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EDVALDO BALBINO ALVES JUNIOR

ATIVIDADE ANTIULCEROGÊNICA E ANTI-INFLAMATÓRIA INTESTINAL DO ESTRAGOL EM MODELOS ANIMAIS

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“Sonhos determinam o que você quer. Ação determina o que você conquista.”

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RESUMO

Os fenilpropanóides são uma classe de compostos produzidos pelas plantas medicinais e são biossintetizados a partir da fenilalanina via ácido cinâmico. O estragol é um fenilpropanoide que está presente como constituinte de óleos essenciais de muitas espécies vegetais, como Ravensara anisata (Madeira), Ocimum basilicum (Manjericão) e Croton zehntneri (Canelinha). Atividades farmacológicas como antioxidante, antimicrobiana, ansiolítica, contrátil do músculo esquelético, relaxante do músculo visceral, anti-inflamatória e gastroprotetora são descritas. O presente trabalho tem como objetivo geral avaliar a atividade antiulcerogênica e anti-inflamatória intestinal do estragol em modelos animais. No modelo de úlcera duodenal induzida por cisteamina a administração oral (v.o) de estragol nas doses de 31; 62; 125 e 250 mg/kg) reduziu ($p<0,001$) a área de lesão ulcerativa (ALU) em duodenos de ratos. A partir do protocolo de úlcera gástrica induzida por ácido acético foi investigada a toxicidade por doses repetidas durante 14 dias. Os resultados demostraram que o tratamento (v.o) com estragol na dose de 250 mg/kg reduziu ($p<0,001$) a ALU, quando comparado ao grupo controle. Esse efeito foi relacionado a um aumento dos níveis de GSH, SOD, IL-10 e TGFβ ($p<0,001$) e uma redução ($p<0,001$) dos níveis de MDA, MPO, IL-1β, TNF-α e NFκB. A administração de estragol durante 14 dias não alterou o peso dos órgãos (coração, fígado, baço e rins), nem os parâmetros bioquímicos e hematológicos avaliados. Além disso, a substância teste protege os animais da redução do consumo de água e de ração. No modelo de indução aguda de inflamação intestinal induzida por TNBS, o estragol reduziu ($p<0,05$) o escore de lesão macroscópica, a ALU, a relação peso/comprimento e o índice diarreico em todas as doses avaliadas. No protocolo sub-crônico de colite ulcerativa com recidiva, o estragol em sua dose mais efetiva (250 mg/kg) reduziu ($p<0,01$) o escore de lesão macroscópica, a ALU e a relação peso/comprimento do cólon. A partir dos protocolos agudo e subcrônico de colite, foi observado que o estragol (250 mg/kg) reduziu ($p<0,001$) os níveis de MDA e MPO e restaurou os níveis de GSH e SOD. Em adição, reduziu ($p<0,001$) os níveis de IL-1β, TNF-α e e NFκB, bem como, aumentou os níveis de TGFβ ($p<0,001$) e restaurou ($p<0,01$) os de IL-10. Na avaliação de função da barreira epitelial foi mostrado um aumento ($p<0,001$) da imunomarcação para zona de oclusão-1 (ZO-1). A partir dos dados analisados, pode-se sugerir que o estragol apresenta atividade antiúlcera duodenal, atividade cicatrizante gástrica está relacionada à indução da reepitelização da lesão e baixa toxicidade por doses repetidas. E atividade anti-inflamatória intestinal na fase aguda e sub-crônica, sendo relacionada à citoproteção da barreira intestinal por meio da preservação das junções comunicantes, sistema antioxidante e da imunomodulação.

Palavras chave: Estragol, atividade cicatrizante gástrica, atividade antiúlcera duodenal, atividade anti-inflamatória intestinal, fenilpropanoides.

ALVES JUNIOR, E.B. ANTIULCEROGENIC AND ANTI-INFLAMMATORY ACTIVITY OF THE ISOMER ESTRAGOLE IN ANIMAL MODELS. 2023. 131 p. Tese de doutorado. Programa de Pós-graduação em Produtos Naturais e Sintéticos Bioativos, CCS/UFPB.

ABSTRACT

Phenylpropanoids are a class of compounds produced by medicinal plants and are biosynthesized from phenylalanine via cinnamic acid. Estravole is a phenylpropanoid that is present as a constituent of essential oils of many plant species, such as Ravensara anisata (Madeira), Ocimum basilicum (Basil) and Croton zehntneri (Cinnamon). Pharmacological activities such as antioxidant, antimicrobial, anxiolytic, skeletal muscle contractile, visceral muscle relaxant, anti-inflammatory and gastroprotective are described. The present work has the general objective to evaluate the intestinal antiulcerogenic and anti-inflammatory activity of trazol in animal models. In the cysteamine-induced duodenal ulcer model, oral (v.o) administration of trazol at doses of 31; 62; 125 and 250 mg/kg) reduced ($p<0.001$) the ulcerative lesion area (ALU) in rat duodenum. From the gastric ulcer protocol induced by acetic acid, the toxicity was investigated by repeated doses for 14 days. The results showed that the treatment (v.o) with 250 mg/kg of trazol reduced ($p<0.001$) the ALU, when compared to the control group. This effect was related to an increase in the levels of GSH, SOD, IL-10 and TGFβ ($p<0.001$) and a reduction ($p<0.001$) in the levels of MDA, MPO, IL-1β, TNF-α and NFκB. The administration of trazol for 14 days did not change the weight of the organs (heart, liver, spleen and kidneys), nor the biochemical and hematological parameters evaluated. In addition, the test substance protects the animals from reduced water and feed consumption. In the acute induction model of TNBS-induced intestinal inflammation, trazol reduced ($p<0.05$) the macroscopic lesion score, ALU, weight/length ratio, and diarrheal index at all doses evaluated. In the sub-chronic protocol of ulcerative colitis with relapse, the most effective dose of trazol (250 mg/kg) reduced ($p<0.01$) the macroscopic lesion score, the ALU and the colonic weight/length ratio. From the acute and sub-chronic colitis protocols, it was observed that trazol (250 mg/kg) reduced ($p<0.001$) MDA and MPO levels and restored GSH and SOD levels. In addition, it reduced ($p<0.001$) levels of IL-1β, TNF-α and NFκB, as well as increased TGFβ levels ($p<0.001$) and restored ($p<0.01$) IL-10 levels. In the evaluation of the epithelial barrier function, an increase ($p<0.001$) of the immunostaining for zone of occlusion-1 (ZO-1) was shown. Based on the data analyzed, it can be suggested that trazol has anti-duodenal ulcer activity, gastric healing activity is related to the induction of re-epithelialization of the lesion and low toxicity by repeated doses. And intestinal anti-inflammatory activity in the acute and sub-chronic phase, being related to the cytoprotection of the intestinal barrier through the preservation of gap junctions, antioxidant system and immunomodulation.

Keywords: Estragole, gastric healing activity, duodenal antiulcer activity, intestinal anti-inflammatory activity, phenylpropanoids.

LISTA DE FIGURAS

LISTA DE ABRVIATURAS, SIGLAS E SÍMBOLOS

AINEs	Anti-inflamatórios não esteroidais
ALU	Área de lesão ulcerativa
AMPc	3',5'- monofosfato de adenosina cíclico
ANOVA	Análise de variância de uma via
CAT	Catalase
CEUA	Comitê de Ética em Uso Animal
COX	Ciclooxigenase
CU	Colite ulcerativa
DC	Doença de Crohn
DII	Doença inflamatória intestinal
DL_{50}	Dose letal 50
d.p	Desvio padrão da média
EGF	Fator de crescimento epidérmico
EROs	Espécies reativas de oxigênio
GI	Gastrintestinal
GPx	Glutationa peroxidase
GSH	Glutationa reduzida
H^+/K^+ ATPase	Bomba de prótons
H_2	Receptor de histamina tipo 2
HCl	Ácido clorídrico
HCO_3^-	Íon bicarbonato
H_2O_2	Peróxido de hidrogênio
HGF	Fator de crescimento de hepatócitos
HPA	Eixo Hipotalâmico-pituitário-adrenal
HSP	Proteínas de choque térmico
IL-1β	Interleucina 1 beta
IL-6	Interleucina 6
IL-8	Interleucina 8
IL-10	Interleucina 10
ILC	Célula linfoide inata
i.p.	Intraperitoneal

IFNγ	Interferon-γ
IGF-1	Fator de crescimento semelhante à insulina-1
iNOS	Sintase do óxido nítrico induzível
M_3	Receptor muscarínico tipo 3
MDA	Malondialdeido
MMP	Metaloproteinase
MPO	Mieloperoxidase
MUC	Mucina
NFκB	Fator nuclear Kb
NK	Natural killer
NLR	Receptor do tipo NOD
NO	Óxido nítrico
NOD	Nucleotide-binding oligomerization domain containing 2
NOS	Sintase do óxido nítrico
NOX	NADPH oxidases
O_2^-	Superóxido
·OH	Hidroxila
$ONOO^-$	Peroxinitrito
PAMP	Padrão molecular associado a patógeno
PG	Prostaglandina
PGE_2	Prostaglandina da série E_2
PGI_2	Prostaciclina
PgPNSB	Programa de Pós-graduação em Produtos Naturais Sintéticos e Bioativos
PRR	Receptor de reconhecimento de padrão
RAGE	Receptor de produtos finais de glicação avançada
RNA	Ácido ribonucleico
ROOH	Hidroperóxidos
-SH	Grupamentos sulfidrilas
SOD	Superóxido dismutase
STAT	Fatores de transcrição ativados por tirosinas quinases
TLR	Receptor do tipo Toll
TFF	Fator trefoil
TGF-β	Fator de crescimento transformante β

TGI	Trato gastrointestinal
TLRs	Receptores Toll-like
TNBS	Ácido trinitrobenzenosulfônico
TNFα	Fator de necrose tumoral-α
UP	Úlcera péptica
v.o.	Via oral
VEGF	Fator de crescimento endotelial vascular

SUMÁRIO

1 INTRODUÇÃO

As afecções do trato gastrintestinal compreendem um conjunto heterogêneo de distúrbios caracterizados por dispepsia, refluxo, inflamação, ulceração, constipação ou diarreia, entre outros, além de apresentar origem complexa e multifatorial. Dentre essas afecções destacam-se as úlceras pépticas, as doenças inflamatórias intestinais e a diarreia (ANANTHAKRISHNAN; XAVIER, 2020)

As úlceras pépticas são caracterizadas pela presença de um processo inflamatório e oxidativo da mucosa gástrica ou intestinal, com presença de lesões necróticas e hemorrágicas. Sua gravidade depende da camada lesionada e pode acometer desde a camada da mucosa, mais superficial, até as camadas tissulares mais profundas, como camada epitelial e muscular da mucosa, com comprometimento da funcionalidade e integridade das células lesionadas (LANAS, CHAN, 2017; ANANTHAKRISHNAN; XAVIER, 2020).

As doenças inflamatórias intestinais (DIIs) também são desordens multifatoriais, que compreendem a colite ulcerativa (CU) e a doença de Crohn (DC). Não possuem etiologia totalmente definida, entretanto, estão associada a distúrbios no sistema imunológico e/ou alterações da microbiota, exposição a certos fatores ambientais e a susceptibilidade genética (MIELE et al., 2018; SORDO et al., 2022).

Embora existam tratamentos específicos disponíveis para as afecções do trato gastrintestinal, algumas limitações como o alto índice de recidiva da doença, a diversidade de efeitos colaterais, acesso limitado aos medicamentos, interações medicamentosas, alto custo de internações dos indivíduos, remetem a necessidade de novas alternativas terapêuticas para o tratamento dessas doenças (HARBORD et al., 2017; CLARKE et al., 2022).

Nessa perspectiva, surgem os produtos naturais, que são compostos químicos de origem animal, mineral, de microrganismos (fungos, bactérias) e vegetal (WANG et al., 2018), em especial as plantas medicinais que apresentam uma riqueza de metabólitos biologicamente ativos de interesse clínico (BOEING et al., 2016). O Brasil possui a maior biodiversidade do planeta e sua

população usa essas fontes naturais para combater doenças de caráter agudo ou crônico. Entretanto, novas pesquisas e tecnologias são necessárias para otimização do uso de fontes naturais como tratamento, com o intuito de isolar componentes responsáveis pelos efeitos biológicos a fim de minimizar possíveis efeitos colaterais (DUTRA et al., 2016; WANG et al., 2018).

A substância selecionada para esse estudo é o Estragol (fenilpropanoide) um composto orgânico aromático presente em óleos essenciais de espécies vegetais, como *Ravensara anisata* (madeira), *Ocimum basilicum* (manjericão/alfavaca), e *Croton zehntneri* (canelinha) cujos estudos farmacológicos relatam sua atividade anti-inflamatória, antioxidante e vasorelaxante, além de atividade gastroprotetora, evidenciada pelo nosso grupo de pesquisa (ALVES JUNIOR et al., 2020)

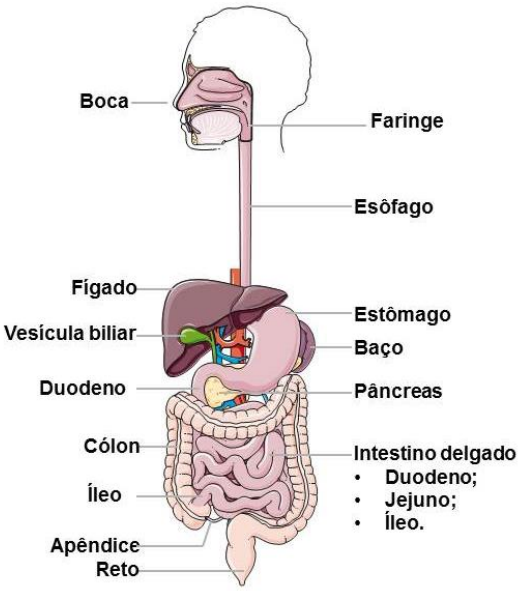
Deste modo, o presente trabalho é uma continuidade dos estudos iniciados por nosso grupo de pesquisa e se propõe avaliar as atividades cicatrizante gástrica, antiúlcera duodenal e anti-inflamatória intestinal dos protocolos de colite aguda e subcrônica do estragol em modelos animais.

2 REVISÃO DA LITERATURA

2.1 Trato Gastrintestinal (TGI)

O Trato gastrintestinal (TGI) é composto por um tubo muscular longo e oco com aproximadamente 6 metros de comprimento que inicia na boca e se estende até o ânus, com diferentes compartimentos (ASHFORD, 2017; GOKULNATH, 2021). Anatomicamente o TGI divide-se em esôfago, estômago, intestino delgado (duodeno, jejuno e íleo) e intestino grosso (cólon, ceco e reto). É em sua maioria composto por camadas musculares (músculo liso), vasos sanguíneos, tecido linfoide e conectivo e glândulas anexas (Figura 1) (CAMPBELL, 2015; IQBAL et al., 2022).

Figura 1. Representação Anatômica do TGI Humano



Fonte: Adaptado de Digestive system, 2017. O TGI consiste em um tubo muscular oco a partir da cavidade oral, continuando pela faringe, esôfago, estômago e intestinos até o reto e ânus. Existem vários órgãos acessórios que auxiliam o TGI secretando enzimas para ajudar a decompor os alimentos em seus nutrientes componentes.

O entendimento do papel do TGI na homeostase está além de sua função digestiva, desse modo, tornou-se objeto de estudos em diversas áreas de conhecimento. Por meio das múltiplas ações das células especializadas presentes em seu revestimento, em conjunto com a microbiota residente em seu lúmen intestinal, promovem processos fisiológicos que resultam no equilíbrio gastrintestinal (OTEIZA; CREMONINI; FRAGA, 2022).

Dentre os múltiplos processos biológicos que ocorrem no TGI, destacam-se: a absorção de nutrientes e bioativos provenientes da alimentação; secreção de hormônios e peptídeos bioativos que coordenam ações locais e sistêmicas; metabolismo de lipídios e carboidratos (IQBAL et al., 2022); exerce função de barreira física que impede a entrada de bactérias, toxinas e outros patógenos na circulação sanguínea; promove a regulação da tolerância aos componentes alimentares e bactérias comensais, bem como a reação à patógenos por meio da presença de células do sistema imune (WELLS et al., 2017). Além disso, proporciona condições ambientais favoráveis ao desenvolvimento da microbiota local em uma relação de simbiose com o indivíduo (OTEIZA; CREMONINI; FRAGA, 2022).

O desequilíbrio dos processos biológicos no TGI são fontes para afecções sistêmicas, incluindo doença cardiovascular, *Diabetes Mellitus* tipo 2 (ODENWALD; TURNER, 2017; BURCELIN et al., 2022) doença do fígado gorduroso não alcóolico (DAMMS-MACHADO et al., 2017) e distúrbios neurológicos (BROWN, 2019), úlceras pépticas e doenças inflamatórias intestinais (DII) (SORDO et al., 2022).

2.2 Úlceras Pépticas

As úlceras pépticas consistem em um conjunto de distúrbios caracterizados pela presença de inflamação e lesões na mucosa, as quais são de origem multifatorial, caracterizadas pelo surgimento de pontos necróticos e lesões hemorrágicas que podem acometer o esôfago, estômago ou duodeno (PASHA et al., 2022).

As úlceras pépticas são classificadas em superficial (submucosa), profunda (muscular externa), perfurante (cavidade peritoneal) e penetrante (órgãos vizinhos) (TAHA et al., 2005; PRASOON et al., 2022).

2.2.1 Etiologia e Fisiopatologia das Úlceras Pépticas

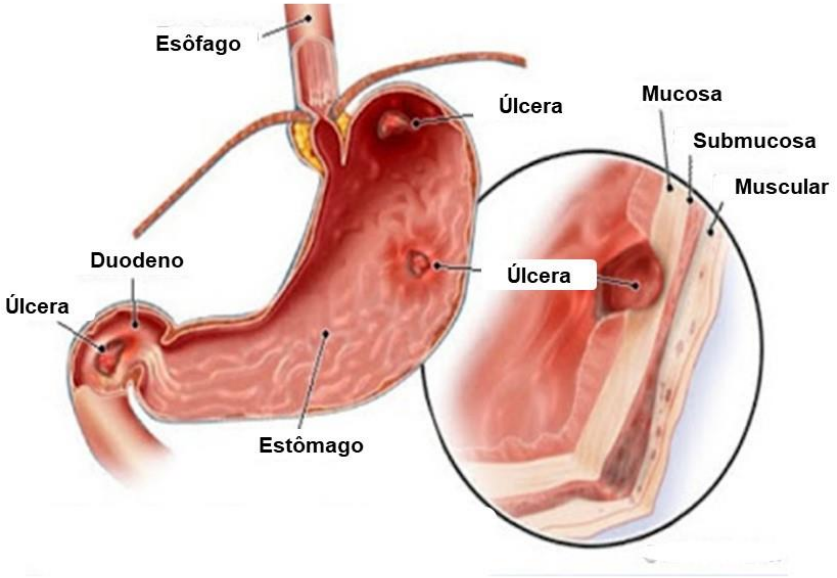
Em condições de homeostase, a secreção ácida gástrica é uma solução isotônica de 150 mmol/L com pH abaixo de 1 e composta majoritariamente pelo ácido clorídrico (HCl), pepsina, íons e pelo fator antianêmico intrínseco (glicoproteína responsável pela absorção da vitamina B12, ferro e cálcio),

sendo secretada pelas células parietais (BROWNLEE, 2014; SCHUBERT, 2015; SAHOO et al., 2018). A regulação desse processo ocorre em quatro fases específicas (SCUBERT, 2016; FEHER, 2017) que compreendem a fase basal, fase cefálica, fase gástrica e fase intestinal (LANDA et al., 2019).

As úlceras pépticas surgem quando o sistema de defesa da mucosa normal é subjugado por agentes nocivos, como HCl, pepsina, bile, isquemia (WOODS; CAREY, 2017), infecção por *H. pylori*, hipergastrinemia, condições de estresse, tabagismo, deficiências nutricionais e uso crônico de AINEs (SEPULVEDA et al., 2016; SIDAHMED et al., 2018; DA SILVA et al., 2018; OLIVEIRA et al., 2022).

As úlceras tendem-se a agravar de acordo com o grau de acometimento da lesão, podendo atingir desde a camada mais superficial da mucosa até as mais profundas, como a muscular, com alteração da funcionalidade e integridade das células lesionadas (TARNAWSKI et al., 2013; LANAS, CHAN, 2017). A denominação da úlcera péptica varia de acordo com o órgão lesionado, que pode ser úlcera esofágica, gástrica ou duodenal, quando o órgão lesionado é o esôfago, estômago ou duodeno, respectivamente (**Figura 2**) (TARNAWSKI et al., 2013; LANAS, CHAN, 2017; ELSHAZLY et al., 2018; KAVITT et al., 2019).

Figura 2. Representação de regiões anatômicas do trato gastrointestinal acometidas pelas úlceras pépticas



As lesões na mucosa gástrica podem ser desenvolvidas por fatores agressivos endógenos (ácido, pepsina e ácidos biliares) associados a fatores exógenos (AINEs, tabagismo, consumo de álcool, estresse emocional e bactéria *H. pylori*) que desestabilizam a integridade da mucosa e inibem a produção de fatores protetores, como o muco, o bicarbonato e as prostaglandinas (TARNAWSKI et al., 2012; YANDRAPU; SAROSIEK, 2015; RIBEIRO, 2017. DA SILVA et al., 2018; MALFERTHEINER; SCHULZ, 2020).

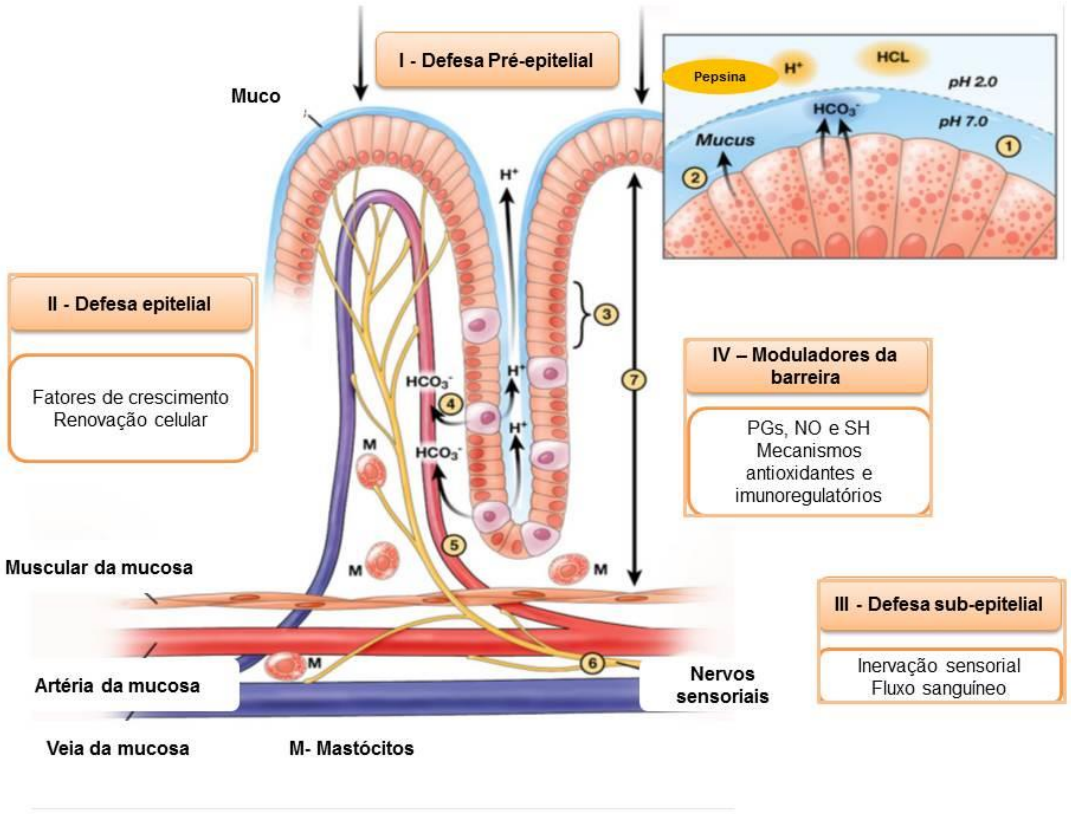
As úlceras gástricas e duodenais apresentam-se arredondadas ou ovaladas com borda regular ou levemente elevadas com fundo normalmente limpo e formação de uma cavidade contendo mediadores inflamatórios, que pode ser de forma aguda e/ou crônica (DA SILVA et al., 2018).

Os processos de hemostasia e os mecanismos de defesa do epitélio estomacal envolvem sistemas locais e neuro-humorais que compreendem os fatores: pré-epiteliais, epiteliais, sub-epiteliais ou endoteliais (TARNAWSKI et al., 2013; QUINTANA-HAYASHI et al., 2018; KAVITT et al., 2019).

A primeira linha de defesa da mucosa gástrica compreende os fatores

pré-epiteliais como o muco, fosfolipídios de membrana e HCl) (QUINTANA-HAYASHI et al., 2018). Os fatores epiteliais representam a segunda linha de defesa e compreendem as células epiteliais, os fatores de crescimento e de renovação celular. Os fatores sub-epiteliais compreendem a inervação sensorial, o fluxo sanguíneo, os fatores reconstrutivos do epitélio e os reguladores da barreira gástrica: prostaglandinas (PG) e prostacicllinas, óxido nítrico, além do sistema antioxidante e imunorregulatório, conforme demonstrado na **Figura 3** (LAINE et al., 2008; TARNAWSKI et al., 2012; TARNAWSKI et al., 2013; YANDRAPU; SAROSIEK, 2015; RIBEIRO, 2017; MAGIEROWSKA et al., 2019).

Figura 3. Mecanismos de Proteção da Mucosa Gástrica



(Fonte: adaptada de TARNAWSKI et al., 2012; TARNAWSKI et al., 2013; YANDRAPU; SAROSIEK, 2015).

O etanol é um dos principais agentes agressores exógenos da mucosa gástrica. Ele exerce uma ação local pela infiltração de mediadores inflamatórios e liberação de radicais livres (BOUTEMINE et al., 2018; DA SILVA et al., 2018); e uma ação sistêmica penetrando rapidamente na mucosa gástrica sendo

convertido em acetaldeído na corrente sanguínea, o que ocasiona redução dos níveis do sistema antioxidante e de prostaglandinas endógenas, além de aumento na expressão de citocinas pró-inflamatórias e produção de radicais livres, o que resulta no estresse oxidativo e aumento dos níveis de malondialdeído (MDA) (DA SILVA et al., 2018). Em geral, o efeito deletério induzido pelo etanol pode se manifestar diretamente via geração de metabólitos reativos, ou indiretamente via ativação de outros mecanismos que finalmente desencadeiam o dano oxidativo (YUN et al., 2017; HUI; FANGYU, 2017; KUMADOH et al., 2021).

Os AINEs são em geral ácidos orgânicos fracos utilizados amplamente na prática clínica pela sua ação analgésica, entretanto tem como efeito colateral a injúria gástrica, já que em contato com o pH neutro da célula epitelial, a molécula do fármaco se ioniza e libera metabólitos lesivos, causando retrodifusão de íons H^+ e formação de lesões superficiais agudas na mucosa do estômago. A perda da integridade da mucosa é seguida pela reação tecidual amplificada pelo conteúdo luminal, como ácido, pepsina, alimento, bile e *H. pylori* (ATHAYDES et al., 2018; HIJOS-MALLADA et al., 2021).

A inibição das prostaglandinas derivadas das ciclooxigenases (COX) reduz os níveis de prostaglandinas, que, consequentemente, diminui a secreção de muco e de bicarbonato, inibe a proliferação celular e diminui o fluxo sanguíneo da mucosa, eventos essenciais para a manutenção da integridade da barreira epitelial. Além disso, a inibição da COX também resulta no aumento de mediadores inflamatórios e biomarcadores apoptóticos, danos epiteliais resultantes de alteração das estruturas microvasculares e aumento da produção de espécies reativas de oxigênio (LANAS; CHAN, 2017; BJARNASON et al., 2018).

O microrganismo *Helicobacter pylori* é uma bactéria Gram-negativa em forma de bacilo que está presente em cerca de 50% da população mundial, destas, 10% estão associadas às úlceras pépticas que pode evoluir para câncer gástrico em 1 a 3% dos casos (SCHUBERT, 2017; CHEN et al., 2021). A relação da presença da bactéria com fatores ambientais e genéticos está relacionada à vulnerabilidade do desenvolvimento de quadros mais graves da

doença ulcerosa (SCHUBERT, 2017; TRAN et al., 2017; HAWKEY., 2022).

O bacilo *H. pylori* tem atividade intrínseca de urease, conferindo-o a capacidade de catalisar a hidrólise da ureia e produzir amônia e gás carbônico (CO_2), resultando em neutralização do ácido gástrico e manutenção da sua sobrevivência (WOODS; CAREY, 2017). Além disso, também estimula processos inflamatórios em resposta à sua infecção, com produção de citocinas pró-inflamatórias, interleucina 1 β (IL-1β) e interleucina 18 (IL-18) (TRAN et al., 2017; SHAH, et al., 2022). Aliado ao fator de imunomodulação, a bactéria *H. pylori* também inibe a secreção ácida gástrica por outros mecanismos, como repressão da atividade da subunidade α-H+/K+-ATPase e da ezerina (papel de tráfego da bomba de prótons), rompimento de barreira da camada de muco e bicarbonato, ruptura das junções comunicantes que culminam em apoptose das células epiteliais, dano ao DNA e simultaneamente estimula produção de somatostatina, com inibição da liberação de secretagogos como gastrina e histamina (SHINDLER-ITSKOVITCH et al., 2018; SHAH, et al., 2022).

Dentre os principais fatores envolvidos na gênese das úlceras gástricas, os fatores ambientais representam grande relevância, com destaque às condições de estresse, uma vez que oferecem risco à integridade de função de barreira da mucosa (CLARKE et al., 2022). Como resposta ao estresse, o organismo libera catecolaminas que favorecem a ativação do eixo hipotálamo-adrenal com liberação de cortisol, agindo diretamente no TGI e resultando em alteração da microcirculação sanguínea gástrica, acúmulo de espécies reativas de oxigênio (EROs), liberação dos grânulos de células inflamatórias contento histamina, aumento da secreção ácida gástrica e surgimento das úlceras (AHMAD et al., 2019).

A endoscopia digestiva alta pode ser usada para diagnosticar a úlcera e é particularmente urgente nos indivíduos com dispepsia e sintomas de alarme concomitantes (idade maior que 60 anos, história familiar de malignidade do trato gastrointestinal superior, perda de peso, saciedade precoce, disfagia, sangramento gastrointestinal, anemia por deficiência de ferro ou vômitos) (SHELL, 2021).

Um estudo prospectivo de pacientes submetidos a uma endoscopia digestiva alta como parte da rotina de manutenção da saúde, determinou que aproximadamente dois terços das pessoas com úlcera são assintomáticas (KAVITT et al., 2019). Entre os pacientes sintomáticos destaca-se a dor epigástrica, que pode estar associada a dispepsia, distensão abdominal, plenitude abdominal, náusea e saciedade precoce (GUPTA et al, 2021). Esses sintomas podem ser de natureza intermitente (KUMADOH et al., 2021; OLIVEIRA et al., 2022).

2.2.2 Cicatrização gástrica

O processo de cicatrização e/ou reparo da úlcera gástrica é complexo e dependente da interposição de vias moleculares e fatores fisiológicos como: fator de crescimento epidérmico (EGF), fator de crescimento transformador α e β (TGF-α e TGF-β), fator de crescimento semelhante à insulina (IGF-1), fatores de crescimento derivados de plaquetas A – D (PDGF), o fator de crescimento de hepatócitos (HGF), o fator de crescimento endotelial vascular A (VEGFA), a anexina-1, fator de crescimento induzível por hipóxia (HIF) -1α e interleucina 10 (IL-10) (SEIWERTH et al., 2018). Além disso, a atividade dos neurônios sensoriais aferentes, bem como as prostaglandinas endógenas, aumenta o fluxo sanguíneo gástrico na margem da úlcera e potencializam a sua cicatrização (MAGIEROWSKA et al., 2019).

A fisiologia da cicatrização da úlcera gástrica tem como base a interconexão entre todos os componentes do sistema de defesa da mucosa gástrica, sendo influenciada por fatores de crescimento, produtos derivados da metabolização pelas COX-1 e COX-2 e neuropeptídeos sensoriais, como o peptídeo relacionado ao gene da calcitonina (CGRP) (FONAI et al., 2011; MAGIEROWSKA et al., 2019).

O processo de cicatrização ocorre em três etapas: inflamatória, proliferativa e remodelação (LINDLEY et al., 2016; HAN; CEILLEY, 2017). A etapa inflamatória da cicatrização está centrada na contenção dos danos em uma área limitada por meio da remoção de agentes patógenos da lesão. A etapa

proliferativa é iniciada quando a microcirculação local sofre vasodilatação e aumenta sua permeabilidade, possibilitando a transcrição leucocitária (em sua maioria neutrófilos e monócitos) (HAN; CEILLEY, 2017).

Na etapa de remodelação, o microambiente gástrico é rico em diferentes citocinas que polarizam a diferenciação dos monócitos em macrófagos, que por sua vez, fagocita restos do tecido, produzem e liberam fatores de crescimento, proliferação e migração celular. O processo de migração é representado pelas células circunvizinhas a exemplo das células epiteliais, queratinócitos e fibroblastos, responsáveis pelo processo de reparo e remodelação do tecido (DA LUZ et al., 2021).

As células epiteliais gástricas possuem alta taxa de renovação celular, necessária para a manutenção da homeostase e integridade da mucosa gástrica pela substituição das células epiteliais danificadas senescentes (FALLAH et al., 2018; ZHANG et al., 2022). A substituição das células lesionadas ou envelhecidas por células progenitoras duram em média de 2 a 4 dias, sendo um processo controlado por fatores de crescimento que norteiam a proliferação celular e posteriormente reepitelizam a área da lesão pela migração celular (YANDRAPU; SAROSIEK, 2015). A reparação tecidual tende a ser eficiente, entretanto, quando ocorre alguma descontinuidade desse processo, o tecido não recupera sua estrutura e funcionalidade, gerando lesões crônicas (braga ET AL., 2016; ZHANG et al., 2022).

As Úlceras péticas se dividem macroscopicamente em duas regiões: a borda da úlcera, formada pela mucosa adjacente sem área necrosada, com presença de componentes epiteliais, formato regular e pouco elevado, que tende a se afunilar à medida que se aprofundam na parede do órgão (BRAGA et al., 2016), e o fundo da úlcera, que é normalmente limpo, mas pode se apresentar coberto de material necrótico esbranquiçado, tecido de granulação avermelhado ou tecido fibroso (RAMOS, 2017). Já microscopicamente as UPs podem ser classificadas em ativas, quando apresentam material necrótico, infiltrado inflamatório (neutrófilos), tecido de granulação e tecido fibrótico cicatricial, ou inativa, quando ocorre uma redução da necrose, do infiltrado neutrofílico e formação da cicatriz tecidual (EL-DAKROURY et al., 2022).

O perfil de expressão de fatores observados na borda da úlcera (zona de cicatrização) de algumas horas até 3 dias após a indução da úlcera, difere significativamente daquele observado após 6 dias e a partir do 14º dia. Desta forma, a cicatrização pode ser caracterizada de acordo com o curso fisiológico da cicatrização e com o perfil expresso em fase inicial, intermediária e tardia (TARNAWSK et al., 2012; AHLUWALIA et al., 2018; SANTOS et al., 2019).

Na borda da úlcera, as glândulas tornam-se dilatadas e suas células adjacentes são desdiferenciadas, expressam o fator de crescimento epidérmico (EGF) e o seu receptor (EGF-R). Após três dias do início da lesão, a proliferação é ativada, essas células migram da borda para o tecido de granulação iniciando assim, a reepitelização do fundo da úlcera (HAN; CEILLEY, 2017). Esse tecido é desenvolvido no período de 48 a 72 horas após a ulceração, sendo composto em sua maioria por macrófagos, fibroblastos e células endoteliais, as quais são responsáveis pela formação de novos microvasos, estimuladas pelo processo de angiogênese (TARNAWSKI; TARNAWSKI, 2012; WU et al., 2022).

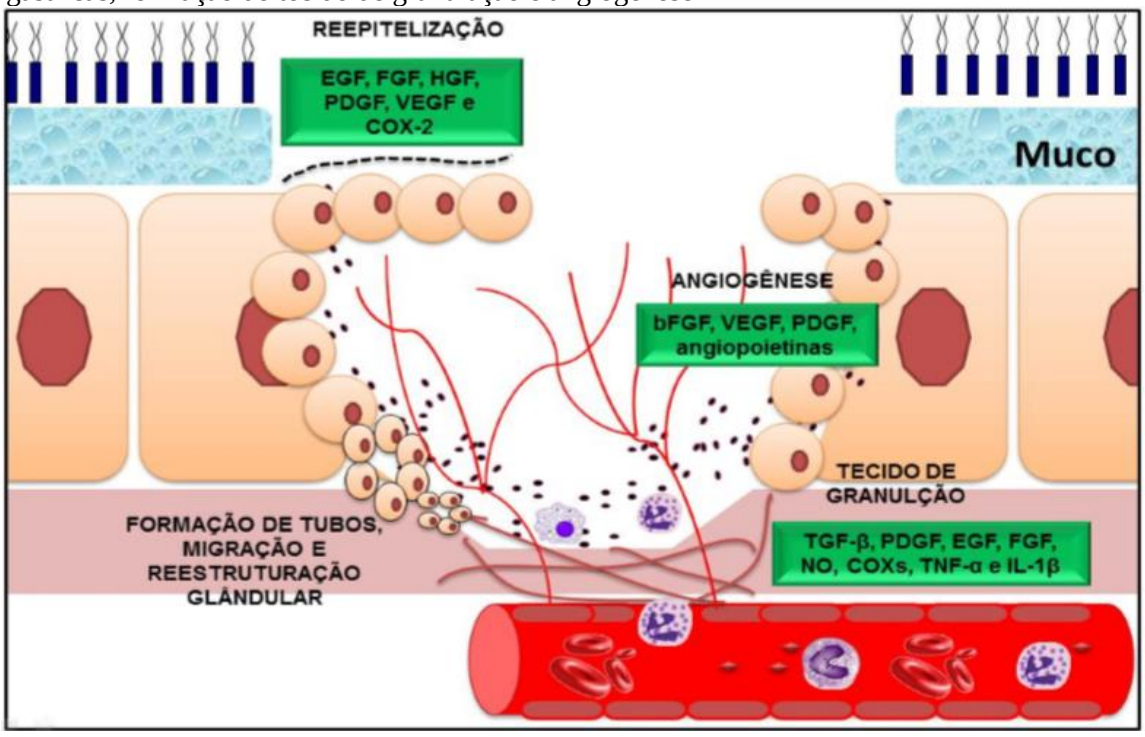
Os fatores de crescimento TGF-β, PDGF, EGF, FGF e as citocinas TNF-α, IL-1β e IL-8, provindas das células inflamatórias, endoteliais e macrófagos, desencadeiam a translocação de fibroblastos e miofibroblastos para o tecido inflamado, o que resulta no aumento da síntese de colágeno e fibronectina (YUN et al., 2017). O TGF-β1 em particular, é protagonista no processo de remodelamento tecidual, na formação da matriz extracelular e na angiogênese, sendo responsável pelo reparo da úlcera instalada (SHAOFANG et al., 2018; BEIRANVAND, 2022).

As metaloproteinases de matriz (MMP) são as enzimas de destaque envolvidas no processo de remodelação tecidual, com participação ativa na síntese e na degradação da matriz extracelular. Elas são grupos de proteases co-dependentes de zinco que atuam na degradação proteolítica de seus componentes. A curva crescente dos níveis de expressão das MMP-2 e MMP-9 produzidas por fibroblastos, queratinócitos e células inflamatórias no tecido gástrico, sugere a instalação e continuidade da úlcera gástrica (MINYAYLO et al., 2022). Diante disto, a regulação dessa expressão está relacionada com o

equilíbrio funcional na degradação da matriz tecidual, que junto ao aumento na expressão de colágenos tipo I, III e IV, facilitam a cicatrização e remodelamento tecidual (FORMIGA et al., 2021; SHIPA et al., 2022; EFTHYMAKIS; NERI, 2022).

A vascularização gástrica é ampliada pelos microvasos sanguíneos (capilares e vênulas coletoras), que são células endoteliais aderentes à membrana basal, com função proliferativa, anastomose e formação de rede capilar em resposta a estímulos angiogênicos (LEE; KATZ 2021), conforme demonstrado na **figura 4**.

Figura 4. Representação dos processos de reepitelização, migração celular, restauração das glândulas gástricas, formação do tecido de granulação e angiogênese



Abreviações – EGF: fator de crescimento epidérmico; FGF: fator de crescimento de fibroblastos; HGF: fator de crescimento de hepatócitos; PDGF: fator de crescimento derivado de plaquetas; VEGF: fator de crescimento do endotélio vascular; TGF: fator transformador do crescimento; NO: óxido nítrico; COX: cicloxigenase; IL: interleucina; TNF: fator de necrose tumoral. Fonte: LFTGI - Adaptado de TARNAWSKI, 2005; TARNAWSKI, 2012.

Com a lesão na mucosa gástrica, ocorre a interrupção do fornecimento de oxigênio, o que resulta na alteração do fenótipo de repouso para o fenótipo angiogênico, com ativação dos genes codificadores de fatores de crescimento

angiogênicos, a exemplo do fator de crescimento endotelial vascular (VEGF). Esse processo é fundamental para reestabelecer a microcirculação sanguínea na mucosa gástrica e recompor o suporte de oxigênio e nutrientes necessários à homeostase do tecido lesionado (SEIWERTH et al., 2018; LEE; KATZ 2021).

O VEGF é o regulador mais efetivo da angiogênese e um mitógeno endotelial específico. Seus efeitos são mediados pela ligação aos receptores VEGF-R1 e VEGF-R2. Os principais indutores da liberação do VEGF são a hipóxia, o PDGF, TGF-β1, bFGF, citocinas, óxido nítrico e a prostaglandinas da série E2 (SEIWERTH et al., 2018; LONGO et al., 2021; MARTININO et al., 2022).

A mucosa gástrica reepitelizada possui anormalidades histológicas, como altura reduzida, dilatação das glândulas gástricas, aumento da conectividade de tecido, rede microvascular desorganizada e um aumento da permeabilidade capilar. Essas desordens, interferem diretamente na defesa da mucosa e pode causar recorrência no desenvolvimento de úlceras. Dessa forma, a qualidade da restauração estrutural da mucosa pode ser o fator mais importante na determinação da recorrência da lesão (TARNAWSKI, 2012; LUZ et al., 2021).

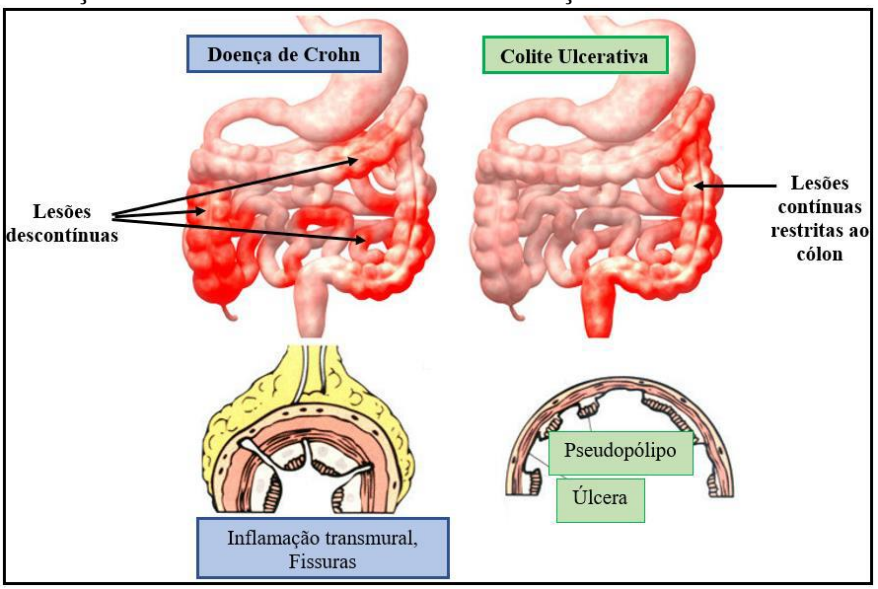
2.3 Doenças Inflamatórias Intestinais (DIIs)

As doenças inflamatórias intestinais (DIIs) constituem desordens inflamatórias e possuem caráter recorrente e debilitante que afetam o trato gastrointestinal. Nas DII, ocorre ativação contínua do sistema imune, liberação de fatores pró-inflamatórios e lesões crônicas (SOUZA; FIOCCHI, 2016).

2.3.1 Etiologia e Fisiopatologia das DIIs

As DIIs classificam-se em Colite Ulcerativa (CU) e Doença de Crohn (DC), que mesmo compartilhando algumas características, como ativação do sistema imune, comprometimento da barreira epitelial e inflamação crônica, diferem em aspectos relacionadas à localização, distribuição das lesões e ao perfil inflamatório ativado (**Figura 5**) (SOUZA; FIOCCHI, 2016).

Figura 5. Representação das características das lesões na doença de Crohn e colite ulcerativa



Fonte:Adaptado de https://www.google.com.br/search?q=inflammatory+bowel+disease

A Doença de Crohn (DC) pode acometer qualquer parte do trato gastrintestinal de forma descontínua, preferencialmente o íleo e cólon, com lesões de natureza transmural, desde a camada mucosa até as camadas musculares (UNGARO et al., 2017). É uma doença inflamatória caracterizada por dor abdominal, febre e sinais clínicos de obstrução intestinal ou diarreia, com passagem de sangue ou muco, ou ambos. No início da lesão é observado uma inflamação superficial que evolui para formação de fístulas e, em formas mais graves, abcessos e estenose, o que dá origem a sua classificação em estenosante ou fistulizante (ANANTHAKRISHNAN et al., 2022).

A Colite Ulcerativa (CU) é uma doença inflamatória crônica que afeta o cólon, sendo caracterizada pela inflamação da mucosa recidivante e remitente, começando no reto e se estendendo para segmentos proximais do cólon (UNGARO et al., 2017). A principal característica da CU é a superficialidade da lesão, sendo restrita a mucosa, e sem infiltração para camadas mais profundas da parede intestinal. As lesões apresentam-se de forma contínua, difusa e simétrica. De acordo com sua localização, podem ser classificadas em: pancolite (ao longo do reto e cólon), colite distal (isolada no lado esquerdo), proctestigmoidite (cólon distal e reto) ou proctite (limitada ao reto). Dentre as complicações da CU, a mais frequente é o sangramento (CHEN

et al., 2022; HAMID et al., 2023).

Os sinais clínicos mais evidenciados em indivíduos com DC são: presença de granulomas intestinais, aumento de macrófagos, eosinófilos e linfócitos teciduais, estenose, espessamento da parede intestinal, lesões aftosas, fístulas, abcessos e fibrose. Em relação aos principais sintomas, incluem diarreia, dor abdominal, sangramento, febre e perda de peso (MARTON et al., 2019). Os sintomas apresentados na CU são principalmente a dor abdominal, a incontinência, a fadiga, o aumento da frequência de evacuações e da secreção de muco, o sangramento retal, e a febre. Um fato importante é a manifestação extra intestinal que ocorre em cerca de metade dos indivíduos acometidos com CU, principalmente nos olhos, na pele e nas articulações (THOMAS et al., 2022).

Existem ainda particularidades entre essas duas afeções no que diz respeito ao perfil inflamatório ativado. Na DC os linfócitos T expressam em grande maioria o fenótipo TH1 e TH17, induzida por um microambiente com presença das citocinas IL-12, IL-6, IL-23 e TGFβ, síntese e liberação de IL-17, IL-22 e IFN-γ. Já na CU, o fenótipo expresso é o TH2 e células T natural Killers (NKT), com envolvimento direto das citocinas IL-4 e IL-13 (ZENG et al., 2022; KANEKO et al., 2023).

Embora as doenças inflamatórias intestinais possuam uma etiologia complexa e multifatorial, existem alguns fatores que favorecem sua gênese, são eles os fatores ambientais, a susceptibilidade genética, as incoerências na resposta imune inata e adaptativa e as alterações na microbiota intestinal (SOUZA; FICCHI, 2016; KIM; CHEON, 2017; KANEKO et al., 2023).

- **Fatores ambientais**

A exposição aos fatores ambientais tem um papel fundamental no surgimento das doenças inflamatórias intestinais. A literatura descreve a interligação de hábitos de vida como determinantes na patogênese das DIIs. Entre os mais discutidos estão o tabagismo, fatores dietéticos, a utilização de alguns medicamentos, estresse e infecções por patógenos entéricos (KOTZE et al., 2022).

Inicialmente o tabagismo foi alvo de estudos que correlacionavam a geração de estresse oxidativo e radicais livres como os causadores de maior predisposição as DIIs, posteriormente também foi associado a alterações imunológicas e da microbiota intestinal como fatores que agravavam essas afecções, com destaque para a DC (THOMAS et al., 2022). Curiosamente, outros estudos mostram que na CU esse hábito exerce um fator protetor, uma vez que estimula a redução de citocinas pró-inflamatórias (IL-8), o aumento da produção de muco e a redução da permeabilidade epitelial do intestino (YADAV et al., 2017).

Outro fator de grande relevância está relacionado a alimentação. Estudos mostram que o consumo elevado de gorduras saturadas, carnes vermelhas, carboidratos e aditivos, associado a um baixo consumo de fibras alimentares, frutas e vegetais favorecem o acometimento das DIIs. Este desbalanço está associado ao aumento de infecções e desencadeamento de um processo inflamatório, bem como, uma disbiose, reduzindo a função de barreira da mucosa intestinal (ANANTHAKRISHNAN et al., 2022).

Medicamentos que reduzem mecanismos de defesa da mucosa intestinal também configuram uma elevada incidência das DIIs, a exemplo dos AINES, em que seu uso contínuo inibe a ciclooxigenase (COX) e a síntese de prostaglandinas citoprotetoras, o que resulta no aumento de leucotrienos e favorece danos à mucosa, aumentando a permeabilidade e iniciando uma resposta inflamatória local (CORRIDONI et al., 2014; MICHAELS; MADSEN, 2023). Os antimicrobianos podem também exercer efeito negativo na mucosa, uma vez que pode causar um desequilíbrio entre os microrganismos comensais e os patogênicos, com produção de metabólitos ativos capazes de iniciar resposta inflamatória (LIMKETKAI et al., 2022).

O estilo de vida interfere diretamente no acometimento das DIIs. Embora ainda permaneça com mecanismos não elucidados, estudos mostram que o estresse crônico, bem como condições psicossociais (depressão), está relacionado ao aumento do índice de atividade da doença (GAO et al., 2018). O provável mecanismo envolvido na depressão se dá pelo aumento da suscetibilidade as doenças mediadas pelo sistema imune, por meio do eixo

hipotálamo-hipófise e a ativação do sistema nervoso autônomo, o que resulta em liberação excessiva de cortisol e citocinas pró-inflamatórias na corrente sanguínea. Além disso, condições crônicas de estresse afetam a microbiota intestinal, com ativação do sistema imunológico (GAO et al., 2018; LIMKETKAI et al., 2022; ASWANI-OMPRAKASH; SHAH, 2022).

- **Fatores genéticos**

A genética é responsável pela susceptibilidade a uma variedade de doenças. Pesquisas recentes investigam genes específicos e sua relação direta com as DIIs, ampliando assim o conhecimento e elucidação do seu desenvolvimento. De acordo com a decodificação específica dos genes, a simbiose dos microrganismos comensais e o hospedeiro são alteradas, resultando em alterações na função de barreira intestinal, processo de reparo tecidual, apoptose, autofagia, sistema imune e resposta a fatores agressores (ALHARBI et al., 2022).

A imunidade inata é responsável pelo início do processo inflamatório intestinal. Estudos indicam que mutações no NOD2 (nucleotide-binding oligomerization domain containing 2), um receptor de reconhecimento padrão que está localizado na superfície de macrófagos e monócitos, aumentam a susceptibilidade da ocorrência de DIIs. O resultado dessas alterações é o desequilíbrio da interação dos microrganismos comensais e o início de uma resposta inflamatória. A expressão desse gene também está relacionada com a microbiota, uma vez que sua presença aumenta a expressão de NOD2 (ALHARBI et al., 2022; DI'NARZO et al., 2022).

Os primeiros genes relacionados à predisposição das DIIs foram os de IL-10 (IL-10RA e IL-10RB), que tem como resposta, inibir a secreção de citocinas pró-inflamatórias. Com isso, sua ausência está relacionada com aumento da resposta inflamatória (MARTIN et al., 2021). As citocinas pró-inflamatórias propagam sinais que resultam em injúrias e cronicidade das DIIS. A expressão dos genes do receptor de IL-23 (IL-23R) e de autofagia ATG16L1 e IRGM estão envolvidos nas DIIs, com exacerbação da resposta TH17 e mutações nos genes de autofagia, com redução da eliminação dos produtos de

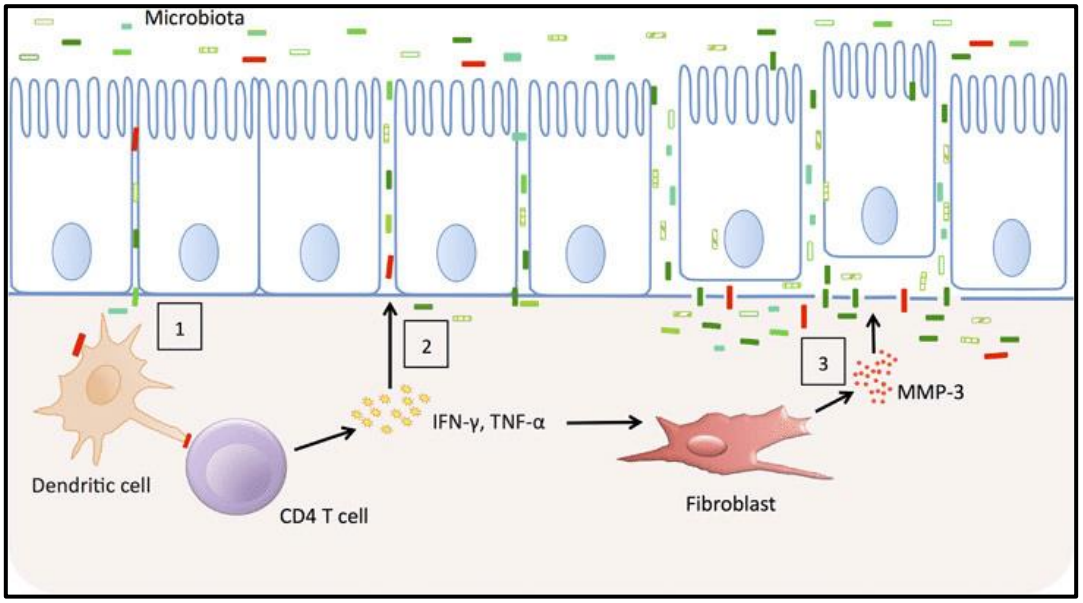
degradação celular e bacterianos, atraindo o sistema imune para o local da inflamação, ampliando a inflamação local (ZHU et al., 2022).

- **Barreira epitelial**

A barreira intestinal é constituída de células epiteliais conectadas, fatores antimicrobianos e muco que separa o lúmen intestinal das células do sistema imunológico interno, e, desta forma, regula as interações da microbiota com o sistema de defesa. O epitélio intestinal é uma barreira física que permite o influxo de nutrientes e solutos essenciais bloqueando microrganismos patogênicos e seus metabólitos (ZHU et al., 2022; TAN, et al 2023).

O epitélio intestinal é composto por uma monocamada de células epiteliais colunares que se conectam por junções de oclusão. Na superfície do epitélio, a camada protetora de muco é composta por mucinas glicosiladas secretadas pelas células caliciformes, além de defensinas liberadas por células de Paneth e células epiteliais. A desregulação da homeostase dessa barreia em indivíduos com DIIs, acarretam no influxo de patógenos e seus metabólitos através da barreira, iniciando a resposta imunológica e inflamação da mucosa (**Figura 6**) (CHENG et al., 2022). Entretanto não está totalmente elucidado se as alterações na barreira epitelial representam uma das origens das DII ou se é danificada em decorrência dos processos inflamatórios locais, mas seu envolvimento na fisiopatologia tanto da DC quanto da CU é consolidado (YANG et al., 2022).

Figura 6. Disfunção na barreira intestinal em indivíduos com DII



Disfunção na barreira intestinal em indivíduos com DII com aumento na captação de antígenos luminais através do epitélio intestinal. Fonte: Adaptado de YU et al., 2018.

Um dos fatores que pode resultar em desregulação da junção apical, é a presença da bactéria *Escherichia coli* aderente invasiva e as enteropatogênicas. Seu mecanismo de indução do processo inflamatório está associado à ativação dos receptores TLR4 em resposta aos lipopolissacarídeos (LPS) presentes nessas bactérias, com produção de citocinas pró-inflamatórias (em grande maioria IFN-γ e TNF-α). Esses mediadores alteram a permeabilidade intestinal ao agir nas proteínas de junção, o que resulta na desregulação da função barreira nas DIIs (ABRAHAM et al., 2022).

As mucinas são glicoproteína protetoras presentes na barreira epitelial, cuja produção é controlada por duas vias, a basal e a estimulada. A via basal constantemente ativa nos processos fisiológicos, enquanto a via estimulada é ativada em resposta a fatores bioativos ou epigenéticos, a exemplo de microrganismos e seus metabólitos, citocinas pró-inflamatórias, neuropeptídeos e fatores de crescimento. Esses estímulos resultam em hiperprodução e glicosilação anormal das mucinas, comum na DC (YANG et al., 2022; TAN, et al 2023).

Dentre as diferentes isoformas da mucina, a MUC2 é predominante na camada de muco expressa por células caliciformes no cólon. Nas DIIs sua

síntese é reduzida, sendo correlacionada com a gravidade da inflamação (YANG et al., 2022).

Estudos recentes têm se concentrado nos fatores imunológicos subjacentes a resposta inflamatória na CU, que é tradicionalmente considerada como tendo um perfil TH_2 com concentrações elevadas de citocinas IL-4 e IL-5. A CU está associada à presença de células T (NKT) natural killer não invariáveis da lâmina própria (Tipo II) que produzem IL-13 e podem mediar a citotoxicidade das células epiteliais. O próprio glicolipídeo foi sugerido como um gatilho para a ativação das células NKT. Uma série de padrões moleculares associados a danos pró-inflamatórios (DAMPs), como calprotectina e caixa de grupo 1 de alta mobilidade (HMGB1), são altamente prevalentes em CU na fase ativa e a liberação descontrolada desses agentes imunogênicos podem ter um papel na perpetuação da natureza não resolvida da inflamação. No geral, a função epitelial desregulada continua sendo o elemento crítico na patogênese da CU (YANG et al., 2022; TAN, et al 2023).

2.4 Epidemiologia

A epidemiologia das afecções que acometem o TGI apresenta um caráter dinâmico, com difícil precisão da estimativa de prevalência devido a subjetividade dos sintomas e a semelhança clínica com outras doenças (HAVENS et al., 2018). A úlcera péptica acomete cerca de 5 a 15% da população mundial, com incidência de 0,1 a 0,3% ao ano, equivalente a 4 milhões de pessoas. Entretanto, esse percentual não é fidedigno considerando que muitos casos são subnotificados (LANAS; CHAN, 2017; SHELL, 2021).

As pesquisas mostram que em relação ao sexo, os homens são mais acometidos pelas úlceras pépticas do que as mulheres, na proporção de 3:1 para úlceras duodenais e 2:1 para úlceras gástricas, dentre as quais as mulheres mais acometidas durante ou após a menopausa. Enquanto a úlcera duodenal acomete mais jovens (20-50 anos), a úlcera gástrica é diagnosticada mais em idoso (a partir dos 55 anos). Além disso, sua incidência está relacionada aos hábitos de vida e associações clínicas com outras doenças (LANAS; CHAN, 2017;).

As úlceras pépticas têm como principais complicações o sangramento,

a perfuração ou a obstrução da saída gástrica, sendo que em 50% dos indivíduos, o sangramento pode ocorrer sem nenhum sintoma de alerta. Embora as internações hospitalares tenham diminuído em todo o mundo, a taxa de letalidade continua estável em 5-10%, e dependendo de agravantes como idade e comorbidade, a mortalidade pode alcançar 20% desses indivíduos (MIN; MIN 2018). As reduções de internações relacionadas às úlceras pépticas podem estar associadas com a utilização de medicamentos gastroprotetores e/ou antissecretórios, uso racional de AINES e terapia mais efetiva frente a bactéria *H. pylori*. Todavia, ainda possuem alta taxa de recorrência, que em sua maioria está relacionada com a não erradicação do bacilo *H. pylori*, com índice de 67% das taxas de reincidência, caindo para 6% quando erradicado (MACHADO et al., 2021).

Os estudos em países da América latina identificaram que a infecção pelo bacilo *H. Pylori*, está presente em úlceras duodenais e gástricas, com prevalência de 74 e 55%, respectivamente (MIN; MIN, 2018). No Brasil, a escassez de dados atuais sobre a prevalência de úlceras pépticas configura uma imprecisão da realidade efetiva. Entretanto, estima-se que a prevalência de úlcera em mulheres e homens foi de 0,1 e 0,2%, respectivamente, com taxa de mortalidade em nível nacional de 3/100 mil habitantes (3,6/100 mil em homens e 2,3/100 mil em mulheres), com tendência a aumentar com o avanço da idade. Esse mesmo estudo mostrou que o Nordeste apresentou índices superiores em comparação com índices nacionais, com taxa de mortalidade de 3,8/100 mil habitantes (4,7/100 mil homens e 2,9/100 mil mulheres) (OLIVEIRA et al., 2015; PIOVANI et al., 2020).

As doenças inflamatórias crônicas impactam diretamente na qualidade de vida da população, apresentando caráter crônico, com fases de remissão e recidiva. Dados recentes indicam que a incidência e a prevalência de DIIs está aumentando ao longo do tempo e em diferentes localizações geográficas. No qual para colite ulcerativa foi reatada uma incidência no norte da Europa (24/100.000), Canadá (19/100.000) e Austrália (17/100.000). Entretanto, as taxas de prevalência são mais alta na Europa (505/100.000), Canadá (248/100.000) e nos EUA (214/100.000).

A prevalência global aumentou de 68,6 casos por 100.000 habitantes em 1990 para 89,6 casos por 100.000 habitantes em 2017, representando um aumento de 31% em 27 anos (PIOVANI et al., 2020; MACHADO et al., 2021).

Com relação a CU, estudos mais recentes relatam o seu aumento em países subdesenvolvidos na América do Sul, África e Ásia (BAI et al., 2020), estando associado às mudanças no estilo de vida, bem como aos hábitos alimentares (WINDSOR; KAPLAN, 2019; MACHADO et al., 2021).

As idades máximas para a ocorrência de DC e CU foram relatadas como sendo de 18-35 anos, sem predominância do sexo na colite ulcerosa. A prevalência de DC e CU está aumentando em nível global. Embora tenha ocorrido avanços na farmacoterapia, mais de 20% dos indivíduos com DC são submetidos a cirurgia e mais de 10% dos acometidos por CU ainda precisam de colectomia (YUKSEL et al., 2017; PIOVANI et al., 2020).

2.5 Microbiota

A microbiota é o conjunto de microrganismo (bactérias, fungos, protozoários e vírus) que colonizam o hospedeiro de modo simbiótico. Dentre suas principais funções destacam-se a metabolização, imunoregulação e função de barreira que dificulta a instalação de outros microrganismos patogênicos (PESSOA et al., 2022).

Estudos recentes evidenciaram a participação da microbiota não só na patogênese das doenças inflamatórias intestinais, mas também na diarreia infecciosa, na síndrome do intestino irritável e nas úlceras pépticas (BAMOLA et al., 2022; CHEN et al., 2023)

As influências microbianas na colite ulcerativa (CU) dizem respeito aos seus efeitos metabólicos na homeostase e função epitelial. O cólon abriga cerca de 106 espécies de bactérias predominantemente anaeróbicas, sendo estas responsáveis pela fermentação dos ácidos graxos de cadeia curta (SCFAs) que representam 70% da energia utilizada pelo epitélio colônico (LI et al., 2022). Uma mudança na microbiota "saudável", como *Bifidobacterium* e Bacteroides, com uma redução simultânea dos SCFAs foi descrita juntamente com um

aumento nas bactérias redutoras de sulfato (SRB) do gênero *Desulfovibrio*. As SRB convertem sulfato em sulfeto, que inibe a oxidação do butirato (principal SCAF) e é diretamente tóxico para as células epiteliais do cólon. Além disso, os SCFAs podem induzir a expansão das células T reguladoras do cólon (DARGAHI et al., 2019; SERAFIM et al., 2020; NIU et al., 2022).

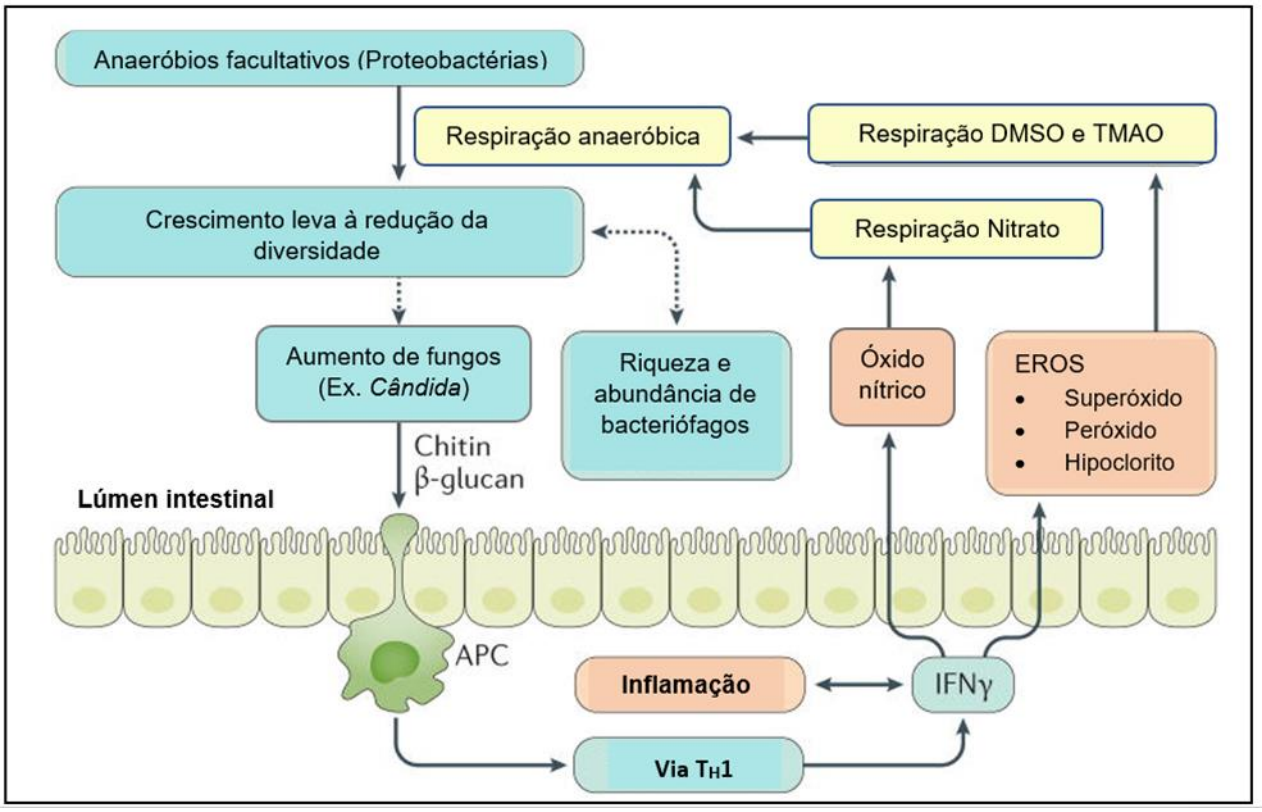
Pesquisas pré-clinicas e clínicas apontam que a microbiota intestinal patogênica e a expressão aumentada de citocinas pró-inflamatórias nas DIIs desempenham um papel central no desenvolvimento e manutenção da doença (SERAFIM ET AL., 2020).

Aproximadamente um terço dos indivíduos com doença de Chron (DC) tem um aumento de *Escherichia coli* invasora aderente associada à mucosa, que permeiam a barreira epitelial da mucosa, aderem e invadem células epiteliais intestinais, sobrevivem e se replicam no interior dos macrófagos, provocando secreção de grandes quantidades de TNF-α. Associado a isso, a permanência intracelular suprime a autofagia, intensificando a gravidade da infecção (AXELRAD et al., 2022; ALEXANDER et al., 2023).

O processo inflamatório do cólon propicia a ativação dos linfócitos do subgrupo TH1, que induzem a liberação de IFNγ e aumentam as espécies reativas de oxigênio (EROs) pelas células imunes fagocíticas da imunidade inata. Esses radicais formam produtos para a respiração anaeróbica que favorecem a replicação de microrganismos anaeróbios facultativos com redução da diversidade bacteriana (**Figura 7**) (NI et al., 2017; ZHOU et al., 2022). Com isso, definir o perfil da microbiota tornou-se uma ferramenta de diagnóstico e monitoramento das doenças inflamatórias intestinais (ZHOU et al., 2022).

O desequilíbrio na composição da microbiota intestinal é denominado disbiose, influencia diretamente na modulação do sistema imunológico, e está relacionada ao desenvolvimento de doenças inflamatórias, autoimunes ou atópicas (SERAFIM et al., 2020; PESSOA et al., 2022).

Figura 7: Inflamação do cólon nas DIIs e as alterações na microbiota intestinal



Fonte: Adaptado de NI et al., 2017.

Os avanços na tecnologia de sequenciamento de DNA (permitindo abordagens independentes de cultura) forneceram uma plataforma para análises aprofundadas do microbioma intestinal. O projeto MetaHIT (Metagenômica do Trato Intestinal Humano) estimou 3,3 milhões de genes bacterianos não redundantes na microbiota luminal. Neste estudo, a abundância relativa de 155 espécies bacterianas pode diferenciar CU, DC e indivíduos saudáveis (DARGAHI et al., 2019; PENG et al., 2021).

A relação da microbiota intestinal, dos fatores genéticos e da resposta inflamatória do hospedeiro embasam tratamentos alternativos com prebióticos, probióticos e transplante de microbiota fecal com possibilidade para desempenhar um papel importante na prevenção e tratamento das DIIs.

Os probióticos utilizados frequentemente são cepas de bactérias formadoras de ácido lático como *Lactobacillus, Bifidobacterium* e *Streptococcus*. As bactérias pertencentes a estes gêneros demonstraram habilidade de inibir o crescimento *in vitro* de diversos patógenos entéricos

como *Salmonella typhimurium*, *Staphylococcus aureus*, *Escherichia coli* e *Clostridium difficile* (HUANG et al., 2022; HOLMAN et al., 2023).

Atualmente, prebióticos têm recebido atenção crescente devido às suas propriedades promotoras da saúde. Os mecanismos subjacentes de sua ação podem incluir inibição de patógenos, produção de metabólitos e modulação da imunidade. É digno de nota que um crescente corpo de evidências revelou sua influência na microbiota intestinal, sugerindo que eles podem desempenhar um papel terapêutico potencial em doenças relacionadas à microbiota, como as DIIs (XIA et al., 2023).

O transplante de microbiota fecal consiste em transplante de microbiota funcional de fezes humanas saudáveis para o intestino de pacientes, que pode tratar doenças intestinais e extraintestinais por meio da reconstrução da microbiota intestinal. As vias de aplicação para o transplante incluem vias do intestino médio (canal endoscópico, tubo nasojejunal, tubo de gastrostomia ou tubo de jejunostomia), tubo transendoscópico colônico, infusão colônica sob colonoscopia, enema, cápsulas e sonda nasogástrica. O uso dessa terapia alternativa para CU e DC foi relatado, mas os achados clínicos baseados em diferentes métodos variam muito. Além disso, na maioria dos estudos clínicos, os materiais fecais são preparados manualmente. Com isso, uma série de pesquisas relatou atitudes negativas entre pacientes, profissionais da saude e candidatos a doadores para o transplante fecal, e essas atitudes são principalmente sobre preocupações estéticas, psicológicas e médicas (como transmissão de patógenos) (DING et al., 2020).

2.6 Sistema antioxidante

O sistema antioxidante é composto por um conjunto de moléculas e enzimas que reagem com espécies reativas de oxigênio (EROs) promovendo a sua inativação para prevenir o estresse oxidativo (SIES; JONES, 2020).

As EROs são formadas em sua maioria pelo metabolismo mitocondrial e de outras organelas como os peroxissomos e retículo endoplasmático por meio do complexo de respiração celular em que são produzidas altas taxas de ATP, oxidação de ácidos graxos e desintoxicação de processos xenobióticos,

respectivamente. Dentre as EROs destacam-se os íons e os peróxidos de oxigênio reativos que em altas concentrações causam danos a biomoléculas como DNA, ácido ribonucléioco (RNA), proteínas e lipídios, o que pode levar ao desequilíbrio homeostásico (SIES; JONES, 2020; TRUONG; JEONG, 2022).

O organismo possui um sistema de defesa antioxidante que pode ser enzimático e não enzimático. O sistema enzimático é composto por proteases que formam a primeira linha de defesa contra o ânion superóxido e o peróxido de hidrogênio, como: a catalase (CAT), a superóxido dismutase (SOD), a glutationa peroxidase (GPx) e a glutationa S-transferase (GST). Já o sistema não enzimático corresponde a segunda linha de defesa e pode ser de produção endógena ou exógena, com destaque para a glutationa reduzida (GSH), tioredoxinas, ácido úrico e bilirrubina que são antioxidantes endógenos, e ácido ascórbico, tocoferóis, vitaminas E e C, carotenoides e compostos fenólicos que são antioxidantes exógenos (ASAKURA; KITAHORA, 2018; SIES, JONES 2020; TRUONG; JEONG, 2022).

A reação espontânea de superóxido (O^{2-}) com óxido nítrico (NO) produz peroxinitrito ($ONOO^{-}$), altamente reativo e desencadeador do estresse oxidativo. A enzima NADPH oxidases (NOX) e as mitocôndrias são fontes potenciais de superóxido, deste modo, o óxido nítrico é gerado pelo óxido nítrico sintases (NOS) e o peróxido de hidrogênio pode ser formado diretamente por várias oxidases (JAESCHKE; RAMACHANDRAN, 2018).

Os mecanismos de defesa antioxidante celular incluem enzimas para o metabolismo rápido de EROs, proteínas de ligação para metais de transição (ex. ferritina) e antioxidantes de quebra de cadeia (ex. vitamina E) para prevenir a peroxidação lipídica. O superóxido pode ser metabolizado pela superóxido dismutase-1 (Cu/Zn-SOD) e dismutase-2 (Mn-SOD), já o peroxinitrito pode ser eliminado pela glutationa (GSH) e o peróxido de hidrogênio é reduzido pela glutationa peroxidase1 (GPx1) ou pela catalase (CAT) (JAESCHKE; RAMACHANDRAN, 2018; PU et al., 2023).

A GSH está localizada, em sua maioria, na mucosa gástrica e fornece

proteção extra frente as EROs. A glutationa possui em sua composição um grupo sulfidrila (-SH) facilmente oxidável, que protege o organismo contra lesões oxidantes, protege a integridade e a permeabilidade da membrana celular, promove a montagem de oligômeros de mucina para manter a fluidez do muco, auxilia na manutenção da função imunológica, na regulação da síntese e degradação de proteínas e na manutenção da estrutura proteica (SHAHINFAR et al., 2021; PU et al., 2023).

O estresse oxidativo protagoniza alterações na fisiopatologia dos processos inflamatórios, pois a peroxidação lipídica exacerba reações de cascata e libera mediadores inflamatórios e células do sistema imune, a exemplo dos neutrófilos, que resulta em aumento da atividade das mieloperoxidase (MPO), uma enzima importante da atividade neutrofílica mediada por citocinas inflamatórias (SHAHINFAR et al., 2021).

Tanto a inflamação crônica quanto a hiperativação do sistema imunológico desencadeiam altos níveis de EROs e reduzem os níveis de antioxidantes endógenos. Esse processo induz a formação de danos na camada mucosa e invasão bacteriana, que resulta na estimulação da resposta imune e favorece a progressão das DIIs (WANG et al., 2022).

As EROs são segundos mensageiros para a ativação de vias de transdução de sinal que resultam na inflamação, a exemplo das vias da cascata de sinalização de proteínas quinases ativadas por mitogênio (MAPKs) e NF-κB. Os antígenos bacterianos, como os lipopolissacarídeos (LPS), ativam os macrófagos por meio do receptor Toll-like-4 (TLR4), que desencadeia a ativação da MAPK exercendo um papel crucial na ativação da resposta imune inata e adaptativa e da via do NF-kB (TABARI et al., 2022; MICHAELS; MADSEN, 2023). Além disso, as EROs mediam a ativação da IκB cinase, que induz a quebra proteasomal de IκBα, liberando a proteína ativa NF-κB que é o fator de transcrição que se liga a promotores de genes-alvo e promove a transcrição de citocinas inflamatórias e quimiocinas (TAN et al., 2023).

O fator 2 relacionado ao fator nuclear eritróide-2 (Nrf2) é responsável por regular a expressão de mais de 300 genes envolvidos na modulação do

estresse oxidativo e da inflamação. Sua ativação suprime a via do NF-κB e melhora o estresse oxidativo observado nas DIIs (BOURGONJE et al., 2023).

O estresse oxidativo sistêmico observado na DC, pode provavelmente contribuir para o desenvolvimento de manifestações extra intestinais, como fístulas perianais, doenças dermatológicas e artrite, que são muito comuns nos indivíduos acometidos por essa doença (BOURGONJE et al., 2023).

2.7 Citocinas

As citocinas compreendem um grupo de proteínas solúveis com baixo peso molecular (6 a 70 kDa) produzidas por diferentes células do sistema imunológico, que atuam como moléculas sinalizadoras pelas vias autócrina, parácrina e endócrina em órgãos e em tecidos inflamados. Deste modo, são mediadores para a manutenção da homeostase da mucosa intestinal e efetivas nas doenças inflamatórias no TGI, além de desenvolver importantes ações no controle e função de células imunes e não imunes, tais como a regulação imunológica, patogênese e modulação de doenças mediadas pelo sistema imune (O'SHEA et al., 2019).

O processo inflamatório é uma resposta biológica de defesa frente a estímulos e infecções que desencadeiam a produção de mediadores inflamatórios, que objetiva cessar o fator desencadeador desse estímulo. Entretanto, quando a inflamação é prolongada ocorre a indução de danos aos tecidos que inicia nova cascata de sinalização, tornando um processo crônico. A sinalização do sistema imune é feita por meio das citocinas, que ao se ligarem aos seus receptores iniciam uma transdução de sinais aumentando a resposta inflamatória (PAVLIDIS et al., 2022).

No início da inflamação, a infecção ou dano tecidual é detectada pelos receptores de reconhecimento de padrões, como os receptores do tipo Toll (TLR), receptores do tipo NOD (NLR) e pelo receptor de produtos de glicação avançada (RAGE), os quais são ativados após a ligação com as moléculas conhecidas como padrões moleculares ativados por patógenos (PAMPs). Em resposta são desencadeadas sinalizações para fatores de transcrição, a exemplo

do fator nuclear κB (NF-κB), que se transloca para o núcleo e ativa vias inflamatórias, como a proteína 1 (AP-1) (ABRAHAM et al., 2022).

A sinalização via NF-κB desempenha um papel fundamental na regulação dos genes relacionados a reação inflamatória aguda. Na sua forma inativa, o NF-κB fica localizado no citoplasma ancorado à proteína IκB-quinase. Quando a enzima IκB-quinase é ativada, causa fosforilação da proteína IκB e posterior degradação do complexo IκB. Essa sinalização resulta em ativação de NF-κB, que se transloca para o núcleo e causa a ativação transcricional de outras citocinas inflamatórias (POTDAR, 2021).

Algumas citocinas exacerbam as respostas inflamatórias, induzindo e intensificando o processo de inflamação (IL-1 β, IL-2, IL-6, IL-7 e TNF), enquanto outras citocinas regulam esse processo por meio da supressão da resposta inflamatória (IL-10, IL-11, e IL-13), modulando dessa forma a resposta imunológica. Na úlcera gástrica as principais citocinas pró-inflamatórias envolvidas são, o TNF-α, IL-1β e IL-6. Estão relacionadas principalmente a resposta à fase aguda da inflamação, sendo característico o infiltrado de neutrófilos na mucosa gástrica com liberação de EROs, causando lesões na mucosa, além de aumento de moléculas de adesão em células endoteliais e leucócitos (THOMAS et al., 2022).

A IL-1β está associada a elevada inflamação na mucosa gástrica, atuando por meio de retroalimentação positiva para induzir a expressão de IL-8 (citocina envolvida na regulação de NFκB). A IL-8 favorece o recrutamento, influxo e ativação de neutrófilos na mucosa gástrica com produção massiva de EROs e potencialização da via de sinalização do NF-κB. Já o TNF-α pode estimular a expressão de ICAM-1, uma molécula de adesão de leucócitos ao endotélio promovendo a diapedese para locais inflamados em células endoteliais vasculares. Além de causar aumento de permeabilidade e edema vascular, o TNF-α, desencadeia também a expressão da COX-2, potencializando os efeitos inflamatórios (PAWLIK et al., 2016; AZIZ et al., 2019).

A IL-6 além de regular a inflamação aguda e crônica, também estimula

as células T e B, os neutrófilos, os macrófagos e os linfócitos. A ativação do receptor de IL-6 dispara uma via de sinalização que ativa o fator de transcrição STAT3, resultando em diferenciação de células T produtoras de IL-17, responsável por promover estímulos de vários produtos nocivos, radicais reativos ao oxigênio e enzimas lisossômicas responsáveis por danos aos tecidos da úlcera gástrica (AZIZ et al., 2019).

A depender do estímulo, as células TH22 desempenham um papel fundamental e dúbio na geração de proteção imunológica ou respostas patológicas, secretando IL-22. O fator decisivo para a sinalização é a presença ou ausência de IL-17A, em que pode desempenhar uma função protetora na ausência de IL-17A, enquanto a sinergia dessas duas citocinas pode levar ao aumento da inflamação (POTDAR, 2021).

A resposta anti-inflamatória representa uma via essencial para a modulação do processo inflamatório, bem como sua duração. A ativação de células T regulatórias (Treg) e secreção de citocinas como TGF-β e IL-10 são essenciais para esse processo. A IL-10 é secretada por monócitos, macrófagos, células do perfil Th1, Th2, Treg e linfócitos B e sua produção aumenta após o início da inflamação, reduzindo a inflamação gástrica por meio da inibição da retroalimentação do TNF-α por monócitos e macrófagos com consequente diminuição das inflamações do tecido gástrico (O'SHEA et al., 2019; POTDAR, 2021).

2.8 Terapêutica

A terapia da úlcera péptica se dá pela utilização de medidas farmacológicas e não farmacológicas. O tratamento farmacológico tem como objetivo reestabelecer o equilíbrio perdido entre a ação dos fatores citoprotetores frente aos fatores agressores e tem como principais funções reduzir ou neutralizar a secreção ácida e/ou estimular o sistema de citoproteção gástrica. O tratamento das úlceras pépticas tem como objetivo reestabelecer o equilíbrio perdido em decorrência da ação dos fatores citoprotetores frente aos fatores agressores (FEITOSA, 2012; BOEING et al., 2016).

Durante um longo período, os principais tratamentos eram centrados

na secreção ácida gástrica, tais como: antiácidos, anticolinérgicos, antagonista dos receptores H_2 de histamina e inibidores de bomba (IBPs) (BOEING et al., 2016).

O efeito local e instantâneo dos antiácidos promove alívio imediato, porém de modo paliativo, já que não agem diretamente na causa da doença, ou pelo menos em um dos mecanismos causadores, como a secreção ácida gástrica. Além de efeitos terapêuticos de curta duração, alguns efeitos colaterais inviabilizaram o uso dos antiácidos para o tratamento de úlceras gástricas, sendo usados apenas para aliviar sintomas dispépticos. Dentre os principais efeitos colaterais têm-se as interações farmacocinéticas na absorção, metabolização e excreção, causadas pela mudança do pH estomacal, inativação de enzimas e formação de quelatos não absorvíveis, os quais resultam na constipação ou diarreia, hipercalemia, insuficiência renal, alcalose metabólica e hipermagnesemia (SINGH; BHIMJI, 2018; PALIWAL et al., 2018).

Uma segunda classe, de medicamentos foi proposta baseada na importância da secreção de acetilcolina pela inervação vagal, os antagonistas dos receptores M_3. Estes foram usados na tentativa de diminuir a secreção ácida gástrica por meio do bloqueio de uma de suas vias, a via neuronal, e foram classificados como antissecretórios, e os protótipos usados foram a atropina e a pirenzepina, logo substituídos pela telenzepina, por apresentar uma grande interação com diferentes tipos de receptores muscarínicos aliada a baixa eficácia a os vários efeitos colaterais (taquicardia, cefaleia, visão turva, constipação severa e confusão mental). Desta forma, o uso dos antagonistas dos receptores M_3 como agente antiulcerogênico se tornou obsoleto (BOEING et al., 2016).

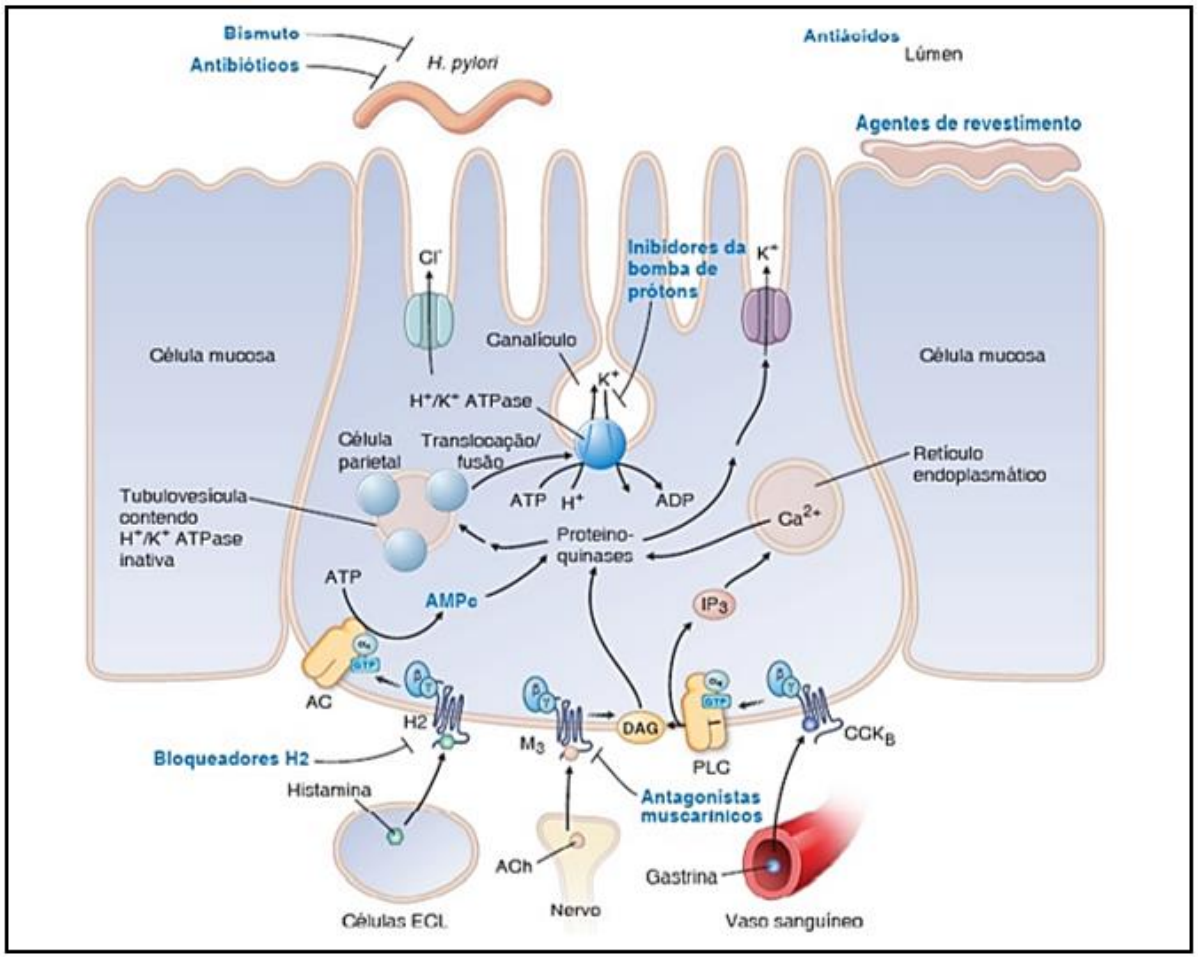
Isso possibilitou o desenvolvimento de outros medicamentos como os antagonista dos receptores H_2, entre os quais cimetidina, ranitidina, famotidina, nizatidina e roxatidina. Esses medicamentos ligam-se ao receptor H2 e impedem a ligação da histamina, bloqueando o estímulo da secreção ácida gástrica, o que leva a redução do volume do suco gástrico e consequentemente da concentração de íons H+ (HSU et al., 2016; RANG; DALE, 2016).

Na tentativa de aumentar a eficácia, os medicamentos desenvolvidos nos anos 80 tinham como alvo a H+/K+-ATPase, inibindo a secreção ácida no final de sua cascata. Estruturalmente, os agentes antissecretórios dessa classe são formados por um anel benzimidazol substituído e um anel de piridina ligados por uma cadeia de metilsulfonilo (NAUNTON et al., 2018; SPECHLER, 2018). Os inibidores de bomba são bases fracas que se tornam ativas em baixo pH nos canalículos estomacais e formam ligações covalentes com resíduos de cisteinas expostos na subunidade α da bomba, causando o seu bloqueio. Entre os inibidores de bomba tem-se o omeprazol, esomeprazol (isômero geométrico do omeprazol), lansoprazol, pantoprazol, rabeprazol e deslansoprazol (SPECHLER, 2018). Embora sejam a classe mais usada hoje em dia, vários efeitos colaterais foram relatados na clínica, o que dificulta a adesão ao tratamento além de seu uso possibilitar o surgimento de outros distúrbios tais como cefaleia, diarreia, erupções cutâneas, surgimento de demência, osteoporose, infecções entéricas e pólipos gástricos (SPECHLER, 2018; NAITO et al., 2018).

Um novo medicamento foi lançado para doenças relacionadas à acidez gástrica, apresentando um início mais rápido de ação e um controle mais prolongado da acidez intragástrica do que os IBPs convencionais (YANG et al., 2018). O Vonoprazan faz parte da classe dos Novos Inibidores da Bomba de Prótons, antagonistas competitivos com o potássio e não requer um ambiente ácido para sua ativação. Seu mecanismo de ação se dá por inibir a bomba de prótons de maneira reversível e competitiva com o sítio de ligação do potássio (KUNA et al., 2019).

Aliado a essa terapia, alguns agentes farmacológicos são capazes de estimular os fatores protetores da mucosa a exemplo dos compostos de bismuto como o sucralfato que, após liberação do metal é capaz de formar géis que se complexam ao muco, diminuindo sua degradação, limitando a retrodifusão de íons H^+, além de inibir a ação da pepsina e estimular a secreção de muco, HCO^{3-} e prostaglandinas (JAIN et al., 2007; BRUNTON et al., 2012) **Figura 8.**

Figura 8: Sítios de ação dos fármacos utilizados no tratamento da doença ulcerosa péptica. antagonistas dos receptores H_2; antagonistas do receptor muscarínico M_3; inibidores da bomba de prótons; Inibidores da Bomba de Prótons antagonistas competitivos com o potássio; antiácidos; agentes de revestimento (citoprotetores) e antibióticos.



Fonte: GOLAN et al., 2014

Quando a úlcera está associada a infecção por *H. pylori* é necessário fazer uso de associações de medicamentos de diferentes classes, tanto pra combater a infecção, quanto para tratar as complicações causadas pela instalação do bacilo (LANAS; CHAN, 2017; KIM et al., 2019).

O tratamento atual para a erradicação do *H. pylori*, consiste em uma terapia tripla que combina dois antimicrobianos e um inibidor da bomba de prótons (IBPs) e também podem ser utilizados medicamentos citoprotetores no auxílio da terapêutica. Além disso, pode ser utilizada uma terapia tripla com metronidazol em casos de resistência ou alergias provocadas pelo uso de

antimicrobianos β-lactâmicos, compostos bismúticos e tetraciclina ou amoxicilina por duas semanas (MALFERTHEINER et al., 2009; LANAS; CHAN, 2017; KIM et al., 2019).

A terapia clássica para o tratamento de *H.* pylori inclui o inibidor de bomba associado com amoxicilina e claritromicina ou metronidazol com claritromicina administrados de 7-14 dias (WANG et al., 2017). Entretanto o bacilo *H. pylori* tem desenvolvido mecanismos de resistência a claritromicina, e sob essa circunstância, foi prosposto como terapêutica uma terapia tripla formada por lansoprazol (Inibidor de bomba de prótons), amoxicilina (antimicrobiano β-lactâmico) e levofloxacino (antimicrobiano pertencentes à classe das quinolonas) que mostrou ter uma menor eficácia que o regime terapêutico clássico (DA SILVA et al., 2016).

As úlceras relacionadas às infecções por *H. pylori* também podem ser tratadas por uma terapia quádrupla (IBP, bismuto, metronidazol e tetraciclina) ou (IBP, claritromicina, amoxicilina e metronidazol), ambos os regimes tem uma taxa de erradicação de mais de 90% (LANAS; CHAN, 2017) e a duração do tratamento varia de 10 a 14 dias, o que pode ocasionar resistência em virtude do uso prolongado (LEE; KIM, 2011; LANAS; CHAN, 2017). Além disso, observa-se que náuseas, dor abdominal, vômito, diarreia, fadiga, dor de cabeça, tonturas e sensações amargas na boca constituem limitações a esta terapêutica (KIM et al., 2017).

A melhora na qualidade de vida dos portadores de úlceras pépticas é bastante evolutiva, estando intrinsicamente ligada ao desenvolvimento de novas alternativas terapêuticas. Entretanto, limitações como o alto índice de recidiva da doença, a diversidade de efeitos colaterais, interações medicamentosas, alto custo de internações dos indivíduos e acesso limitado aos medicamentos, remetem a necessidade de novas alternativas terapêuticas para o tratamento das úlceras, a exemplo dos produtos naturais, que têm demonstrado uma ampla diversidade de composição química e biologicamente ativa (LI et al., 2015; BOEING et al., 2016).

Quanto ao tratamento das DIIs, ele está centrado em terapias

farmacológicas (aminossalicilatos, imunossupressores e os imunobiológicos), intervenções cirúrgicas e alterações nos hábitos de vida (DASSOPOULOS et al., 2015). Os aminosalicilatos representam a terapia de primeira linha em pessoas com CU leve a moderada e compreendem todos os medicamentos que tem como metabólito ativo o ácido 5-aminossalicílico (5-ASA). As preparações atuais com base nos 5-ASA incluem a sulfasalazina, mesalasina, olsalazina e basalazina (ANNAHÁZI; MOLNÁR, 2015; UNGARO et al., 2017).

Os corticoides são eficazes em induzir a remissão da DC e CU e os mais utilizados na clínica são a prednisolona, budesonida e a beclometasona (WALJEE et al., 2016). O mecanismo de ação desses medicamentos é centrado em inibir o recrutamento de células imunes, a produção de citocinas pró-inflamatórias e a ativação e proliferação de células T. Contudo, o seu uso prolongado pode ocasionar efeitos adversos, tais como infecções, síndrome metabólica, doenças cardiovasculares (SALES-CAMPOS et al., 2015), dentre outros. Além disso, é relatado que esses medicamentos não promovem uma cicatrização efetiva da mucosa, o que é crucial para o restabelecimento das funções fisiológicas do intestino, facilitando a ocorrência de recidivas.

Na terapia de manutenção em longo prazo, os pró-fármacos imunossupressores 6-mercaptopurina e a azatioprina, pertencente ao grupo das tiopurinas, tem sido utilizado com um intervalo de tempo de vários meses em indivíduos com DIIs. Seu efeito terapêutico está relacionado à inibição da proliferação das células participantes da resposta imune e diminuição na síntese de prostaglandinas (HO et al., 2015).

A identificação de citocinas específicas responsáveis pela patogênese das DIIs possibilitou o desenvolvimento de agentes terapêuticos que têm como alvo citocinas pró-inflamatórias, proteínas cinases responsáveis pela sinalização e transdução de sinal em células T e moléculas de adesão responsáveis pela migração endotelial, os chamados fármacos imunobiológicos (COSKUN et al., 2016). Atualmente, são utilizados seis agentes imunobiológicos para o tratamento das DIIs, quatro anti-TNF (infliximab, adalimumab, golimumab e certolizumab pegol) e dois agentes anti-integrinas (natalizumab e vedolizumab) (DANESE et al., 2015; COSKUN et al., 2016;

UNGARO et al., 2017).

As terapias biológicas, como infliximabe e adalimumabe, tornaram-se os principais medicamentos para o tratamento das DIIs. Estudos sugerem que a terapia combinatória do infliximabe e um imunomodulador, como a azatioprina, podem ajudar a otimizar a farmacocinética, minimizar a imunogenicidade e melhorar os resultados das substâncias administrada de forma isolada (SULTAN et al., 2017). Entretanto, evidências do uso dos agentes imunobiológicos no tratamento das DIIs não dão uma indicação da eficácia esperada para além de um ano (DANESE et al., 2015; MOSS, 2015).

Ainda não existe nenhum tratamento curativo para as DIIs, porém as terapias disponíveis são capazes de suprimir a ativação imune anormal, levar a remissão da doença e evitar recaídas em alguns casos, permitindo-lhes levar um estilo de vida normal. No entanto, nenhum dos medicamentos incluídos nessa terapia é livre de possíveis efeitos colaterais, e a maioria deles são de alto custo (SULTAN et al., 2017).

2.10 Produtos naturais

Os produtos naturas são compostos químicos de origem animal, mineral, vegetal e de microrganismos (fungos, bactérias) (DUTRA et al., 2016; WANG et al., 2018). Apresentam uma diversidade de moléculas biologicamente ativas com grande capacidade de se tornarem novas alternativas terapêuticas para o tratamento convencional existente, o que pode superar suas limitações, seja de modo individual ou por meio de sinergismo (AHN, 2017).

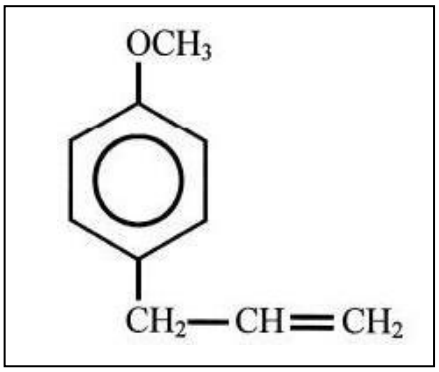
O Brasil possui a maior biodiversidade do planeta. Em seu território são encontradas cerca de 20% de todas as plantas que se tem conhecimento no mundo, e sua população usa diversas fontes naturais como forma de combater doenças de caráter agudo ou crônico, porém novas pesquisas e tecnologias são necessárias para otimização do uso de fontes naturais para o tratamento, como forma de isolar componentes responsáveis pelos efeitos biológicos, a fim de minimizar possíveis efeitos colaterais provindos da complexidade dos compostos (DUTRA et al., 2016).

Os fenilpropanóides são uma classe de compostos produzidos pelas plantas medicinais e são biossintetizados a partir da fenilalanina via ácido cinâmico. Em sua composição encontra-se um anel benzeno ligado a uma cadeia de 3 átomos de carbono, sendo responsáveis pelos aromas característicos de algumas plantas (SÁ et al., 2014). Estudos anteriores já demonstraram atividade gastroprotetora (ALVES JUNIOR et al., 2020) frente a diferentes agentes ulcerogênicos, em que a atividade está relacionada à ação antissecretória, citoprotetora, imunomoduladora e antioxidante (RIBEIRO, 2016).

2.10.1 Estragol

É um fenilpropanoide que consiste em um anel de benzeno substituído por um grupo metoxi e um grupo propenil (**Figura 9**) e está presente como constituinte de óleos essenciais de muitas espécies vegetais, como *Ravensara anisata*, *Ocimum basilicum* e *Croton zehntneri*. Estas espécies são amplamente utilizadas tanto na medicina popular quanto na culinária (SILVA-ALVES et al., 2013). Seus óleos essenciais são muito empregados em aromaterapia, sendo também utilizado como agente aromatizante nas indústrias farmacêutica, cosmética e alimentícia como conservante (SILVA-ALVES et al., 2013).

Figura 9. Representação química do Estragol



Fonte: https://pubchem.ncbi.nlm.nih.gov/compound/8815#section=Top

O estragol possui a fórmula molecular $C_{10}H_{12}O$, cuja sinonímia são metil-chavicol, *p*-alilanisol, chavicol metil éter e 4-metoxialilbenzeno (R&D CHEMICALS, 2017).

Estudos farmacocinéticos afirmam que o estragol possui rápida

absorção por via oral e sua metabolização é dependente de dose, sendo que em baixas doses o radical metóxi é metabolizado e em altas doses são necessárias oxidações na cadeia lateral (SMITH et al., 2002). O estragol apresenta três vias de metabolização, sendo a primeira é pela via de O-desmetilação, a qual favorece a formação de um metabólito que pode ser excretado com ácido glicurônico sendo considerada a principal via quando ingerido em baixas doses; a segunda via é da epoxidação, a qual é conduzida pela enzima hidrolase epóxida; e a terceira via é pela 1'hidroxilação, sendo considerada uma sinalização tóxica do estragol em altas doses, de caráter hepatotóxico por formar o metabólito tóxico sulfato de 1'hidrociestragol (SMITH et al., 2002).

Algumas atividades biológicas já foram descritas na literatura, entre as quais, atividade antioxidante, antimicrobiana (MORAIS et al., 2006), ansiolítica, contrátil do músculo esquelético, relaxante do músculo visceral (SOARES et al, 2007) e anti-inflamatória nas doses de 3-30 mg/kg (PONTE et al., 2012) e apresenta DL50 de 2800 mg/kg para ratos e 2250 mg/kg para camundongos (SILVA-ALVES et al, 2013).

Após estudos conduzidos pelo nosso grupo de pesquisa, foi evidenciada a atividade gastroprotetora do estragol (ALVES JUNIOR et al., 2020) com envolvimento das vias antioxidante e imunomoduladora. A partir disto, essa substância foi selecionada para dar continuidade aos estudos descritos no presente trabalho.

3. OBJETIVOS

3.1 Objetivo geral

- Avaliar a atividade antiulcerogênica/cicatrizante gástrica e anti-inflamatória intestinal do estragol em modelos animais.

3.2 Objetivos específicos

- ❖ Investigar o efeito cicatrizante do estragol em estômagos de ratos submetidos ao modelo de úlcera gástrica induzida por ácido acético;
    - Realizar uma análise dos mecanismos cicatrizantes (proliferação celular, angiogênese e remodelamento da matriz extracelular).

- ❖ Avaliar o efeito antiulcerogênico do estragol em modelo de indução de úlcera duodenal em ratos;

- ❖ Investigar o efeito do estragol nas doenças inflamatórias intestinais em modelo de colite ulcerativa aguda em ratos;

- ❖ Estudar o efeito anti-inflamatório intestinal do estragol em modelo que mimetiza a recidiva da retocolite ulcerativa;

    - Realizar uma análise dos mecanismos citoprotetores da barreira intestinal (camada de muco e junções comunicantes).

A partir destes modelos, os tecidos retirados de cada protocolo específico serão utilizados para:

- Analisar por histologia e imunohistoquímica os estômagos, duodenos e cólons;
- Avaliar uma possível atividade antioxidante;
- Verificar o efeito sobre a produção de citocinas pró e anti-inflamatórias

4 RESULTADOS E DISCUSSÃO

4.1 Artigo 1: Estragole prevents cysteamine-induced duodenal ulcers and accelerates healing by re-epithelialization of gastric ulcers dependent on antioxidant and immunomodulatory mechanisms in rats. Artigo a ser submetido a revista Frontiers in Pharmacology

Estragole prevents cysteamine-induced duodenal ulcers and accelerates healing by re-epithelialization of gastric ulcers dependent on antioxidant and immunomodulatory mechanisms in rats

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Keywords: Estragole, gastric ulcer, duodenal ulcers, healing, phenylpropanoid, immunomodulatory

Abstract

Background: Estragole (phenylpropanoid) an aromatic organic compound present in essential oils from plant species, such as *Ravensara anisata* (madeira), *Ocimum basilicum* (manericão), and *Croton zehntneri* (canelinha) whose pharmacological studies report its anti-inflammatory, antioxidant, vasorelaxant and gastroprotective activity.

Hypothesis/Purpose: To evaluate their duodenal antiulcer activity, gastric healing, and repeated dose toxicity of phenylpropanoid estragole in rats.

Methods: Preventive antiulcer effects were assessed using oral pre-treatment on cysteamine-induced duodenal lesions models. Gastric healing, the underlining mechanisms, and toxicity after repeated doses were carried out using the acetic acid-induced gastric ulcer rat model and oral treatment for 14 days.

Results: In the cysteamine-induced duodenal ulcer, estragole (31–250 mg/kg) decreased significantly the ulcer area and so prevented lesions formation. In the acetic acid-induced ulcer model, Estragole (250 mg/kg) reduced ($p<0.001$) the ulcerative injury. These effects were related to an increase in the levels of reduced glutathione (GSH), superoxide dismutase (SOD), interleukin (IL)-10, and transforming growth factor beta (TGF-β). In contrast, reduced ($p<0.001$) malondialdehyde (MDA), myeloperoxidase (MPO), interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), and nuclear transcription factor kappa B (NF-κB). Oral toxicity investigation for 14 days revealed no alterations in heart, liver, spleen, and kidney weight nor the biochemical and hematological assessed parameters. Treatment with Estragole protected animals from body weight loss maintaining feed and water intake.

Conclusion: Estragole presents high safety and low toxicity when administered in repeated doses for 14 days, prevents duodenal ulcers and a potential pro-healing effect on gastric lesions with involvement of antioxidant and immunomodulatory pathways seem to be involved in the curative effect.

1 Introduction

Peptic ulcers consist of a set of disorders characterized by the presence of inflammation and lesions in the mucosa, which are of multifactorial origin, characterized by the appearance of necrotic points and hemorrhagic lesions that can affect the esophagus, stomach or duodenum (Pasha et al., 2022). Its etiology is related to the imbalance between the protective agents of the gastric mucosa and the aggressive agents (Boeing et al., 2016; Malfertheiner; Schulz, 2020). The epidemiology of the conditions that affect the GI tract is dynamic, with difficult to accurately estimate the prevalence due to the subjectivity of the symptoms and the clinical similarity with other diseases. Peptic ulcer affects approximately 5 to 15% of the world's population, with an incidence of 0.1 to 0.3% per year, equivalent to 4 million people. However, this percentage is not reliable considering that many cases are underreported (Havens et al., 2018).

Pharmacological treatment of peptic ulcer aims to reestablish the lost balance between the action of cytoprotective factors against aggressive factors and has as main functions to reduce or neutralize acid secretion and/or stimulate the gastric cytoprotection system (Naunton et al., 2018; Spechler, 2018). However, limitations such as the high rate of disease recurrence, the variety of side effects, drug interactions, high cost of hospitalization for individuals and limited access to drugs, point to the need for new therapeutic alternatives for the treatment of ulcers, such as natural products, that have demonstrated a wide diversity of chemical composition and biologically active (Li et al., 2015; Boeing et al., 2016).

In recent years, many plant species and their isolated compounds has shown promising effects in the context of peptic ulcers in studies using animal models (Venzon et al., 2018). Phenylpropanoids are a class of compounds produced by medicinal plants and are biosynthesized from phenylalanine via cinnamic acid. These compounds are widely used in the industry as fragrances, agrochemicals, and nutraceuticals (Cox-Georgian et al., 2019).

Estragole is a phenylpropanoid consisting of a benzene ring substituted by a methoxy group and a propenyl group and is present as a constituent of essential oils of many plant species such as *Ravensara anisata*, (madeira), *Ocimum basilicum* (manjericão), and *Croton zehntneri* (canelinha). These species are widely used both in folk medicine and in cooking (Silva-Alves et al., 2013). Its essential oils are widely used in aromatherapy and it is used as a flavoring agent in

the pharmaceutical, cosmetic and food industries as a preservative. Some biological activities have already been described in the literature, including antioxidant and antimicrobial activity (Morais et al., 2006), anxiolytic, skeletal muscle contractile and visceral muscle relaxant (Soares et al, 2007) and anti-inflammatory at doses of 3-30 mg/kg (Ponte et al., 2012). It presents LD50 of 2800 mg/kg for rats and 2250 mg/kg for mice (Silva-Alves et al, 2013).

After studies conducted by our research group, the gastroprotective activity of Estragole was evidenced (Alves Junior et al., 2020) with involvement of antioxidant and immunomodulatory pathways.

Thus, considering the previous promising results, this study aimed to evaluate the gastric healing activity using the acetic acid induction model, the duodenal anti-ulcer activity induced by cysteamine, and oral toxicity in repeated doses for 14 days, as well the mechanisms of action of Estragole.

2 Materials and methods

2.1. Animals

Male Wistar rats (*Rattus norvegicus*) (180−250 g) were provided by the Animal Production Unit (APU) of the Institute for Research on Drugs and Medicines of the Federal University of Paraiba (IPeFarM/UFPB). The animals were acclimated to the conditions of the local vivarium at a temperature of 23 ± 2°C and a light-dark cycle of 12 h, fed with industrial pellet food and water *ad libitum*. All experimental protocols followed international principles for the study with laboratory animals (Zimmermann, 1983) and were approved by the Institutional Ethics Commission on Animal Use (CEUA/UFPB) under the registration number: 4120010819/20. All efforts were to reduce the number of animals used, and their pain, and stress.

2.2. Reagents

The following substances were used in this study: Estragole (SIGMA Chemical Co, U.S.A) solubilized in 5% Tween 80, sodium acetate (SIGMA Chemical Co, U.S.A), acetonitrile (SIGMA Chemical Co, U.S.A), 5,5‘-ditiobis-2-nitrobenzoic acid (DTNB) (SIGMA Chemical Co, U.S.A), ethylenediaminetetraacetic acid (EDTA) (SIGMA Chemical Co, U.S.A), glacial acetic acid (SIGMA Chemical Co, U.S.A), trichloroacetic acid (SIGMA Chemical Co, U.S.A), anti-rat antibodies for IL-1β, TNF-α and IL-10 (R&D systems), biotinylated sheep polyclonal antibodies (anti-IL-1β, anti-TNF-α or an-ti-IL-10) (R&D Systems), immunohistochemical polyclonal antibodies rats (NFκB or TGFβ) (Cloud-clone Corp), sodium carbonate (MERK, Germany), potassium chloride (SIGMA Chemical Co, USA), magnesium chloride (SIGMA Chemical Co, USA), Cysteamine hydrochloride (SIGMA Chemical Co, USA), sodium chloride PA (QUIMEX-MERCK, Brazil), ethyl ether (MERCK, Germany),, monobasic sodium phosphate (SIGMA Chemical Co, USA), bibasic sodium phosphate (SIGMA Chemical Co, USA), lansoprazole (SIGMA Chemical Co, USA), Methyl-Phenylindole (SIGMA Chemical Co, USA), ketamine 5 % (VETANARCOL), Tris Buffer (Vetec ®), Trizma Buffer (SIGMA Chemical Co, U.S.A) and xylazine 2 % (DORCIPEC).

2.3. Preventive antiulcer activity evaluation on cysteamine-induced duodenal ulcer model

Rats (N = 6-7) were fasted for 12 h and treated orally (10 mL/kg) with lansoprazole 30 mg/kg (standard control), 5% Tween 80 (negative controls), Estragole ((31; 62; 125 or 250 mg/kg). After 1 h from pretreatment, cysteamine hydrochloride (300 mg/kg) was given twice orally (5 mL/kg)

in a 4-h interval induction. After 24 h, animals were euthanized and the small intestine was removed to be opened in the antimesenteric region. They were kept between glass plates to obtain images with the aid of a digital camera for lesion determination. Ulcer area (UA, mm2) quantification was carried out using AVSoft Bioview® software version 4.0.1.

2.4. Healing assessment on acetic acid-induced gastric ulcer model

Wistar rats (N = 8–10), after a 12-h fasting, were randomly assigned to 6 groups: Sham (healthy group), positive control (lansoprazole 30 mg/kg), negative control (5% Tween 80), Estragole (250 mg/kg). Estragole's most effective dose was selected from the cysteamine-induced duodenal ulcer protocol. The criteria used were based on the highest lesion inhibition percentages among evaluated doses. Animals were anesthetized with ketamine and xylazine and after that, subjected to laparotomy. Stomachs were exposed and the serosa outer face received 100 µl of absolute acetic acid, soaked in a cotton pellet delimited by a 5.0 mm circular diameter plastic tube for 1 min. Then, the stomachs were washed with destilled water, and the abdominal wall was sutured with cotton thread. After 48 h of gastric lesion induction, animals were treated orally (10 mL/kg) once a day for 14 days, as described above. This process was carried out except for the Sham group, which was only subjected to laparotomy and immediately sutured. On the fifteenth day, animals were euthanized, stomachs were removed and opened along the great curvature for determination of the Ulcerative Lesion Area - (mm2) with the aid of a digital caliper and also to state healing percentage (%), as described below.

ULA (mm2) = (ulcer length) × (ulcer width)

Healing (%) = (Negative control ULA − Treated group ULA /Negative control ULA) × 100

After ULA determination, tissue samples circumscribing gastric injuries were collected and stored in preserved in a 10% buffered formaldehyde solution for histological and immunohistochemical analysis. For biochemical assays, tissues were stripped, weighed, and frozen at − 80 ºC.

2.4.1.1. Histological and morphometric analysis

Fragments of gastric tissue from the acetic acid-induced gastric ulcer protocol and the duodenal tissue from the cysteamine-induced ulcer protocol were preserved in a 10% buffered formaldehyde solution until histological processing. The tissues were embedded in histological paraffin and sectioned with a thickness of 4 µm. From the sectioned tissues, 2 stains were performed: hematoxylin and eosin (HE) and Masson’s trichrome (MT). The histological sections stained by Masson's trichrome were visualized through the 40x objective of the Olympus microscope (Tokyo, Japan) and 20 random images were digitalized through the same microscope and Q-Color3 microscope, in each image, all pixels with shades of blue (Masson's trichrome) were selected to create a binary image, digitally processed and calculate the area in μm^2.

2.4.2 Determination of reduced glutathione (GSH), malondialdehyde (MDA), myeloperoxidase (MPO), and Superoxide dismutase (SOD)

2.4.2.1. Non-protein sulfhydryl group (GSH) determination

The tissue samples were suspended in 0.02M 1:10 (v/v) EDTA. From this homogenate, 400 µL were removed and 320 µL of distilled water and 80 µL of 50% trichloroacetic acid were added, being centrifuged at 3000 rpm at 4°C for 15 minutes. Then, 100 µL of the resulting supernatant

was pipetted into a 96-well microplate and 200 µL of Tris and 25 µL of DTNB were added. This microplate was incubated at room temperature and after 15 minutes, reading on a spectrophotometer (Polaris) was performed 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 nmol GSH/mg of protein (Faure and Lafond, 1995).

2.4.2.2. Malondialdehyde (MDA) determination

The samples were suspended in Trizma® buffer (Tris HCl) 1:5 (w/v), homogenized, and centrifuged at 11.000 rpm at 4°C for 10 minutes. Soon after, 300 µL of the supernatants were transferred to Eppendorf tubes and 750 µL of the chromogenic compound (10.3 mM of 1-methyl-2-phenylindol) and 225 µL of hydrochloric acid (37%) were added and incubated at 45ºC in a water bath for 40 minutes and again centrifuged at 11.000 rpm at 4ºC for 5 minutes. Then, 300 µL of the supernatant was transferred to a 96-well microplate, and absorbance was determined by colorimetry (586 nm), using a plate reader (Polaris' spectrophotometer). The data were interpolated with the standard curve and the results were expressed as nmol MDA/g tissue (Esterbauer and Cheeseman, 1990).

2.4.2.3. Myeloperoxidase (MPO) determination

The tissue sample fragments were homogenized in the hexadecyltrimethylammonium bromide (HTAB) buffer, which has a detergent function, lysing the granules of the neutrophils that contain the myeloperoxidase. After homogenization, the material was subjected to sonication for 5 minutes. Then, the sample was subjected to a double freezing and thawing process to facilitate the disruption of cellular structures and consequently the release of the enzyme. The homogenate was centrifuged at 5.000 rpm at 4ºC for 20 minutes and concentrated for 24 hours. On the following day, 7 µL of the supernatant were collected, to which 200 µL of the reading solution (o-dianisidine hydrochloride, potassium phosphate buffer, and 1% H2O2) were added. The reading was performed using a spectrophotometer at 450 nm wave, at times 0 and 1 minute. The results were expressed as units of myeloperoxidase per gram of tissue (Krawisz et al., 1984).

2.4.2.4. Superoxide dismutase (SOD) determination

The tissue samples were homogenized in phosphate buffer (0.4 M, pH 7.0) and centrifuged for 15 minutes 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 (15W) for 10 minutes. After this period, the material was taken to the 630 nm spectrophotometer (Sun et al., 1988).

2.4.5. Immunomodulatory activity

2.4.5.1. Cytokines determination

The levels of pro-inflammatory (IL-1β and TNF-α) and immunoregulatory (IL-10) cytokines were determined through an enzyme-linked immunosorbent assay (ELISA - sandwich type). The capture antibodies for each interleukin were sensitized in a 96-well microplate (flat bottom). After 18h, the plate was washed with 0.05% tween 20 solution (wash buffer), blocked with a 1% bovine serum albumin solution, and washed with the wash buffer. Soon after, a tissue macerate was prepared in phosphate-buffered saline (PBS) in the proportion of 100 mg of tissue to 600 µL of

PBS, homogenized, and centrifuged at 4000 rpm for 10 minutes at 4°C. Supernatants (100 µL) were pipetted in a 96-well plate and made the standard curve. The biotinylated secondary antibody (100 µL) was added to each well, followed by incubation for 2 hours and 3 washes. The plate was incubated with streptavidin for 20 minutes, washed 3 times, and the substrate for development was added (DuoSet Kit © - R & DSystems Catalog - DY999) and incubated for 20 minutes. After this time, the reaction was stopped by adding 50 µL of the stop solution and the reading was performed on a spectrophotometer at 450 nm. The results were obtained by interpolation with the standard curve and expressed in pg/mL (Kendall et al., 1983).

2.4.6. Immunohistochemical analysis

Stomach sections (5 µm) of 5 animals per group were obtained with a microtome and transferred to silanized slides (Dako, Glostrup Denmark), and subjected to dewaxing and hydration. Then, the slides are subjected to the simple indirect method of blocking with Anti Peroxidase Peroxidase (APP), where the primary antibody is directed against the antigen (protein) to be detected and the secondary antibody serves as a bridge for binding to the APP complex, and incubated overnight at 4°C with the following primary antibodies a: nuclear transcription factor kappa B (NF-κB) and transforming growth factor beta (TGF-β). After being washed with distilled water, the slides were incubated with a secondary for 60 min and 3,3'-diaminobenzidine (DAB, Biocare Medical, CA, USA) was used as the chromogen and the specimens were counterstained with hematoxylin. The samples were visualized under an optical microscope (Olympus microscope, Tokyo, Japan) with coupled to a camera (Nikon DS-Ri2).

2.5. Evaluation of toxicity after repeated doses

During the course of the acetic acid-induced ulcer model, water and feed intake, and body weight of the animals were evaluated for 14 days. On the 15th day, animals were fasted for 12 h and anesthetized for blood collection via arterial puncture. To perform the biochemical analysis, blood was collected in tubes containing a gel separator and centrifuged for 10 min at 1000 g. Urea, creatinine, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose, cholesterol, and triglycerides were measured using the animal serum. For hematological analyzes, blood was collected in tubes containing EDTA, followed by determination of red blood cell, hematocrit, hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), global and differential leukocyte counts. Blood smears were also collected and stained to confirm and control differential counts of leukocytes (neutrophils, lymphocytes, monocytes, and eosinophils). Those parameters were determined using specific kits for automatic biochemical analyzer and automatic hematological analyzer. The blood smears were stained and analyzed under optical microscopy. After blood collection, animals were euthanized and organs (heart, liver, spleen, and kidneys) were collected for macroscopic and weight evaluation.

2.6. Statistical analysis

Data were expressed as mean ± standard deviation (SD) or mean ± standard error (SE) for parametric values or expressed as median (minimum value - maximum value) for non-parametric data. One-way ANOVA (parametric data) or Kruskal-Wallis test (non-parametric data) was performed, followed by Dunnet and Tukey or Dunn post-tests, respectively. The results were considered significant when $p < 0.05$. All data were analyzed using GraphPad® 5.0 software.

3 Results

3.1. Effect of Estragole oral pre-treatment on duodenal ulcer formation

Results for the cysteamine-induced duodenal ulcer induction model in rats showed that oral administration of lansoprazole (30 mg/kg) or estragole at doses of 31, 62, 125 or 250 mg/kg significantly reduced ULA ($p<0.001$) when compared to the negative control group (5% tween 80), with injury inhibition percentages of 52, 53, 76, 87 and 77 %, respectively (Figure 1). This result can also be seen in Figure 2, where there are representative photographs of the duodenum of animals treated with the vehicle, the standard substance and the substance tested at different doses.

3.1.1. Histological analysis of duodenal ulcer formation

Histological sections of the duodenum stained with hematoxylin and eosin belonging to the Normal, lansoprazole and Estragole groups (Figure 3A, C, D), the integrity of the villi (V) and enterocytes without cytostructural alterations is observed. In the negative control group (5% tween 80) (B) there is a loss of epithelial cells and an increase in the leukocyte infiltrate. In sections stained with Masson's trichrome, it was found that Estragole (Figure 3G) reduced extracellular ($p<0.01$) 7.00 (5-8) matrix deposition compared to the control group (5% tween 80) 10.00 (9-12) (3F) (Figure 3).

3.2. Effect of Estragole on gastric healing after 14 days of oral treatment

The results for the acetic acid-induced gastric ulcer model in rats showed that estragole at the dose (250 mg/kg) and lansoprazole (30 mg/kg) significantly reduced ALU ($p<0.001$) to 18.8 ± 1.2 and 12.6 ± 2.4 mm^2 when compared to the negative control group (5% tween 80) with 47.0 ± 4.0 mm^2, presenting percentages of gastric healing of 60 and 73%, respectively (Figure 4).

3.2.1. Histological analysis of gastric healing

In the histological sections of the stomach stained with hematoxylin and eosin (Fig. 5 A-D), the stomach mucosa is observed (M). In the animals of the Sham (A) lansoprazole (C) or Estragole (D) group, the integrity of the mucosa and gastric glands is observed. Unlike animals in the negative control group (5% tween 80), intense mucosal destruction is observed, with ulcer formation, which is characterized by necrosis of the organ surface and intense acute inflammatory reaction (*).

In the histological sections stained with Masson trichrome (Fig.5 E-H), in the Sham (E) 12.00 (10-19), lansoprazole 15.00 (11-19) (G) or Estragole 18.00 (10-22) (H) groups, the integrity of the stomach mucosa (M) and gastric glands is observed. However, in the animals of the negative control group (5% tween 80) ($p<0.001$) 45.00 (31-57) (F) exuberant scar tissue (∞) is observed in the area of the mucosal ulcer.

3.2.3 Effect of Estragole in the modulation of antioxidant and anti-inflammatory properties during gastric healing

3.2.3.1. Reduced Glutathione (GSH)

In determining the antioxidant activity of Estragole, it was possible to verify that the control group (5% tween 80) showed a reduction in GSH levels to 71.1 ± 4.3 nmol of GSH/mg protein compared to the Sham group (93.6 ± 4.3 nmol GSH/mg protein). However, previous administration of lansoprazole (30 mg/kg) (92.9 ± 6.3 nmol of GSH/mg of proteins) or Estragole (250 mg/kg) (90.7 ± 8.8 nmol GSH / mg protein) restored baseline GSH levels (Fig. 6 A).

3.2.3.2. Superoxide Dismutase (SOD)

Animals in the control group (5% tween 80) demonstrated a significant reduction in the activity of the SOD enzyme to 0.2 ± 0.1 U of SOD/mg of protein compared to the Sham group (2.8 ± 0.4 U SOD/mg protein). However, previous administration of lansoprazole (30 mg/kg) or Estragole (250 mg/kg) increase ($p < 0.001$) activity of SOD to 3.2 ± 0.4 and 2.6 ± 0.4 U of SOD/mg of protein, respectively (Fig. 6 B).

3.2.3.3. Myeloperoxidase (MPO)

The results showed that in the control group (5% tween 80) there was an increase in MPO levels to 64.5 ± 4.3 units of MPO / g of tissue when compared to the Sham group (14.9.1 ± 2.3 units MPO/g tissue). However, the groups treated with lansoprazole (30 mg/kg) or Estragole (250 mg/kg) significantly reduced MPO levels to 35.3 ± 3.6 and 35.5 ± 2.2 unit of MPO/g of tissue, respectively in comparison with to the control group (Fig. 6 C).

3.2.3.3. Malondialdehyde (MDA)

The control group showed an increase ($p<0,001$) in MDA levels to 83.6 ± 2.8 nmol MDA/g tissue compared to the Sham group (47.3 ± 3.3 nmol MDA/g tissue). However, the administration of lansoprazole (30 mg/kg) (68.2 ± 3.6 nmol of MDA/g tissue) or Estragole (250 mg/kg) (56.0 ± 2.2 nmol MDA/g of tissue) restored MDA levels to baseline conditions, compared to the Sham group (Fig. 6 D).

3.2.3.5. Levels of cytokines IL-1β, TNF-α and IL-10

In the evaluation of immunomodulatory effects by measuring the levels of cytokines. Estragole significantly decreased ($p < 0.001$) the levels of IL-1β to 341.5. ± 35.1 pg/mL compared to the control group (710.1 ± 83.1 pg/mL) (Fig 7 A). TNF-α levels were also decreased ($p<0,001$) after treatment with Estragole to 753.3 ± 118.9 pg/mL, when compared to the control group (2246 ± 134.5 pg/mL) (Fig 7 B). Furthermore, Estragole prevented ($p<0.001$) the reduction of IL-10 (pg/mL) (176.3 ± 17.5 pg/mL) in comparison the control group (68.0 ± 10.1 pg/mL) (Fig 7 C).

3.2.3.6. Immunohistochemical analysis for NFκB and TGF-β in gastric samples submitted to acetic acid-induced gastric ulcer model

Stomachs were submitted to acetic acid and treated with Estragole showed the presence of NF-κB (brown color positivity) unevenly distributed and focal similar to the Sham group, significantly reduced immunostaining to 57 (48–58) μm^2 when compared to the control group 69.0 (62-75) μm^2, in which there is the presence of these irregularly and multifocally marked, especially in the ulcer area (Fig. 8). In the evaluation of immunostaining for TGF-β results showed that treatment with Estragol significantly increased positive cells marked for TGF-β to 149.0 (201.0–224.0) μm^2 when compared to the control group 104.0 (100.0–107.0) μm^2 (Fig. 9).

3.2.4. Effect of Estragole on low toxicity after repeated doses

Results showed that animals treated with Estragole (250 mg/kg) increased ($p<0.01$) water intake (mL) 241.9 ± 10.3 compared to the control group (204.2 ± 11.1). Besides, treatment with Estragole also increased ($p<0,01$) feed intake (g) to 165.7 ± 17.3 and prevented ($p< 0.001$) body weight (g) loss (56.0 ± 2.7) compared to the control group (31.0 ± 3.6) (Table 1).

In the macroscopic evaluation of the heart, liver, spleen, and kidneys, there was no evidence of alterations between Sham, control, and treated groups. Furthermore, no significant changes ($p>0.05$) were observed in the organ index across all analyzed groups (Table 2). There were no significant changes ($p>0.05$) in hematological and biochemical parameters when compared to their control group (Table 2).

4 Discussion

Natural products and their secondary metabolites presents diverse biological activities, making them objects of interest for research, with the aim of developing new alternatives for safer and more effective therapy for the treatment of various pathological processes (Newman; Cragg, 2016). In the context of peptic ulcers and inflammatory bowel diseases, available therapy induces and maintains disease remission for a limited period but does not modify or reverse the underlying pathogenic mechanism, most often leading to disease recurrence (Danese et al., 2015; Marild et al., 2022).

In view of this, new therapeutic approaches are needed for the treatment of these diseases and, for this, it is important to use animal models to mimic their characteristics (Jiminez et al., 2015). In previous studies conducted by our research group, the gastroprotective effect of Estragole related to cytoprotective, antioxidant and anti-inflammatory mechanisms was demonstrated (Alves Júnior et al., 2020). These results stimulated the continuity of studies with these substances, now evaluating the gastric healing activity, duodenal antiulcer and intestinal anti-inflammatory activity in experimental models *in vivo*.

Duodenal ulcer is the most prevalent type of peptic ulcer in the world population, being strongly associated with infection by the bacteria *H. pylori*. Its pathogenesis is related to an increase in acid-peptic secretion and other proteases along with a reduction in mucosal protective factors (Choi et al., 2012). Cysteamine (β-mercaptoethylamine) is a low molecular weight aminothiol widely distributed in organisms, being characterized as a natural product resulting from the metabolism of coenzyme A (Khomenko et al., 2009). It causes rapid ulceration and is one of the best agents to mimic acute and chronic duodenal ulcer in animal models by reducing somatostatin bioavailability (Besouw et al., 2013; Mishra; Bajpai; Chandra, 2019) and elevating serum gastrin levels (Adinortey et al., 2013), which generates an increase in gastric acid secretion, resulting in a decrease in the ability to neutralize low pH in the proximal duodenum (Adler et al., 1983). In addition, it reduces dopamine levels in the glandular portion of the stomach and duodenum (Szabo et al., 1987), alters gastrointestinal emptying and motility (Adinortey et al., 2013; Mishra; Bajpai; Chandra, 2019).

Estragole at the doses evaluated (31, 62, 125 and 250 mg/kg) significantly reduced duodenal ulcerative lesions when compared to negative controls, suggesting a potent duodenal antiulcerogenic activity that may be related to an antioxidant effect, maintenance of mucosal protective factors, reduced infiltration of cells involved in the inflammatory process and/or regulation of blood flow. A similar protective effect on the duodenal mucosa was demonstrated in studies with the phenolic compounds geraniol (Carvalho et al., 2014), β-myrcene (Bonamin et al., 2014) and *p*-cymene and rosmarinic acid (Formiga et al., 2021) in the same model. These effects were related to a decrease in MPO levels and an increase in GSH levels.

Histomorphological analysis showed pre-treatment with Estragole promoted a decrease in cysteamine-induced lesions in the tissue layers of the duodenum, as well as in the migration of inflammatory cells. Previous studies showed a similar protective effect on the duodenal mucosa in studies with phenolic compounds carried out by Carvalho et al. (2014) with geraniol, and by

Bonamin et al. (2014) with β-myrcene in the same model. The most effective dose of Estragole (250 mg/kg) was selected for evaluation of gastric healing and toxicity after repeated doses.

One of the most relevant aspects of peptic ulcers is the chronicity of the disease, which is characterized by repeated episodes of healing and recurrence (Adinortey et al., 2013). The gastric ulcer induction model by acetic acid is used in the perspective of studying the healing process of the lesions for prolonged intervals after the induction of the ulcer, which was considered chronic due to its permanence for a long period and due to its similarity with the ulcer. gastric ulcers in humans, both macroscopically and histologically.

The model reliably produces rounded and deep ulcers in the stomach of rats and other animal models as it leads to the development of thrombi in the vessels of the tissue layers, microvascular stasis and the ischemic necrosis of the gastric mucosa with consequent ulcer formation (Tarnawski; Ahluwalia, 2012; Adinortey et al., 2013).

In the present study, it can be seen that the most effective dose of Estragole (250 mg/kg) accelerated the healing process when treated for 14 days, considering the occurrence of a significant reduction in the wound ulcerative lesion area (ULA), when compared to the group treated with the vehicle alone. Similar effects have already been reported in the literature for the phenolic compound 1,8-cineol and the polyphenol quercetin, showing a healing effect on the gastric mucosa in this same experimental protocol (Susuki et al., 1998; Caldas et al., 2015). The data from this study also corroborate the results for the phenolic compounds thymol (30 and 100 mg/kg) (Ribeiro et al., 2017), geraniol (3 mg/kg) (Venzon et al., 2018) and and *p*-cymene (Formiga et al., 2021) had a healing effect on the gastric mucosa in this experimental protocol.

Histomorphological study conducted from the reading of slides stained with HE showed that the treatment with Estragole promoted the protection of tissue architecture and gastric glands, with a decrease in the inflammatory infiltrate and preservation of the gastric glands. In the Masson's trichrome staining, the integrity of the stomach mucosa and gastric glands was observed, different from the control group that presented abundant scar tissue. These results corroborate with studies reported in the literature with 1,8-cineol, quercetin and *p*-cymene (Rocha Caldas et al., 2015a; Rocha Caldas et al., 2015b; Formiga et al., 2021). These data suggest that the restoration of the epithelium promoted by the test substance occurs through the preservation of the gastric mucosa due to the increase in cell proliferation, a very important process in the reconstitution of the gastric mucosa. Thus, the duodenal anti-ulcer activity shown in this study may be related to antioxidant effects, the reinforcement of mucosal protection factors, as well as the regulation of acid secretion and blood flow (Spechler, 2018).

In this context, these data provide strong evidence of the healing properties exerted by Estragole. Thus, from this point onwards, healing mechanisms were evaluated using gastric tissue samples from an acetic acid-induced gastric ulcer model.

Excessive production of reactive oxygen species in gastric tissue disrupts the integrity and permeability of cell membranes, leading to cell death and favoring ulcer formation (Dunnill et al., 2017; Dudzińska et al., 2018). In this sense, the antioxidant activity was evaluated by measuring the levels of GSH (mg of non-protein sulfhydryl groups per g of tissue), SOD (unit. per g of protein), MDA (nmol per g of tissue) in gastric samples, and MPO (unit. per g of tissue). The antioxidant system acts on gastric mucosal cells to prevent or minimize the cytotoxicity promoted by oxidative stress. This system involves the action of enzymes such as SOD and compounds with the potential to scavenge free radicals such as GSH (do Carmo et al., 2018).

Daily administration of Estragole for 14 days demonstrated an antioxidant effect by increasing the levels of GSH and SOD and reducing the levels of MDA and MPO. Thus, the curative effect found in our study may be related to the inhibition of free radicals with consequent reduction of toxic lipoperoxides and/or due to the promotion of the synthesis of endogenous antioxidants to maintain the cellular redox state. Previous studies with this same substance also confirm the participation of the antioxidant system in its mechanism of action in acute gastric lesions (Alves Junior et al., 2020). Compounds phenolics 1,8-cineole (Rocha Caldas et al., 2015a; Rocha Caldas et al., 2015b), thymol (Ribeiro et al., 2016) and limonene (de Souza et al., 2019) were shown to increase antioxidant defenses. These results corroborate with these studies. Antioxidant property found in the present work and thus suggest that the decrease in ROS activity by the substance under study may be responsible for reducing the extent of the injury induced by acetic acid, promoting a more favorable environment for the process of healing.

Gastric healing is a multifactorial and complex process involving inflammation, cell migration, proliferation, re-epithelialization, angiogenesis, tissue remodeling and deposition of the extracellular matrix (ECM). All these processes are controlled by growth factors and transcription factors activated by tissue injury, acting in a coordinated manner (Tarnawski, 2005; Tarnawski and Ahluwalia, 2012). The ulcer healing mechanism depends on the involvement of many molecular pathways, compounds that presents therapeutic properties such as increased growth factors and anti-inflammatory activity are strong candidates for the prevention and treatment of human peptic ulcers (Magierowski et al., 2017; Viana et al., 2019).

In the injured gastric mucosa, activation of the inflammatory process occurs with an increase in mediators, such as nuclear transcription factor kappa B (NF-kB), which upregulates the expression of inflammatory responses with increased release of IL-1, IL-6 and TNF (Liu et al., 2016; Wang et al., 2018). Overexpression of IL-1β contributes to ulcer formation and stimulates the production of more TNF-α (Boshtam et al., 2017), high levels of IL-1 and TNF are related to neutrophil infiltration and epithelial cell apoptosis, which causes reduced gastric microcirculation around the ulcer region and delays in ulcer healing (Wang et al., 2018).

IL-10 acts as a protective cytokine of the gastric mucosa, and signals an anti-inflammatory response, reducing the immune response mediated by inflammatory cells. This effect occurs through the inhibition of TNF-α feedback and the consequent reduction of the inflammatory process in the gastric tissue (Mollazadeh et al., 2019).

Treatment with Estragole reduced the levels of inflammatory cytokines (TNF-α and IL-1β) and kept the IL-10 cytokine close to the baseline, suggesting an immunomodulatory effect. Previous studies with this same substance also confirm the participation of the anti-inflammatory system in its mechanism of action in acute gastric lesions (Alves Junior et al., 2020). The compound phenolics limonene (de Souza et al., 2019) and carvacrol (Souza et al., 2017) have demonstrated similar immunomodulatory effects that corroborate our findings.

The results found in the present study demonstrate that treatment by repeated doses for 14 days with Estragole reduced immunostaining for NF-kB when compared to the negative control groups. The NF-κB pathway plays a key role in the regulation of genes related to an acute inflammatory reaction, after exposure to various inflammatory stimuli, NF-κB is activated, translocate to the nucleus, and causes transcriptional activation of other pro-inflammatory cytokines (Akanda et al., 2018; Aziz et al., 2019). The healing activity of this substance seems to be, at least in part due to the downregulation of the NFκB pathway, a key player in pro-inflammatory cytokine production. Furthermore, treatment with Etragole increased the immunostaining of TGF-β, a growth factor involved in the migration of fibroblasts and

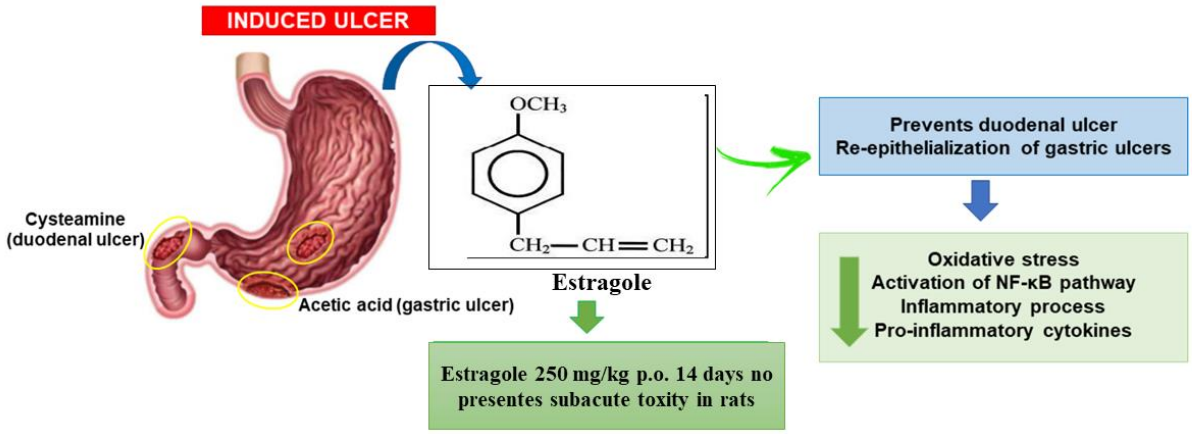
miofibroblocks, stimulation of angiogenesis, and collagenization in gastric tissues (Tarnawski, 2010; Akanda et al., 2018). These results suggest an immunomodulatory activity of this compound, due to its properties in maintaining basal levels of anti-inflammatory cytokines and decreasing levels of inflammatory cytokines.

These results indicate that the substance under study stimulates endogenous healing mechanisms via induction of multiple factors involved in gastric ulcer healing such as induction of cell proliferation and angiogenesis. Results corroborate a study conducted with the phenylpropanoid Rosmarinic acid in ulcers induced by acetic acid, in which the treatments had a healing effect due to the increase in growth factors that induce the re-epithelisation of the gastric lesion (Moraes et al., 2013; Formiga et al., 2021).

In this model of acetic acid-induced gastric ulcers, we also evaluated the safety use of Estragole treatment after 14 consecutive oral administrations once a day. Data from Sigma Aldrich, supplier of the substance, show that Estragole has low acute toxicity LD50 Oral in rats (female) 2.000 mg/kg (OECD Test Guideline 423), with no mortality observed at this dose (Alves Junior et al., 2020). Seeking to corroborate previous studies and expand toxicological information, toxicity was evaluated with the most effective dose of Estragole (250 mg/kg). No changes in body mass, weights of the organs, hematological and biochemical parameters were noted throughout the treatment. We found that Estragole 250 mg/kg treatment did not alter any of the biochemical or hematologic parameters analyzed. Taken together, these findings indicate that 14 d oral treatment with Estragole (250 mg/kg daily) has no subacute toxicity in rats.

Based on the results of the evaluation of the gastric healing activity and duodenal anti-ulcer activity of Estragole and the safety obtained by the toxicological evaluations. Curative mechanisms seem to be due to a reduction in oxidative stress (increase in GSH and SOD with a reduction in MDA and MPO) and immunomodulatory (reduction in TNF-α, IL-1β, NFκB and increase in TGF-β and IL-10 levels). These results are accompanied by the safety of their administration evaluated in a 14-days treatment.

Graphical abstract



Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

Alves Júnior EB, Araruna MEC, Serafim CAL, Pessoa MLS, Alves VP, and Batista LM contributed to the conception of the study, execution of the experiments, and writing of the manuscript. Alves AF and Nunes MKS contributed to the histomorphological and immunohistochemical analysis of this study and Araujo AA contributed to the in vitro experiments of this article. Silva MS and Sobral MV provided technical support and the drugs evaluated in this study. All authors reviewed and approved the final version of this manuscript.

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FIGURES

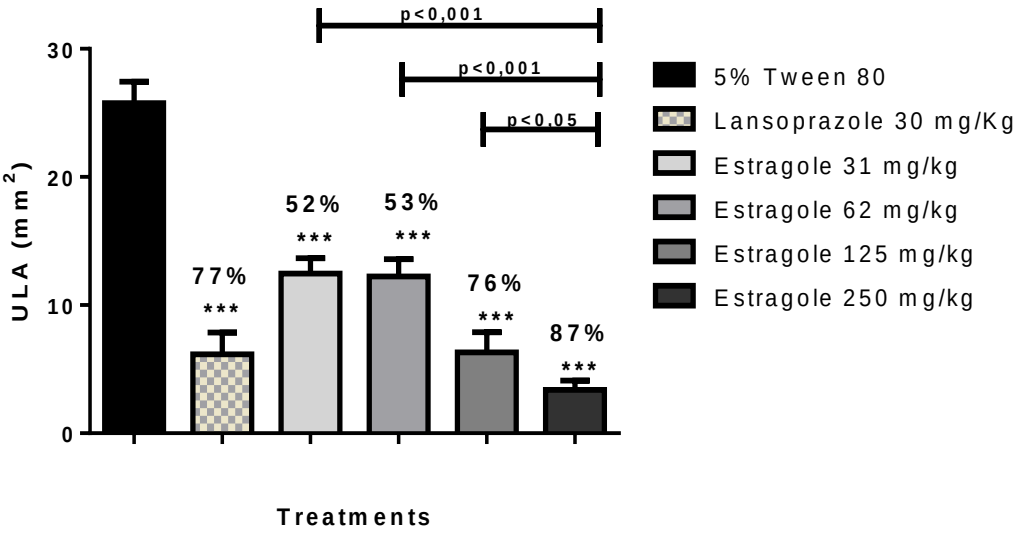


Fig. 1: Effect of oral administration of estragole on cysteamine-induced duodenal ulcer. Results are expressed as mean ± s.d. One-way analysis of variance (ANOVA) was used: F(5.27) = 4.780, followed by Dunnett's or Tukey's test. ***p<0.001, compared with the negative control group (5% tween 80); (n= 5-7). ULA = Ulcerative Lesion Area.

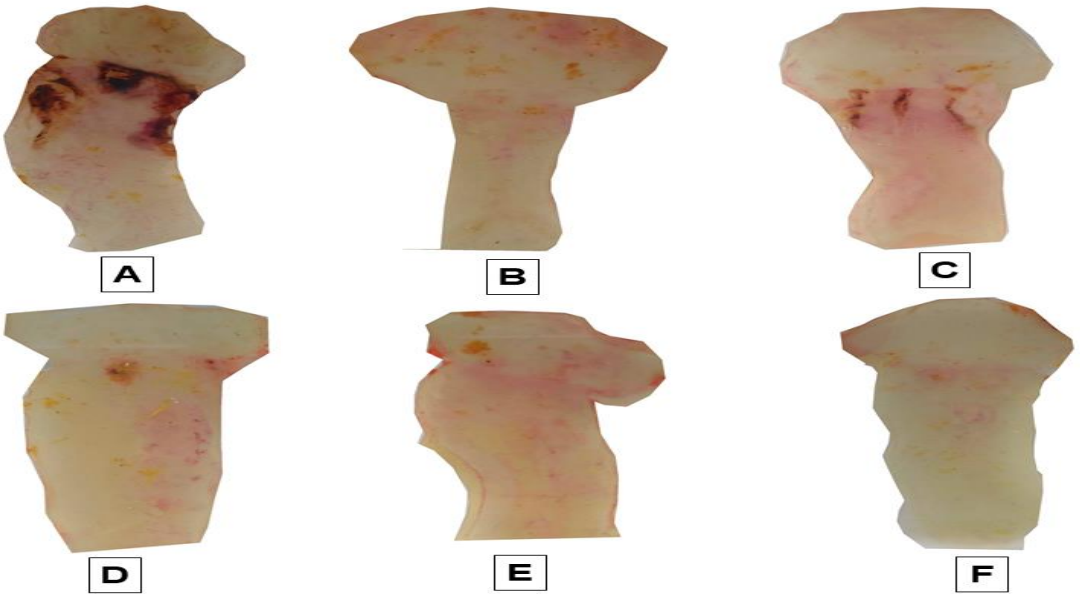


Fig. 2: Macroscopic aspects of the duodenal mucosa of male Wistar rats pretreated (p.o.) with 5% tween 80 (A); lansoprazole 30 mg/kg (B); Estragole 31 mg/kg (C), 62 mg/kg (D), 125 mg/kg (E) and 250 mg/kg (F) in the cysteamine-induced duodenal ulcer mode.

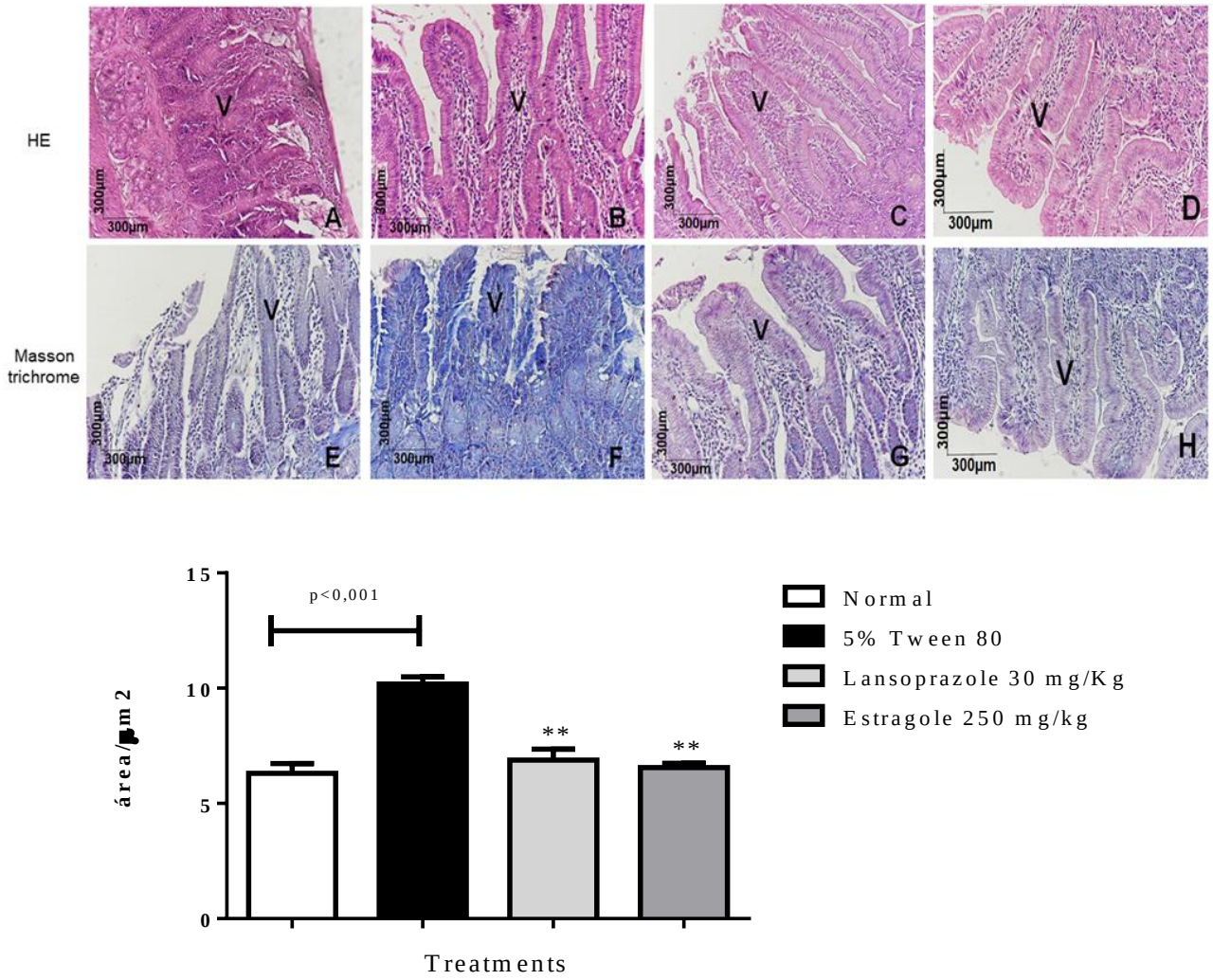


Fig. 3: Microscopic effects of the duodenal mucosa of rats submitted cysteamine-induced duodenal ulcer and pretreated or not with Estragole. Representative photomicrographs of the animals' duodene from the experimental groups: Normal (A,E), 5% tween 80 (B,F), lansoprazole 30 mg/kg (C,G) and Estragole 250 mg/kg (D,H). HE staining (A,B,C,D) and Masson trichrome (E,F,G,H). Intestinal villi and enterocytes (V). Results are expressed as median (minimum-maximum) of the parameters analyzed (n=5, 3 sessions per animal). Kruskall Walis test and Dunn's posterior test was performed using the software Graph Pad Prism. **p < 0.01 compared to the control group (5 % tween 80).

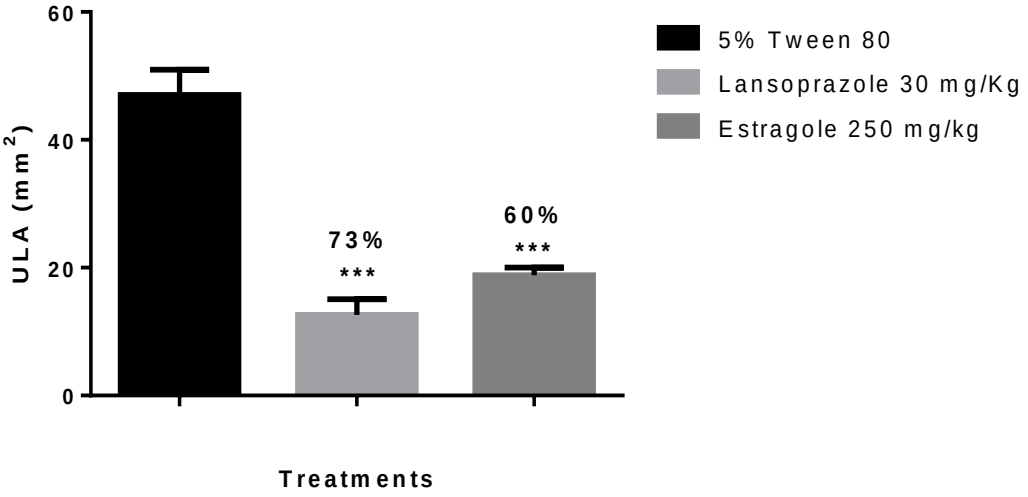


Fig. 4: Effect of oral administration Estragole and lansoprazole on acetic acid-induced gastric ulcer. Results are expressed as mean ± s.d. One-way analysis of variance (ANOVA) was used: F(3.23) =211.6, followed by the Dunnett and Tukey test. ***p<0.001, compared with the negative control group (Tween 80 5%) (n= 6-8).ULA = Ulcerative Lesion Area.

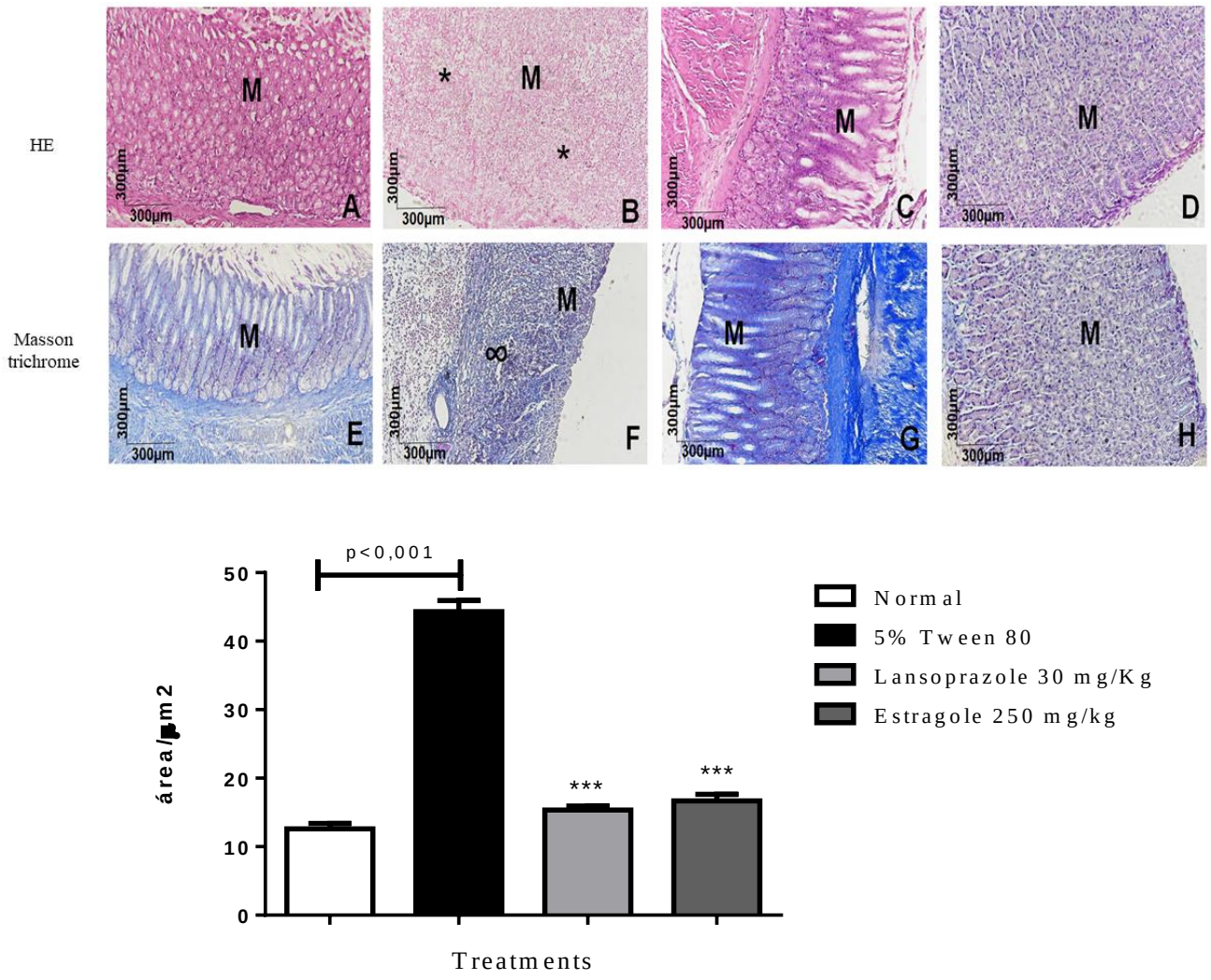
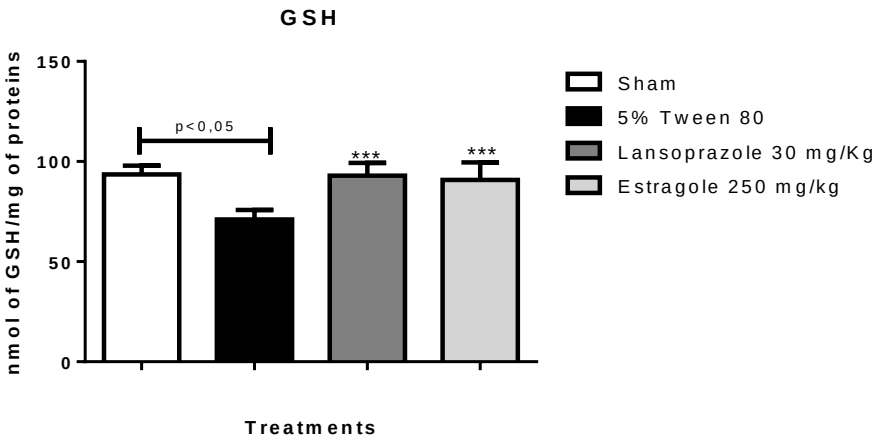
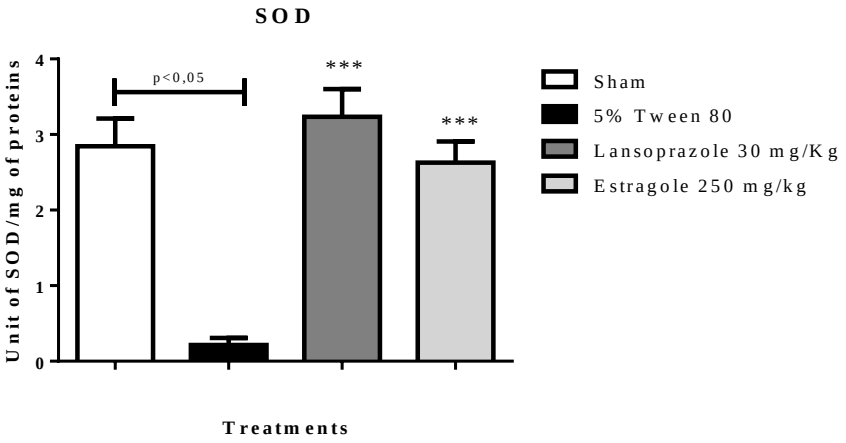


Fig. 5: Microscopic effects of the gastric mucosa of rats submitted acetic acid-induced gastric ulcer and treated or not with Estragole. Representative photomicrographs of the animals' stomachs from the experimental groups: Normal (A,E), 5% tween 80 (B,F), lansoprazole 30 mg/kg (C,G) and Estragole 250 mg/kg (D,H). HE staining (A, B, C, D) and Masson trichrome (E,F,G,H). Stomach mucosa (M), acute inflammatory reaction(*), scarring tissue (∞). Morphometric analysis by Masson's trichrome. Results are expressed as median (minimum-maximum) of the parameters analyzed (n=5, 3 sessions per animal). Kruskall Walis test and Dunn's posterior test was performed using the software Graph Pad Prism. ***p < 0.001 vs. negative control group (5 % tween 80).

A


GSH
nmol of GSH/mg of proteins
150
100
50
0
p<0,05
***
***
Sham
5% Tween 80
Lansoprazole 30 mg/Kg
Estragole 250 mg/kg
Treatments

B


SOD
Unit of SOD/mg of proteins
4
3
2
1
0
p<0,05
***
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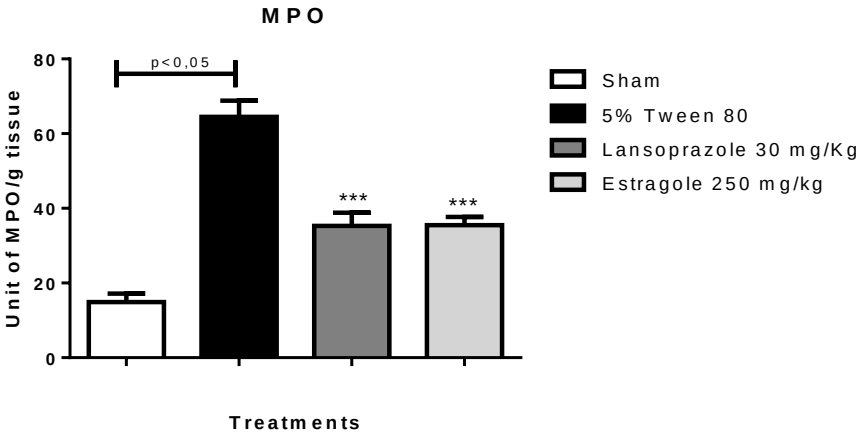
Sham
5% Tween 80
Lansoprazole 30 mg/Kg
Estragole 250 mg/kg
Treatments

C


MPO
Unit of MPO/g tissue
80
60
40
20
0
p<0,05
***
***
Sham
5% Tween 80
Lansoprazole 30 mg/Kg
Estragole 250 mg/kg
Treatments

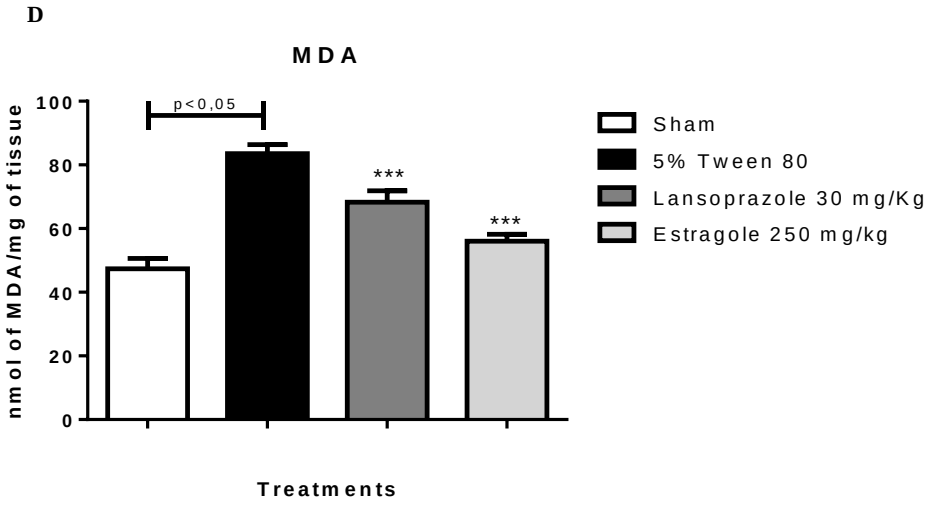


Fig 6. Effect of oral administration of Estragole and lansoprazole in A) GSH, B) SOD C) MDA and D) MPO levels form gastric ulcer model induced by acetic acid in rats. Results expressed as mean ± SE of the parameters analyzed (n = 5-8). The analysis of variance of one via (ANOVA) followed by the Dunnett's and Tukey's test. ***p <0.001 vs control group.

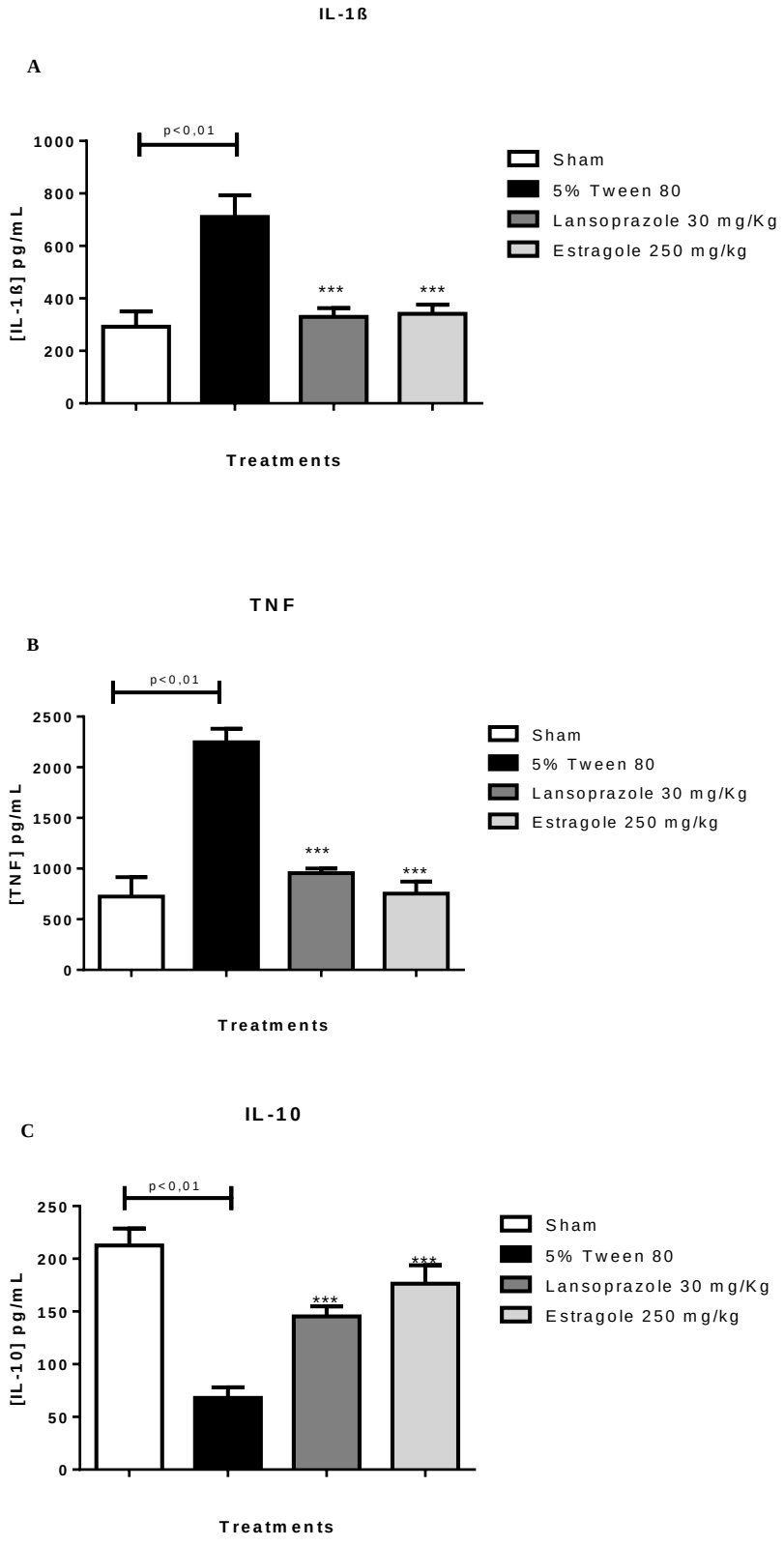


Fig 7. Effect of Estragole and lansoprazole in A) IL-1β, B) TNF-α and C) IL-10 levels form gastric ulcer model induced by acetic acid in rats. Results expressed as mean ± SE of the parameters analyzed (n = 6–8). The analysis of variance of one via (ANOVA) followed by the Dunnett's and Tukey's test. ***p <0.001 vs control group.

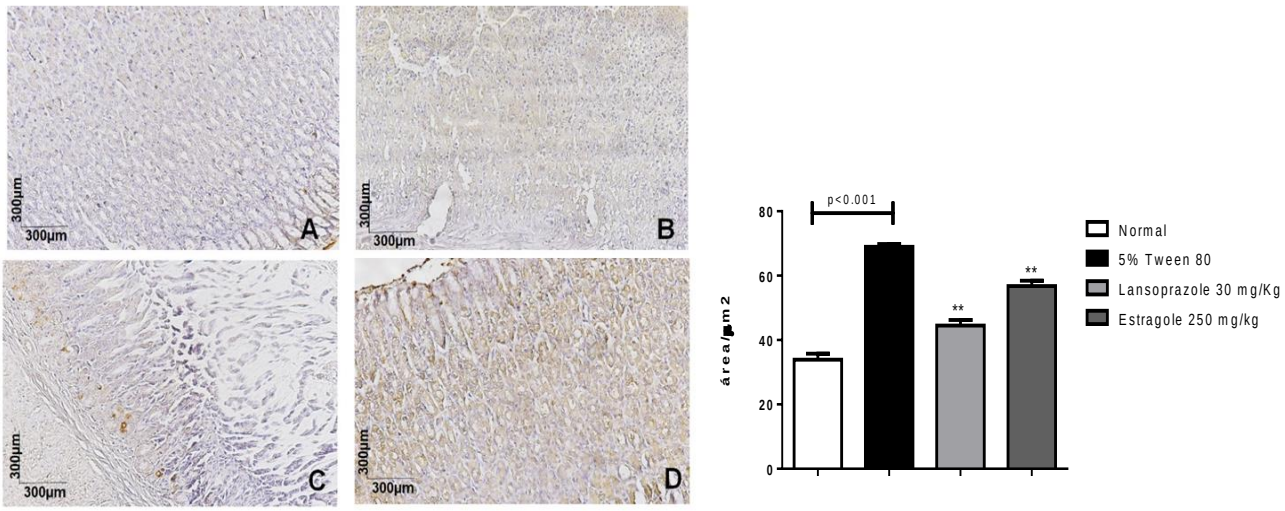


Fig 8. Photomicrographs with immunohistochemical marking for nuclear transcription factor kappa B (NF-κB) in stomach samples from rats. A - normal; B- 5 % Tween 80 (10 mL/kg); C- Lansoprazole (30 mg/kg); D- Estragole (250 mg/kg). Results are expressed as median (minimum-maximum) of the parameters analyzed (n=5, 3 sessions per animal). Kruskall Walis test and Dunn's posterior test was performed using the software Graph Pad Prism. **p < 0.01, compared to the control group (5 % tween 80).

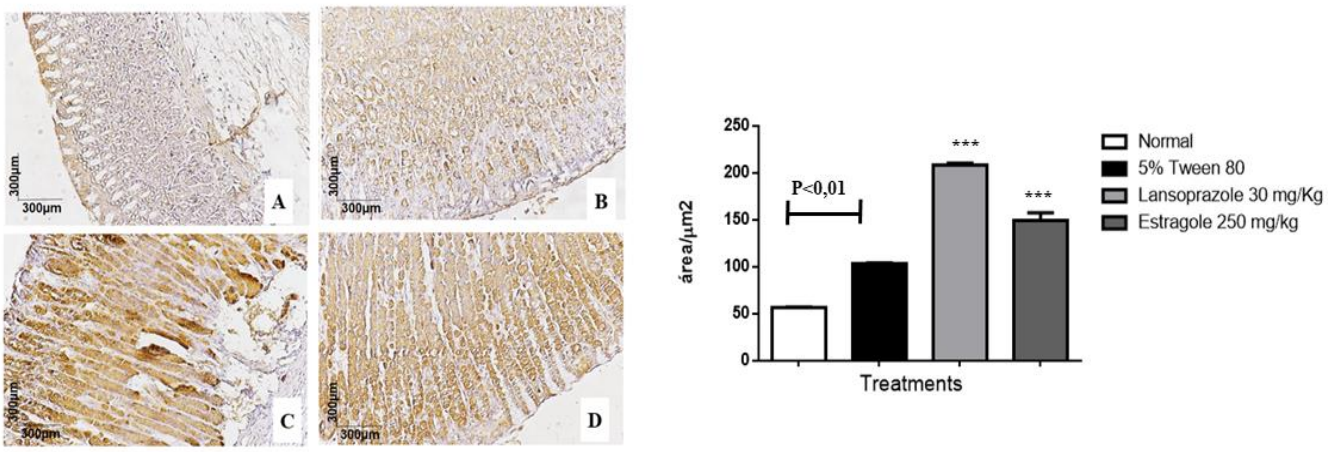


Fig 9. Photomicrographs with immunohistochemical marking for transforming growth factor beta (TGF-β) in stomach samples from rats. A - normal; B- 5 % Tween 80 (10 mL/kg); C- Lansoprazole (30 mg/kg); D- - Estragole (250 mg/kg). Results are expressed as median (minimum-maximum) of the parameters analyzed (n=5, 3 sessions per animal). Kruskall Walis test and Dunn's posterior test was performed using the software Graph Pad Prism. **p < 0.01, compared to the control group (5 % tween 80).

Table 1. Effect of oral administration of Estragole for 14 days on water, feed, weight evolution and organ index of rats in the acetic acid-induced ulcer model

Parameters	**Treatments**			
	Sham	**5% Tween 80**	**Lansoprazole (30mg/kg)**	**Estragole (250mg/kg)**
Water intake (mL)	247.2 ± 12.9	204.2 ± 11.1$^{###}$	231.9 ± 11.3***	241.9 ± 10.32***
Feed intake (g)	175.5 ± 16.5	145.4 ± 9.6$^{###}$	167.8 ± 17.4**	165.7 ± 17.3**
Weight evolution (g)	52.3 ± 3.9	31.0 ± 3.6$^{###}$	56.0 ± 2.7***	50.0 ± 4.0***
Organ index				
Liver	34.9 ± 1.8	33.5 ± 2.2	33.6 ± 2.6	36.6 ± 1.9
Heart	4.3 ± 0.1	4.1 ± 0.3	4.2 ± 0.2	3.9 ± 0.3
Kidneys	8.2 ± 0.2	8.5 ± 0.6	8.4 ± 0.2	8.1 ± 0.5
Spleen	2.8 ± 0.2	2.7 ± 0.1	3.0 ± 0.1	3.0 ± 0.5

Values expressed as mean ± SD (n=6-8). One-way Analysis of Variance (ANOVA): F(3.52) =19.61, followed by the Dunnet and Tukey post-test. *p<0.05 and ***p<0.001 compared to the 5%Tween 80 group; ###p< 0.001 compared to the Sham group. . For organ evaluation, values were expressed as organs index corresponding to the division of body (mg) by weight of animals (g).

Table 2. Hematological and biochemical parameters of rats treated for 14 days with lansoprazole or Estragole, in the acetic acid-induced ulcer model.

Parameters	Treatments			
	Sham	**5% Tween 80**	**Lansoprazole (30mg/kg)**	**Estragole (250mg/kg)**
Hematological				
Red blood cells (10^6/mm^3)	7.7 ± 0.4	7.3 ± 0.4	7.1 ± 0.4	8.1 ± 0.3
Hemoglobin (g/dL)	14.8 ± 0.4	14.3 ± 1.0	14.0 ± 1.0	15.7 ± 0.4
Hematocrit (%)	39.7± 1.5	39.6 ± 2.9	38.3 ± 2.6	43.3 ± 1.0
MCV (μ^3)	52.1 ± 2.0	55.4 ± 2.1	52.3 ± 3.9	53.5 ± 1.4
HCM (μg)	19.5 ± 0.7	19.9 ± 0.7	19.1 ± 1.3	19.4 ± 0.6
CHCM (%)	36.6 ± 1.0	36.0 ± 0.8	36.2 ± 1.1	36.2 ± 0.4
Leukocytes (10^3/mm^3)	8.2 ± 2.9	9.3 ± 4.2	11.6 ± 5.0	8.8 ± 1.3
Neutrophils (%)	6.1 ± 2.8	8.3 ± 1.5	8.8 ± 3.4	7.1 ± 1.7
Lymphocytes (%)	78.9 ± 5.5	77.1 ± 4.9	75.0 ± 5.9	73.0 ± 8.6
Monocytes (%)	14.8 ± 1.6	13.1 ± 2.1	13.3 ± 2.1	14.22 ± 5.9
Biochemicals				
Glucose (mg/dL)	150.7 ± 15.5	147.0 ± 15.3	160.7 ± 23.1	158.4 ± 13.7
Cholesterol (mg/dL)	67.2 ± 6.1	75.0 ± 6.3	71.1 ± 3.9	70.6 ± 2.1
Urea (mg/dL)	47.8 ± 5.6	47.0 ± 4.0	49.1 ± 5.1	31.8 ± 2.4
Creatinine (mg/dL)	0.6 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.4 ± 0.06
FAL (mg/dL)	269.2 ± 17.4	313.2± 44.3	302.1 ± 35.9	296.8 ± 16.9
AST (U/I)	138.8 ± 6.3	124.2 ± 10.8	129.0 ± 12.8	122.8 ± 14.2
ALT (U/I)	58.7 ± 7.4	56.3 ± 13.8	54.6 ± 11.0	44.6 ± 4.4

Values were expressed as mean ± standard deviation (n=6-8). One-way Analysis of Variance (ANOVA): F(3.25) =2.050, followed by the Dunnet.and Tukey post-test. α

4.2 Artigo 2: *Estragole ameliorate TNBS-induced colitis in rats via antioxidant, immunomodulatory and cytoprotective mechanisms. Artigo a ser submetido a revista Frontiers in Pharmacology*

Estragole ameliorate TNBS-induced colitis in rats via antioxidant, immunomodulatory and cytoprotective mechanisms

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Keywords: Estragole, ulcerative colitis, antioxidant, immunomodulatory, cytoprotection.

Abstract

Background: Estragole is an aromatic organic compound belonging to the class of phenylpropanoids derived from cinnamic aldehydes present in essential oils of plant species such as *Ravensara anisata* (Madeira), *Ocimum basilicum* (Manjericão) and *Croton zehntneri* (Canelinha). Some pharmacological studies report its anti-inflammatory, antioxidant, vasorelaxant and gastroprotective activity.

Hypothesis/Purpose: To evaluate intestinal anti-inflammatory activity of Estragole in the trinitrobenzene sulphonic acid (TNBS)-induced colitis model acute and chronic in rats.

Methods: Intestinal anti-inflammatory effects were assessed using TNBS-induced colitis model acute and chronic in rats. The mechanisms were evaluated from colonic tissue fragments of the acute and chronic models.

Results: Estragole (31–250 mg/kg) acute phase or (250 mg/kg) chronic phase oral administration reduced ($p<0.01$ and $p<0.001$) the macroscopic lesion score, ulcerative area, intestinal weight/length ratio and diarrheal index in TNBS-treated animals. At dose (250 mg/kg) acute and chronic phase decreased ($p<0.001$) malondialdehyde (MDA) and myeloperoxidase (MPO), restored ($p<0.001$) glutathione (GSH) levels, and superoxide dismutase (SOD). In addition, Estragole decreased immunomarking for factor nuclear kappa B (NF-κB) and levels interleukin

-1 (IL-1) and tumor necrosis fator α (TNF-α) and maintained IL-10 and TGFβ basal levels. Furthermore, increased immunostaining for and zonula occludens 1 (ZO-1) was observed.

Conclusion: Estragole presents intestinal anti-inflammatory activity in acute colitis and remission-phase models related to cytoprotection of the intestinal barrier, antioxidant and immunomodulatory effects.

Introduction

Inflammatory bowel diseases (IBDs) constitute inflammatory disorders and have a recurrent and debilitating character that affect the gastrointestinal tract (Ananthakrishnan et al., 2022), being classified into ulcerative colitis (UC) and Crohn's disease (CD) (Torrres et al., 2017; Huo; Sun, 2021).

IBDs directly impact the quality of life of the population, presenting a chronic character with phases of remission and relapse. Although the incidence is stabilizing in many Western countries, the number of cases remains high, as the estimated prevalence exceeds 0.3% of the population in North America and 0.7% in Europe (NG et al., 2018; COWARD et al., 2019).

Cronhn’s disease (CD) can discontinuously affect any part of the gastrointestinal tract, preferably the ileum and colon, with lesions of a transmural nature, from the mucous layer to the muscle layers. While Ulcerative colitis (UC) is a chronic inflammatory disease that affects the colon, being characterized by relapsing and remitting mucosal inflammation, starting in the rectum and extending to proximal segments of the colon (Yao et al, 2021). The pathogenesis of UC is multifactorial, involving genetic abnormalities, altered dietary patterns, intestinal barrier dysfunction, microbiota dysbiosis, and abnormal host immune reactions (Kobayashi et al., 2020).

Current pharmacotherapy includes the use of corticosteroids, immunosuppressants, 5-aminosalicylates, and biological therapies to reduce the inflammatory process through the immune system and sometimes surgical interventions (corticosteroids, mesalamine compounds, azathioprine, methotrexate, TNF-α and IL-17A inhibition) (Weisshof, et al., 2018; Ahluwalia et al., 2018). The main goal of UC treatment involves inducing and maintaining a long and stable remission, decreasing the incidence of relapses, and preventing further development (Yao et al, 2021). However, current therapy consistently fails to prevent relapse of the active phase of IBD, in addition, it is associated with several side effects that contribute to non-adherence to treatment (Triantafillidis, 2011; Liverani, 2016; Actis; Pellicano, 2017).

In the search for new therapeutic alternatives, natural products have emerged, in the last two centuries, the pharmaceutical industry has used naturally occurring chemical compounds as active principles in themselves, as well as a basis for the development of new molecular architectures (DUTRA et al., 2016; KHAN, 2018). Terpenes are the most abundant group of secondary metabolites obtained from plants and the main constituents of essential oils (BARBOSA et al., 2017). Monoterpenes are natural compounds derived from geranyl diphosphate (GPP) and constitute 90% of essential oils (SÁ et al., 2013). These compounds have several biological activities already evaluated in experimental models such as anti-inflammatory (SOMENSI et al., 2019), antibacterial (HOUDKOVA et al., 2017), gastroprotective and gastric healing (VENZON et al., 2018).

Estragole is a phenylpropanoid consisting of a benzene ring substituted by a methoxy group and a propenyl group and is present as a constituent of essential oils of many plant species such as *Ravensara anisata* (Madeira), *Ocimum basilicum* (Manjericão) and *Croton zehntneri*

(Canelinha). These species are widely used both in folk medicine and in cooking (Silva-Alves et al., 2013). Essential oils derived from these species are widely used in the food industry, as well as in aromatherapy. The literature describes some biological activities of Estragole, such as antioxidant and antimicrobial activity (Morais et al., 2006), anxiolytic, skeletal muscle contractile and visceral muscle relaxant (Soares et al, 2007), anti-inflammatory at doses of 3-30 mg/kg (Ponte et al., 2012), gastroprotective activity (Alves Junior et al., 2020) with involvement of antioxidant and immunomodulatory pathways.

Considering the pharmacological potential of Estragole and the lack of studies evaluating the effects of these compounds isolated in IBD models, we aimed to evaluate their effects in the trinitrobenzene sulfonic acid (TNBS)-induced ulcerative colitis model.

2 Materials and methods

2.1. Animals

Male Wistar rats (*Rattus norvegicus*) (180−250 g) were provided by the Animal Production Unit (APU) of the Institute for Research on Drugs and Medicines of Federal University of Paraiba (IPeFarM/UFPB). The animals were acclimated to the conditions of the local vivarium in a temperature of 23 ± 2°C and a light-dark cycle of 12 h, fed with industrial pellet food and water *ad libitum*. All experimental protocols followed international principles for the study with laboratory animals (Zimmermann, 1983) and were approved by the Institutional Ethics Commission on Animal Use (CEUA/UFPB) under registration number: 4120010819/20. All efforts were to reduce the number of animals used, their pain, suffering and stress.

2.2. Reagents

The following substances were used in this study: Estragole (SIGMA Chemical Co, U.S.A), prednisolone (SIGMA Chemical Co, U.S.A), sodium acetate (SIGMA Chemical Co, U.S.A), acetonitrile (SIGMA Chemical Co, U.S.A), 5,5‘-ditiobis-2-nitrobenzoic acid (DTNB) (SIGMA Chemical Co, U.S.A), ethylenediaminetetraacetic acid (EDTA) (SIGMA Chemical Co, U.S.A), bovine serum albumin (BSA) (SIGMA Chemical Co, U.S.A), trinitrobenzene sulfonic acid (TNBS) (SIGMA Chemical Co, U.S.A), trichloroacetic acid (SIGMA Chemical Co, U.S.A), biotinylated sheep polyclonal antibodies (anti-IL-1β, anti-TNF-α or anti-IL-10) (R&D systems), immunohistochemical polyclonal antibodies rats (NFκB, TGFβ or ZO-1) (Cloud-clone Corp), sodium carbonate (MERK, Germany), potassium chloride (SIGMA Chemical Co, USA), magnesium chloride (SIGMA Chemical Co, USA), sodium chloride PA (QUIMEX-MERCK, Brazil), ethyl ether (MERCK, Germany), phenolphthalein (RIEDELDE HAËN, Germany), monobasic sodium phosphate (SIGMA Chemical Co, USA), bibasic sodium phosphate (SIGMA Chemical Co, USA), prednisolone (SIGMA Chemical Co, USA), Methyl-Phenylindole (SIGMA Chemical Co, USA), ketamine 5 % (VETANARCOL), Tris Buffer (Vetec ®), Trizma Buffer (SIGMA Chemical Co, U.S.A) and xylazine 2 % (DORCIPEC).

2.3. Trinitrobenzene sulfonic acid (TNBS)-induced intestinal acute inflammation in rats

The experimental protocol described by Morris et al. (1989) was conducted with some modifications. Each group of animals was pre-treated orally with 5% Tween 80 (colitic group), 2 mg/kg prednisolone (standard control group; Estragole (31, 62, 125 or 250 mg/kg), 48, 24, and 1 h before TNBS administration and 24 h after inflammation induction. After fasting for 24 h, rats were anesthetized with 2% xylazine hydrochloride and 5% ketamine hydrochloride for rectal administration of TNBS (10 mg per animal dissolved in 0.25 mL of 50% ethanol) with the aid of

a 2 mm diameter probe, which was inserted around 8 cm inside the rectum. Following the administration of TNBS, animals were kept for 15 min upside down. Then, after 48 h of TNBS administration, rats were anesthetized and euthanized. Colonic segments were collected and photographed for ulcerative area (UA) quantification and macroscopic score determination. General parameters such as diarrhea and colon weight/length ratio were also evaluated. An untreated non-colitic group was added to the experiment whose animals were not submitted to inflammation induction. Intestinal injury score was assessed according to a scale previously described by Bell et al. [96]: (1) focal hyperemia, no ulcer; (2) ulceration, no hyperemia/bowel wall thickening; (3) ulceration, inflammation at one site; (4) ulceration, inflammation at 2 or more sites; (5) major injury > 1 cm; 6–10 major damage > 2 cm.

The percentage of injury inhibition (%) was calculated as described:

$$\% \text{ Lesion inhibition} = \left[\frac{Sample\ UA\ x\ 100}{Colitic\ group\ UL}\right] - 100$$

To calculate the weight/length ratio parameter, the following formula was used:

$$\frac{\text{Colon weight (g)}}{\text{Colon lenght (cm)}}$$

Diarrheal status was determined from the Evacuation Index (EI) for 24 h until euthanasia. Stools were counted and classified as solids, semisolids, and liquids. The EI was calculated as follows:

$$EI = S\ (n^o \text{ of solid stools x } 1) + (n^o \text{ of watery stools x } 2) + (n^o \text{ of liquid stools x } 3)$$

2.4 Chronic phase with recurrence of the intestinal inflammatory process trinitrobenzene sulfonic acid (TNBS)-induced intestinal inflammation in rats

The experimental protocol described by Morris et al. [1989] was conducted with some modifications. After fasting for 24 h, rats were anesthetized with 2% xylazine hydrochloride and 5% ketamine hydrochloride for rectal administration of TNBS (10 mg/Kg dissolved in 0.25 mL of 50% ethanol) with the aid of a 2 mm diameter probe, which was inserted around 8 cm inside the rectum. Following the administration of TNBS, animals were kept for 10 min upside down. 24 h after the initial induction, the groups of animals will be treated orally with 5% tween 80 (colitic group), 2 mg/kg prednisolone (standard control group) or Estragole 250 mg/kg. The animals were treated once a day for 21 days. On the 14th day after the first induction, the second administration (recurrence) of TNBS (10 mg/0.25 mL 50% v/v, rectally) was performed to mimic the recurrent relapses in inflammatory bowel diseases in humans. On the 21st day rats were anesthetized and euthanized. Colonic segments were collected and photographed for ulcerative area (UA) quantification and macroscopic score determination. General parameters such as diarrhea and colon weight/length ratio were also evaluated. An untreated non-colitic group was added to the experiment whose animals were not submitted to inflammation induction. Intestinal injury score was assessed according to a scale previously described by Bell et al. [96]: (1) focal hyperemia, no ulcer; (2) ulceration, no hyperemia/bowel wall thickening; (3) ulceration, inflammation at one site; (4) ulceration, inflammation at 2 or more sites; (5) major injury > 1 cm; 6–10 major damage > 2 cm.

After ULA determination, tissue samples of colon injuries were collected and stored in preserved in a 10% buffered formaldehyde solution for histological and immunohistochemical analysis. For biochemical assays, tissues were stripped, weighed and frozen at − 80 ºC.

2.4.1.1. Histological analysis

2.4.1.2. Morphometric analysis

2.4.2 Determination of reduced glutathione (GSH), malondialdehyde (MDA), myeloperoxidase (MPO) and Superoxide dismutase (SOD)

2.4.2.1. Non-protein sulfhydryl group (GSH) determination

The protocol according to Faure and Lafond, (1995) was followed to determine the GSH levels. The tissue samples were suspended in 0.02M 1:10 (v/v) EDTA. From this homogenate, 400 µL were removed and 320 µL of distilled water and 80 µL of 50% trichloroacetic acid were added, being centrifuged at 3000 rpm at 4°C for 15 minutes. Then, 100 µL of the resulting supernatant was pipetted into a 96-well microplate and 200 µL of Tris and 25 µL of DTNB were added. This microplate was incubated at room temperature and after 15 minutes, a reading on a spectrophotometer (Polaris) was performed 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 nmol GSH/mg of protein.

2.4.2.2. Malondialdehyde (MDA) determination

The samples were suspended in Trizma® buffer (Tris HCl) 1:5 (w/v), homogenized and centrifuged at 11.000 rpm at 4°C for 10 minutes. Soon after, 300 µL of the supernatants were transferred to Eppendorf tubes and 750 µL of the chromogenic compound (10.3 mM of 1-methyl-2-phenylindol) and 225 µL of hydrochloric acid (37%) were added and incubated at 45ºC in a water bath for 40 minutes and again centrifuged at 11.000 rpm at 4ºC for 5 minutes. Then, 300 µL of the supernatant was transferred to a 96-well microplate and absorbance was determined by colorimetry (586 nm), using a plate reader (Polaris' spectrophotometer). The data were interpolated with the standard curve and the results were expressed as nmol MDA/g tissue (Esterbauer and Cheeseman, 1990).

2.4.2.3. Myeloperoxidase (MPO) determination

The tissue samples fragments were homogenized in the hexadecyltrimethylammonium bromide (HTAB) buffer, which has a detergent function, lysing the granules of the neutrophils that contain the myeloperoxidase. After homogenization, the material was subjected to sonication for 5 minutes. Then, the sample was subjected to a double freezing and thawing process to facilitate the disruption of cellular structures and consequently the release of the enzyme. The homogenate was centrifuged at 5.000 rpm at 4ºC for 20 minutes and concentrated for 24 hours. On the following day, 7 µL of the supernatant were collected, to which 200 µL of the reading solution (o-dianisidine hydrochloride, potassium phosphate buffer and 1% H_2O_2) were added. The reading was performed using a spectrophotometer at 450 nm wave, at times 0 and 1 minute. The results were expressed as units of myeloperoxidase per gram of tissue (Krawisz et al., 1984).

2.4.2.4. Superoxide dismutase (SOD) determination

The tissue samples were homogenized in phosphate buffer (0.4 M, pH 7.0) and centrifuged for 15 minutes 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 (15W) for 10 minutes. After this period, the material was taken to the 630 nm spectrophotometer (Sun et al., 1988).

2.4.5. Immunomodulatory activity

2.4.5.1. Determination of IL-1, TNF-α and IL-10 Levels

The levels of pro-inflammatory (IL-1β and TNF-α) and immunoregulatory (IL-10) cytokines were determined by means of an ELISA immunoenzymatic assay (sandwich type). The capture antibodies for each interleukin were sensitized in a 96-well microplate (flat bottom). After 18h, the plate was washed with 0.05% tween 20 solution (wash buffer), blocked with a 1% bovine serum albumin solution and washed with the wash buffer. Soon after, a tissue macerate was prepared in phosphate buffered saline (PBS) in the pro-portion of 100 mg of tissue to 600 µL of PBS, homogenized and centrifuged at 4000 rpm for 10 minutes at 4°C. Supernatants (100 µL) were pipetted in a 96-well plate and made the standard curve. The biotinylated secondary antibody (100 µL) was added to each well, followed by incubation for 2 hours and 3 washes. The plate was incubated with streptavidin for 20 minutes, washed 3 times, the substrate for development was added (DuoSet Kit © - R & DSystems Catalog - DY999) and incubated for 20 minutes. After this time, the reaction was stopped by adding 50 µL of the stop solution and the reading was performed on a spectrophotometer at 450 nm. The results were obtained by interpolation with the standard curve and expressed in pg/mL (Kendall et al., 1983).

2.4.6. Immunohistochemical analysis

Colons sections (5 µm) of 5 animals per group were obtained with a microtome and transferred to silanized slides (Dako, Glostrup Denmark), and subjected to dewaxing and hydration. Then, the slides subjected to the simple indirect method of blocking with Anti Peroxidase (APP), where the primary antibody is directed against the antigen (protein) to be detected and the secondary antibody serves as a bridge for binding to the APP complex, and incubated overnight at 4°C with the following primary antibodies a: nuclear transcription factor kappa B (NF-κB), transforming growth factor beta (TGF-β) and zonula occludens 1 (ZO-1). After being washed with distilled water, the slides were incubated with a secondary for 60 min and 3,3'-diaminobenzidine (DAB, Biocare Medical, CA, USA) was used as the chromogen and the specimens were counterstained with hematoxylin. The samples were visualized under an optical microscope (Olympus microscope, Tokyo, Japan) with coupled to a camera (Nikon DS-Ri2).

2.6. Statistical analysis

Data were expressed as mean ± standard deviation (SD) or mean ± standard error (SE) for parametric values or expressed as median (minimum value - maximum value) for non-parametric data. One-way ANOVA (parametric data) or Kruskal-Wallis test (non-parametric data) was performed, followed by Dunnet and Tukey or Dunn post-tests, respectively. The results were considered significant when $p < 0.05$. All data were analyzed using GraphPad® 5.0 software.

Results

3.1. Effect of Estragole in intestinal acute inflammation in a TNBS-induced colitis model

The parameters ulcerative lesion area (ULA), lesion score, weight/length ratio, and diarrhea were evaluated 48 h after TNBS administration, as shown in Table 1 and Figure 1 respectively. TNBS

administration to colitic groups led to the development of large lesions and intense signs of inflammation with gross ulcerative areas. These effects were followed by an increased weight/length ratio of the colonic segment and the diarrhea score, which was determined by the evacuation index (EI), when compared to non-colitic group ($p < 0.001$). The treatments with Estragole (31-250 mg/kg) ($p< 0.01$; $p < 0.001$) or prednisolone ($p < 0.001$) reduced those parameters in all evaluated doses. The most effective dose of Estragole (250 mg/kg) were selected for the following experiments.

3.1.1. Histological analysis

Histological sections of the colon stained with hematoxylin and eosin belonging to the non-colitic, prednisolone and Estragole groups (Figure 3A, C, D), the integrity of the mucosa (M) and goblet cells are observed. In the colitic group (5% tween 80) (B) intense mucosal destruction is observed, with ulcer formation, which is characterized by organ surface necrosis and intense acute inflammatory reaction (*) and increased leukocyte infiltrate. In sections stained with Masson's trichrome, it was found that Estragole (Figure 2H) reduced extracellular matrix deposition ($p<0.001$) 9.00 (2-15) compared to the colitic group (5% tween 80) who presented scar tissue (∞) in the area of the mucosal ulcer ($p<0.001$) 26.58 (20-35) (2F) (Figure 2).

3.1.2 Effect of Estragole in the modulation of antioxidant and anti-inflammatory properties in acute colitis

3.1.2.1. Reduced Glutathione (GSH)

In determining the antioxidant activity of Estragole, it was possible to verify that colitic group (5% tween 80) showed a reduction in GSH levels to 71.1 ± 4.8 nmol of GSH/mg protein compared to the normal group (93.6 ± 4.3 nmol GSH/mg protein). However, administration of Estragole (250 mg/kg) restore ($p<0,001$) to 90.7 ± 6.5 nmol GSH/mg protein (Fig. 3 A).

3.1.2.2. Superoxide Dismutase (SOD)

Colitic group (5% tween 80) demonstrated a significant reduction in the activity of the SOD enzyme to 0.22 ± 0.09 U of SOD/mg of protein compared to the normal group (2.85 ± 0.37 U SOD/mg protein). However, SOD activity was significantly increased ($p <0.001$) with administration of Estragole (250 mg/kg) to 2.62 ± 0.28 of SOD/mg of protein, respectively, when compared to the colitic group (5% tween 80) (Fig. 3 B).

3.1.2.3. Myeloperoxidase (MPO)

The results showed that in the colitic group (5% tween 80) there was an increase in MPO levels to 64.5 ± 4.3 units of MPO/g of tissue when compared to the normal group (14.9 ± 3.5 units MPO/g tissue). However, the groups treated with prednisolone (2 mg/kg) or Estragole (250 mg/kg) significantly reduced MPO levels to 35.5 ± 5.54 and 35.3 ± 6.90 unit of MPO/g of tissue, respectively, in relation to the colitic group (Fig. 3 C).

3.1.2.4. Malondialdehyde (MDA)

Colitic group (5% tween 80) showed an increase in MDA levels to 83.6 ± 2.7 nmol MDA/g tissue compared to the normal group (47.3 ± 2.7 nmol MDA/g tissue). However, the administration of Estragole significantly reduced MDA levels to 58.1 ± 2.3b nmol MDA/g of tissue), when compared to the colitic group (Fig. 3 D).

3.1.2.5. Levels of IL-1β, TNF-α and IL-10

Next, was evaluated the immunomodulatory effects by measuring the levels of cytokines. Estragole significantly decreased ($p< 0.001$) the levels of IL-1β (pg/ml) to 333.0 ± 36.6 compared to the colitic group (5% tween 80) (730.1) (Fig 4 A). TNF-α levels (pg/ml) were also decreased after treatment with Estragole to 867.7 ± 63.8), when compared to the colitic group (5% tween 80) (2246 ± 47.77) (Fig 4 B). Furthermore, Estragole prevented ($p<0.01$) the reduction of IL-10 (pg/ml) (161.3 ± 29.8) in comparation the control group (68.0 ± 9.4) (Fig 4 C).

3.1.3. Immunohistochemical analysis for NFκB, TGF-β and ZO-1 in colon samples submitted to acute inflammation in a TNBS-induced colitis acute model

Colons submitted to TNBS and treated with Estragole showed presence of NF-κB (brown color positivity) focal and concentrated labeling in the distal enterocytes close to the lumen of the organ, next to the goblet cells, significantly reduced immunostaining to 20.0 (19.0-25.0) μm^2 when compared to the colitic group 26.8 (24.0-29.0) μm^2, in which there is the presence of these irregularly and multifocally marked, especially in the ulcer area (Fig. 5). In the evaluation of immunostaining for TGF-β results showed that treatment with Estragole significantly increased positive cells marked for TGF-β to 81.5 (70.0–121.0) μm^2 when compared to the control group 104.0 (85.0-120.0) μm^2 (Fig. 6). In the evaluation for ZO-1, the treatment with Estragole increased the marking 107.5 (102.0-112.0) μm^2 (brown color positivity) which was distributed in a regular and multifocal way, similar to the normal group 200.0 (150.0-222.0) μm^2. In the colitic group, partial and irregular marking is observed in the colonic mucosa 41.5 (25.0-58.0) μm^2 (Fig. 7).

3.2. Effect of Estragole in chronic phase with recurrence of the intestinal inflammatory process in a TNBS-induced colitis model

The parameters ulcerative lesion area (ULA), lesion score and weight/length ratio were evaluated 21 days after TNBS administration, as shown in Table 2 and Fig. 8. TNBS administration to colitic groups led to the development of large lesions and intense signs of inflammation with gross ulcerative areas. These effects were followed by an increased weight/length ratio of the colonic segment, when compared to non-colitic group ($p < 0.001$). The treatments with Estragole (250mg/kg) ($p < 0.001$) or prednisolone ($p < 0.001$) reduced those parameters.

3.2.1. Histological analysis

Histological sections of the colon stained with hematoxylin and eosin belonging to the non-colitic, prednisolone and Estragole groups (Figure 9A, C, D), the integrity of the mucosa (M) and goblet cells is observed. In the colitic group (5% tween 80) (9B) intense mucosal destruction is observed, with ulcer formation, which is characterized by organ surface necrosis and intense acute inflammatory reaction (*) and increased leukocyte infiltrate. In sections stained with Masson's trichrome, it was found that Estragole (Figure 9H) reduced extracellular matrix deposition ($p<0.001$) 8.00 (5-14) compared to the colitic group (5% tween 80) who presented scar tissue (∞) in the area of the mucosal ulcer 26.50 (20-30) (9F) (Fig. 9).

3.2.2 Effect of Estragole in the modulation of antioxidant and anti-inflammatory properties in chronic phase colitis with recurrence of the intestinal inflammatory process

3.2.2.1. Reduced Glutathione (GSH)

In determining the GSH, it was possible to verify that colitic group (5% tween 80) showed a reduction in GSH levels to 69.8 ± 4.3 nmol of GSH / mg protein compared to the normal group (93.6 ± 5.7 nmol GSH/mg protein). However, administration of Estragole (250 mg/kg) restore to 83.2 ± 4.3 nmol GSH/mg protein) restored baseline GSH levels (Fig. 10 A).

3.2.2.2. Superoxide Dismutase (SOD)

Animals in the colitic group (5% tween 80) demonstrated a significant reduction in the activity of the SOD enzyme to 0.22 ± 0.30 U of SOD/mg of protein compared to the normal group (2.88 ± 0.72 U SOD/mg protein). However, SOD activity was significantly increased ($p < 0.001$) with administration of Estragole (250 mg/kg) to 5.14 ± 0.8 of SOD/mg of protein, respectively, when compared to the colitic group (5% tween 80) (Fig. 10 B).

3.2.2.3. Myeloperoxidase (MPO)

The results showed that in the colitic group (5% tween 80) there was an increase in MPO levels to 64.5 ± 2.03 units of MPO/g of tissue when compared to the normal group (14.9 ± 0.82 units MPO/g tissue). However, the groups treated with Estragole (250 mg/kg) significantly reduced ($p<0.001$) MPO levels to 23.0 ± 3.5 unit of MPO/g of tissue, in relation to the colitic group (Fig. 10 C).

3.2.2.4. Malondialdehyde (MDA)

The colitic group (5% tween 80) showed an increase in MDA levels to 83.5 ± 2.7 nmol MDA/g tissue compared to the normal group (47.3 ± 3.2 nmol MDA/g tissue). However, the administration of Estragole 250 mg/kg) reduced ($p<0.001$) MDA levels (58.2 ± 4.6 nmol MDA/g of tissue) when compared to the colitic group (Fig. 10 C).

3.2.2.5. Levels of IL-1β, TNF-α and IL-10

Next, we evaluated the immunomodulatory effects by measuring the levels of cytokines. Estragole significantly decreased ($p< 0.001$) the levels of IL-1β (pg/ml) to 333.2 ± 38.3 compared to the colitic group (5% tween 80) (729.0 ± 317.1) (Fig. 11 A). TNF-α levels (pg/ml) were also decreased after treatment with Estragole ($p< 0.001$) (867.3 ± 36.64), when compared to the colitic group (5% tween 80) 2246.2 ± 43.6) (Fig. 11 B). Furthermore, Estragole prevented ($p<0.001$) the reduction of IL-10 (pg/ml) (161.3 ± 24.8) in comparation the control group (68.5 ± 20.83) (Fig. 11 C).

3.2.3. Immunohistochemical analysis for NFκB, TGF-β and ZO-1 in colon samples submitted to chronic phase colitis with recurrence of the intestinal inflammatory process

Colons submitted to TNBS and treated with Estragole showed presence of NF-κB focal and concentrated labeling in the distal enterocytes close to the lumen of the organ, significantly reduced immunostaining to 3.00 (1.0-5.0) μm^2 when compared to the colitic group 11.00 (9.0-14.0) μm^2 (Fig. 12). In the evaluation of immunostaining for TGF-β results showed that treatment with Estragole significantly increased positive cells marked for TGF-β to 11.00 (10.0–15.0) μm^2 when compared to the control group 3.0 (2.0-5.0) μm^2 (Fig. 13). In the evaluation for ZO-1, the treatment with estragole increased the marking 104.00 (100.0-121.0) μm^2 which presence of regular and evenly distributed staining in the mucosal colon, in the colitic group, partial and irregular marking is observed in the colonic mucosa 48.5 (35.0-54.0) μm^2 (Fig. 14).

Discussion

Ulcerative colitis (UC) is a chronic inflammatory disease that affects the colon being characterized by relapsing and remitting mucosal inflammation, starting in the rectum and extending to proximal segments of the colon. The main characteristic of UC is the superficiality of the lesion, being restricted to the mucosa and not infiltrating deeper layers of the intestinal wall (VINDIGNI, et al., 2016). In the absence of timely and effective treatment, progressive and cumulative intestinal injury can lead to complications such as strictures, fistulas, loss of intestinal function, surgical interventions and cancer (TORRES et al., 2017; AGRAWAL; COLOMBEL, 2019).

The experimental model used was the chemical one, using TNBS, which mimics inflammatory events in humans from the induction of T cell reactivity (Antoniou et al., 2016). TNBS administration induces transmural colitis that is driven by a TH1-mediated immune response that is characterized by the infiltration of $CD4^+$ cells, neutrophils, and macrophages into the lamina propria and the secretion of cytokines (Kiesler et al., 2015; Silva et al., 2022). The model employs intrarectal administration of TNBS hapten, solubilized in ethanol, which breaks the protective colonic mucus barrier, allowing the hapten to penetrate the lamina propria. TNBS will then haptenize the self or microbial proteins/peptides, making them immunogenic, and triggering both innate and adaptive immune responses in the host (MORRIS et al., 1989; Kiesler et al., 2015; Antoniou et al., 2016). Additionally, the TNBS-induced model can mimic the acute and chronic stages of IBD (Silva et al., 2022).

In the acute model Estragole at the doses evaluated, significantly reduced ulcerative lesions when compared to colitic group, with a significant reduction in diarrhea and in the severity and extent of the lesion which was reflected in the macroscopic lesion score. Diarrhea is an important parameter for evaluating the intestinal anti-inflammatory effect, since its increase indicates loss of the absorptive capacity of the colon (BInder, 2009; Orsi et al., 2014). In addition, reports in the literature demonstrate an association between diarrhea and epithelial barrier dysfunction, through failures in the integrity of gap junctions (BARMEYER et al., 2017). Histomorphological evaluation of tissues from rats subjected to Estragole treatment showed decreased lesion area and the inflammatory infiltrate. Similar effects were previously found for Caffeic acid (ESPINDOLA et al., 2019).

In view of the promising results obtained in the acute model of ulcerative colitis, most effective dose (250 mg/kg) was selected to investigate its effect in the 21-day chronic ulcerative colitis model with recurrence on the 14th day and to investigate the antioxidant and immunomodulatory effect of Estragole. The relapsing ulcerative colitis model is considered to mimic this disease in humans and can be used to evaluate new treatments potentially applicable to IBD (YANG et al., 2012).

When administered in the chronic model with recurrence of the inflammatory process, Estragole 250 mg/kg significantly reduced ulcerative lesions when compared to the colitic group, with a significant reduction in the severity and extent of the lesion, in the weight/weight ratio, length and macroscopic score of the lesion, thus evidencing the anti-inflammatory effect of the administration during the period of 21 days of treatment. The weight/length ratio is a parameter for evaluating the inflammatory effect, in which the increase in this ratio in the colonic tissues is associated with the respective increase in edema, one of the first markers of inflammation (JONES et al., 2016).

The histomorphological evaluation confirms the reduction of the inflammatory infiltrate in the group treated with Estragole. These results are related to a decrease in the migration of inflammatory cells.

IBD both in humans and in animal models are characterized by the activation of polymorphonuclear cells, which increase the levels of reactive oxygen species (ROS) and generate oxidative stress (HENDRICKSON et al., 2002; MATRICON et al., 2010). In this context, the next step of the present study was to investigate whether the intestinal anti-inflammatory effect of Estragole is related to its antioxidant capacity.

Chronic inflammation causes overactivation of the immune system and triggers high levels of ROS that reduce levels of endogenous antioxidants. This process induces the formation of damage in the mucosal layer and bacterial invasion, which in turn stimulates the immune response and contributes to the progression of IBDs (LUCERI et al., 2019). In both acute and chronic models, Estragole (250 mg/kg) increased GSH and SOD levels and restore baseline levels. SOD is part of the enzymatic antioxidant system that forms the first line of defense against superoxide anion and hydrogen peroxide (KIVRAK et al., 2017; SARANGARAJAN et al., 2017). GSH participates in the non-enzymatic system that protects the integrity and permeability of the cell membrane, promotes the assembly of mucin oligomers, helps in the maintenance of immune function and in the maintenance of protein structure (ROBACZEWSKA et al., 2016; PÉREZ et al., 2017).

Oxidative stress leads to changes in the pathophysiology of inflammatory processes, as lipid peroxidation exacerbates cascade reactions and releases inflammatory mediators and immune system cells, such as neutrophils, which results in increased activity of myeloperoxidase (MPO), an important enzyme in the inflammatory cytokine-mediated neutrophil activity. Oral administration of Estragole reduced ($p<0.001$) the levels of MDA and MPO compared to the colitic group, these enzymes participate in the lipid peroxidation process, activate the epithelium favoring the infiltration of neutrophils, the synthesis and release of cytokines by macrophages, among other mediators that contribute to oxidative stress (PEREZ et al., 2017). Thus, the anti-inflammatory effect found in our study may be related to the inhibition of toxic lipoperoxides and/or due to the promotion of the synthesis of endogenous antioxidants. These results corroborate a study with the phenylpropanoid Cinnamaldehyde (Qu et al., 2019) have been shown to reduce MPO-mediated lipoperoxidation and increase antioxidant defenses.

Reative Oxigen Species (ROS) are second messengers for the activation of signal transduction pathways that induce inflammation, such as the signaling pathways of mitogen-activated protein kinases (MAPKs) and NF-κB, a transcription factor that binds to gene promoters, target and trigger the transcription of inflammatory cytokines and chemokines (SONG et al., 2014; AKANDA et al., 2018). Treatment with Estragole in both the acute and chronic phases significantly reduced immunostaining for NF-κB, as well as reduced levels of inflammatory cytokines IL-1β and TNF-α, these cytokines participate in the innate immune response and play a key role in disrupting the state of intestinal homeostasis and are involved in the recruitment of granulocytes and increased TH17 activity (Danese et al., 2017; Ahluwalia et al., 2017). These results followed the same patterns found in the phenylpropanoids from *Dendropanax dentiger* (Yang et al., 2021) and *Lilium Asiatic* hybrid flowers (Thi et al., 2017).

The main mechanism for controlling responses in the gut is the activation of regulatory T cells (Treg) that play an important role in the maintenance of gastrointestinal homeostasis and secrete immunoregulatory cytokines such as IL-10 and TGF-β, these cytokines suppress macrophage-mediated inflammatory responses and effector T cells (Yamada et al., 2016; Ahluwalia et al., 2018). In our study, treatment with Estragole restored levels ($p<0.001$) of IL-10 and increased immunostaining ($p<0.001$) for TGF-β when compared to the colitic group. In IBDs, a reduced

expression of TGF-β in the mucosa has been observed, which leads to inadequate conditioning of dendritic cells and the perpetuation of the inflammatory state (Ahluwalia et al., 2018). This study corroborates the literature described by Choi and collaborators (2010), in which the Methyleugenol Increased expression of IL-10 and TGF-β.

In present study, treatment with Estragole in the acute and chronic phases reduced the levels of inflammatory cytokines (TNF-α and IL-1β) considered responsible for amplifying and maintaining chronic inflammation in IBDs and kept the cytokines IL-10 and TGF-β close to baseline, suggesting a immunomodulatory effect. Zeng et al., (2020) studied the Chlorogenic acid (CGA), a polyphenol, and demonstrated that its intestinal anti-inflammatory effect is related to a decrease in TNF-α concentrations via inhibition of the NF-kB pathway. Studies with geraniol (Soubh et al., 2015) demonstrated inhibition of IL-1β and the NF-κB pathway and corroborated this study.

The intestinal epithelium is composed of a monolayer of columnar epithelial cells that are connected by tight junctions (VARGAS-ROBLES et al., 2019). On the surface of the epithelium, the protective layer of mucus is composed of glycosylated mucins secreted by goblet cells, in addition to defensins released by Paneth cells and epithelial cells. Deregulation of this barrier homeostasis in individuals with IBD leads to the influx of pathogens and their metabolites through the barrier, initiating the immune response and mucosal inflammation (Vancamelbeke, Vermeire, 2017; Cheng et al., 2022). An increase in immunostaining for ZO-1 was observed for the groups treated with Estragole, these findings may be related to the increased expression of Mucin type 2 (MUC-2), which can lead to pre-epithelial protection and hinder the development of inflammation. In a study with the *p*-cymene monoterpene (Formiga et al, 2020), a similar result was shown.

Our data demonstrated that the monoterpene Estragole has intestinal anti-inflammatory activity related to antioxidant (increase in GSH and SOD with reduction in MDA and MPO) and immunomodulatory (reduction in NF-κB, TNF-α and IL-1β, and increase in IL-10 and TGF effects, as well as cytoprotective mechanisms, acting on the pre-epithelial or epithelial barrier. Thus, these results suggest a potential use of this substance for the treatment of intestinal inflammation.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

Alves Júnior EB, Araruna MEC, Serafim CAL, Pessoa MMB, Alves VP, Silva MS and Batista LM contributed to the conception of the study, execution of the experiments and writing of the manuscript. Silva MS provided the substance under study, Alves AF and Sousa MCP contributed to the histomorphological and immunohistochemical analysis of this study and Araujo AA contributed to the in vitro experiments of this article. All authors reviewed and approved the final version of this manuscript.

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FIGURES

Table 1. Effect of Estragole in intestinal acute inflammation in a TNBS-induced colitis model.

Groups	Dose (mg/kg)	Ulcerative Area (mm^2)	% Lesion Inhibition	Lesion Score	Weight/Length (mg/cm)	Evacuation Index (EI)
Non-colitic	-	ND	ND	ND	74.9 ±5.9	12.0 ±3.0
Colitic (5% Tween 80)	-	217.1 ± 5.7	-	6.0± 0.9	184.7± 10.4 $^{###}$	30.0 ±2.4$^{\#}$
Prednisolone	2	84.4 ± 5.5***	61%	3.0± 0.5 ***	107.5± 5.0 ***	9.0 ±3.0***
Estragole	31	119.7 ± 12.7**	44%	4.5±1.9$^{*.}$	16.7± 10.9*	9.0 ±1.1*
	62	80.0 ± 6.5***	63%^{a}	4.0±1.2*	147.5± 12.6**	8.0 ±0.7***
	125	63.0 ± 5.8***	70%^{a,b}	3.0±0.8**	130.1± 8.5**	6.0 ±1.1***
	250	49.5 ± 4.9***	77%^{a,b,c}	2.0±0.5**	112.8± 9.7***	4.0 ±1.3***

Data are expressed as mean ± SD or median (minimum/maximum values). For parametric data, we used one-way ANOVA followed by Dunnett's and Tukey's post-tests. For non-parametric data, we used Kruskal–Wallis test and Dunn's test. *p < 0.05, **p < 0.01, ***p < 0.001 compared to colitic groups; $^{###}$p < 0.001 compared to non-colitic group. (n = 7–10). ^{a}p< o.005 compared to Estragole 31 mg/kg.). ^{b}p< o.005 compared to Estragole 62 mg/kg.). ^{c}p< o.005 compared to Estragole 125 mg/kg ND = not detectable.

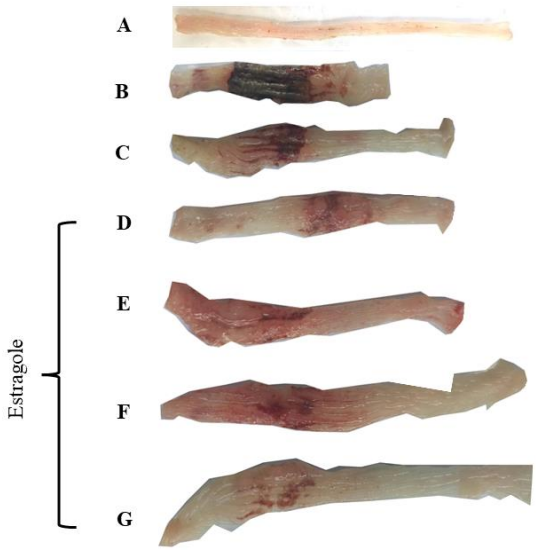


Fig. 1. Representative images of rat colons from non-colitic group (A); colitic groups (5% Tween 80); prednisolone 2 mg/kg (C); Estragole 31 mg/kg (D), 62 mg/kg (E), 125 mg/kg (F), and 250 mg/kg (G) subjected to acute TNBS-induced ulcerative colitis model.

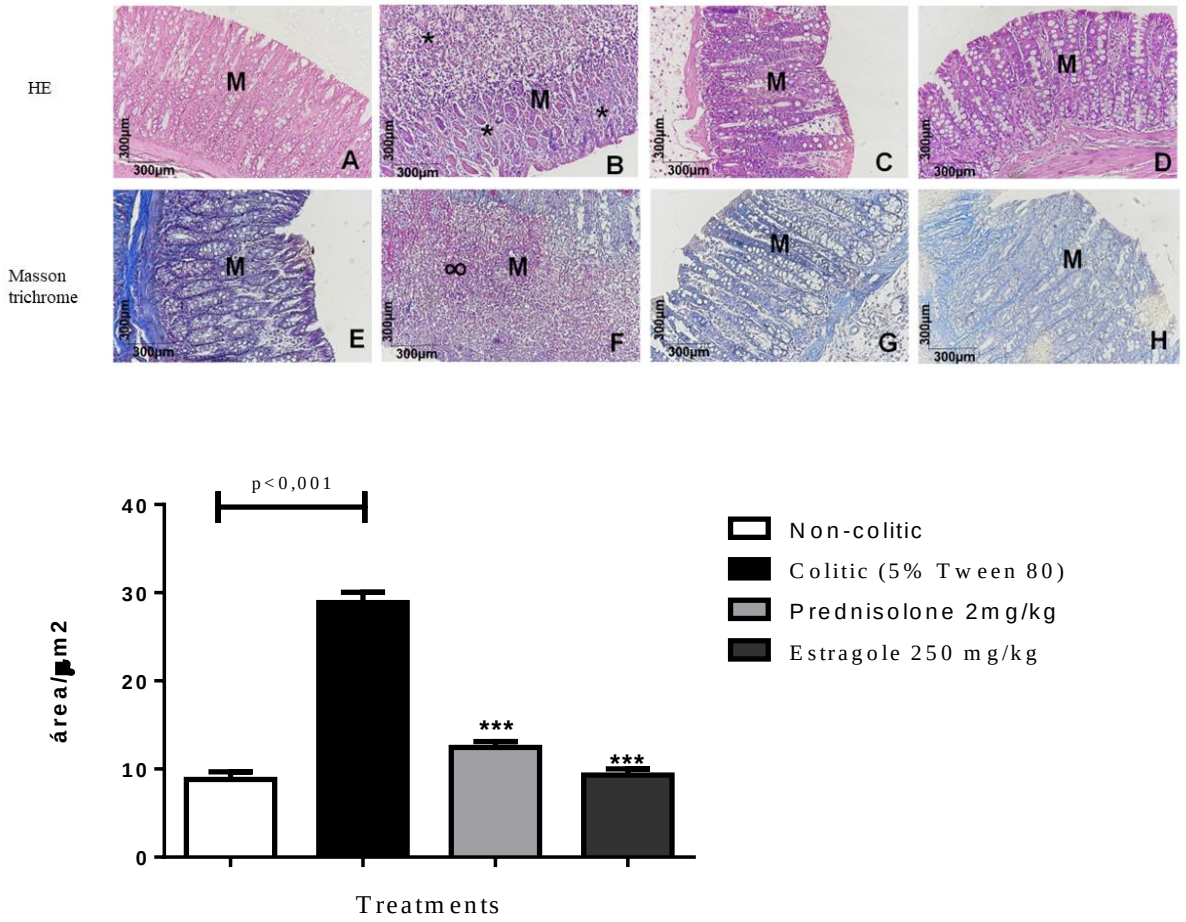
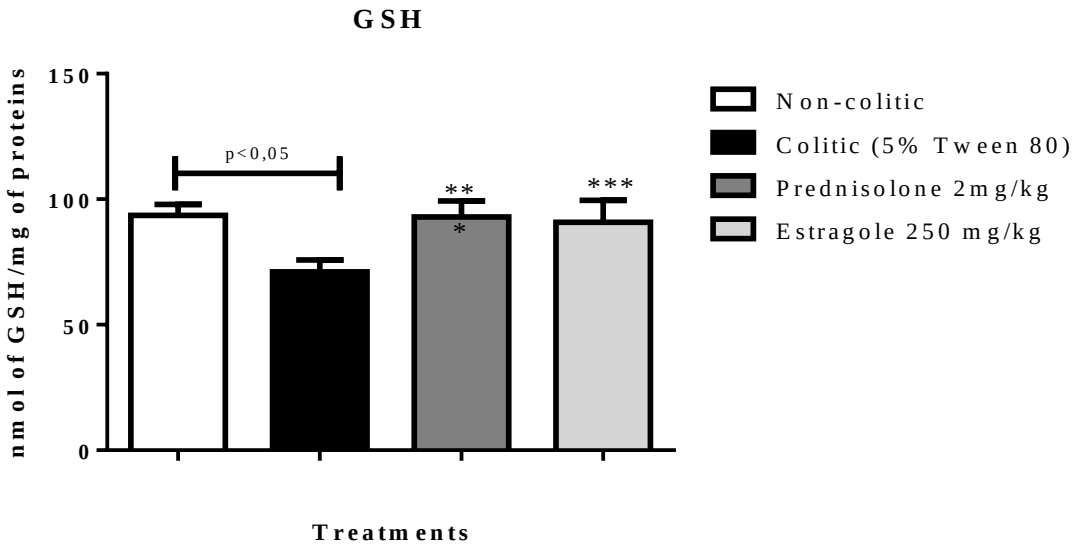
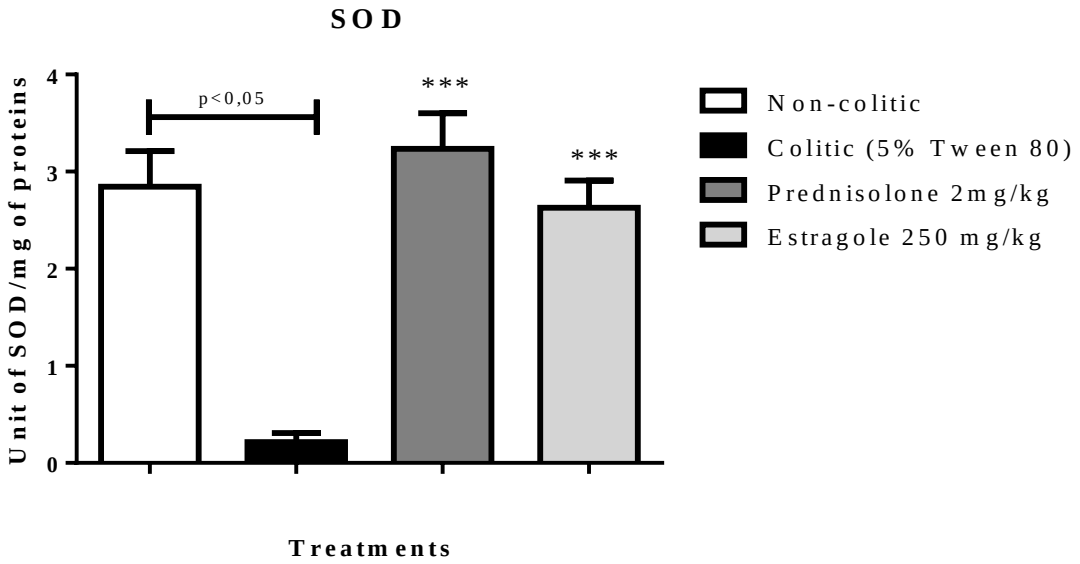


Fig. 2: Microscopic effects of the colon of rats submitted to acute TNBS-induced ulcerative colitis model and treated or not with Estragole. Representative photomicrographs of the animals' colon from the experimental groups: Non-colitic (A,E), Colitic (5% tween 80) (B,F), prednisolone 2 mg/kg (C,G) and Estragole 250 mg/kg (D,H). HE staining (A, B,C,D) and Masson trichrome (E,F,G,H). Colonic mucosa (M), acute inflammatory reaction (*), scarring tissue (∞). Results are expressed as median (minimum-maximum) of the parameters analyzed (n=5, 3 sessions per animal). Kruskall Walis test and Dunn's posterior test was performed using the software Graph Pad Prism. ***p < 0.001 compared to the colitic group (5 % tween 80).

A


GSH
nmol of GSH/mg of proteins
150
100
50
0
p<0,05
**
*
***
Treatments
Non-colitic
Colitic (5% Tween 80)
Prednisolone 2mg/kg
Estragole 250 mg/kg

B


SOD
Unit of SOD/mg of proteins
4
3
2
1
0
p<0,05
***
***
Treatments
Non-colitic
Colitic (5% Tween 80)
Prednisolone 2mg/kg
Estragole 250 mg/kg

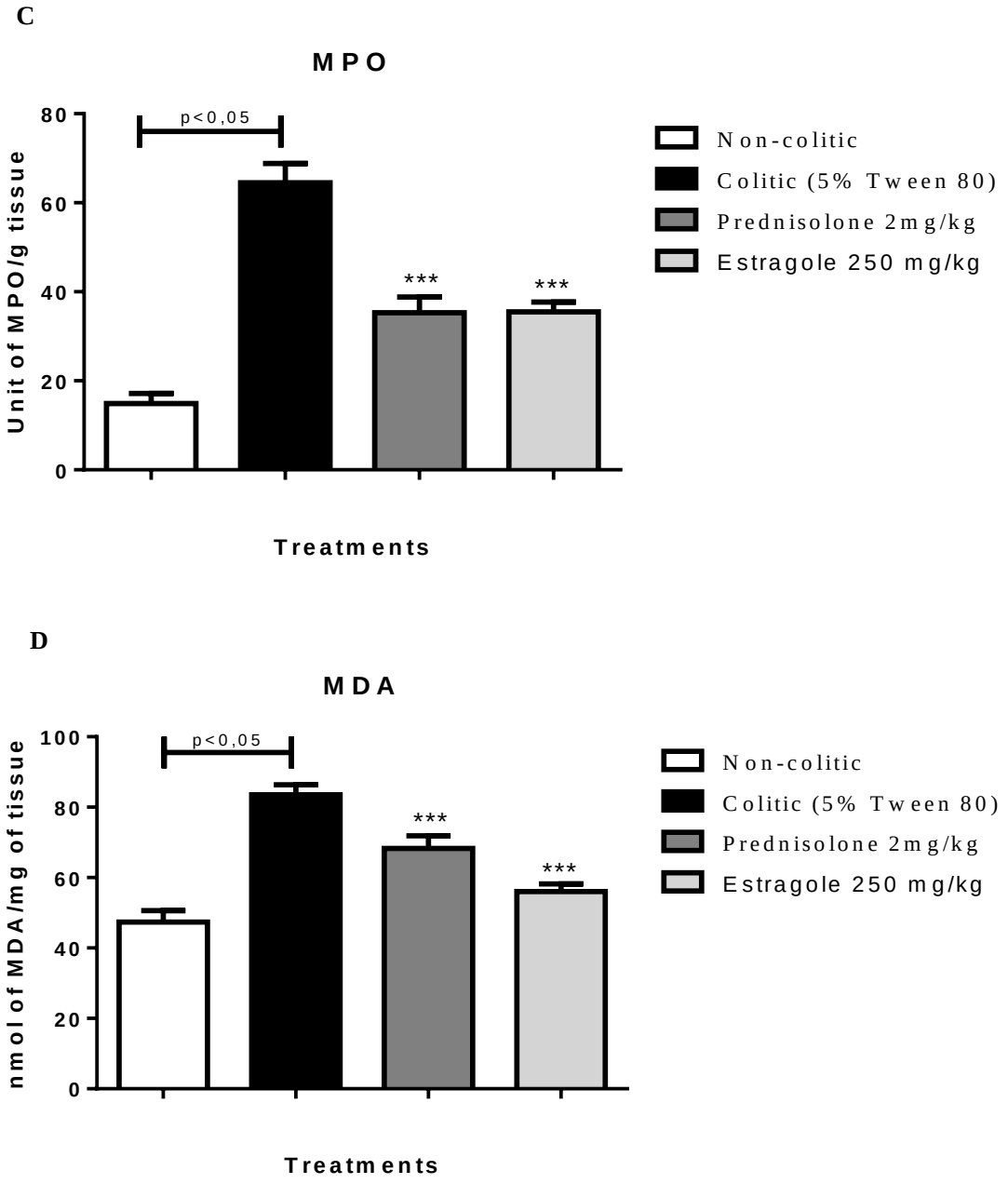


Fig 3. Effect of oral administration of Estragole and prednisolone in A) GSH, B) SOD C) MDA and D) MPO levels form acute TNBS-induced ulcerative colitis model in rats. Results expressed as mean ± SE of the parameters analyzed (n = 5-8). The analysis of variance of one via (ANOVA) followed by the Dunnett's and Tukey's test. ** p <0.01 or ***p <0.001 vs non-colitic group.

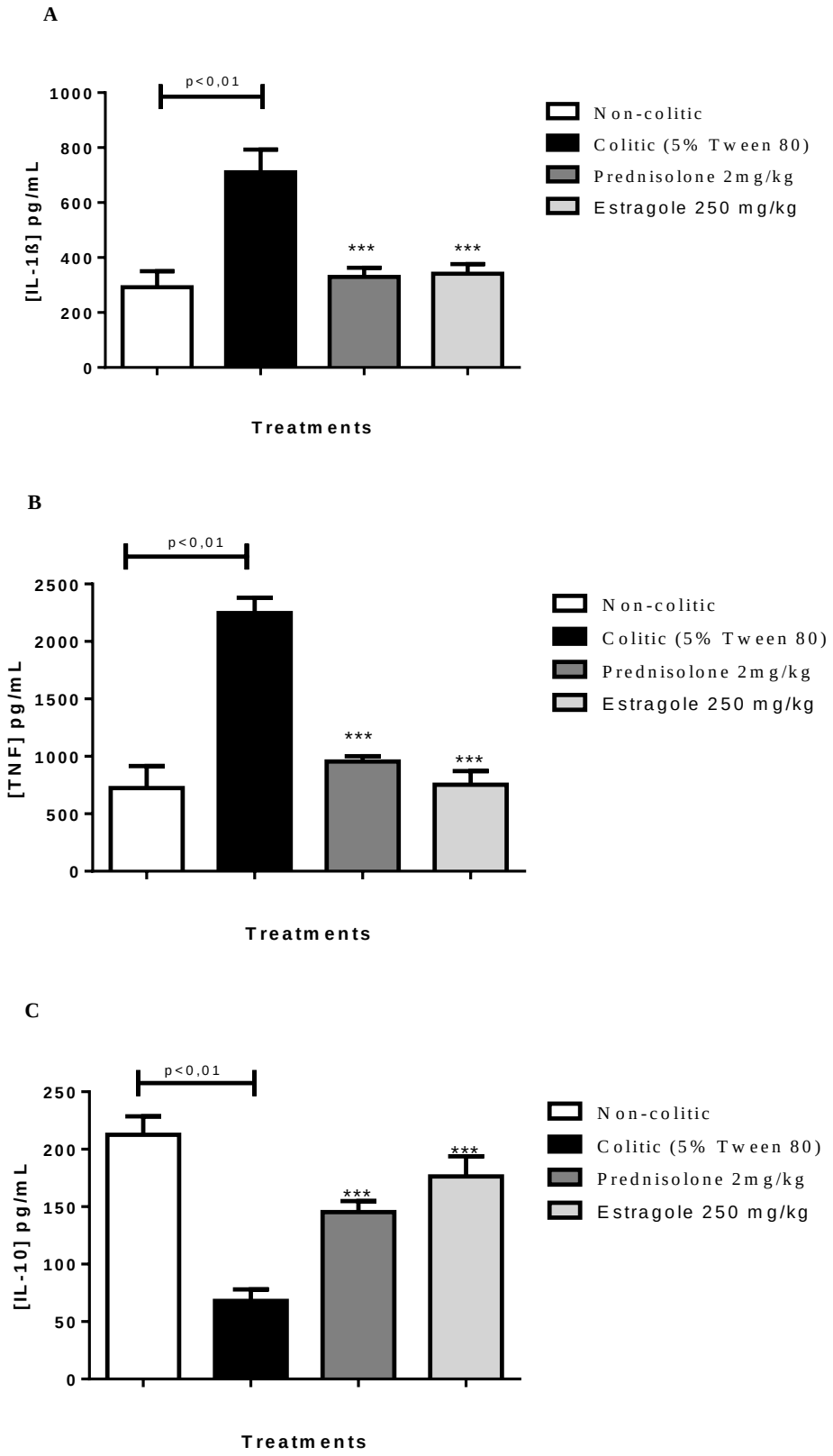


Fig 4. Effect of Estragole and prednisolone in A) IL-1β, B) TNF-α and C) IL-10 levels form acute TNBS-induced ulcerative colitis model in rats. Results expressed as mean ± SE of the parameters analyzed (n = 6–8). The analysis of variance of one via (ANOVA) followed by the Dunnett's and Tukey's test. ***p <0.001 vs control group.

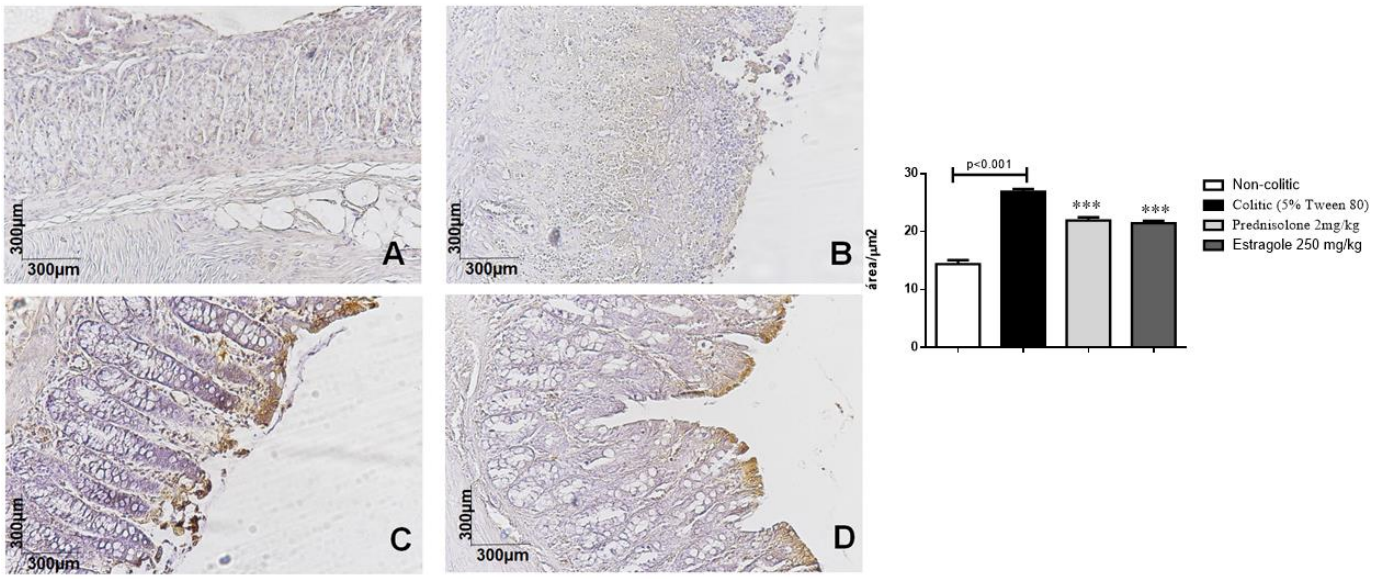


Fig 5. Photomicrographs with immunohistochemical marking for nuclear transcription factor kappa B (NF-κB) in colon samples from rats. A – non-colitic; B- colitic (5 % Tween 80 -10 mL/kg); C- Prednisolone (2 mg/kg); D- Estragole (250 mg/kg). Results are expressed as median (minimum-maximum) of the parameters analyzed (n=5, 3 sessions per animal). Kruskall Walis test and Dunn's posterior test was performed using the software Graph Pad Prism. **p < 0.01, ***p < 0.001 compared to the colitic group (5 % tween 80).

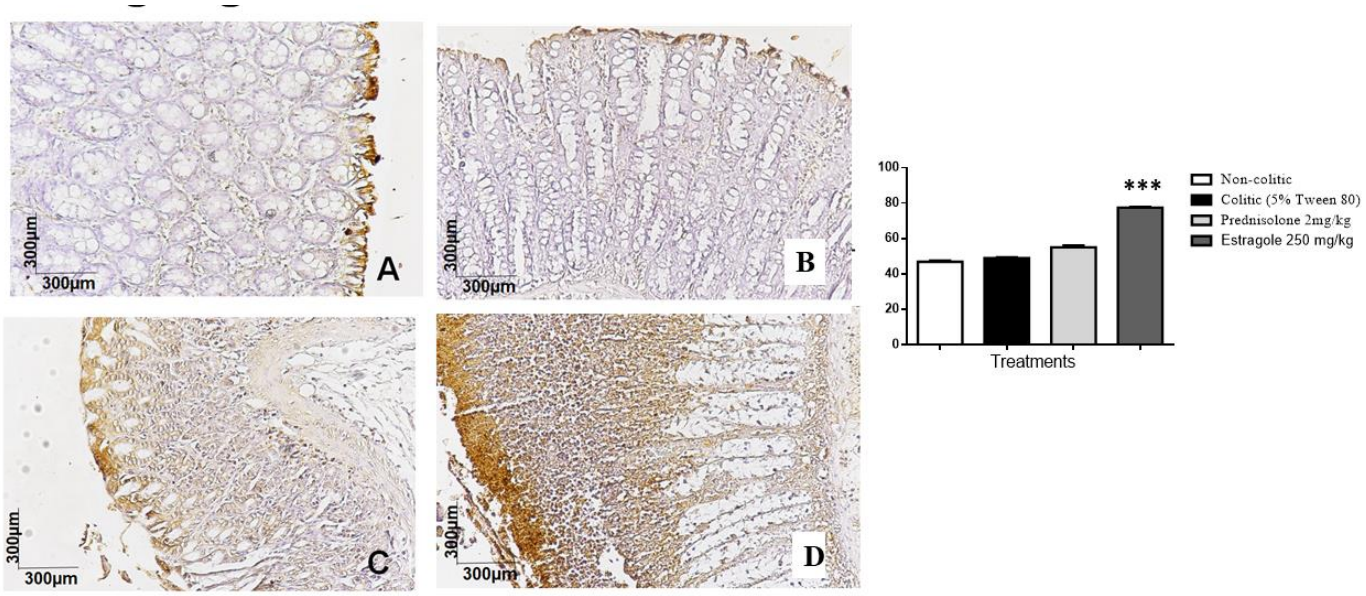


Fig 6. Photomicrographs with immunohistochemical marking for transforming growth factor beta (TGF-β) in colon samples from rats. A – non-colitic; B- colitic (5 % Tween 80 -10 mL/kg); C- Prednisolone (2 mg/kg); D- Estragole (250 mg/kg). Results are expressed as median (minimum-maximum) of the parameters analyzed (n=5, 3 sessions per animal). Kruskall Walis test and Dunn's posterior test was performed using the software Graph Pad Prism. ***p < 0.001 compared to the colitic group (5 % tween 80).

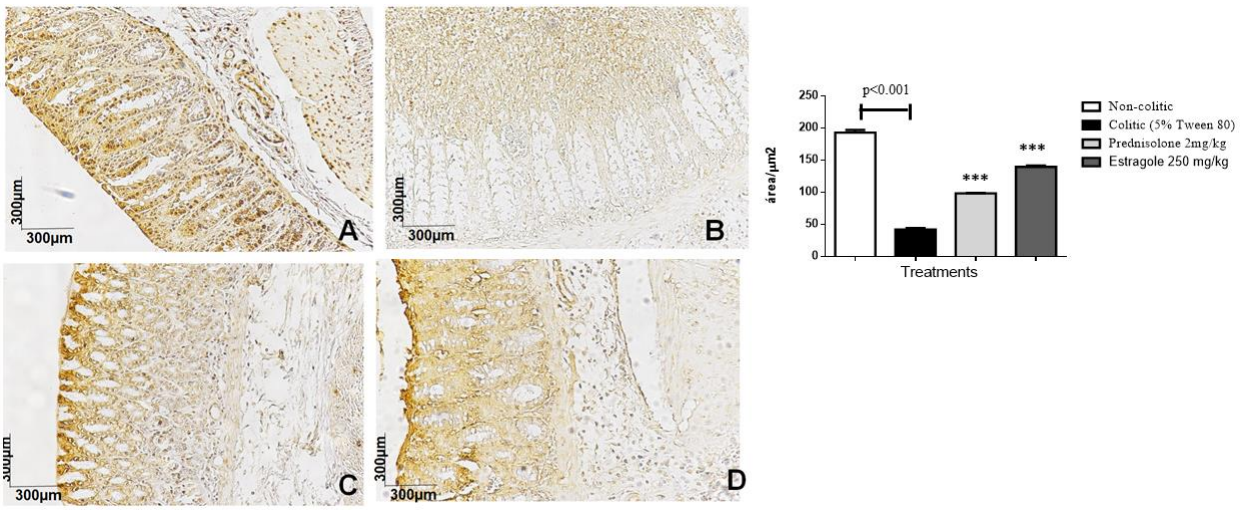


Fig 7. Photomicrographs with immunohistochemical marking for zonula occludens 1 (ZO-1) in colon samples from rats. A – non-colitic; B- colitic (5 % Tween 80 -10 mL/kg); C- Prednisolone (2 mg/kg); D- Estragole (250 mg/kg). Results are expressed as median (minimum-maximum) of the parameters analyzed (n=5, 3 sessions per animal). Kruskall Walis test and Dunn's posterior test was performed using the software Graph Pad Prism. ***p < 0.001 compared to the colitic group (5 % tween 80).

Table 2. Effect of Estragole in chronic phase with recurrence of the intestinal inflammatory process in a TNBS-induced colitis model. Data are expressed as mean ± SD or median (minimum/maximum values).

Groups	Ulcerative Area (mm^2)	% Lesion Inhibition	Lesion Score	Weight/Length (mg/cm)
Non-colitic	ND	ND	ND	91.4 ± 3.5
Colitic (5% Tween 80)	96.17 ± 4.3 ###	0	3.0 ± 0.7###	157.9 ± 7.5 ###
Prednisolone (2mg/kg)	11.00 ± 2.1***	81%	1.0 ± 0.6 ***	127.5 ± 13.7 **
Estragole (250mg/kg)	15.50 ±2.9 ***	79%	1.5± 0.7 **	133.5 ± 4.3 **

For parametric data, we used one-way ANOVA followed by Dunnett's and Tukey's post-tests. For non-parametric data, we used Kruskal–Wallis test and Dunn's test. ** p < 0.01, *** p < 0.001 compared to colitic groups; ### p < 0.001 compared to non-colitic group. (n = 7–10). ND = not detectable.

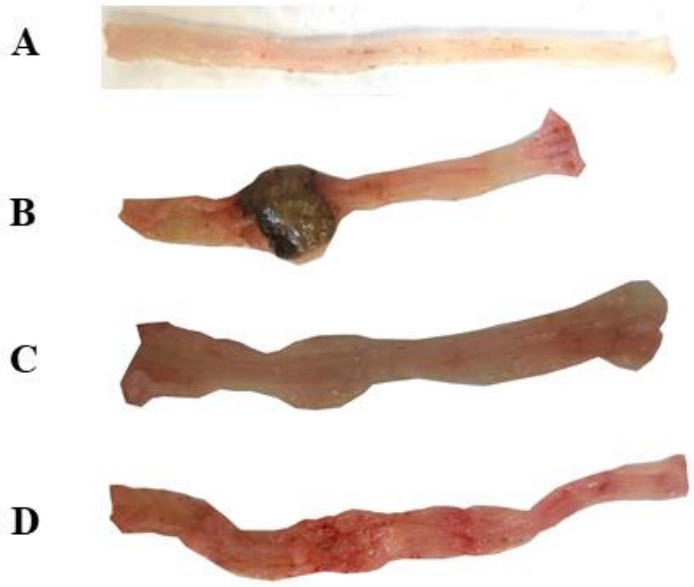


Fig 8. Representative images of rat colons from non-colitic group (A); colitic groups (5% Tween 80); prednisolone 2 mg/kg (C); Estragole 250 mg/kg (D) subjected to chronic phase with recurrence of the intestinal inflammatory process in a TNBS-induced colitis model.

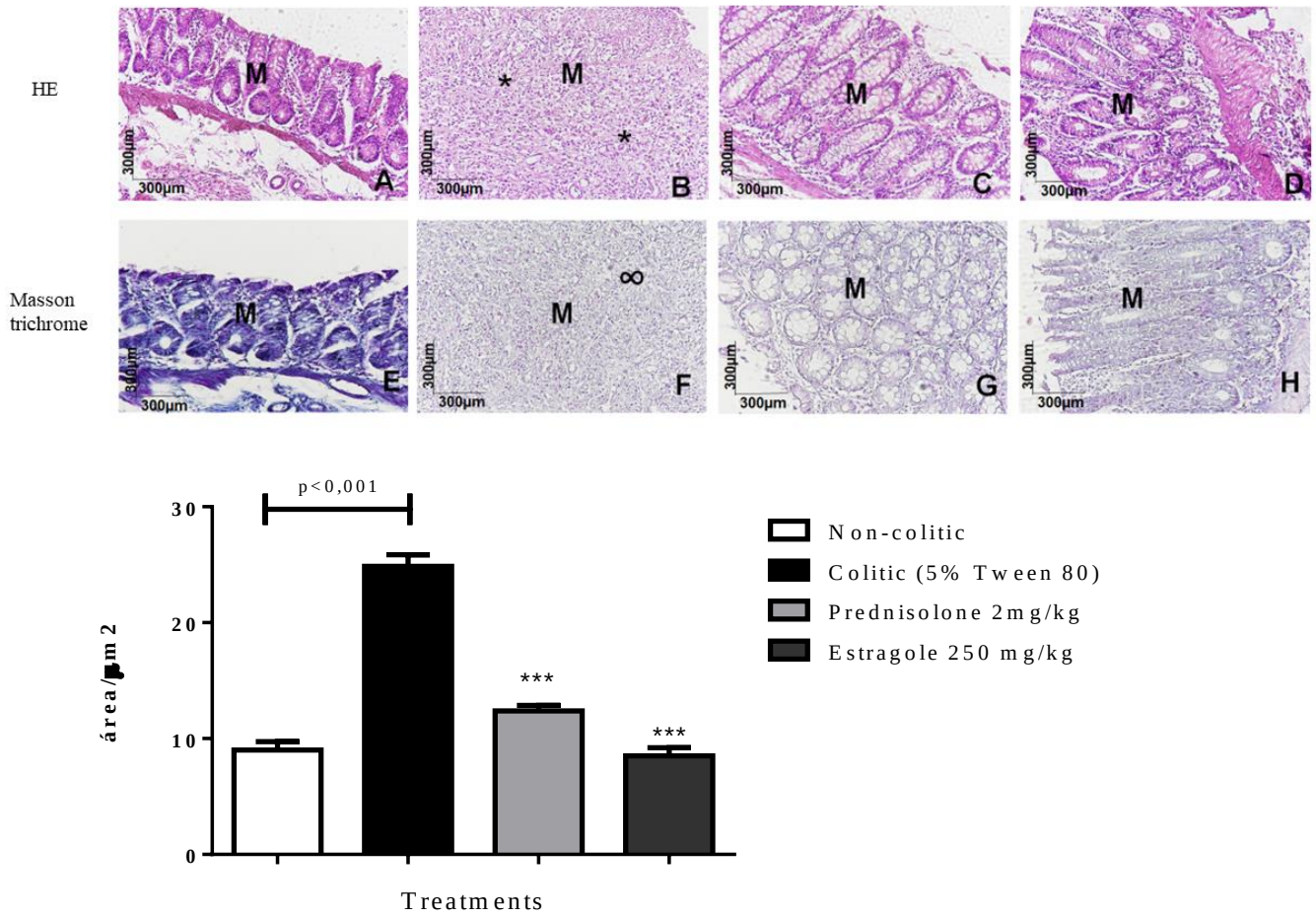
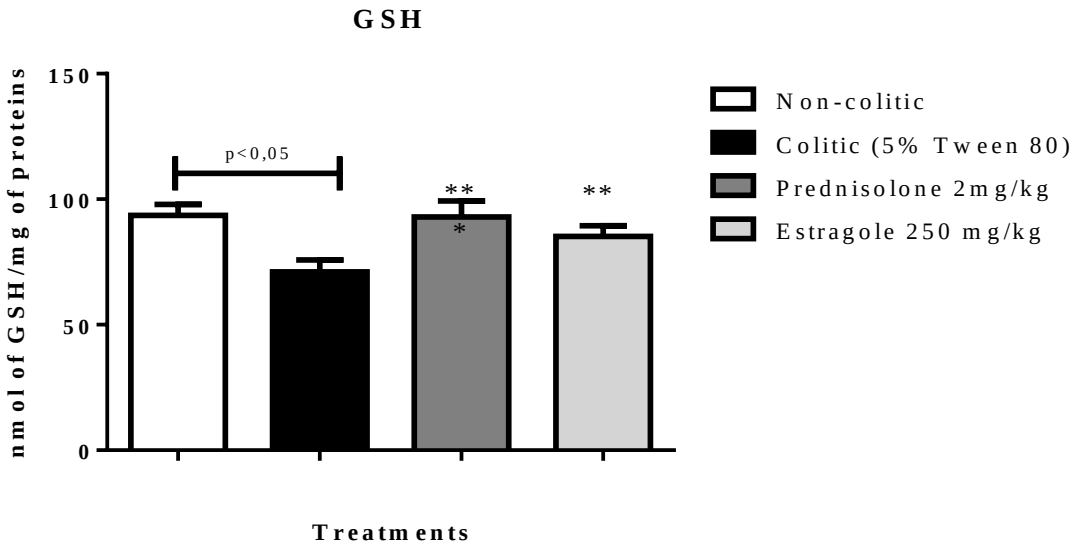
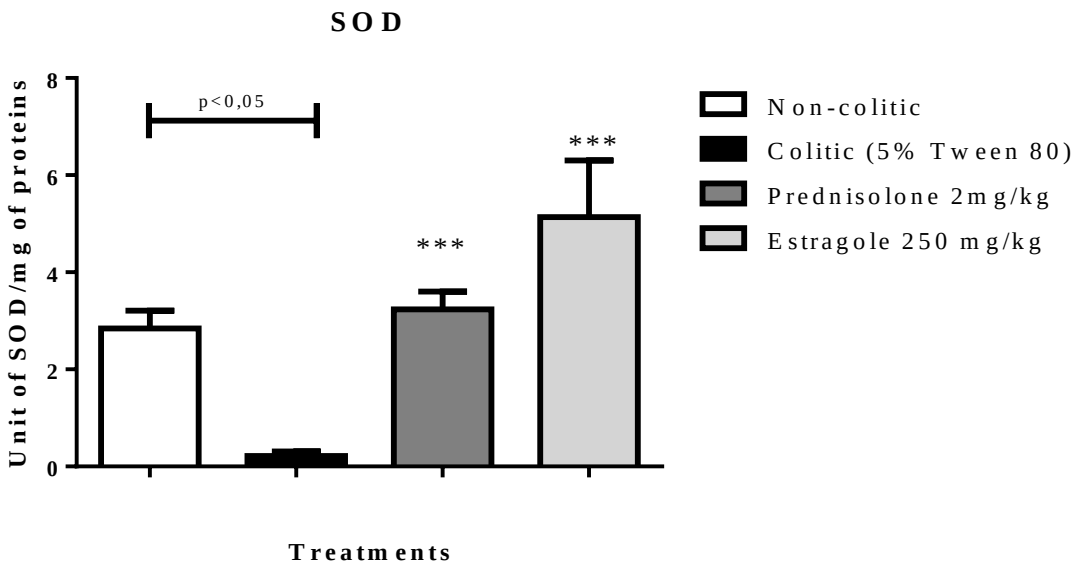


Fig. 9: Microscopic effects of the colon of rats submitted to in chronic phase with recurrence of the intestinal inflammatory process in a TNBS-induced colitis model and treated or not with Estragole. Representative photomicrographs of the animals' colon from the experimental groups: Non-colitic (A,E), Colitic (5% tween 80) (B,F), prednisolone 2 mg/kg (C,G) and Estragole 250 mg/kg (D,H). HE staining (A,B,C,D) and Masson trichrome (E,F,G,H). Colonic mucosa (M), acute inflammatory reaction (*), scarring tissue (∞). Results are expressed as median (minimum-maximum) of the parameters analyzed (n=5, 3 sessions per animal). Kruskall Walis test and Dunn's posterior test was performed using the software Graph Pad Prism. ** p,0,01 or ***p < 0.001 compared to the non-colitic group (5 % tween 80).

A


GSH
nmol of GSH/mg of proteins
150
100
50
0
p<0,05
**
*
**
Treatments
Non-colitic
Colitic (5% Tween 80)
Prednisolone 2mg/kg
Estragole 250 mg/kg

B


SOD
Unit of SOD/mg of proteins
8
6
4
2
0
p<0,05
***
***
Treatments
Non-colitic
Colitic (5% Tween 80)
Prednisolone 2mg/kg
Estragole 250 mg/kg

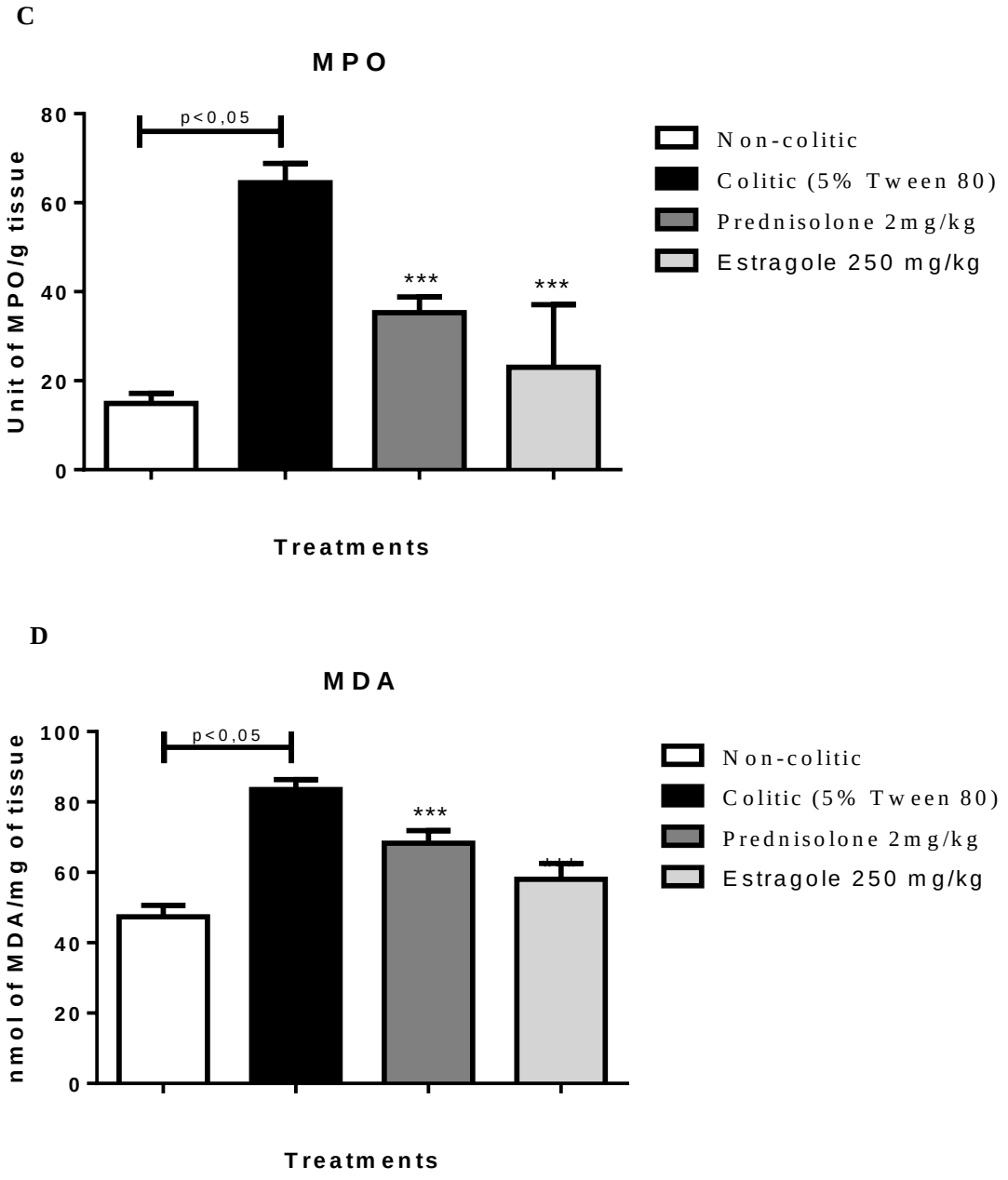
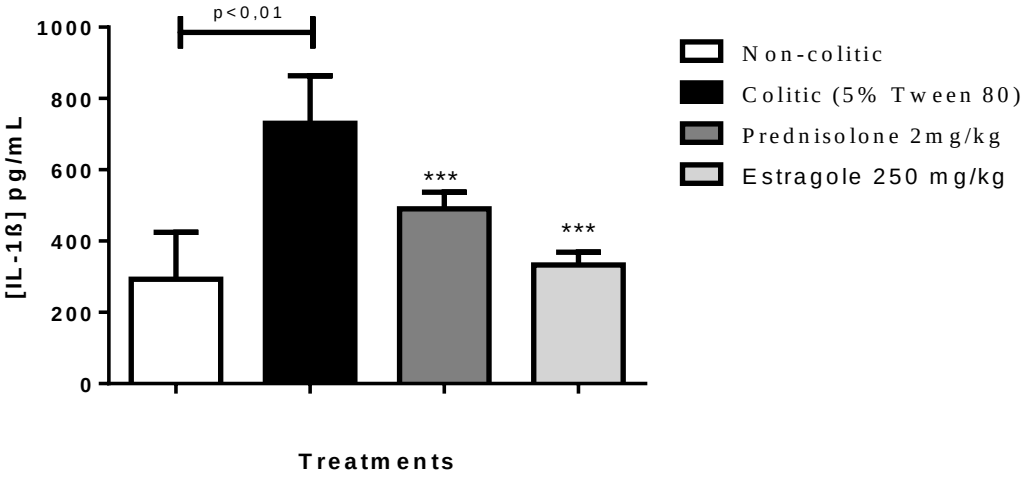
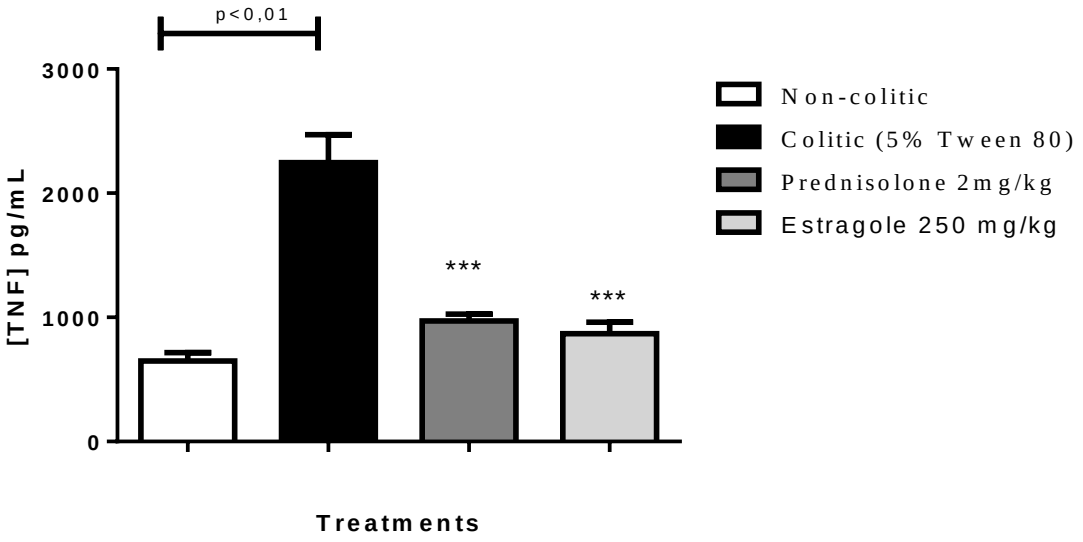


Fig 10. Effect of oral administration of Estragole and prednisolone in A) GSH, B) SOD C) MDA and D) MPO levels form in chronic phase with recurrence of the intestinal inflammatory process in a TNBS-induced colitis model in rats. Results expressed as mean ± SE of the parameters analyzed (n = 5-8). The analysis of variance of one via (ANOVA) followed by the Dunnett's and Tukey's test. ** p <0.01 or ***p <0.001 vs non-colitic group.

A


p<0,01
1000
800
600
400
200
0
[IL-1ß] pg/mL
***
***
Treatments
Non-colitic
Colitic (5% Tween 80)
Prednisolone 2mg/kg
Estragole 250 mg/kg

B


p<0,01
3000
2000
1000
0
[TNF] pg/mL
***
***
Treatments
Non-colitic
Colitic (5% Tween 80)
Prednisolone 2mg/kg
Estragole 250 mg/kg

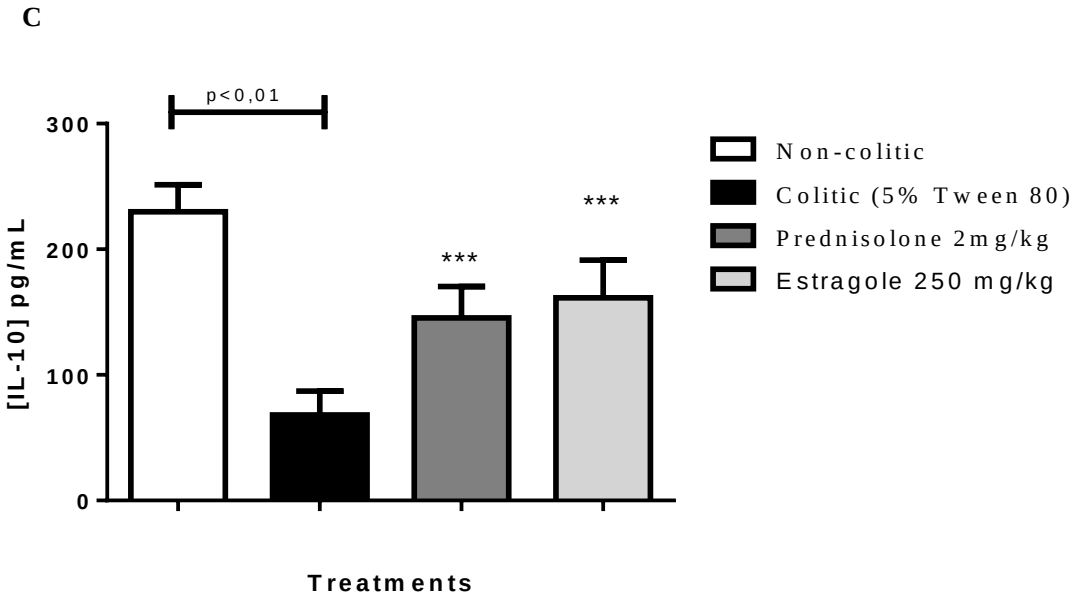


Fig. 11. Effect of Estragole and prednisolone in A) IL-1β, B) TNF-α and C) IL-10 levels form in chronic phase with recurrence of the intestinal inflammatory process in a TNBS-induced colitis model in rats. Results expressed as mean ± SE of the parameters analyzed (n = 6–8). The analysis of variance of one via (ANOVA) followed by the Dunnett's and Tukey's test. ** p <0.01 or ***p <0.001 vs control group.

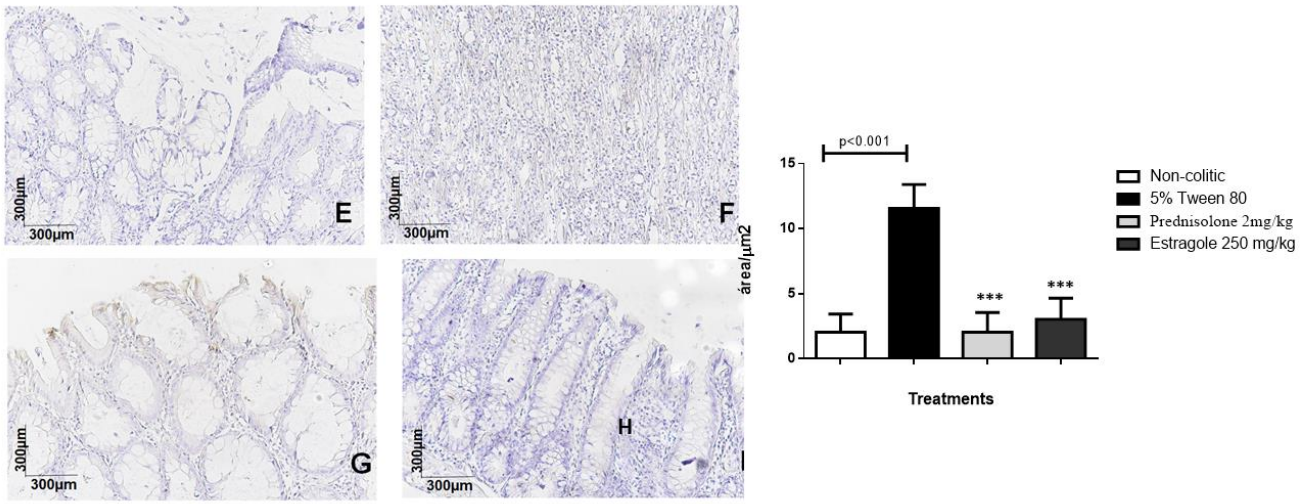


Fig 12. Photomicrographs with immunohistochemical marking for nuclear transcription factor kappa B (NF-κB) in colon samples from in chronic phase with recurrence of the intestinal inflammatory process in a TNBS-induced colitis model in rats. E – non-colitic; F- colitic (5 % Tween 80 -10 mL/kg); G- Prednisolone (2 mg/kg); H- Estragole (250 mg/kg). Results are expressed as median (minimum-maximum) of the parameters analyzed (n=5, 3 sessions per animal). Kruskall Walis test and Dunn's posterior test was performed using the software Graph Pad Prism. ***p < 0.001 compared to the colitic group (5 % tween 80).

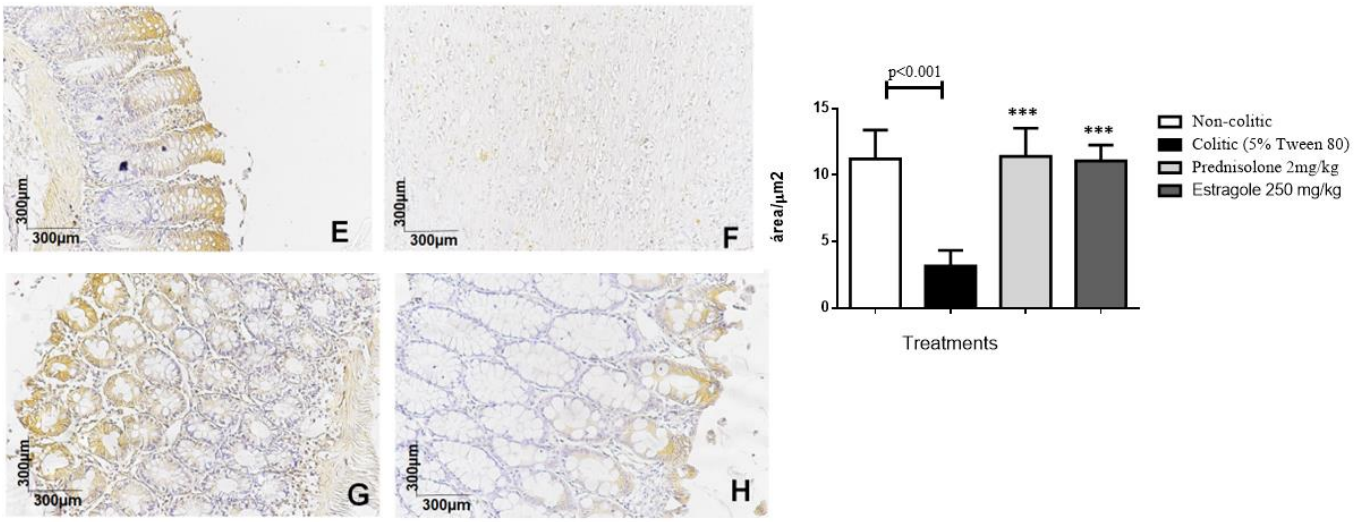


Fig. 13. Photomicrographs with immunohistochemical marking for transforming growth factor beta (TGF-β in colon samples from in chronic phase with recurrence of the intestinal inflammatory process in a TNBS-induced colitis model in rats. E – non-colitic; F- colitic (5 % Tween 80 -10 mL/kg); G- Prednisolone (2 mg/kg); H- Estragole (250 mg/kg). Results are expressed as median (minimum-maximum) of the parameters analyzed (n=5, 3 sessions per animal). Kruskall Walis test and Dunn's posterior test was performed using the software Graph Pad Prism. ***p < 0.001 compared to the colitic group (5 % tween 80).

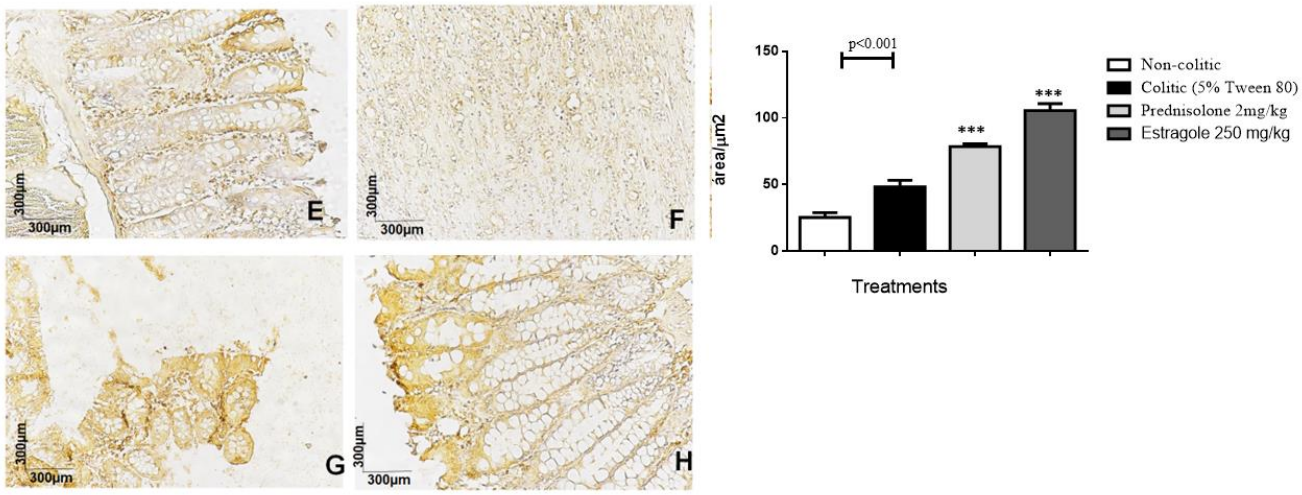


Fig. 14. Photomicrographs with immunohistochemical marking for zonula occludens 1 (ZO-1) in colon samples from in chronic phase with recurrence of the intestinal inflammatory process in a TNBS-induced colitis model in rats. E – non-colitic; F- colitic (5 % Tween 80 -10 mL/kg); G- Prednisolone (2 mg/kg); H- Estragole (250 mg/kg). Results are expressed as median (minimum-maximum) of the parameters analyzed (n=5, 3 sessions per animal). Kruskall Walis test and Dunn's posterior test was performed using the software Graph Pad Prism. ***p < 0.001 compared to the colitic group (5 % tween 80).

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CONCLUSÃO

A partir da análise dos resultados obtidos para o estragol, frente a modelos que mimetizam a úlcera gástrica, duodenal e a colite ulcerativa, é possível concluir que essa substância apresenta:

- Atividade anti-úlcera duodenal;
- Baixa toxicidade por doses repetidas durante 14 dias a partir do modelo de úlceras gástricas induzidas por ácido acético;
- Atividade cicatrizante gástrica relacionada a mecanismos antioxidantes (restauração dos níveis de GSH e SOD e redução dos níveis MDA e MPO), imunomoduladores (redução dos níveis de IL-1β e TNF-α e aumento de IL-10; redução da imunomarcação para NFκB e aumento para TGFβ);
- Atividade anti-inflamatória intestinal (modelo agudo e sub-crônico com recidiva do processo inflamatório induzido por TNBS) relacionada a mecanismos antioxidantes (restauração dos níveis de GSH e SOD e redução dos níveis MDA e MPO), imunomoduladores (redução dos níveis de IL-1β e TNF-α; aumento de IL-10; redução da imunomarcação para NFκB e aumento para TGFβ) e citoprotetores da barreira intestinal epitelial (aumento da expressão de ZO-1).

APÊNDICES

APÊNDICE A- Artigo publicado na revista Biomedicine & Pharmacotherapy

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Original article

Estragole prevents gastric ulcers via cytoprotective, antioxidant and immunoregulatory mechanisms in animal models

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ARTICLE INFO

Keywords:
Estragole
Gastric ulcer
Gastroprotection
Cytoprotection
Antioxidant
Immunoregulatory

ABSTRACT

Background: Estragole is an aromatic organic compound belonging to the class of phenylpropanoids derived from cinnamic aldehydes and present in essential oils of plant species, such as*Ravensara anisata* (madeira), *Ocimum basilicum* (manjericão/alfavaca) and *Croton zehntneri* (canelinha). Pharmacological studies report its anti-inflammatory, antioxidant and vasorelaxant activity.

Hypothesis/Purpose: This study aimed to evaluate the acute non-clinical toxicity, gastroprotective activity and the related mechanisms of action.

Methods: Acute toxicity was assessed according to OECD guide 423 in mice. Ethanol, stress, piroxicam and pylorus ligation-induced gastric ulcer models were used to investigate antiulcer properties. The related mechanisms of action were using the ethanol-gastric lesions protocol.

Results: In the acute oral toxicity assay, doses of 300 or 2000 mg/kg of estragole administered orally in Swiss mice did not induce any behavioral changes. However, the dose of 2000 mg/kg showed a decrease in water and feed intake. Lethal dose 50 % (LD50) was set to be equal to or greater than 2500 mg/kg, according to OECD. In all evaluated protocols, estragole (31.25, 62.5, 125 and 250 mg/kg) significantly reduced the area of ulcerative lesion when compared to control groups. To investigate the mechanisms involved in the gastroprotective activity, the antisecretory or neutralizing of gastric secretion, cytoprotectant, antioxidant and immunoregulatory effects were evaluated. Results showed that treatment with estragole (250 mg/kg) reduced ($p < 0.05$) the volume of the gastric juice. Besides, sulfhydryl groups, nitric oxide, mucus and prostaglandins seems to be involved in the gastroprotective property. Treatment also increased ($p < 0.001$) levels of reduced glutathione (GSH), interleukin-10 (IL-10) and positive cells marked for glutathione peroxidase (GPx) and cyclooxygenase 2

APÊNDICE B- Artigo publicado na revista Phytomedicine

ARTICLE IN PRESS

Phytomedicine xxx (xxxx) xxx

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Phytomedicine

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Original Article

Effect of *p*-cymene and rosmarinic acid on gastric ulcer healing – Involvement of multiple endogenous curative mechanisms

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ARTICLE INFO

Keywords:
p-Cymene
Rosmarinic acid
Gastroduodenal anticulcer
Gastric healing
Oral toxicity after repeated doses

ABSTRACT

Background: *p*-Cymene and rosmarinic acid are secondary metabolites found in several medicinal plants and spices. Previous studies have demonstrated their anti-inflammatory, antioxidant, and cytoprotective effects.
Purpose: To evaluate their gastroduodenal antiulcer activity, gastric healing and toxicity in experimental models.
Methods: Preventive antiulcer effects were assessed using oral pre-treatment on HCl/ethanol-induced gastric lesions and cysteamine-induced duodenal lesions models. Gastric healing, the underlining mechanisms and toxicity after repeated doses were carried out using the acetic acid-induced gastric ulcer rat model and oral treatment for 14 days.
Results: In the HCl/ethanol-induced gastric ulcer and cysteamine-induced duodenal injury, *p*-cymene and rosmarinic acid (50–200 mg/kg) decreased significantly the ulcer area, and so prevented lesions formation. In the acetic acid-induced ulcer model, both compounds (200 mg/kg) markedly reduced the ulcerative injury. These effects were related to an increase in the levels of reduced glutathione (GSH) and interleukin (IL)-10, and due to a decrease in malondialdehyde (MDA), IL-1β, tumor necrosis factor (TNF)-α, total and mitochondrial reactive oxygen species (ROS) levels. Downregulation of factor nuclear kappa B (NFκB) and enhanced expression of suppressor of cytokine signaling (SOCS)3 were also demonstrated. Furthermore, positive vascular endothelial growth factor (VEGF), metalloproteinase (MMP)-2, and cyclooxygenase (COX-2)-stained cells were increased in treated groups. Treatment also upregulated the platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), transforming growth factor (TGF)-β and epidermal growth factor receptor (EGFR) in gastric tissues. In isolated gastric epithelial cells this healing effect seems to be linked to a modulation of apoptosis,

Abbreviations: ALP, alcaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; bFGF, basic fibroblast growth factor; BSA, bovine

APÊNDICE C- Artigo publicado na revista International Journal of Molecular Science

International Journal of
Molecular Sciences

MDPI

Article

p-Cymene and Rosmarinic Acid Ameliorate TNBS-Induced Intestinal Inflammation Upkeeping ZO-1 and MUC-2: Role of Antioxidant System and Immunomodulation

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APÊNDICE D- Artigo publicado na revista Journal of Ethnopharmacology

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Hyptis suaveolens (L.) Poit protects colon from TNBS-induced inflammation via immunomodulatory, antioxidant and anti-proliferative mechanisms

Flávia Danniele Frota Machado [a], Rodrigo de Oliveira Formiga [a], Gedson Rodrigues de Morais Lima [a], Neyres Zínia Taveira de Jesus [a], Edvaldo Balbino Alves Júnior [a], Alexsandro Fernandes Marinho [a], Josean Fechine Tavares [a], Flávia Almeida Santos [b], Ana Flávia Seraine Custódio Viana [b], Aurigena Antunes Araújo [c], Raimundo Fernandes de Araújo Júnior [d], Cláudia Helena Pellizzon [e], Leônia Maria Batista [a,*]

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ARTICLE INFO

Keywords:
Hyptis suaveolens
Medicinal plant
Intestinal anti-inflammatory activity

ABSTRACT

Ethnopharmacological relevance: In folk medicine *Hyptis suaveolens* (Lamiaceae) has been reported to relieve respiratory and gastrointestinal infections, indigestion, cold, pain, fever, cramps, skin diseases, gastric ulcer and inflammatory disorders. This study investigated the effects and the mechanisms of action of *Hyptis suaveolens* (L.) Poit (Lamiaceae) ethanol extract (Hs-EtOH) and hexane phase (Hs-HexF) against intestinal inflammation.

Material and methods: Acute and relapse TNBS-induced ulcerative colitis protocols were used to evaluate intestinal anti-inflammatory activity. Damage evaluations, biochemical, histological and immunostaining parameters were determined.

Results: Both extracts decreased macroscopic colonic inflammation and the area of lesion induced by TNBS. Nevertheless, only Hs-HexF was able to reduce colonic wall thickness, edema and diffuse inflammatory cell

APÊNDICE E- Artigo publicado na revista Naunyn-Schmiedeberg's Archives of Pharmacology

Naunyn-Schmiedeberg's Archives of Pharmacology
https://doi.org/10.1007/s00210-020-01894-2

ORIGINAL ARTICLE

Rosmarinic acid prevents gastric ulcers via sulfhydryl groups reinforcement, antioxidant and immunomodulatory effects

Raphaela Francelino do Nascimento[1] · Rodrigo de Oliveira Formiga[1] · Flávia Danielle Frota Machado[1] · Igor Rafael Praxedes de Sales[1] · Gedson Moraes de Lima[1] · Edvaldo Balbino Alves Júnior[1] · Giciane Carvalho Vieira[1] · Raquel Fragoso Pereira[1] · Aurigena Antunes de Araújo[2] · Raimundo Fernandes de Araújo Junior[2] · José Maria Barbosa Filho[1] · Leônia Maria Batista[1,3]

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Abstract
Rosmarinic acid (RA) is a secondary metabolite present in several plant species that has already demonstrated antioxidant, antiallergic, anticancer, antimicrobial, neuroprotective, and hepatoprotective effects experimentally. Due to the promising pharmacological properties found previously, this study aimed to assess the oral acute toxicity and the gastroprotective effect of RA using animal models. Acute toxicity was assessed according to OECD guide 423. Ethanol, stress, NSAIDs, and pylorus ligature-induced gastric ulcer models were used to investigate antiulcer properties. The related mechanisms of action were also evaluated from ethanol-induced gastric lesions protocol. RA (300 and 2000 mg/kg) showed no changes in behavioral, water and food intake, body and organs weight parameters with LD_{50} set around 2500 mg/kg. RA presented gastroprotective activity in all assessed doses (25, 50, 100, and 200 mg/kg) using different animal models. Besides, it was observed that this effect is not related to the modulation of gastric juice parameters (pH, volume, and $[H^+]$), the participation of nitric oxide, mucus, and prostaglandins. However, increased sulfhydryl groups, GSH and IL-10 levels as well as reduced of proinflammatory cytokine (TNF-α and IL-1β) levels were found for RA-treated groups. RA presents low acute toxicity and gastroprotective activity, preventing ulcer formation via cytoprotective, antioxidant, and anti-inflammatory mechanisms.

APÊNDICE F- Artigo publicado na revista World Journal of Gastroenterology

World Journal of Gastroenterology

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ORIGINAL ARTICLE

Basic Study

Antifungal activity and antidiarrheal activity *via* antimotility mechanisms of (-)-fenchone in experimental models

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APÊNDICE G- Artigo publicado na Molecules

molecules

MDPI

Review

A Review of the Role of Flavonoids in Peptic Ulcer (2010–2020)

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Abstract: Peptic ulcers are characterized by erosions on the mucosa of the gastrointestinal tract that may reach the muscle layer. Their etiology is multifactorial and occurs when the balance between offensive and protective factors of the mucosa is disturbed. Peptic ulcers represent a global health problem, affecting millions of people worldwide and showing high rates of recurrence. *Helicobacter pylori* infection and the use of non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most important predisposing factors for the development of peptic ulcers. Therefore, new approaches to complementary treatments are needed to prevent the development of ulcers and their recurrence. Natural products such as medicinal plants and their isolated compounds have been

APÊNDICE H- Artigo publicado na revista Molecules

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MDPI

Review

Intestinal Anti-Inflammatory Activity of Terpenes in Experimental Models (2010–2020): A Review

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Abstract: Inflammatory bowel diseases (IBDs) refer to a group of disorders characterized by inflammation in the mucosa of the gastrointestinal tract, which mainly comprises Crohn's disease (CD) and ulcerative colitis (UC). IBDs are characterized by inflammation of the intestinal mucosa, are highly debilitating, and are without a definitive cure. Their pathogenesis has not yet been fully elucidated; however, it is assumed that genetic, immunological, and environmental factors are involved. People affected by IBDs have relapses, and therapeutic regimens are not always able to keep symptoms in remission over the long term. Natural products emerge as an alternative for the

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ORIGINAL RESEARCH
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(-)-Carveol Prevents Gastric Ulcers via Cytoprotective, Antioxidant, Antisecretory and Immunoregulatory Mechanisms in Animal Models

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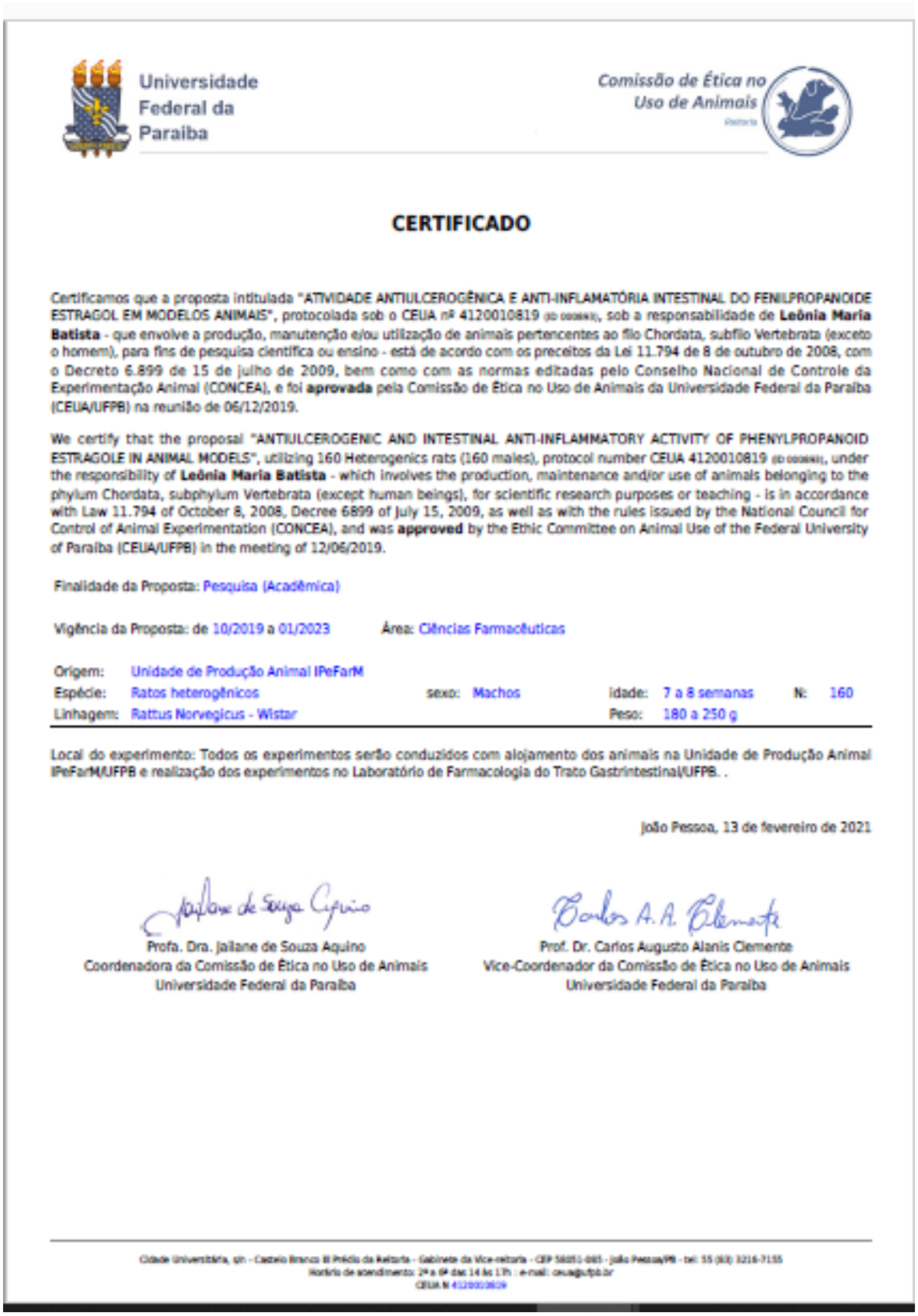
Background: (-)-Carveol (*p*-Mentha-6,8-dien-2-ol) is a monocyclic monoterpenic alcohol, present in essential oils of plant species such as *Cymbopogon giganteus*, *Illicium pachyphyllum* and in spices such as *Carum carvi* (cumin). Pharmacological studies report its antitumor, antimicrobial, neuroprotective, vasorelaxant, antioxidant and anti-inflammatory activity.

Hypothesis/Purpose: The objective of this study was to evaluate the acute non-clinical oral toxicity, gastroprotective activity of monoterpene (-)-Carveol in animal models and the related mechanisms of action.

Methods: Acute toxicity was assessed according to OECD guide 423 in mice. Ethanol, stress, NSAIDs and pylorus ligation-induced gastric ulcer models were used to investigate antiulcer properties. The related mechanisms of action were using the ethanol-gastric lesions protocol.

ANEXO

ANEXO A- - Certificado de aprovação da Comissão de Ética no Uso de Animais – CEUA



Universidade Federal da Paraíba

Comissão de Ética no Uso de Animais

CERTIFICADO

Certificamos que a proposta intitulada "ATIVIDADE ANTIULCEROGÊNICA E ANTI-INFLAMATÓRIA INTESTINAL DO FENILPROPANOIDE ESTRAGOL EM MODELOS ANIMAIS", protocolada sob o CEUA nº 4120010819 (ID [illegible]), sob a responsabilidade de **Leônia Maria Batista** - 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 06/12/2019.

We certify that the proposal "ANTIULCEROGENIC AND INTESTINAL ANTI-INFLAMMATORY ACTIVITY OF PHENYLPROPANOID ESTRAGOLE IN ANIMAL MODELS", utilizing 160 Heterogenics rats (160 males), protocol number CEUA 4120010819 (ID [illegible]), under the responsibility of **Leônia Maria Batista** - 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 12/06/2019.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 10/2019 a 01/2023 Área: Ciências Farmacêuticas

Origem:	Unidade de Produção Animal IPeFarM				
Espécie:	Ratos heterogênicos	sexo: Machos	idade:	7 a 8 semanas	N: 160
Linhagem:	Rattus Norvegicus - Wistar		Peso:	180 a 250 g	

Local do experimento: Todos os experimentos serão conduzidos com alojamento dos animais na Unidade de Produção Animal IPeFarM/UFPB e realização dos experimentos no Laboratório de Farmacologia do Trato Gastrintestinal/UFPB. .

João Pessoa, 13 de fevereiro de 2021

Profa. Dra. Jailane de Souza Aquino
Coordenadora da Comissão de Ética no Uso de Animais
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Prof. Dr. Carlos Augusto Alanis Clemente
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