Dra. Islania Gisela Albuquerque Gonçalves
Coordenadora da Comissão de Ética no Uso de Animais
Universidade Federal da Paraíba

Prof. Dr. Ricardo Romão Guerra
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade Federal da Paraíba

Cidade Universitária, s/n - Castelo Branco III Prédio da Reitoria - Gabinete da Vice-reitoria - CEP 58051-085 - João Pessoa/PB - tel: 55 (83) 3216-7155
Horário de atendimento: 2ª a 6ª das 14 às 17h : e-mail: ceua@ufpb.br
CEUA N 6061090318

Fonte: Comissão de Ética no Uso de Animais-UFPB, 2018.

ANEXO D – ARTIGO PUBLICADO EM REVISTA QUALIS A1 COM FI: 6,706.

nutrients
MDPI

Article

Supplementation with *Spirulina platensis* Prevents Uterine Diseases Related to Muscle Reactivity and Oxidative Stress in Rats Undergoing Strength Training

Paula Benvindo Ferreira [1], Anderson Fellyp Avelino Diniz [1], Francisco Fernandes Lacerda Júnior [1], Maria da Conceição Correia Silva [1], Glêbia Alexa Cardoso [2], Alexandre Sérgio Silva [2] and Bagnólia Araújo da Silva [3,*]

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Abstract: Strength training increases systemic oxygen consumption, causing the excessive generation of reactive oxygen species, which in turn, provokes oxidative stress reactions and cellular processes that induce uterine contraction. The aim of this study was to evaluate the possible protective effect of *Spirulina platensis* (SP), an antioxidant blue algae, on the contractile and relaxation reactivity of rat uterus and the balance of oxidative stress/antioxidant defenses. Female Wistar rats were divided into sedentary (CG), trained (TG), and T + supplemented (TG50, TG100) groups. Reactivity was analyzed by AQCAD, oxidative stress was evaluated by the malondialdehyde (MDA) formation, and the antioxidant capacity was measured by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Strength training increased contractile reactivity and decreased the pharmaco-mechanical component of relaxing reactivity in rat uterus. In addition, training decreased oxidation inhibition in the plasma and exercise increased oxidative stress in the uterine tissue; however, supplementation with algae prevented this effect and potentiated the increase in antioxidant capacity. Therefore, this study demonstrated that food supplementation prevents changes in reactivity and oxidative stress induced by strength training in a rat uterus, showing for the first time, that the uterus is a target for this exercise modality and antioxidant supplementation with *S. platensis* is an alternative means of preventing uterine dysfunction.

Keywords: *Spirulina platensis*; physical exercise; uterus; oxidative stress; muscle reactivity

Citation: Ferreira, P.B.; Diniz, A.F.A.; Lacerda Júnior, F.F.; Silva, M.d.C.C.; Cardoso, G.A.; Silva, A.S.; da Silva, B.A. Supplementation with *Spirulina platensis* Prevents Uterine Diseases Related to Muscle Reactivity and Oxidative Stress in Rats Undergoing Strength Training. *Nutrients* **2021**, *13*, 3763. https://doi.org/10.3390/nu13113763

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1. Introduction

Regular training has numerous beneficial effects on human health through the induction of homeostatic adaptations in different physiological systems such as the cardiorespiratory and muscle systems [1]. However, the magnitude of the effect of a specific training regime can vary significantly between individuals, as well as in individuals undergoing training who may not respond as expected [2]. This is due to factors such as the characteristics of the training regime, environmental conditions and individual factors, such as habitual physical activity, previous physical fitness level, genetics, psychological factors, age and sex [3].

In the past few decades, women have become increasingly physically active and evidence demonstrates that physical training can increase self-esteem, cardiorespiratory fitness, ovulation, and menstrual regularity while decreasing insulin resistance and body

Fonte: Revista Nutrients, 2021.

ANEXO E – ARTIGO PUBLICADO EM REVISTA QUALIS A1 COM FI: 5,988.

frontiers in Pharmacology

REVIEW
published: 24 July 2020
doi: 10.3389/fphar.2020.01120

Ionic Channels as Potential Therapeutic Targets for Erectile Dysfunction: A Review

Anderson Fellyp Avelino Diniz[1†], Rafael Carlos Ferreira[1†], Iara Leão Luna de Souza[2] and Bagnolia Araujo da Silva[1*]

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Erectile dysfunction (ED) is a prevalent condition, especially in men over 40 years old, characterized by the inability to obtain and/or maintain penile erection sufficient for satisfactory sexual intercourse. Several psychological and/or organic factors are involved in the etiopathogenesis of ED. In this context, we gathered evidence of the involvement of Large-conductance, Ca^{2+}-activated K^+ channels (BK_{Ca}), Small-conductance, Ca^{2+}-activated K^+ channels (SK_{Ca}), KCNQ-encoded voltage-dependent K^+ channels (K_V7), Transient Receptor Potential channels (TRP), and Calcium-activated Chloride channels (CaCC) dysfunctions on ED. In addition, the use of modulating agents of these channels are involved in relaxation of the cavernous smooth muscle cell and, consequent penile erection, suggesting that these channels are promising therapeutic targets for the treatment of erectile dysfunction.

Keywords: canalopathies, erectile dysfunction, Ca^{2+}-activated K^+ channels, KCNQ-encoded voltage-dependent K^+ channels, transient receptor potential channels, calcium-activated chloride channels

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INTRODUCTION

Erectile dysfunction (ED) is a persistent inability to achieve and/or maintain a penile erection enough for satisfactory sexual intercourse (McCabe et al., 2016). Predominantly a vascular disorder, ED affects both physical and psychological health, having a direct impact on men's life quality and their sexual partners, mainly due to a reduction in self-esteem and impairment of interpersonal

Abbreviations: ED, Erectile dysfunction; BK_{Ca}, Large-conductance, Ca^{2+}-activated K^+ channels; TRP, Transient Receptor Potential; CaCC, Calcium-activated Chloride channels; K_V7, KCNQ-encoded voltage-dependent K^+ channels; $SK_{Ca}{}^{2+}$, Small-conductance, Ca^{2+}-activated K^+ channels; ROS, Reactive Oxygen Species; eNOS; RhoA, Endothelial nitric oxide synthase; Small G protein GTP-binder; ROCK, Rho-associated protein kinase; MS, Metabolic Syndrome; NO, Nitric Oxide; CNS, Central Nervous System; NA, Norepinephrine; NANC, Non-adrenergic, Non-cholinergic; PGI_2, Prostacyclin; $PGE_{1/2}$, Prostaglandins E type 1 and 2; CaM, Calmodulin; Ca_V, Voltage-dependent Ca^{2+} channels; AC, Adenylyl cyclase; PKA, cAMP dependent protein kinase; PKG, cGMP-dependent protein kinase; ATP, Adenosine triphosphate; GTP, Guanosine triphosphate; cAMP, Cyclic adenosine monophosphate; cGMP, Cyclic guanosine monophosphate; IP_3, Inositol 1,4,5-triphosphate; SR, Sarcoplasmic reticulum; SERCA, Sarco/endoplasmic reticulum Ca^{2+} ATPase; MLCK, Myosin light chain kinase; NCX, Na^+/Ca^{2+} exchanger; PMCA, Plasma membrane Ca^{2+}-ATPase; SHIM, Male Sexual Health Inventory; IIFE, International Index of Erectile Function; PKC, Protein kinase C; $RCK_{1/2}$, Regulator of potassium conductance; TRPA, Transient Receptor Potential Ankyrin; TRPC, Transient Receptor Potential Canonical; TRPM, Transient Receptor Potential Melastatin; TRPML, Transient Receptor Potential Mucolipine; TRPP, Transient Receptor Potential Polycystin; TRPV, Transient Receptor Potential Vanilloid; AA, Arachidonic acid; TMEM16, Transmembrane protein with unknown function 16A; DNDS, 4,4-dithitrostylbene-2,2-disulfonic acid; NFA, Niflumic acid; A9C, Anthracene-9-carboxylic acid.

Fonte: Revista Frontiers in Pharmacology, 2020.

ANEXO F – ARTIGO PUBLICADO EM REVISTA QUALIS A1 COM FI: 7,310.

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Research Article

Potential Therapeutic Role of Dietary Supplementation with *Spirulina platensis* on the Erectile Function of Obese Rats Fed a Hypercaloric Diet

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Spirulina platensis, an important source of bioactive compounds, is a multicellular, filamentous cyanobacterium rich in high-quality proteins, vitamins, minerals, and antioxidants. Due to its nutrient composition, the alga is considered a complete food and is recognized for its anti-inflammatory, antioxidant, antiobesity, and reproprotective effects. All of which are important for prevention and treatment of organic and metabolic disorders such as obesity and erectile dysfunction. The aim of this study was to investigate the modulatory role of *Spirulina platensis* food supplementation and the mechanisms of action involved in reversing the damage caused by a hypercaloric diet on the erectile function of rats. The animals were divided into a standard diet group (SD, $n = 5$); a hypercaloric diet group (HCD, $n = 5$); a hypercaloric diet group supplemented with *S. platensis* at doses of 25 (HCD+SP25, $n = 5$), 50 (HCD+SP50, $n = 5$), and 100 mg/kg (HCD+SP100, $n = 5$); and a hypercaloric diet group subsequently fed a standard diet (HCD+SD, $n = 5$). In the rats fed a hypercaloric diet, dietary supplementation with *S. platensis* effectively increased the number of erections while decreasing latency to initiate penile erection. Additionally, *S. platensis* increases NO bioavailability, reduces inflammation by reducing the release of contractile prostanoids, enhances the relaxation effect promoted by acetylcholine (ACh), restores contractile reactivity damage and cavernous relaxation, reduces reactive oxygen species (ROS), and increases cavernous total antioxidant capacity (TAC). Food supplementation with *S. platensis* thus restores erectile function in obese rats, reduces production of contractile prostanoids, reduces oxidative stress, and increases NO bioavailability. Food supplementation with *S. platensis* thus emerges as a promising new therapeutic alternative for the treatment of erectile dysfunction as induced by obesity.

Fonte: Revista Oxidative Medicine and Cellular Longevity, 2020.

ANEXO G – ARTIGO PUBLICADO EM REVISTA QUALIS A1 COM FI: 7,310.

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Research Article

Spirulina platensis Consumption Prevents Obesity and Improves the Deleterious Effects on Intestinal Reactivity in Rats Fed a Hypercaloric Diet

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The consumption of hypercaloric diets is related to the development of obesity, favoring the etiology of gastrointestinal disorders. In this context, *Spirulina platensis* (SP), some blue-green algae with antioxidant action, appears as a potential therapeutic alternative to prevent obesity and associated intestinal disorders. Thus, the present study is aimed at evaluating the deleterious effects of the hypercaloric diet on the contractile and relaxing reactivity of the ileum of rats, as well as the possible preventive mechanisms of dietary supplementation with SP. Wistar rats were divided into three groups: fed a standard diet (SD), a hypercaloric diet (HCD), and/or supplemented with 25 mg/kg SP (HCD + SP25) for 8 weeks. The hypercaloric diet was effective in promoting obesity in rats, as well as decreasing potency and ileal relaxing and contractile efficacy. In contrast, dietary supplementation with SP was able to prevent some of the parameters of experimental obesity. In addition, SP prevented the reduction of intestinal reactivity, possibly due to a positive modulation of voltage-gated calcium channels (Ca_V) and negative regulation of muscarinic receptors (M3). Thus, food supplementation with *Spirulina platensis* becomes a promising alternative in the prevention of gastrointestinal diseases induced and/or aggravated by obesity.

Fonte: Revista Oxidative Medicine and Cellular Longevity, 2021.

ANEXO H – ARTIGO PUBLICADO EM REVISTA QUALIS A1 COM FI: 6,623.

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Arthrospira platensis prevents oxidative stress and suppresses IL-1β expression in the ileum of rats fed a hypercaloric diet

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ABSTRACT

Obesity is characterized by an energy imbalance caused by caloric intake and expenditure, being a risk factor associated with a wide range of pathophysiological conditions including gastrointestinal diseases. In recent years, it has been shown that overexpression of oxidative stress and pro-inflammatory cytokines is a mechanistic link between obesity and cellular functions in animals and humans. Some natural products are reported to be effective in counteracting the adverse effects of oxidative stress. Thus, *Arthorpira platensis (AP)* stands out, an alga with anti-inflammatory and antioxidant activities. Thus, the objective is to evaluate the preventive effects of AP supplementation on oxidative stress and interleukin-1β (IL-1β) levels in the ileum of rats fed a hypercaloric diet. The rats were divided into a group fed a standard diet (SD), a hypercaloric diet (HCD) and/or fed a hypercaloric diet and supplemented simultaneously with AP 25 mg/Kg (HCD + AP25), after 8 weeks of treatment the ileum was collected for The analyses. It was observed that in the DHC group there was underproduction of antioxidant enzymes as well as overexpression of oxidative stress in the ileum of rats, interestingly this damage was prevented by the alga. Furthermore, the DHC group showed high levels of IL-1β (940.6 ± 34.5) such an increase was prevented in the HCD + AP25 group (597.2 ± 33.3). It is evident, therefore, that AP prevents the increase in oxidative stress and the inflammatory profile in the ileum of obese rats, making it a promising therapeutic alternative in the treatment of inflammatory bowel diseases aggravated by obesity.

Fonte: Revista Journal of Functional Foods, 2023.