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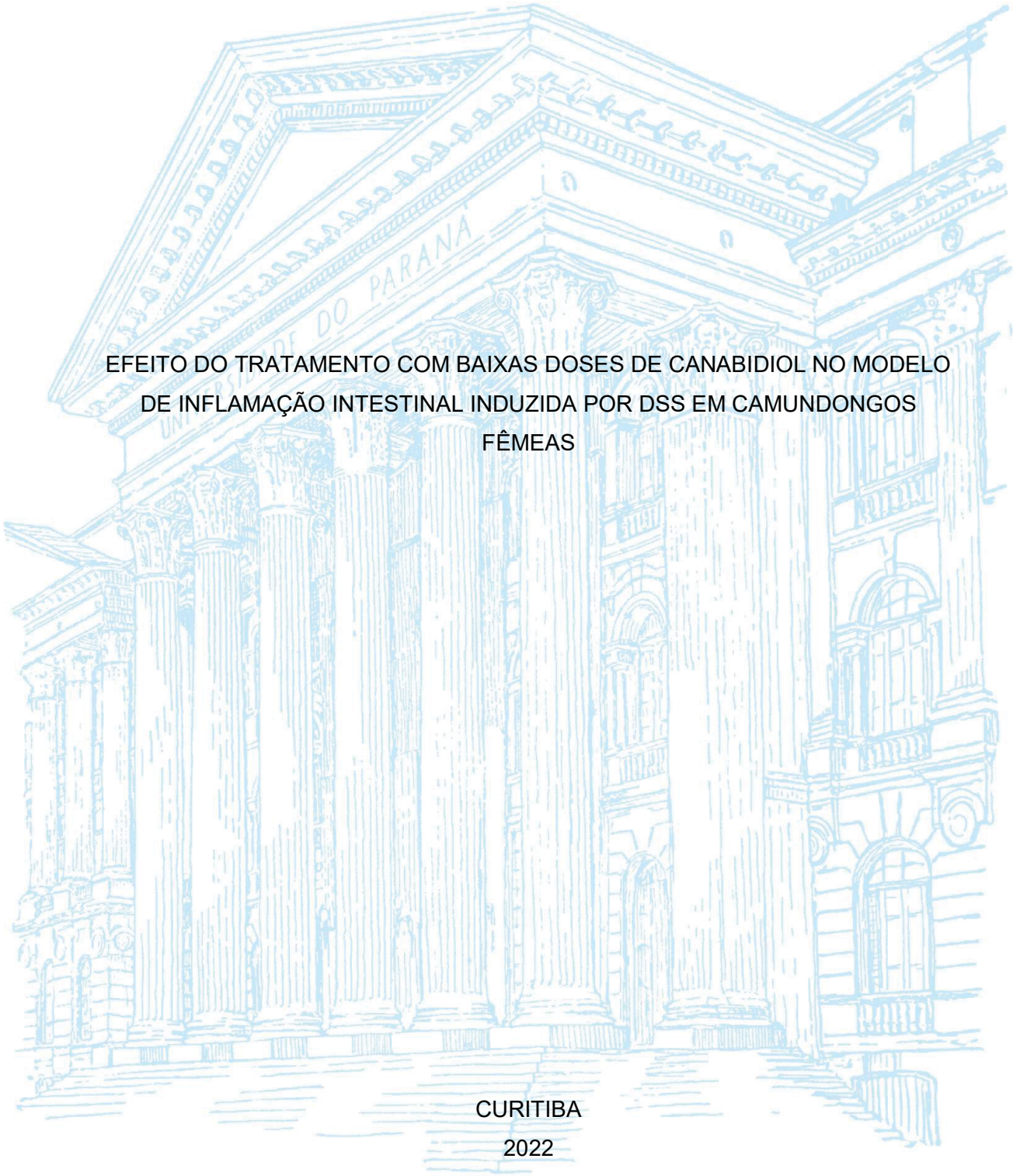
ANDRE FELIPE NAIDEK



EFEITO DO TRATAMENTO COM BAIXAS DOSES DE CANABIDIOL NO MODELO DE INFLAMAÇÃO INTESTINAL INDUZIDA POR DSS EM CAMUNDONGOS FÊMEAS

CURITIBA

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ANDRE FELIPE NAIDEK

EFEITO DO TRATAMENTO COM BAIXAS DOSES DE CANABIDIOL NO MODELO DE INFLAMAÇÃO INTESTINAL INDUZIDA POR DSS EM CAMUNDONGOS FÊMEAS

Dissertação apresentada ao curso de Pós-Graduação em Farmacologia, Setor de Ciências Biológicas, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Mestre em Farmacologia.

Orientadora: Profa. Dra. Maria Fernanda Werner
Co-Orientadora: Profa. Dra. Cristina Aparecida Jark Stern

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ATA Nº314

ATA DE SESSÃO PÚBLICA DE DEFESA DE MESTRADO PARA A OBTENÇÃO DO GRAU DE MESTRE EM FARMACOLOGIA

No dia quatro de março de dois mil e vinte e dois às 14:30 horas, na sala TEAMS, Sala virtual no TEAMS, foram instaladas as atividades pertinentes ao rito de defesa de dissertação do mestrando **ANDRE FELIPE NAIDEK**, intitulada**: Efeito do tratamento com baixas doses de canabidiol no modelo de inflamação intestinal induzida por DSS em camundongos fêmeas**, sob orientação da Profa. Dra. MARIA FERNANDA DE PAULA WERNER. A Banca Examinadora, designada pelo Colegiado do Programa de Pós-Graduação FARMACOLOGIA da Universidade Federal do Paraná, foi constituída pelos seguintes Membros: MARIA FERNANDA DE PAULA WERNER (UNIVERSIDADE FEDERAL DO PARANÁ), FERNANDO LOPES (55001394), ROBERTO ANDREATINI (UNIVERSIDADE FEDERAL DO PARANÁ). A presidência iniciou os ritos definidos pelo Colegiado do Programa e, após exarados os pareceres dos membros do comitê examinador e da respectiva contra argumentação, ocorreu a leitura do parecer final da banca examinadora, que decidiu pela APROVAÇÃO. Este resultado deverá ser homologado pelo Colegiado do programa, mediante o atendimento de todas as indicações e correções solicitadas pela banca dentro dos prazos regimentais definidos pelo programa. A outorga de título de mestre está condicionada ao atendimento de todos os requisitos e prazos determinados no regimento do Programa de Pós-Graduação. Nada mais havendo a tratar a presidência deu por encerrada a sessão, da qual eu, MARIA FERNANDA DE PAULA WERNER, lavrei a presente ata, que vai assinada por mim e pelos demais membros da Comissão Examinadora.

CURITIBA, 04 de Março de 2022.

Assinatura Eletrônica
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MARIA FERNANDA DE PAULA WERNER
Presidente da Banca Examinadora

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FERNANDO LOPES
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ROBERTO ANDREATINI
Avaliador Interno (UNIVERSIDADE FEDERAL DO PARANÁ)

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TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação FARMACOLOGIA da Universidade Federal do Paraná foram convocados para realizar a arguição da Dissertação de Mestrado de **ANDRE FELIPE NAIDEK** intitulada: **Efeito do tratamento com baixas doses de canabidiol no modelo de inflamação intestinal induzida por DSS em camundongos fêmeas**, sob orientação da Profa. Dra. MARIA FERNANDA DE PAULA WERNER, que após terem inquirido o aluno e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de mestre está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 04 de Março de 2022.

Assinatura Eletrônica
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Sou grato a todos que fizeram parte desta jornada!

NOTA EXPLICATIVA

Esta dissertação é apresentada em formato alternativo – artigo para publicação – de acordo com as normas do Programa de Pós-Graduação em Farmacologia da Universidade Federal do Paraná e do Sistema de Bibliotecas (SiBi), constando de uma Introdução, objetivos do trabalho, um artigo científico, conclusão e referências. O artigo cientifico inclui Introdução, materiais e métodos, resultados, discussão, conclusão e referências.

RESUMO

A colite ulcerativa é uma doença inflamatória intestinal (DII) que afeta o cólon, causando dor, inflamação e diarreia, podendo levar à ansiedade e depressão. Devido à falta de tratamentos eficazes para a colite ulcerativa, são necessários estudos em busca de novos tratamentos. Estudos sugerem que o canabidiol (CBD), um fitocanabinóide, tem efeitos analgésicos, anti-inflamatórios, antioxidantes e ansiolíticos. Este estudo investigou se tratamento com baixas doses de CBD é capaz aliviar os sinais colônicos da colite experimental e melhorar o bem-estar dos animais, através dos possíveis efeitos do CBD. A colite ulcerativa foi induzida com 5% de Dextran Sulfato de Sódio (DSS) na água potável durante 5 dias, sendo substituído por somente água potável nos últimos 2 dias. Camundongos fêmeas foram tratados oralmente com CBD (0,01 mg/kg, 0,1 mg/kg e 1 mg/kg) durante os sete dias de experimentos. A hipersensibilidade abdominal mecânica foi medida com os filamentos de Von Frey em dias intercalados. Labirinto em cruz elevado, teste de campo aberto e teste de construção de ninho foram utilizados entre o sétimo e oitavo dia do protocolo. No oitavo dia, os tecidos do cólon e do córtex pré-frontal dos camundongos foram extraídos. Os níveis de mieloperoxidase (MPO), TNF-α, IL-10 e glutationa reduzida (GSH) foram medidos no cólon e os níveis de serotonina (5-HT) foram analisados por cromatografia líquida de alta eficiência (HPLC-ED) no córtex pré-frontal. O tratamento com CBD foi capaz de reduzir a hipersensibilidade abdominal, MPO e TNF-α, e foi capaz de normalizar os níveis de GSH e 5-HT quando comparado ao grupo DSS. O CBD atenuou a inflamação, a dor abdominal e o estresse oxidativo; e foi capaz de melhorar o bem-estar dos animais em relação ao grupo DSS. Nossos resultados auxiliam em novas perspectivas de abordagens terapêuticas em relação ao uso de CBD na colite ulcerativa. Além dos efeitos anti-inflamatórios, analgésicos e antioxidantes, baixas doses de CBD também promovem o bem-estar, possivelmente através da regulação dos níveis de serotonina no córtex pré-frontal. Mais estudos são necessários para entender completamente o mecanismo de ação do CBD neste modelo de DII.

Palavras-chave: Canabidiol; Doenças Inflamatórias Intestinais; Inflamação; Dor; Ansiedade; Serotonina;

ABSTRACT

Ulcerative colitis (UC) is an inflammatory bowel disease (IBD) that affects the colon, causing pain, inflammation, and diarrhea that could potentially lead to anxiety and depression. Due to the lack of effective treatments for ulcerative colitis, studies seeking new treatments are necessary. Studies suggest that cannabidiol (CBD), a phytocannabinoid, has analgesic, anti-inflammatory, anti-oxidative and anxiolytic effects. In this study investigated whether the treatment with of low doses of CBD is able to help improve the colonic symptoms of the experimental colitis, and improve the well-being of the animals, evaluating the possible CBD effects. Colitis was induced with 5% Dextran Sulphate Sodium (DSS) in the drinking water for 5 days, being replaced by drinking water in the last 2 days. Female Mice were orally treated with three different doses of CBD (0.01 mg/kg, 0.1 mg/kg and 1 mg/kg) during the seven days of experiments. Mechanical abdominal hypersensitivity was measured with von Frey hairs in intercalated days. Elevated plus Maze (EPM), Open field test (OFT) and nest building test were used between the seventh and eighth days of the protocol. On the eighth day, the mice's colon and prefrontal cortex tissue were extracted. Myeloperoxidase (MPO), TNF-α, IL-10. Glutathione (GSH) levels were measured in the colon and serotonin (5-HT) levels were analyzed using high-performance liquid chromatography (HPLC-ED) in the prefrontal cortex. CBD treatment was able to reduce abdominal hypersensitivity, MPO and TNF-α levels, and it was capable of normalizing GSH and serotonin levels when compared to the DSS group. CBD attenuated inflammation, abdominal pain, and oxidative stress; and it was able to improve the welfare of the animals compared to the DSS group. Our results assist in new perspectives of therapeutic approaches regarding the use of CBD in UC colitis. Beyond anti-inflammatory, analgesic, and antioxidant effects, low doses of CBD also promote welfare, possibly through the regulation of serotonin levels in the prefrontal cortex. Further studies are required to fully understand the mechanism of CBD action in this model of IBD.

Keywords: Cannabidiol; Intestinal Bowel Disease; Inflammation; Pain; Anxiety; Serotonin;

LISTA DE FIGURAS

ARTIGO CIENTÍFICO

LISTA DE SIGLAS

2-AG - 2-araquinoglicerol

5-HT1A - Receptor da serotonina 1A

CB_1 - Receptor Canabinoide 1

CB_2 - Receptor Canabinoide 2

CBD – Canabidiol

DII - Doenças Inflamatórias Intestinais

DSS - Dextran Sulfato de sódio

GSH - Glutationa

HPLC - Cromatografia liquida de alta eficiência

MAGL - Monoacilglicerol Lipase

MPO – Mieloperoxidase

PPAR-γ - Receptor ativado por proliferadores de peroxissoma gama

TGI - Trato Gastrointestinal

THC - Delta-9-Tetrahidrocanabidiol

TRPV1 - Receptor de potencial transiente vanilóide 1

ARTIGO CIENTIFICO

5-ASA - 5-aminosalicylic acid

5-HT – Serotonin

H&E - Hematoxylin & Eosin stain

HTAB - Hexadecyltrimethylammonium bromide

DAI - Disease Activity Index

ELISA - Enzyme-linked immunosorbent assay

EPM - Elevated Plus Maze

FAAH - Fatty acid amide hydrolase

GPR55 - Orphan G-protein-coupled receptor 55

IBD - Inflammatory Bowel Disease

OFT- Open Field Test

PAS - Periodic acid-Schiff

SCFAs - Short-chain fatty acids

TMB - 3,3′,5,5′ - tetramethylbenzidine

TRPVA - Transient receptor potential ankyrin

UC - Ulcerative Colitis

SUMÁRIO

1 INTRODUÇÃO

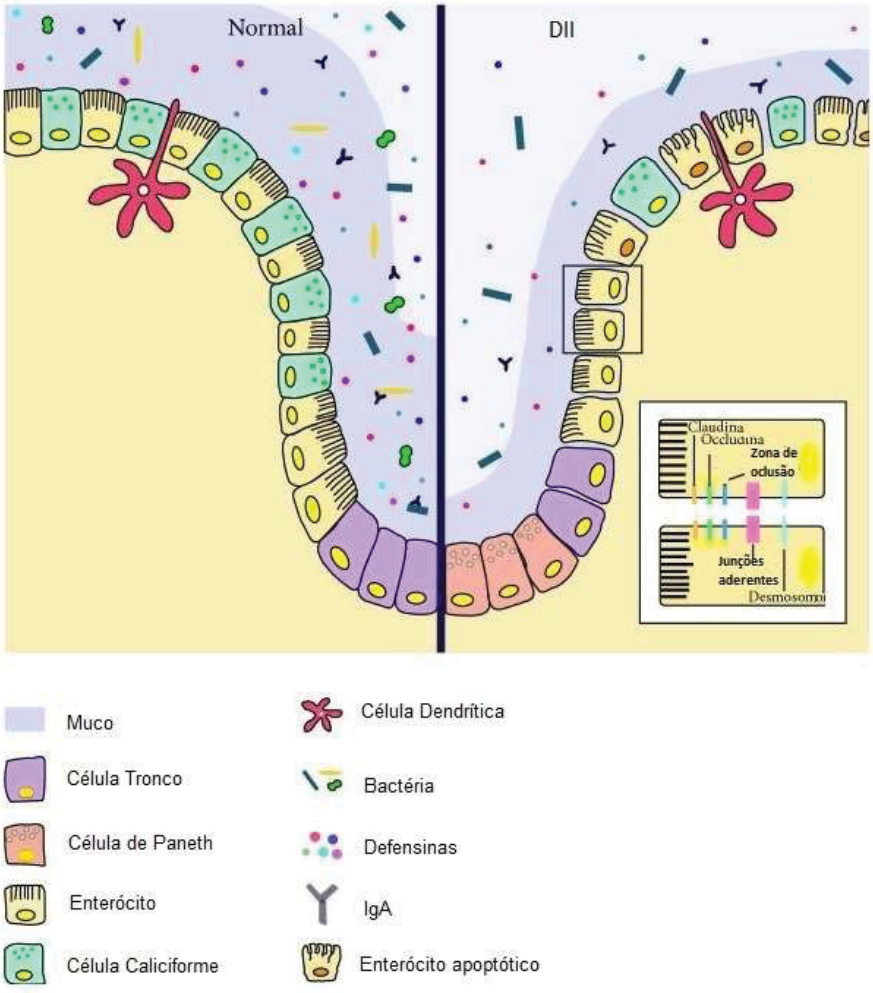
1.1 DOENÇAS GASTROINTESTINAIS E COLITE ULCERATIVA

As patologias que acometam o trato gastrointestinal (TGI) se apresentam como um grande problema da sociedade moderna, afetando grande parte da população mundial. Pelo TGI ser o principal responsável pela digestão, absorção e excreção das substâncias ingeridas, o seu mau funcionamento leva a um declínio na qualidade de vida, podendo desencadear outras doenças, principalmente pela absorção e excreção alterada de diversas substâncias, além do desconforto abdominal, dor e estresse (Greenwood-Van Meerveld et al., 2017). Diversas doenças que acometem o TGI apresentam caráter inflamatório, como a doença de Crohn e a colite ulcerativa, as chamadas doenças inflamatórias intestinais (DII). A colite ulcerativa é uma inflamação crônica no cólon, que causa diversos sintomas no paciente como, transito intestinal alterado e absorção desregulada de nutrientes necessários para saúde humana (Ordás et al., 2012; Feuerstein et al., 2019). Na colite ulcerativa, a integridade da barreira protetora intestinal fica comprometida devido a diminuição da secreção de mucina no cólon, levando à um aumento na permeabilidade a patógenos (Michielan; D'Incà, 2015). As citocinas pró-inflamatórias tem papel importante no desenvolvimento da doença, pois são responsáveis pelo recrutamento das células do sistema imune, ampliando e agravando a inflamação no foco da doença, aumentado a apoptose e necrose, consequentemente levando a perda da barreira protetora no TGI (Du; Ha, 2020) (Figura 1).

Vários estudos mostram que, fatores de risco como predisposição genética, estresse, alimentação inadequada, infecção com *Salmonella* ou *Campylobacter* e o uso indiscriminado de anti-inflamatórios não esteroidais tem relação com o aparecimento da colite ulcerativa (Adams et al., 2013). Dados epidemiológicos sobre as DII indicam que no mundo ocidental há um aumento significativo destas doenças nas últimas décadas. Os países do hemisfério sul e leste tem taxas mais baixas de DII, enquanto existe uma ascensão na Europa, Ásia e países em desenvolvimento (Malik, 2015). Além disto, existe uma tendência de estabilização das DII na América do Norte e norte europeu. Estas regiões são conhecidas por terem as maiores taxas do mundo de incidência (9 a 20 casos por 100,000 pessoas por ano) e prevalência (35 a 250 casos por 100,000 pessoas por ano) para colite ulcerativa (Malik, 2015). Em

regiões mais desenvolvidas do Brasil, as taxas de incidência da colite ulcerativa aumentaram muito nas últimas décadas (Kotze; Damião, 2020). A incidência aumentou principalmente nos países que adotaram um estilo de vida industrializado, o que sugere que fatores ambientais podem ser cruciais no desencadeamento do início da doença. De uma maneira geral, a prevalência de DII é maior em mulheres do que em homens, onde a prevalência da doença de Crohn é cerca de 20 a 50% maior em mulheres quando comparado aos homens (Betteridge et al., 2013).

FIGURA 1 – PATOFISIOLOGIA SIMPLIFICADA DA COLITE ULCERATIVA



FONTE – IMAGEM MODIFICADA DE Michielan; D'Incà (2015)

Os sintomas da colite ulcerativa incluem dor abdominal, diarreia, sangue nas fezes e perda de peso acentuada (Ho et al., 2020). Além disto, pacientes com colite

ulcerativa podem desenvolver transtornos de humor, como ansiedade e depressão. De maneira geral, a ansiedade decorrente da colite ulcerativa acomete mais mulheres que homens (Barberio et al., 2021). Os pacientes que sofrem com uma forma mais grave da doença, sofrem com hemorragias e possíveis necroses, que necessitam de tratamentos mais invasivos como o uso de corticoides e até mesmo imunossupressores, para diminuir a inflamação espontânea causada pela colite ulcerativa (Singh et al., 2018). Apesar disto, os tratamentos disponíveis não são totalmente satisfatórios e não trazem a cura da doença, afetando cronicamente a qualidade de vida e bem-estar dos pacientes. Os tratamentos disponíveis além de caros e ineficientes, apresentam diversos efeitos colaterais que colaboram para um declínio ainda maior no bem-estar das pessoas afetadas (Baumgart et al., 2007).

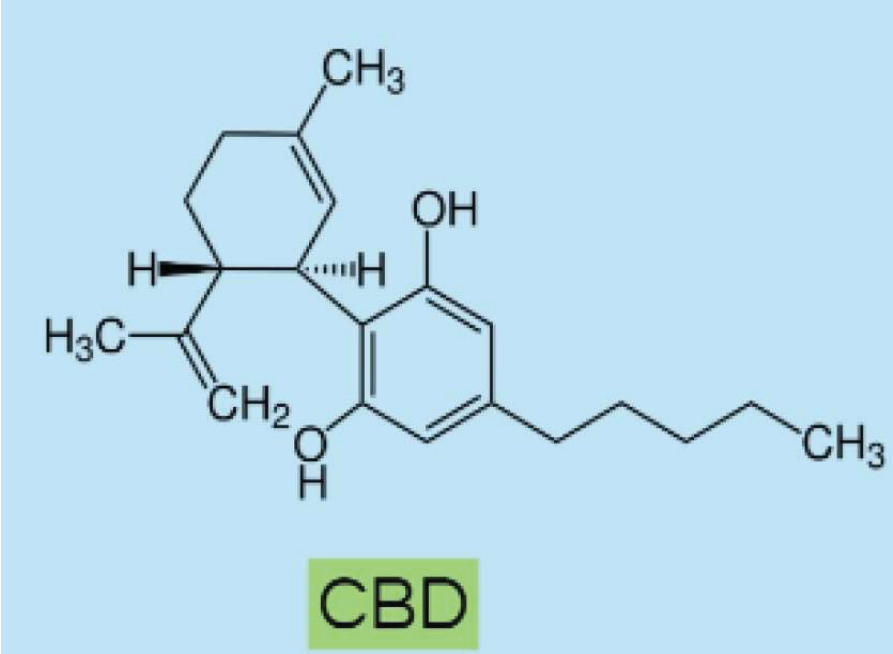
Pela falta de tratamentos ideais para a colite ulcerativa, novos estudos e tratamentos alternativos se mostram necessários. Além de alvos para a cura da doença, não existem dúvidas da necessidade de tratamentos que aliviem os sintomas e aumentem a qualidade de vida e bem-estar destes pacientes.

1.2 PRODUTOS NATURAIS

Desde a antiguidade os produtos naturais são utilizados na tentativa de curar doenças. Na Grécia antiga, foi documentado por Teofrasto, discípulo de Aristóteles, o uso da planta *Papaver somniferum* que tem a morfina como substância ativa (Duarte, 2005). Hoje, muitos dos medicamentos disponíveis são extraídos e coletados a partir de produtos naturais, sendo o uso de plantas medicinais uma prática bem estabelecida e difundida mundialmente (Dias et al., 2012). Assim, cada vez mais pesquisas têm como objetivo usufruir dos benefícios dos produtos naturais como na busca por menor incidência de efeitos colaterais (Viegas Jr et al., 2006). No Brasil, aproxidamente 25% do lucro das indústrias farmacêuticas vem de extratos de plantas, mostrando a grande utilidade destes compostos. Por isso, nota-se um aumento significativo nas pesquisas com plantas medicinais nas últimas décadas, pois tais plantas possuem um potencial farmacológico alto (Ferreira et al., 2014). Entre estas plantas que apresentam interesse medicinal, inclui-se, a *Cannabis sativa*, que tem um grande potencial farmacológico e por isso, diversos estudos buscam entender melhor seus efeitos.

1.3 CANABINOIDES E CANABIDIOL

FIGURA 2 – ESTRUTURA QUIMICA DO CANABIDIOL



FONTE – IMAGEM RETIRADA DE Gonçalves et al. (2020).

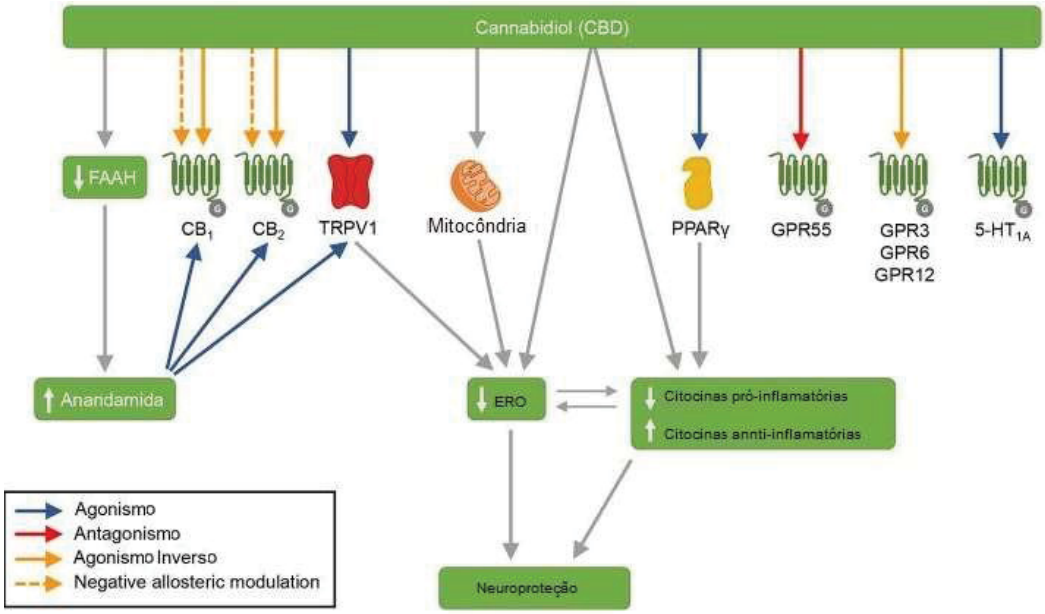
A *Cannabis sativa* L. é constituída por cerca de cinco mil fito constituintes, entre eles flavonoides, esteróis, alcanos e ácidos graxos, além da possível presença de cerca de 120 componentes específicos da família dos canabinoides (Qian *et al.,* 2019). Alguns registros antigos em textos chineses remetem que o uso medicinal da *Cannabis sativa* já ocorria há aproximadamente cinco mil anos (Amin *et al.,* 2019). Apesar disso, o uso mais comum da *Cannabis* nos dias atuais continua sendo recreativo, nos Estados Unidos da América, o número de usuários aumentou significativamente de 14 milhões para aproximadamente 26 milhões de pessoas (Qian *et al.,* 2019). O uso milenar da planta, associado ao uso recreativo e observação cientifica de seus efeitos, tem levantado a possibilidade e o interesse no ambiente científico para uso terapêutico dos canabinoides, causando aumento no número de estudos com estas substâncias nos últimos 15 anos (Amin et al., 2019). Até abril de 2019, mais de 30 estados americanos permitiram o uso medicinal dos canabinoides. Esse uso medicinal da *Cannabis*, aborda o tratamento alternativo de uma vasta

quantidade de doenças onde os tratamentos convencionais falharam, como por exemplo, tratamento da epilepsia, alguns tipos de câncer e dor crônica (Qian et al., 2019). Estas funções dos canabinoides, só são possíveis principalmente através do sistema endocanabinoide. Este, consiste em receptores para canabinoides, e ligantes endógenos específicos, denominados endocanabinoides, além de enzimas ligadas a síntese e metabolização destes componentes. Basicamente, os principais responsáveis pelas respostas do sistema endocanabinoide no organismo, são os receptores canabinoides, denominados 1 e 2 (CB1 e CB2). Estes receptores são ligados a proteína G, ou metabotrópicos, mais especificamente acoplados a proteína G inibitória. Por isso, a atividade destes receptores está ligada à uma atividade diminuída da adenilato ciclase, posteriormente reduzindo a quantidade de AMP cíclico. A ativação destes receptores está ligada a diversos efeitos fisiológicos, como memória, analgesia, alterações no apetite e no sistema imune (Amin et al., 2019). Os receptores CB1 estão localizados principalmente em neurônios centrais e viscerais, no sistema nervoso entérico. No sistema nervoso central, os endocanabinoides atuam principalmente nos neurônios pré-sinápticos, modulando a atividade de neurônios glutamatérgicos e GABAérgicos. Já os receptores CB2 estão principalmente expressos em células do sistema imune, como os neutrófilos e macrófagos. Basicamente, os receptores CB2 estão relacionados a funções imunes, enquanto os CB1 têm sua função mais ligada a neuromodulação (DiMarzo et al, 2015). Já quando falamos das principais substâncias endógenas ativas nestes receptores, os chamados endocanabinoides primários são aqueles de maior relevância sendo eles a anandamida e o 2-araquinoglicerol (2-AG), mediadores lipídicos que são sintetizados sob demanda, em vez de armazenados em vesículas nas células (Lu; Mackie, 2021). Após a entrada de cálcio nos neurônios pós-sinápticos e a ativação da fosfolipase D e da diacilglicerol lipase, respectivamente no caso da anandamida, e do 2-AG, a conversão de fosfolipídios é completada, ocorrendo a síntese sob demanda para estes dois endocanabinoides. Estes endocanabinoides primários são degradados por hidrólise enzimática. A anandamida é hidrolisada em ácido araquidônico e etanolamida pela enzima amida hidrolase de ácidos graxos, também conhecida como *Fatty acid amide hydrolase* (FAAH). Já o 2-AG sofre a degradação pela monoacilglicerol lipase (MAGL), sendo transformado em ácido araquidônico e glicerol (Saito et al, 2010). Acredita-se que os efeitos terapêuticos dos canabinoides, vem principalmente através do sistema de endocanabinoides, apesar de outras vias

também estarem envolvidas. Além dos ligantes endocanabinoides, a *Cannabis sativa* apresenta mais de oitenta fitocanabinoides em sua composição que apresentam papel no sistema endocanabinoide, com destaque para os proeminentes Delta-9-Tetrahidrocanabidiol (THC) e canabidiol (CBD) (Figura 2). O THC, um composto psicotomimético, se liga principalmente ao receptor CB_1, causando sensações de euforia, relaxamento e outras características de substâncias psicoativas. Enquanto isso, o CBD não apresenta atividade psicotomimética, mas assim como o THC, apresenta diversas utilidades farmacológicas, atuando em ambos os receptores CB (DiMarzo et al., 2015). O CBD tem a habilidade de funcionar regulando indiretamente ambos os receptores CB_1 e CB_2. Além disto, um dos principais papéis do CBD no organismo ocorre de maneira indireta. O CBD atua como inibidor da FAAH, enzima responsável pela degradação do endocanabinoide primário anandamida. Desta forma, o CBD indiretamente aumenta os níveis desta substância no organismo (DiMarzo et al., 2015). Além disto, o CBD tem atividade promiscua, isto é, se liga em diferentes tipos de receptores para promover seus efeitos terapêuticos. Entre os alvos do CBD, podemos destacar o receptor de potencial transitório vaniloide tipo 1 (TRPV1), receptor ativado por proliferadores de peroxissoma gama (PPAR-γ) e o receptor de serotonina 5-HT1A (Figura 3) (Gonçalves et al., 2020). O TRPV1 ou receptor da capsaicina, é conhecido por sua ação na detecção e regulação da temperatura. Sua modulação está ligada a mecanismos de nocicepção e inflamação (Iftinca et al., 2021). Outro receptor que tem sua modulação relacionada a inflamação, é o PPAR-γ. Este receptor nuclear é encontrado principalmente no tecido adiposo, colón e macrófagos, com sua ação ligada principalmente ao metabolismo de ácidos graxos. Porém, estudos mostram que a regulação deste receptor, está relacionada a resposta inflamatória e mecanismos antioxidantes (De Carvalho et al., 2021). Já, o receptor pré-sináptico 5-HT1A, onde o CBD atua como agonista, tem sua ativação relacionada a regulação deste neurotransmissor e a neuromodulação. Diversos fármacos que atuam como agonista deste receptor, como a buspirona, são usados de forma terapêutica no tratamento da depressão e ansiedade. Além disto, os níveis gerais de serotonina estão correlacionados aos mecanismos de nocicepção (Haleem, 2018). Até por isto, diversas atividades terapêuticas para os dois fitocanabinoides são estudadas. Para o CBD, estudos sugerem a habilidade de funcionar como analgésico, antiemético, anti-inflamatório, ansiolítico e como protetores na neurodegeneração (Amin et al., 2019). Outras potenciais utilidades e efeitos já descritos para o CBD, são

como, tratamento alternativo para epilepsia, diminuição de espasmos musculares, esclerose múltipla, dor neuropática, e como tratamento para alguns tipos de câncer (Bruni et al*.,* 2018). Além disto, a presença do sistema endocanabinoide no TGI está relacionado a uma regulação depressora nas suas funções como, a diminuição de secreções e menor mobilidade no TGI (Pesce et al., 2018). Neste contexto, os canabinoides estão sendo investigados como tratamento para diversas doenças, entre elas doenças inflamatórias, e doenças gastrointestinais, foco deste estudo. Como mostrado anteriormente, esta classe de fármacos possui boa resposta como analgésicos, anti-inflamatórios e relaxantes. As perspectivas deste tipo de substância no tratamento da colite são promissoras, e por isto, merecem atenção especial. Com isso, o sistema canabinoide surge como um potencial alvo para investigar novos tratamentos que apresentem maior eficácia. Pela grande presença do sistema endocanabinoide no trato gastrointestinal, a ativação dos receptores CBs podem suprimir alguns dos sintomas da colite ulcerativa, como, por exemplo, a diarreia associada a doença e a hipersensibilidade abdominal (Gyires *et al.,* 2016). Alguns estudos de inflamação intestinal in vitro mostraram que os receptores canabinoides têm papel na inflamação, descrevendo uma melhora na restituição da barreira epitelial intestinal em resposta a distúrbios inflamatórios, que sugerem que os receptores canabinoides podem atuar de maneira protetora no epitélio intestinal. Com Base nestes fatos, o CBD aparece como uma interessante alternativa no tratamento de doenças inflamatórias intestinais.

FIGURA 3 – MECANISMOS DE AÇÃO DO CANABIDIOL



FONTE – IMAGEM MODIFICADA DE Peres et al. (2018).

2 OBJETIVOS

2.1 OBJETIVO GERAL

Investigar os efeitos do tratamento com baixas doses de CBD no modelo animal de colite induzida por Dextran Sulfato de Sódio (DSS) em camundongos fêmeas através da utilização técnicas farmacológicas, histológicas e bioquímicas. Analisar os efeitos do tratamento com baixas doses de CBD associados a redução da dor, ansiedade e inflamação no trato gastrointestinal no modelo de colite, bem como a possível melhora no bem-estar animal.

2.2 OBJETIVOS ESPECÍFICOS

Avaliar os efeitos da administração oral de baixas doses de CBD na colite induzida por DSS em camundongos:

1. Avaliar os efeitos do tratamento com CBD nos parâmetros gerais da colite;
2. Avaliar os efeitos do tratamento oral com CBD nos parâmetros histológicos colônicos;
3. Avaliar o efeito do tratamento oral com CBD na hipersensibilidade abdominal à estimulação mecânica;

4. Avaliar os efeitos do tratamento oral com CBD nos parâmetros inflamatórios (níveis de mieloperoxidase (MPO); níveis de TNF-α e IL-10) e nos níveis de glutationa (GSH) colônicos;
5. Avaliar os efeitos do tratamento oral com CBD nos parâmetros de mobilidade, ansiedade e bem-estar através de testes comportamentais (Campo aberto, Labirinto em cruz elevado e teste da formação de ninho);
6. Avaliar os efeitos do tratamento oral com CBD nos níveis totais de serotonina no córtex pré-frontal através de cromatografia liquida de alta eficiência (HPLC);

3 ARTIGO CIENTÍFICO

Low doses of Cannabidiol reduces inflammation and pain, improves mice welfare and regulates cortical serotonin levels on DSS-induced colitis model

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ABSTRACT

Introduction: Ulcerative colitis (UC) is an inflammatory bowel disease (IBD) that affects the colon, causing pain, inflammation, and diarrhea that could potentially lead to anxiety and depression. Due to the lack of effective treatments for ulcerative colitis, studies seeking new treatments are necessary. Studies suggest that cannabidiol (CBD), a phytocannabinoid, has analgesic, anti-inflammatory, anti-oxidative and anxiolytic effects. Here, we intended to investigate whether the use of low doses of CBD is able to help improve the colonic symptoms of the disease, and the well-being of the animals, evaluating the possible CBD effects.

Methods: Colitis was induced with 5% Dextran Sulphate Sodium (DSS) in the drinking water for 5 days, being replaced by drinking water in the last 2 days. Female Mice were orally treated with three different doses of CBD (0.01 mg/kg, 0.1 mg/kg and 1 mg/kg) during the seven days of experiments. Mechanical abdominal hypersensitivity was measured with von Frey hairs in intercalated days. Elevated plus Maze (EPM), Open field test (OFT) and nest building test were used between the seventh and eighth days of the protocol. On the eighth day, the mice's colon and prefrontal cortex tissue were extracted. Myeloperoxidase (MPO), TNF-α, IL-10 and Glutathione (GSH) levels were measured in the colon tissue and serotonin (5-HT) levels were analyzed using high-performance liquid chromatography (HPLC-ED) in the prefrontal cortex.

Results: CBD treatment was able to reduce abdominal hypersensitivity, MPO and TNF-α levels, and it was capable of normalizing GSH and serotonin levels when compared to the DSS group. CBD attenuated inflammation, abdominal pain, and oxidative stress; and it was able to improve the welfare of the animals compared to the DSS group.

Conclusion: Our results assist in new perspectives of therapeutic approaches regarding the use of CBD in UC. Beyond anti-inflammatory, analgesic, and antioxidant effects, low doses of CBD also promote welfare, possibly through the regulation of serotonin levels in the prefrontal cortex. Further studies are required to fully understand the mechanism of CBD action in this model of IBD.

Keywords: Cannabidiol; Intestinal Bowel Disease; Inflammation; Pain; Anxiety; Serotonin;

1. INTRODUCTION

Ulcerative colitis (UC) is an inflammatory bowel disease (IBD), characterized by colonic mucosal inflammation, more prevalent in females [1, 2] The pathophysiology involves genetic, environmental, and psychological factors. UC patients can present a diversity of symptoms including abdominal pain, diarrhea, bloody stool and weight loss. Further, abdominal pain negatively affect the quality of life of patients, leading to anxiety and depression [3, 4, 5].

The treatment strategy includes corticosteroids, 5-aminosalicylic acid (5-ASA), immunosuppressive drugs, and anti-TNF-α antibodies, and so far, no treatment promotes the cure of the disease [6]. Natural products emerge as the source of good medicine to treat different diseases, including IBD [7]. Cannabidiol (CBD), the non-psychoactive component of *Cannabis sativa*, has gained great recognition due to its potential therapeutic in several disorders such as anxiety, depression, chronic pain, and IBD [8,9]. CBD exerts its molecular and behavioral effects because of its anti-inflammatory, antioxidant, and analgesic properties through various molecular targets, acting over the endocannabinoid system receptors namely cannabinoid type 1 (CB_1) and type 2 receptor (CB_2), serotonin 5-HT1A receptor, transient receptor potential vanilloid type 1 (TRPV1) receptor, peroxisome proliferator-activated receptor-γ (PPAR-γ) and the orphan G-protein-coupled receptor 55 (GPR55) [10]. On that account, serotonin (5-HT), a neurotransmitter, is related modulation of pain, depression, and anxiety. Moreover, serotonin signaling in the prefrontal cortex through several receptors modulating cognitive, emotional and pain processes in the brain make CBD an interesting therapeutic choice for a handful of diseases, including UC [11,12,13]. However, the effects of CBD in IBD has not been fully explored.

This study aimed to determine whether low doses of CBD can reduce inflammation, abdominal pain, and anxiety-like behavior in a female mice model of DSS-induced colitis. Furthermore, the relationship between anti-inflammatory, analgesic, anti-oxidant, anxiolytic and wellbeing effects of CBD together with cortical levels of 5-HT was established to find the possible underlying mechanism.

2. MATERIAL AND METHODS

2.1 Animals

All experiments were conducted following approval by the Committee of Animal Experimentation of the Federal University of Paraná (CEUA/BIO–UFPR, 1353) and were rigorously performed in congruence with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011). Adult female Swiss mice (22–32 g) were used in all experiments and were maintained in a controlled environment at 22 ± 2 °C, 12 h light/dark cycle, 10 animals per cage with wood shaving bedding and free access to tap water and food (Nuvilab CR-1, Quimtia S/A, Brazil).

2.2 Pharmacological treatments

Mice were divided into five different groups during experiments: control group, DSS group treated with vehicle (water and tween 20 10% 1 mL/kg, per oral (p.o.)) and DSS group treated with three different doses of CBD (0.01 mg/kg, 0.1 mg/kg or 1 mg/kg, p.o.; dissolved in 10% of Tween 20 and then subsequently homogenized in water, HempMeds®, Campinas, Brazil). The low doses were selected based on previous works due to the fact that CBD produces inverted U-shaped dose-response curves [14].

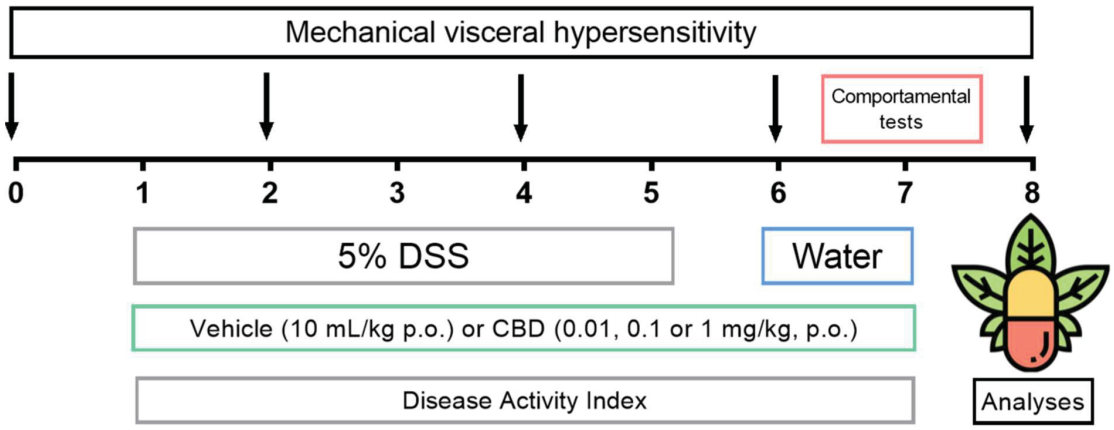

Fig. 1. Timeline of the entire experiment.

2.3 DSS colitis induction and Disease Activity Index (DAI)

For five consecutive days 5% DSS (Dextran Sulphate Sodium, Molecular weight: 40000 Da, TdB Consultancy) were added to drinking water. After the fifth day, the 5% DSS solution was replenished by regular drinking water for two additional days and on the eighth day, all animals were euthanized with overdoses of inhaled isoflurane via a vaporizer in a closed container. Then, their colons were extracted, the lengths were measured and the colon tissue was either immediately stored at −80 °C for future analysis or fixed for later histological investigation. The control group received only tap water during experiment [15].

The Disease Activity Index (DAI) was determined by changes related to colitis. Here, we analyzed weight loss and alterations in the feces such as stool consistency and blood presence, as previously reported [15]. The DAI score was calculated considering the weight loss (scored as 0 if bodyweight increased or remained within 1% of the baseline; 1 for a 1–5% loss; 2 for a 5–10% loss; 3 for a 10–15% loss; or 4 for weight loss >15%) The stool consistency (graded as 0 for no diarrhea; 2 for loose

stool that did not stick to the anus; and 4 for liquid stool that did stick to the anus) and the presence of occult blood (value of 0 when assigned for none, 2 for the presence of blood).

2.4 Histological analysis

To analyze the microscopic changes caused by DSS colitis, a portion of the mice colon was extracted and fixed for 24 h with 4% formaldehyde and then transferred to 70% ethanol. After that, the tissue was dehydrated with alcohol and xylene, embedded in paraffin wax and then sectioned with a microtome in slides (5 µm). Hematoxylin & Eosin stain (H&E) stain were made to analyze the histopathological changes. Alcian Blue and Periodic acid-Schiff (PAS) stain were performed to analyze the acidic mucin (Alcian Blue pH 2.5) and neutral mucin (PAS) in the colon tissue [16]. Staining pixels of PAS and Alcian blue staining areas were quantified by the intensity at ImageJ software.

2.5 Evaluation of abdominal hypersensitivity to mechanical stimuli

To evaluate the abdominal pain, mechanical abdominal hypersensitivity was measured with von Frey hairs (0.008 - 0.6 g, North Coast Medical, Morgan Hill, CA, USA). The basal mechanical nociceptive threshold was evaluated by mechanical stimulation of mice's abdomen. The control group and the animals treated with vehicle or CBD (0.01; 0.1 or 1 mg/kg, p.o.) were placed in individual boxes and acclimatized for 60 min before each measurement. On the eighth day, mice were no treated with CBD. Mechanical abdominal hypersensitivity was evaluated on days 0, 2, 4, 6, and 8 of the experiments 1h after CBD treatments as the frequency response of withdrawal

and/or licking the abdominal elicited by 5 consecutive applications of different von Frey filaments [15].

2.6 Quantification of myeloperoxidase (MPO) levels

For the determination of MPO levels, the colon tissue was homogenized in potassium phosphate buffer (80 mM, pH 5.4) containing 0.5% hexadecyltrimethylammonium bromide (HTAB), and then centrifuged at 11000g for 20 min at 4 °C. The supernatant was mixed with 0.017% H_2O_2 and 18.4 mM 3,3′,5,5′ - tetramethylbenzidine (TMB). The enzymatic MPO level absorbance was measured by a spectrophotometer at 620 nm and the results expressed as optical density (O.D.)/mg of protein, as previously reported [15].

2.7 Quantification of Glutathione (GSH) levels

The colon tissue was homogenate with 12.5% trichloroacetic acid and centrifuged for 15min at 9000g at 4 °C. After that, the supernatant was mixed with TRIS buffer (400 mM, pH 8.9) and 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB, 10 mM), which react with GSH. The absorbance was determined by a spectrophotometer at 415 nm. The values were interpolated into a standard curve of GSH (0.375 - 3 µg), corrected for the tissue weight, and expressed as µg/g of tissue, as previously reported [15].

2.8 Determination of TNF-α and IL-10 levels

Supernatants of colon samples were used to evaluate the cytokines TNF-α and IL-10 levels using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's recommendations (Peprotech EC Ltd, London UK). Absorbance was measured using a microplate reader (Bio-Tek Instruments Inc., Winooski, VT, USA) at

450 nm with a wavelength correction set at 620 nm. Recombinant mice TNF-α standard curve (0 - 2000 pg/mL) and recombinant mice IL-10 standard curve (0 - 1000 pg/mL) were used to interpolate concentrations of all the samples and the results were expressed as pg/mg of protein.

2.9 Nest Building Test

Between the seventh and eighth days of the experiment at the end of the light cycle, animals were placed one per cage (391 × 199 × 160 mm) with clear wood shaving bedding and with free access to pelleted food and tap water overnight with a cotton nestled (24 x 13 x 4 cm ~ 95 g). The following morning, at the beginning of the light cycle, the cotton piece used for nest construction was scored (1 to 5; graded 1 for very poor or no nest building and 5 for optimal nest building) to evaluate the general well-being and animal mood and behaviors associated with psychiatric disorders such as depression [17].

2.10 Open Field Test (OFT)

On the seventh day of the protocol, all mice were placed individually in the center of a circular arena for 5 min to evaluate spontaneous locomotor activity. The animals were treated with vehicle or CBD (0.01; 0.1 or 1 mg/kg, p.o.), and after 60 min their mobility was evaluated. The circular arena (42 cm diameter × 24 cm high) was sectioned into 25 different sectors. All animals were recorded and evaluated manually counting all sectors crossed with the four paws. The place was cleaned with a 10% ethanol solution, in order to eliminate any odor left by the other animals [18].

2.11 Elevated Plus Maze (EPM)

On the seventh day of the experiments, all mice were treated 60 min before the test with vehicle or CBD (0.01; 0.1 or 1 mg/kg, p.o.), placed individually in the center of the EPM (30 cm × 5 cm each arm connected in the middle at a 5 × 5 cm open center with the closed arm wall at 15 cm high) allowing them to explore for 5 min. All animals were recorded. The total time spent in the open/closed arms and the total time they entered each arm were assessed to evaluate the potential anxiolytic-like effect and the spontaneous locomotor activity [18].

2.12 High-performance liquid chromatography

Samples of the prefrontal cortex were collected on the eighth day of the experiment and stored at −80 °C. Serotonin (5-HT) level was quantified by high-performance liquid chromatography (HPLC-ED) with electrochemical detection in the prefrontal cortex tissue. The samples were homogenized by ultrasound (Sonics) in 0.1 M perchloric acid, containing 0.02% sodium metabisulfite (Sigma) and 50 ng/ml internal standard 3,4-dihydroxybenzylamine hydrobromide (Sigma). Then centrifugated at 12,298 g for 20 minutes at 4 °C. The sample supernatant was injected onto the HPLC (Shimadzu) with a reverse phase C-18 column (Synergi Fusion-RP C-18; 150 x 4.6 mm id, 4 µm particles - Phenomenex) with guard column (Security Guard Cartridges Fusion-RP, 4 x 3.0 mm) and electrochemical detector (ESA Coulochem III) equipped with a 350 mV guard cell (ESA 5011A) and injection pump LC-20AT (Shimadzu). The column was kept at a controlled temperature (25°C). The mobile phase used was injected at a rate of 1 mL/min changed to the following composition: 20 g of citric acid monohydrate (Merck), 200 mg of 1-octane sulfonic acid (Merck), and 40 mg of ethylenediaminetetraacetic acid (Sigma) in 900 mL of HPLC grade water. The pH of the running buffer was set to 4.0 and then filtered through a filter with a 0.45

μm pore diameter. Then methanol (Merck) was added until a final concentration of 10% (v/v) was reached. The prescriptions of the neurotransmitter was calculated by the area under the curve (current vs time) interpolated to a standard curve. The units used to express the result was ng/g tissue weight [19].

2.13 Statistical analysis

Results were expressed as means ± S.E.M when the criteria were met for performing parametric tests in the Kolmogorov-Smirnov normality test. One-way ANOVA followed by Newman-Keuls test or two-way ANOVA followed by Bonferroni test were used for parametric data. Non-parametric data were expressed as Median with 95% CI. Statistical differences between groups were determined using Kruskal–Wallis followed by Dunn's test for non-parametric data. All data analyses were performed using GraphPad Prism 6 software (GraphPad Software Inc., San Diego, CA, USA). Differences with $p < 0.05$ were considered statistically significant.

3. RESULTS

3.1 Disease Activity Index (DAI) and Colon size

At the end of the colitis protocol, DAI and colon size were measured. Two-way ANOVA showed a significant difference of the following factors: interaction ($F(24, 448) = 1.869$, $P=0.0081$), time ($F(4.745, 265.7) = 294.3$, $P < 0.0001$), Column factor ($F(3, 56) = 5.630$, $P=0.0019$) and subject ($F(56, 448) = 3.044$, $P < 0.0001$), time ($F(4.745, 265.7) = 294.3$, $P < 0.0001$), when the DAI was measured. Bonferroni's post hoc test showed that although all groups besides control developed the DSS-induced colitis represented by the weight loss, diarrhea, and blood in the stool, mice treated with CBD were capable of showing a decrease in DAI in all CBD treatments when compared to

DSS group ($P < 0.05$). This amelioration of DAI was also seen on the seventh day, in the CBD 0.01 and CBD 0.1 treatment ($P < 0.05$) and no difference between the CBD 1 group and DSS group ($P > 0.05$). On the eighth day of experiments, CBD 0.1 and 1 groups had no difference from the DSS group ($P > 0.05$). Only the CBD 0.01 group was able to show a decrease in DAI by on the eighth day when compared to the DSS group ($P < 0.05$) (Fig. 2).

After the end of the protocol, the mice colon were extracted and the lengths measured. One-way ANOVA demonstrated significant effects in the length of the colon ($F(4,70) = 28.05$, $P < 0.0001$) (Fig. 3 A and 3 B). Newman-Keuls post hoc test revealed that all groups presented a shortened colon when compared to the control group ($P < 0.05$). CBD was not able to prevent shortening in the colon, caused by the DSS-induced colitis.

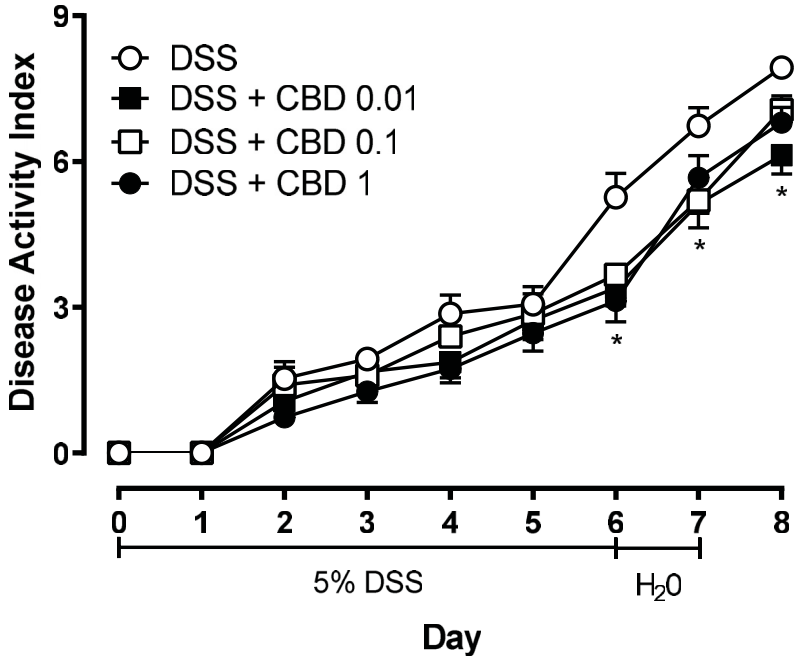

Fig. 2. Effect of CBD on DAI in DSS-induced colitis in mice. The animals received 5% DSS in water for 5 days followed by 2 days of tap water. Animals were treated once a day, DSS group (Vehicle) or DSS + CBD (0.01; 0.1 or 1 mg/kg, p.o.). The results are expressed as mean ± standard error of means (n = 15). * $P < 0.05$ when compared to the DSS group (Two-way ANOVA followed by Bonferroni test).

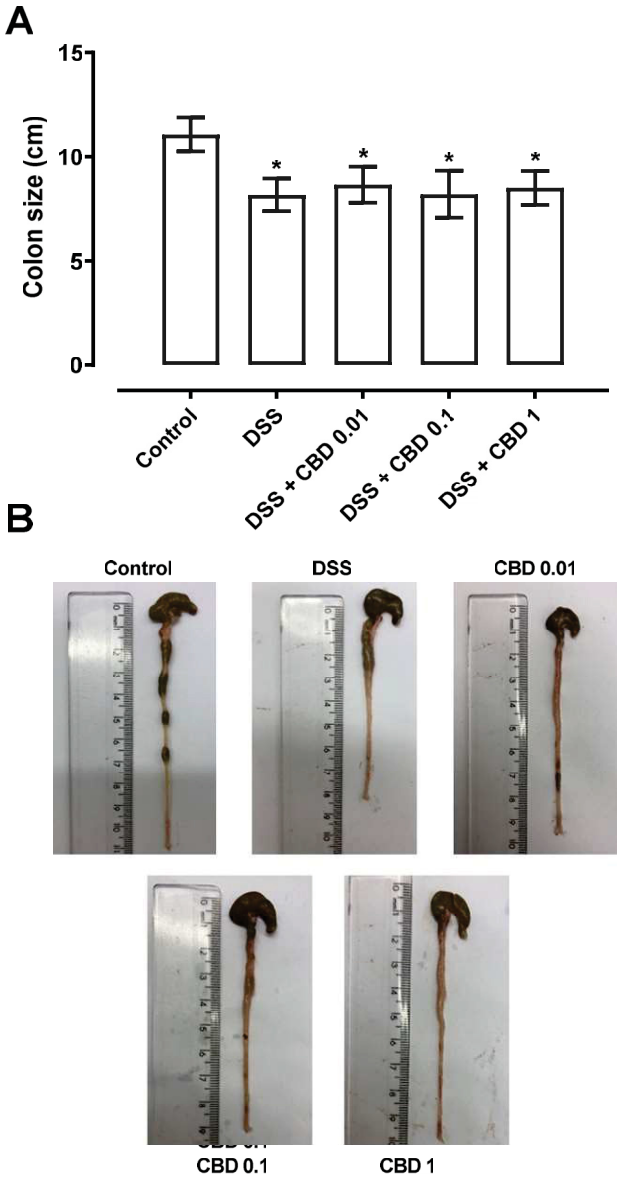

Fig. 3. Effect of CBD on colon size in DSS-induced colitis in mice (A and B). The animals received 5% DSS in water for 5 days followed by 2 days of tap water. The control group received only tap water. Animals were treated once a day (Vehicle or CBD (0.01; 0.1 or 1 mg/kg, p.o.)). The results are expressed as mean ± standard error of means (n = 15). * $P < 0.05$ when compared to the Control group (One-way ANOVA followed by Newman-Keuls test).

3.2 Histological analyses

We next evaluated the protective effect of CBD in DSS-induced colitis in the colonic tissue. For this, colon samples were used for histological analysis. Both H&E, PAS, and alcian blue stains showed that CBD and DSS groups had destruction in colonic tissue with histopathological changes in the mucosal, submucosal, muscular layer of the colonic wall when compared to the control group, but no difference between the CBD group and DSS group was found (Fig. 4). The histological analyses showed that CBD was not able to protect the colonic architecture nor preserved the colonic mucins against DSS-induced colitis.

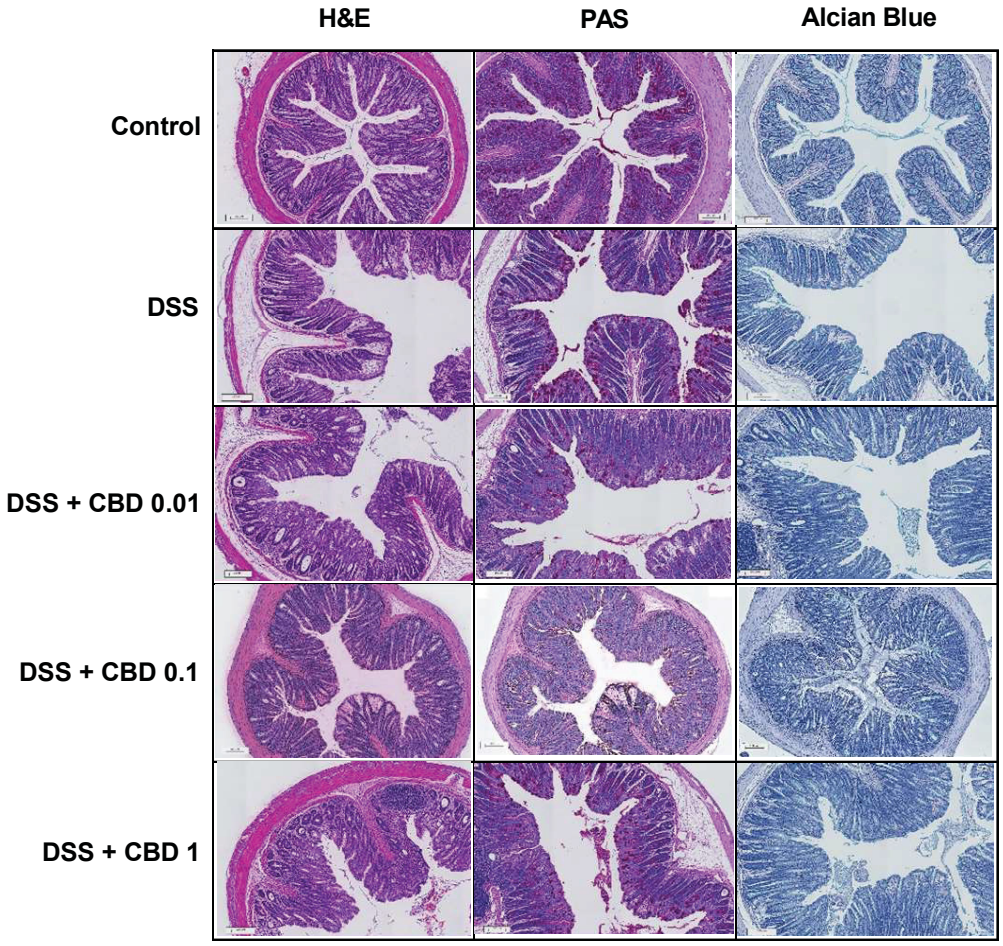

Fig. 4. Effect of CBD administration at the microscopical level for histochemical staining of colons (H&E) neutral mucin like-glycoproteins (PAS) and acid mucin (Alcian Blue pH 2.5). 10x magnification, (scale = 100 μm). Mice received 5% DSS in water for 5 days followed by 2 days of tap water. The control group received only tap water. Animals were treated once a day, DSS group (Vehicle) or DSS + CBD (0.01; 0.1 or 1 mg/kg, p.o.).

3.3 Evaluation of abdominal hypersensitivity to mechanical stimuli

Animals treated with vehicle developed a mechanical abdominal hypersensitivity, showing an increased frequency response to mechanical stimulation

throughout the experiment. This result was represented by the increase in the frequency response of withdrawal and/or licking the abdominal region starting on day 2 and throughout all days tested. The DSS group had an increase in the frequency response when compared to the control group. All mice with DSS colitis treated with CBD showed a decrease in frequency response to mechanical stimulation when compared to the DSS group. Starting on day 2, the CBD groups had a decrease in frequency response in the 0.16 filament by around 45% when compared to the DSS group (62.67 ± 6.72 %) (Fig. 5 B). By day 4, the CBD 0.01 (39 %) and 0.1 (45 %) treatment maintained the decrease frequency response in the 0.16 filament when compared to the DSS group (65.33 ± 4.96 %) (Fig. 5 C). DSS group had an increase by 75% in frequency in the 0.16 filament when compared to the control group (37.33 ± 6.13 %) (Fig. 5 C). On day six, CBD 0.01 (37%), 0.1 (35%) and 1 (27%) treatment maintained the decrease in frequency response in the 0.16 filament when compared to the DSS group (65.33 ± 4.70 %) (Fig 5 D). On the eighth day, CBD 0.01 (29%), 0.1 (33%) and 1 (43%) treatment were able to maintain a decrease in frequency response in the 0.16 filament when compared to the DSS group (72 ± 4.70 %) (Fig. 5 E). DSS group had an increase by 59% in frequency in the 0.16 filament when compared to the control group (45.33 ± 3.63 %) (Fig. 5 E).

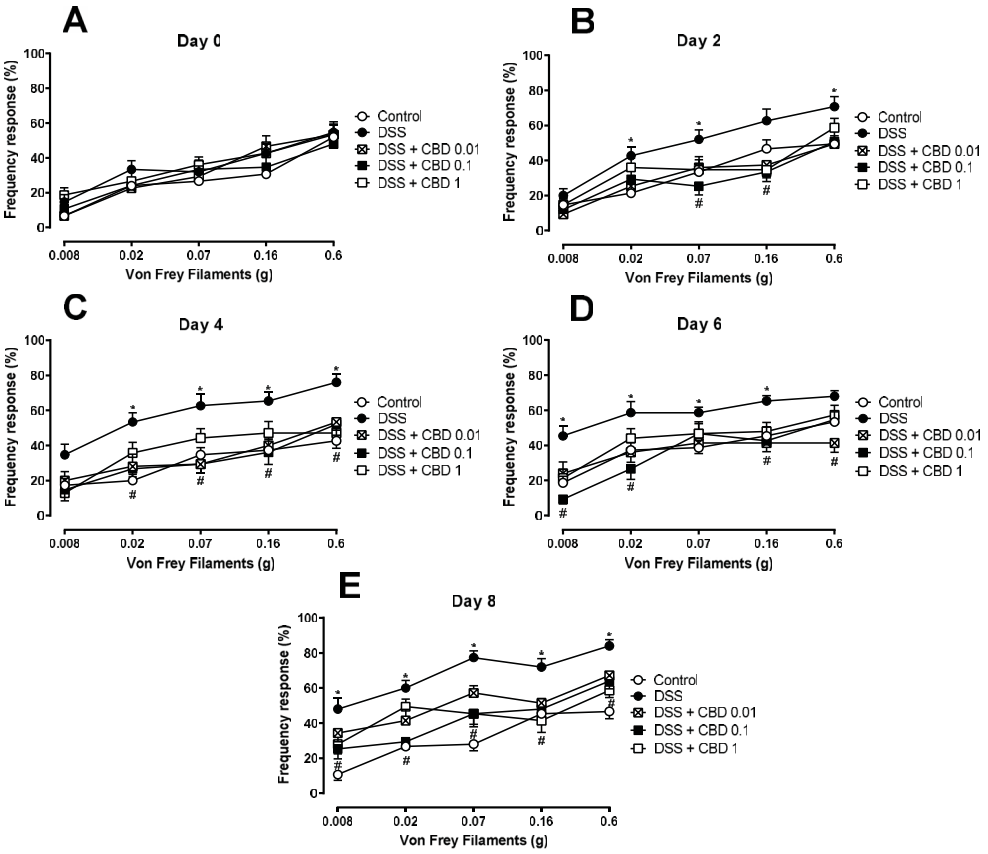

Fig. 5. Effect of CBD on DSS-induced abdominal hypersensitivity. Mice received 5% DSS in water for 5 days followed by 2 days of tap water. The control group received only tap water. Animals were treated once a day, DSS group (Vehicle) or CBD (0.01; 0.1 or 1 mg/kg, p.o.). On day zero (Fig. 5 A) and every two days after starting DSS treatment the animals were tested with von Frey filaments (Fig. 5 B to Fig. 5 E). The results are expressed as mean ± standard error of means (n = 15). * $P < 0.05$ when compared to the Control group; # $P < 0.05$ when compared to the DSS group (Two-way ANOVA followed by Bonferroni test).

3.4 Evaluation of inflammatory and antioxidant parameters

MPO levels increase is an inflammatory marker in DSS-induced colitis mice as it is associated with infiltration of neutrophils into colonic tissue. The MPO levels in the colon demonstrated significant difference in the One-way ANOVA ($F (4, 25) = 12,19$, $P < 0.0001$) (Fig. 6 A). Newman-Keuls test revealed that the DSS and the CBD 1 group had an increase in MPO levels in the colonic mucosa when compared to the control group ($P < 0.05$). CBD 0.01 and CBD 0.1 treatment decreased neutrophil infiltration when compared to the DSS group ($P < 0.05$).

In the DSS-induced colitis mice, GSH levels in the colon tissue were significant different in the One-way ANOVA ($F (4, 25) = 4.029$, $P = 0.0117$). The Newman-Keuls test showed that, the DSS group decreased GSH levels in comparison to the control group ($P < 0.05$). While CBD 1 treatment showed no significant difference when compared to the control group ($P > 0.05$), CBD 0.01 and CBD 0.1 groups were able to prevent GSH depletion when compared to the DSS group ($P < 0.05$) (Fig. 6 B).

Moreover, One-way ANOVA demonstrated significant effects in the TNF-α ($F (4, 25) = 4.418$, $P=0.0077$) and IL-10 levels ($F (4, 25) = 4.630$, $P=0.0062$) in the colon. Newman-Keuls post hoc test showed that only the DSS group showed difference (increase) in TNF-α levels when compared to the control group ($P < 0.05$). All three CBD treatments showed a decrease in TNF-α levels when compared to the DSS group ($P < 0.05$) (Fig. 6 C). All groups had a significant decrease ($P < 0.05$) in IL-10 levels when compared to the control group (FIG. 6 D).

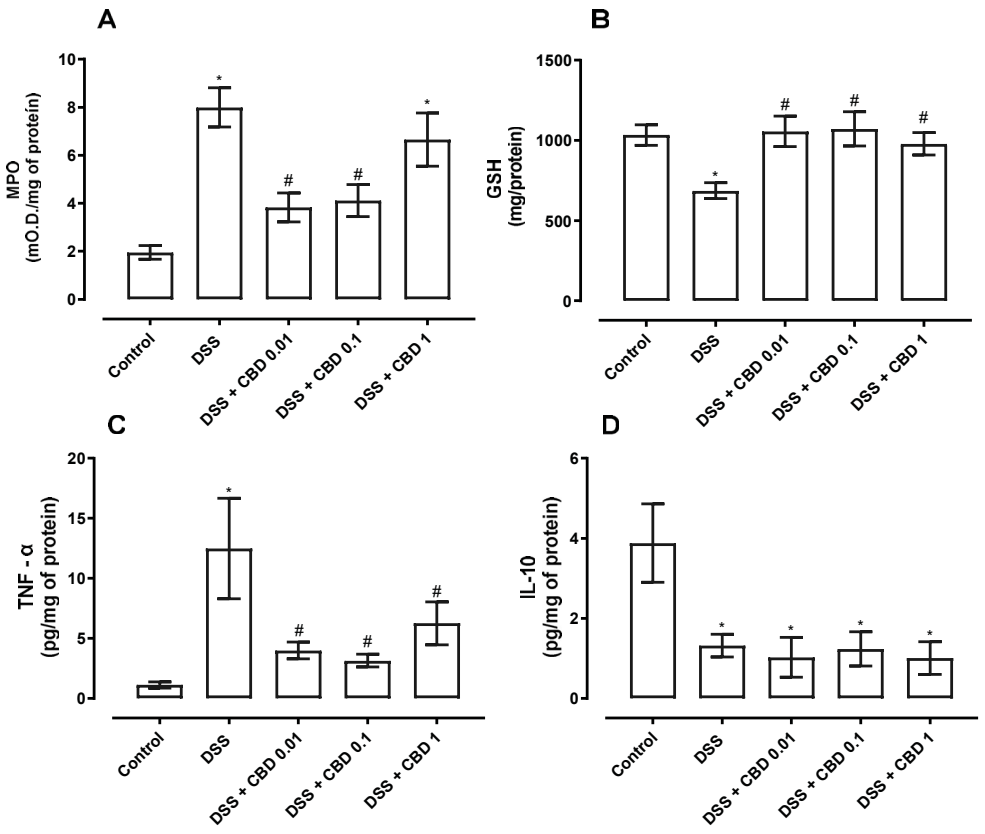

Fig. 6. Effect of CBD administration on MPO levels (Fig. 6 A), GSH activity (Fig. 6 B) TNF-α (Fig. 6 C) and IL-10 (Fig. 6 D) levels. Mice received 5% DSS in water for 5 days followed by 2 days of tap water. The control group received only tap water. Animals were treated once a day, DSS group (Vehicle) or CBD (0.01; 0.1 and 1 mg/kg, p.o.). The results are expressed as mean ± standard error of means (n = 5-6). * $P < 0.05$ when compared to the Control group; # $P < 0.05$ when compared to the DSS group (One-way ANOVA followed by Newman-Keuls test).

3.5 Evaluation of Nest Building, Open Field and Elevated Plus Maze Tests

Next, the nest-building behavior was analyzed to evaluate the welfare of the animals. The nest score was significantly different in the Kruskal–Wallis test (P <

0.0005). The DSS group had a lower score in nest building when compared to the control group ($P < 0.05$). All CBD doses showed an increased score in the nest building test when compared to the DSS group ($P < 0.05$) with no difference when compared to the control group ($P > 0.05$) (Fig. 7 A).

On the seventh day of the colitis protocol, spontaneous locomotor activity was measured in the OFT to understand the CBD and DSS-induced colitis effects on it. The spontaneous locomotor activity showed no significant difference in the One-way ANOVA ($F (4, 70) = 1.867$, $P > 0.05$) (Fig. 7 B).

In order to evaluate anxiety-like behavior and mobility on DSS colitis mice, the elevated plus-maze test was also performed on the seventh day of the colitis protocol. The One-way ANOVA demonstrated significantly different only in the percentage of time at the open arm ($F (4, 70) = 6.274$, $P=0.0002$). The Newman-Keuls post hoc test showed that all CBD groups had an increased percentage of time at the open arm when compared to the DSS group ($P < 0.05$). The DSS group decreased the percentage of time at the open arm when compared to the control group ($P < 0.05$) (Fig. 7 C, D and E).

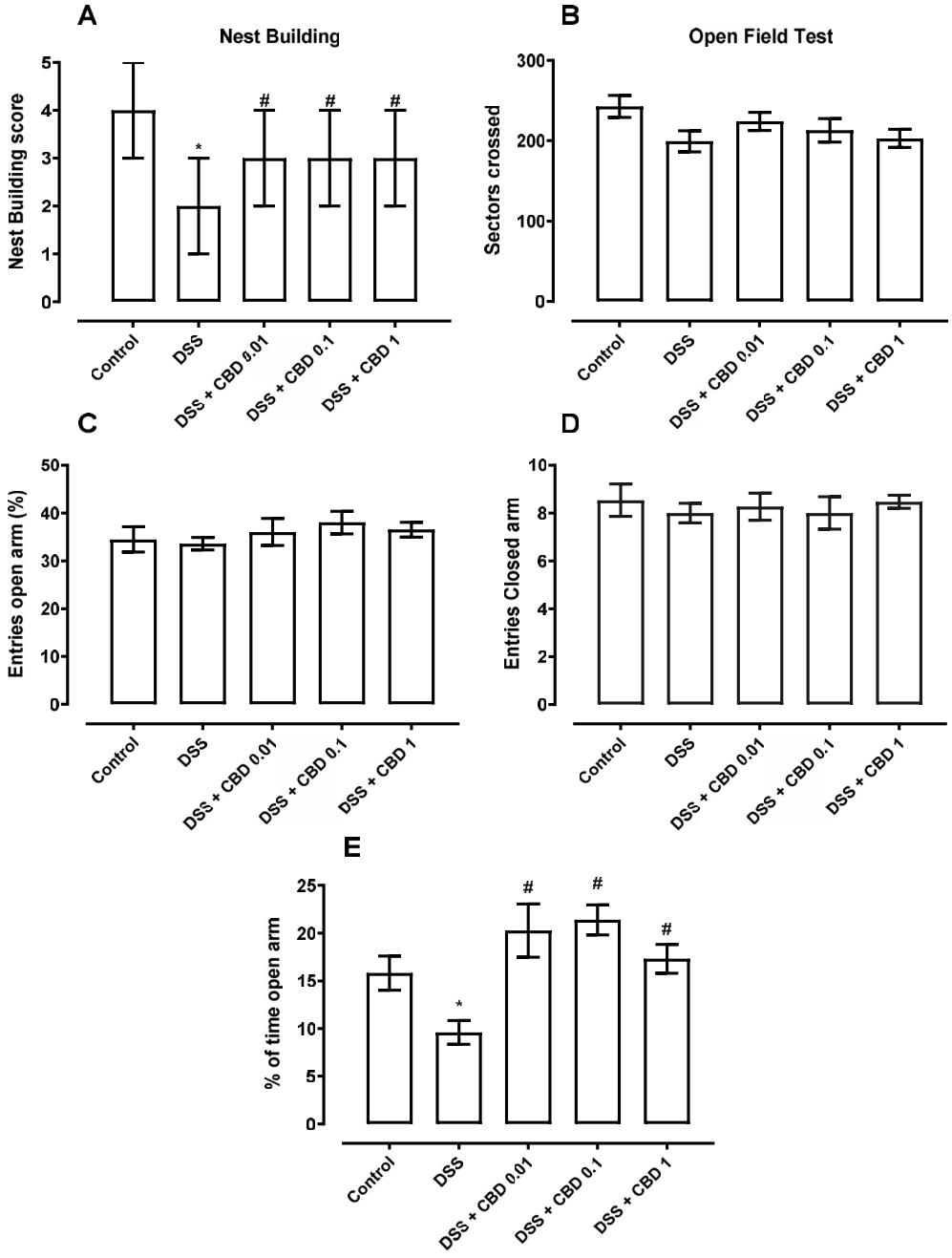

Fig. 7. Effect of CBD administration on the nest building test (Fig. 7 A) and OFT (Fig. 7 B) on DSS-induced colitis. Effect of CBD administration on EPM (Fig. 7 C, D and E) on DSS-induced colitis. Mice received 5% DSS in water for 5 days followed by 2 days of tap water. The control group received only tap water. Animals were treated

once a day, DSS group (Vehicle) or CBD (0.01; 0.1 and 1 mg/kg, p.o.). Results are expressed as mean ± standard error of means (n = 15). * P < 0.05 when compared to the Control group; # P < 0.05 when compared to the DSS group (One-way ANOVA followed by Newman-Keuls test for parametric data and Kruskal–Wallis followed by Dunn's test for non-parametric data).

3.6 Evaluation of Serotonin levels

The 5-HT levels in the prefrontal cortex demonstrated a significant difference in the One-way ANOVA ($F(4, 40) = 7.597$, $P=0.0001$) (Fig. 8). Newman-Keuls test revealed that the DSS had a significant decrease in 5-HT levels in the prefrontal cortex when compared to the control group ($P < 0.05$). All CBD treatments had an increase in the serotonin levels when compared to DSS group ($P < 0.05$) (Fig.8).

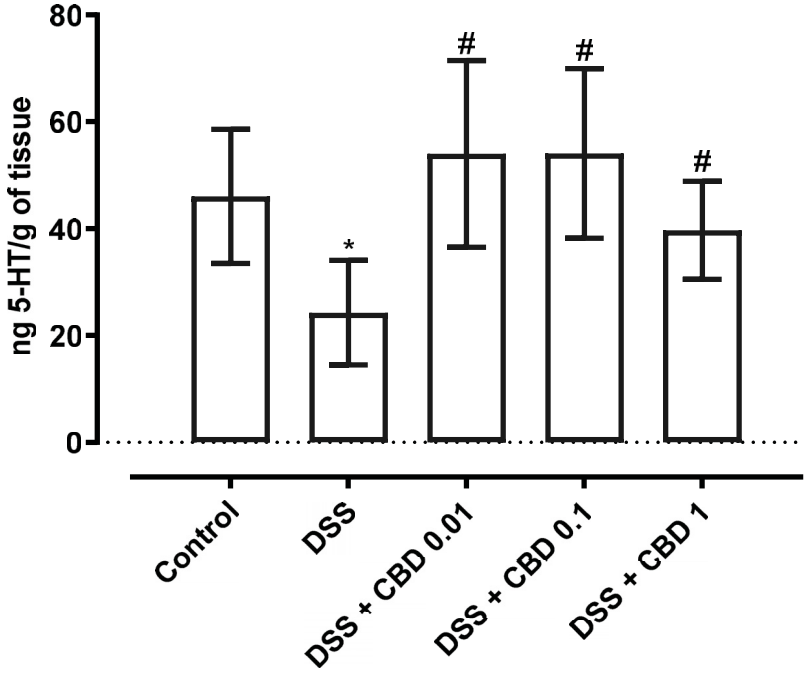

Fig. 8. Effect of CBD administration on 5-HT levels in the prefrontal cortex on DSS-induced colitis. Mice received 5% DSS in water for 5 days followed by 2 days of tap water. The control group received only tap water. Animals were treated once a day, DSS group (Vehicle) or CBD (0.01; 0.1 and 1 mg/kg, p.o.). Results are expressed as mean ± standard error of means (n = 9-10). * $P < 0.05$ when compared to the Control group; # $P < 0.05$ when compared to the DSS group (One-way ANOVA followed by Newman-Keuls test).

4. DISCUSSION

UC is a chronic relapsing-remitting disease in which no effective pharmacological treatment can completely cure this pathological condition in addition to carrying significant side effects. As a result, CBD, one of the main non-psychomimetic

phytocannabinoids emerges as a viable treatment opportunity to be studied. Data from literature already demonstrated that CBD has major properties in pain, inflammation, and anxiety, forms of distress that are experienced by UC patients [20]. Here, we showed that low doses of CBD reduced abdominal pain, inflammation, and oxidative stress and promoted welfare in DSS-induced colitis mice. Besides *Cannabis sativa* being one of the most studied plants, there are few studies relating the benefits of the whole plant and its cannabinoids in IBD, and in this sense, this study adds important findings of CBD in a well-characterized experimental colitis model.

DSS-induced colitis is a noted sulfated polysaccharide-induced model that resembles UC. The acute colitis was induced in mice following DSS regimen administration in drinking water. During the three last days of the experiment, animals displayed significant weight loss, diarrhea, and fecal bleeding, which were similar to those previously reported by our group [15]. All these DAI parameters were used here to assess and evaluate the clinical progression of disease that was significantly reduced by CBD 0.1 mg/kg treatment. Again, the severity of colitis was macroscopically scored based on the shortening of colon length, which was accompanied by DAI in vehicle DSS-treated mice. However, the three doses of CBD not prevented colonic shortness. A recent study focusing on the ability of combined Fish Oil/CBD administration improves inflammation and dysbiosis in the dextran sulphate sodium (DSS) model of mouse colitis, published as the current one was being completed, reported that CBD alone at 0.3-30 mg/kg given by oral route did not affect DAI, colon weight/length ratio, body weight, and MPO in the DSS-induced colitis mice [21]. In this regard, we speculate that this apparent discrepancy may be attributed to a sex and strain-related difference, since we used female Swiss mice vs. male CD1 mice. Notably, Salviato et al, 2021 pointed out that females may be more sensitive than males to the effects of

cannabinoids, studying the sensitivity to the low Δ^9-tetrahydrocannabinol doses in anxiety-like behavior. Sexual dimorphism is a relevant factor that needs to be taken into account since the prevalence of UC is higher in women [1]. Moreover, mice treated with CB1R and CB2R selective agonists required higher doses at 2 times a day to reduce macroscopic and microscopic parameters in the DSS colitis model when compared to the acute model induced by the transient receptor potential ankyrin 1 (TRPA1) agonist (mustard oil) administrated by the intracolonic route [23]. As previously reported, other authors observed that a peripherally restricted synthetic mixed CB1/CB2R agonist treatment did not affect the body weight, DAI, and MPO in colitis induced by DSS [24].

In addition to important macroscopic changes, histochemical alterations are useful markers of the severity of the aggression promoted by the DSS in the colon. Here, DSS was also effective in generating histological changes in the microstructure of mice colon tissue. H&E colon staining revealed extensive tissue damage, which was not improved by CBD treatment. Moreover, the colonic mucin carbohydrate content, namely neutral (PAS+) and acidic (Alcian Blue+) mucins, were reduced by DSS and remained unchanged followed by low CBD doses treatment. Recently, the oral administration of higher doses of a cannabidiol-rich cannabis extract (61.5 – 615 mg/kg) for 10 days in normal mice promoted a serious dysbiosis, represented by an increase in the relative abundance in *Akkermansia muciniphila*, a mucin-degrading bacterium, which in turn promoted a severe decrease in Muc2 expression, resulting in negative gut health reflex [25]. Despite we do not evaluate the gut microbiome or Muc 2 gene expression, it is well established that the reduction in the mucin content may contribute to the disruption of the intestinal tight junctions, impairing the intestinal barrier function, and favoring the inflammatory response observed in UC. Furthermore,

to our knowledge, this is the first study demonstrating the macro and microscopic effects (including mucin staining) of CBD in a colitis model in mice.

UC patients often experience moderate to severe abdominal pain. Interestingly, in a controlled study, women with UC that received dried cannabis flowers cigarettes (~16% THC, 0.1% CBD among others cannabinoids) displayed a significant reduction of abdominal pain, a symptom that was not modified in the control group [26]. Again, our data showed a significant reduction in abdominal mechanical sensitivity in DSS-induced colitis female mice promoted by CBD on all assessment days. To further understand the mechanisms of CBD analgesia, it is interesting to know that the TRP receptor family is expressed in the mucosa and the colon muscle layers and is known to play an important role in pain, regulation of motility, absorption and secretion processes, as well as homeostasis of the intestinal mucosa [27]. TRPV1 regulation induces amelioration of the inflammatory process during DSS-induced colitis and TRPV1 upregulation has been observed in both colitis animal models and IBD patients. Moreover, the literature presents a wide range of studies indicating that CBD could interact with TRPV1 and then modulate nociceptive response [28].

Although the mechanism that DSS induces coltis is not clear, the resulting colonic inflammation from ingestion of DSS has been shown to include polymorphonuclear cells colonic infiltration. Indeed, MPO activity is an inflammatory marker used to quantify the neutrophil migration in whole-tissue colons. Considering the administration of the chemical irritant DSS, the resulting colonic inflammation could modulate the abdominal hypersensitivity in the DSS-induced colitis model. Because MPO levels were significantly reduced by all tested doses of CBD, we suggest that the severity of colonic inflammation was also reduced by the CBD treatment. Again, contrary to our data, higher doses of CBD (0.3-10 mg/kg, p.o.) did not attenuated MPO activity [21].

Here, together with the reduction of colonic leukocyte infiltration, CBD 0.1 mg/kg also changed another inflammatory marker, represented by the reduction of the TNF-α levels. This anti-inflammatory effect promoted by CBD has been demonstrated previously in colitis as well as in other chronic inflammatory diseases [29, 30]. Although not evaluated here, all these anti-inflammatory effects can be explained by CBD promiscuous activity and its complex mechanism of action. CBD can act through PPARγ, a nuclear receptor that can be expressed in the gastrointestinal tract, which could interact with NFκB, leading to a downregulation of pro-inflammatory gene expression, such as TNF-α [31]. In addition, CBD can act modulating the CB2R, which is known to be more expressed in the immune system [32]. Of note, CB1R and CB2R were found in the normal human colonic biopsies through western blot and immunohistochemical analysis, reinforcing the roles of modulation of colonic neuronal input and secretion and colonic immunomodulation, respectively. Moreover, in mild- and moderate-scored UC patient samples, the authors found higher levels of CB2R in the mucosa epithelium [33]. It is well known that besides CBD displaying a low affinity for CB1 and CB2R, this phytocannabinoid can interact with both CBR at low concentrations, in addition to the other non-CB pharmacological targets to promote its effects [34].

Oxidative stress is highly associated with the inflammatory process and therefore it is involved in UC disease [35], as well as is linked to DNA damage and cancer [36]. CBD can block free radical chain reactions and transform them into less active forms and/or protecting enzymes related to antioxidant activity, modulating the level and activity of oxidants and antioxidants targets, such as increasing GSH levels after CBD incubation in mouse microglial cells [37]. Our results demonstrated that CBD at 0.01 and 0.1 mg/kg is capable of normalizing colonic GSH levels when compared to the control and DSS

group. In fact, CBD can prevent oxidation by protecting non-enzymatic antioxidants, as in the case of GSH, showing significant positive correlations with the mucosal anti-inflammatory effect. Altogether, here CBD 0.1 mg/kg reduced DAI score abdominal pain, inflammatory and oxidative stress parameters, but this data did not demonstrate a positive correlation with histopathological features.

UC patients can develop anxiety and depression in consequence of symptoms or due to the side effects of pharmacological treatments [38]. Considering the strong comorbidity between chronic pain and anxiety, here we highlighted the ability of low doses of CBD to promote analgesic and anxiolitic-like effects in DSS-induced colitis female mice. All tested doses of CBD increased open arms exploration in the EPM, suggesting an anxiolytic-like effect of CBD. Moreover, in the same animals, the general wellbeing promoted by CBD was confirmed by nesting behavior. Notably, low doses of CBD normalize general serotonin levels in the prefrontal cortex tissue of female mice in DSS-induced colitis. Anxiety and depression have been associated with low levels of serotonin, with most antidepressants acting by increasing the activity of the serotonin system. In addition, 5-HT1A receptors are expressed in the prefrontal cortex and hippocampus neurons and are related to the 5-HT response on fear, anxiety, and stress [39]. Previous studies showed that CBD could modulate anxiety responses through the 5-HT1A receptor and positive effects against anxiety during public speaking [40].

In this way, we suggest that low doses of CBD improved pain, distress and suffering in female mice in DSS-induced colitis. In line with these findings, low doses of CBD (0.3-3 mg/kg, i.p.) were able to reduce pain and anxious behaviors in a model of neuropathic chronic pain in rats, as well as modulated the CBR1 and TRPV1R expression in cortical and limbic structures [41]. In the same direction, CBD at low doses

(0.1-1 mg/kg, i.v.) promotes analgesia and reduces anxiety through TRPV1 and 5-HT1A receptor activation, respectively, and restoring the 5-HT neurotransmission under neuropathic pain conditions in rats [42]. The results of the study discussed highlight the complexity of the CBD action during UC. DAI results were able to show that low doses of CBD can alleviate colitis symptoms, and abdominal analgesia together with anxiolytic-like effect and improvement in well-being present a positive correlation with the 5-HT levels in the prefrontal cortex of female DSS-induced colitis mice.

Taken together, this study demonstrated several benefiting effects of low doses of CBD treatment in DSS-induced colitis in mice. Beyond anti-inflammatory, analgesic, and antioxidant effects, low doses of CBD also promote welfare, possibly through the regulation of serotonin levels in the prefrontal cortex.

5. CONCLUSION

We examined the effects of low doses of CBD in modulating inflammation, abdominal pain, anxiety-like behavior, and welfare in a mice model of colitis. The current findings are relevant and support new perspectives of therapeutic approaches regarding the efficacy of CBD for treating UC and comorbid mood disorders. Further studies are required to fully understand the mechanism of CBD action in this model of IBD.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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4 CONCLUSÃO

As descobertas realizadas neste estudo auxiliam no maior entendimento dos efeitos do CBD na colite e trazem novas perspectivas de abordagens terapêuticas. Os resultados aqui presentes sugerem que o CBD em doses mais baixas tem efeitos anti-inflamatórios e analgésicos. Além disto, o CBD apresentou uma boa ação antioxidante. Em somatória a estes efeitos, o CBD foi capaz de normalizar os níveis de serotonina, indicando um efeito ansiolítico. Todos estes efeitos aqui citados permitem afirmar que o CBD em doses baixas, foi capaz de induzir o bem-estar geral dos animais. Se não como principal abordagem terapêutica, o CBD deve ser estudado ainda mais como terapia adjuvante ao tratamento convencional. Mais estudos são necessários para entender completamente os mecanismos de ação do CBD neste modelo de DII.

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