PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
MESTRADO EM CIÊNCIAS DA SAÚDE

KARINE NADER GERVASONI

EFEITO PROTETOR DA PIPERINA NO DANO INTESTINAL INDUZIDO POR INDOMETACINA

Presidente Prudente – SP
2023

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Dissertação apresentada à Pró-Reitoria de Pesquisa e Pós-Graduação, Universidade do Oeste Paulista, como parte dos requisitos para obtenção do título de Mestre em Ciências da Saúde

Orientadora:
Profa. Dra. Lizziane Kretli Winkelstroter Eller

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Presidente Prudente, 18 de dezembro de 2023.

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RESUMO

Efeito protetor da piperina no dano intestinal induzido por indometacina

Anti-inflamatórios não esteroidais (AINES) são medicamentos largamente prescritos na prática clínica para o tratamento da inflamação e dor, principalmente para casos de dor crônica, osteoartrite, artrite reumatoide, pós-operatório e cólicas menstruais. O mecanismo de ação dos AINES é baseado na inibição não seletiva da enzima Cicloxigenase (COX) com inibição da conversão do ácido araquidônico em Prostaglandinas (PGs) que consiste em uma substância mediadora de reações inflamatórias no organismo. O uso crônico de AINES está associado a eventos adversos e danos aos órgãos, especialmente mucosa gástrica, intestino delgado, sistema cardiovascular, rins, fígado, cérebro e trato respiratório. O objetivo deste trabalho foi avaliar o efeito protetor da piperina no dano intestinal induzido por Indometacina. Foram utilizados 18 camundongos machos linhagem *Mus musculus,* de 6-8 semanas de idade. Foi realizada indução de dano intestinal com indometacina (10mg/ml) e co-tratamento com piperina (20mg/ml) ambos via oral. Após 14 dias, os animais foram eutanasiados. Foi realizada análise sorológica bioquímica. A inflamação intestinal foi avaliada com base em análises macroscópicas, histopatológicas e metagenômicas. A análise histopatológica demonstrou uma redução da inflamação do intestino delgado ($p<0,05$) e desaparecimento da necrose da parede intestinal do intestino grosso. As medições das criptas e vilosidades demonstraram aumento dos valores no grupo tratado com piperina ($p<0,05$). Foi observado um valor de Aspartato aminotransferase (AST) cerca de seis vezes maior no grupo Indometacina ($p<0,05$). Em relação a microbiota intestinal, foi possível observar aumento da diversidade de gênero no grupo tratado com piperina ($p<0,05$). Houve redução de 50% na formação de micronúcleo com a administração de piperina 20mg/kg ($p<0,05$). Concluiu-se que o co-tratamento com piperina tem grande potencial na remediação dos efeitos adversos causados pelos AINES.

Palavras-chave: Indometacina; AINES; Piperina; Intestinos; Anti-Inflamatórios não Esteroides.

ABSTRACT

Protective effect of piperine on indomethacin-induced intestinal damage

Non-steroidal anti-Inflammatory drugs (NSAIDs) are medications widely prescribed in clinical practice for the treatment of inflammation and pain, mainly in cases of chronic pain, osteoarthritis, rheumatoid arthritis, post-surgery and menstrual cramps. The mechanism of action of NSAIDs is based on the non-selective prevention of the enzyme Cyclooxygenase (COX) with protection from the conversion of arachidonic acid into Prostaglandins (PGs), which consist of a substance that mediates inflammatory reactions in the body. The classic use of NSAIDs is associated with adverse events and organ damage, especially gastric mucosa, small intestine, cardiovascular system, kidneys, liver, brain and respiratory tract. The objective of this work was to evaluate the protection of piperine in intestinal damage induced by Indomethacin. 18 male Mus musculus mice, 6-8 weeks old, were used. Intestinal damage was induced with indomethacin (10mg/ml) and co-treatment with piperine (20mg/ml), both orally. After 14 days, the animals were euthanized. Biochemical serological analysis was performed. Intestinal inflammation was evaluated based on macroscopic, histopathological and metagenomic analyses. Histopathological analysis demonstrated a reduction in inflammation of the small intestine ($p<0.05$) and disappearance of necrosis of the intestinal wall of the large intestine. The proportions of crypts and villi caused increased values in the group treated with piperine ($p<0.05$). An Aspartate aminotransferase (AST) value approximately six times higher was observed in the Indomethacin group ($p<0.05$). Regarding the intestinal microbiota, it was possible to observe an increase in gender diversity in the group treated with piperine ($p<0.05$). There was a 50% reduction in micronucleus formation with the administration of piperine 20mg/kg ($p<0.05$). It is concluded that co-treatment with piperine has great potential in remedying the side effects caused by NSAIDs.

Keywords: Indomethacin; NSAIDs; Piperine; intestinal damage; Intestines; Anti-Inflammatory Agents, Non-Steroidal.

LISTA DE SIGLAS

AINES	Anti-inflamatórios Não Esteroidais
ALT	Alanina aminotransferase
AST	Aspartato aminotransferase
CAT	Catalase
COX	Cicloxigenase
CYP450	Citocromo P450
DII	Doença Inflamatória Intestinal
EMT	Transcrição Eptelial Mesenquimal
FDA	Food and Drug Administration
FOS	Frutooligossacarídeo
GES-1	Células de eptélio gástrico Humano induzidas por etanol
GSH	Glutationa
GSH-Px	Glutationa Peroxidase
IL	Interleucina
IL-1β	Interleucina-1β
IL-6	Interleucina-6
IL-8	Interleucina-8
INDO	Indometacina
iNOS	Óxido nítrico Sintase Induzível
MDA	Malondialdeído
MPO	Mieloperoidase
NF-κβ	Fator Nuclear Kappa-β
P38-MPK	Proteína quinase ativada po mitógeno p38
PG	Prostaglandina
ROS	Espécies Reativas do Oxigênio
SOD	Superóido Desmutase
STAT3/SNAIL	Transdutores de Sinal e Ativadores de Transcrição-3
TBARS	Substância reativa ao ácido tiobarbitúrico
TNBS	Ácido trinitrobenzenosufônico
TNF-α	Fator de Necrose Tumoral-α
UV-B	Ultravioleta-B

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1 Introdução

Anti-inflamatórios Não Esteroidais (AINES) são medicamentos largamente prescritos na prática clínica para tratamento de inflamação e dor como osteoartrite, artrite reumatoide, pós-operatório e cólicas menstruais (Bindu et al., 2020; Lázár et al., 2021). O mecanismo de ação dos AINES é baseado na inibição não seletiva da enzima Cicloxigenase (COX) com inibição da conversão do ácido araquidônico em Prostaglandinas (PGs) que consiste em uma substância mediadora de reações inflamatórias no organismo (Gliszczyńska; Nowaczyk, 2021).

Descoberta em 1963, a Indometacina (ácido 1-(p-clorobenzoil)-5-metoxi-2-metilindol-3-acético) é um AINE considerado mais potente em relação ao ácido acetilsalicílico, fenilbutazona, ibuprofeno e naproxeno (Gliszczyńska; Nowaczyk, 2021). Possui aprovação pela *Food and Drug Administration* (*FDA*) como um medicamento com propriedades anti-inflamatórias, analgésicas e antipiréticas, sendo atualmente prescrito para osteoartrite, artrite reumatoide, artrite gotosa, espondilite anquilosante e dor aguda nos ombros (Villar-Martínez et al., 2021; Gliszczyńska; Nowaczyk, 2021).

O uso crônico de AINES está associado a eventos adversos e danos aos órgãos, especialmente mucosa gástrica, intestino delgado, sistema cardiovascular, rins, fígado, cérebro e trato respiratório (Bindu et al., 2020; Lázár et al., 2021).

Os danos provocados pelo uso de AINES ocorrem, principalmente, devido a redução da secreção de PGs, o que impossibilita sua função fisiológica no organismo, como por exemplo, a proteção da mucosa gastrintestinal. Além disso, acredita-se que, a interação direta da droga com a superfície hidrofóbica da mucosa também contribua com o dano (Gliszczyńska; Nowaczyk, 2021).

Ainda que apresente segurança e eficácia, El-Demerdash et al. (2021) demonstraram em estudo que a indometacina administrada em dose única (48mg/Kg), pode estar associada a estresse oxidativo na mucosa gástrica por meio da redução do níveis de glutationa (GSH) e catalase (CAT), além da peroxidação lipídica. Foi observado também, indução da inflamação no tecido gástrico por meio do aumento dos marcadores inflamatórios como Fator de Necrose Tumoral-α (TNF-α), Interleucina-1β (IL-1β), Fator Nuclear Kappa-β (NFκβ) , Caspase-3 e do gene proteína quinase ativada por mitógeno (p38-MAPK) (El-Demerdash et al., 2021).

As complicações das úlceras pépticas gastroduodenais são cinco vezes mais frequentes em pacientes que fazem uso de AINES, e dentre estas complicações pode-se destacar a hemorragia digestiva alta aguda (Tai; McAlindon, 2021; Kavitt et al., 2019). Até o momento não existe nenhuma estratégia comprovada capaz prevenir lesões no intestino delgado induzidas por AINES (Monteros et al., 2021). Entretanto, a medicina tradicional se destacou nos últimos tempos com a validação científica das propriedades

de produtos a base de ervas medicinais (Kondapalli et al., 2022). Além da ampla aplicação, os fitocompostos têm sido bastante ressaltados devido a menor presença de efeitos colaterais e ao baixo custo (Direito et al., 2023).

Pimenta do reino ou Pimenta preta (*Piper nigrum*) possui a piperina em sua constituição, um alcaloide que apresenta múltiplos benefícios a saúde (Tripathi et al., 2022). Esse composto é amplamente conhecido por inibir enzimas metabolizadoras, especialmente a Citocromo P-450 (CYP450) e UDP-glucoronil transferase, levando ao aumento da biodisponibilidade de nutrientes e fármacos, como curcumina, betacaroteno, ciprofloxacino e nimesulida. Ademais, atividades antioxidante, antitumoral, anti-inflamatória, antiespasmódica, hepatoprotetora, antibacteriana, imunomoduladora, dentre outras, são encontradas na literatura (Haq et al., 2021; Tripathi et al., 2022).

O efeito anti-inflamatório da piperina ainda não está bem elucidado. Jaisin et al. (2020) analisaram células de queratinócitos humanos irradiados por ultravioleta-B (UV-B). Naquele estudo, os autores sugeriram que o efeito anti-inflamatório da piperina ocorreu devido eliminação de radicais livres e diminuição da expressão de citocinas inflamatórias como p-38, p-JNK, COX-2, óxido nítrico sintase induzível (iNOS), IL-6 e IL-8 (Jaisin et al., 2020). Outro possível mecanismo de ação da piperina é a inibição da via da NFκβ, uma vez que a ativação dessa via controla a regulação de proteínas inflamatórias, como por exemplo a IL-6, COX e TNFα (Al-Johani et al., 2022; Laurindo et al., 2023).

Acredita-se também que a piperina pode aumentar a viabilidade celular (dose dependente) e proteger a mucosa gástrica da lesão por meio do aumento da atividade antioxidante e redução de espécies reativas de oxigênio (ROS) e mieloperoxidase (MPO) (Duan et al., 2022).

Pouco se sabe a respeito do impacto da piperina na microbiota intestinal. A microbiota intestinal tem papel importante no desenvolvimento da imunidade por aumentar a tolerância das bactérias benéficas e promover resposta adequada aos patógenos (Widhani et al., 2022). A função da microbiota tem sido discutida nas doenças metabólicas uma vez que as suas alterações podem estar relacionadas a sensibilidade a insulina, secreção de incretinas, homeostase energética e saúde intestinal (Kanazawa et al., 2021). Kondapalli et al. (2022), avaliaram o potencial efeito de extratos de três plantas (*Ocimum sanctum* (850 mg/Kg), *Zingiber officinale* (500 mg/Kg) *e Piper nigrum* (100mg/Kg)), administradas durante 30 dias. No estudo, os resultados demostraram que níveis cecais de *Lactobacillus* e *Bifidobacterium* aumentaram nos grupos tratados com extratos isolados e associados (Kondapalli et al., 2022).

Diante do exposto, testar a piperina como estratégia terapêutica para exercer proteção intestinal é de grande valia uma vez que são escassos trabalhos que avaliem

seu efeito protetor no dano intestinal em decorrência ao uso de AINES. Nesse contexto, este trabalho teve como objetivo avaliar o efeito protetor da piperina em relação ao dano intestinal induzido por indometacina.

2.MATERIAIS E MÉTODOS

2.1.Experimentação animal

Foram utilizados 18 camundongos machos linhagem *Mus musculus* (C57BL/6), de 6-8 semanas de idade (peso entre 18- 22 g). Os animais foram acomodados em gaiolas, num total de até 5 animais/gaiola e mantidos em condições controladas de temperatura (24 ± 2 ° C) e luz (ciclo: claro e escuro). Eles receberam ração e água *ad libitum*.

O projeto foi submetido à Comissão de Ética no Uso de Animais (CEUA) da Universidade do Oeste Paulista (UNOESTE) e aprovado sob o protocolo nº 7631. Foram adotados os princípios éticos em experimentação animal, preconizados pelo Colégio Brasileiro de Experimentação Animal (COBEA) e a Legislação Brasileira de Animais de Experimentação, LEI AROUCA - LEI No 11.794, DE 8 DE OUTUBRO DE 2008, onde o número de animais por grupo experimental foi o mínimo necessário para se obter resultados estatísticos.

Os animais foram divididos aleatoriamente em 2 grupos de 9 camundongos para uma etapa de adaptação por 7 dias, pré-indução por 10 dias, seguidos de indução de dano intestinal + tratamento por 4 dias, conforme tabela 1 (Peng et al., 2021; Shu et al., 2019).

Tabela 1. Descrição dos grupos experimentais

Grupos experimentais	Fase pré-indução (10 dias)	Fase de indução + tratamento (4 dias)
Grupo 1: I	Os animais receberam via oral 0,5% de carboxilmetilcelulose (veículo)	Os animais receberam por via oral indometacina (10 mg/kg)
Grupo 2: IP	Os animais receberam por via oral solução de piperina (20mg/kg)	Os animais receberam por via oral solução indometacina (10 mg/kg) + piperina (20mg/kg)

Na etapa de adapatação ambos os grupos receberam ração e água. Na etapa de pré-indução o grupo I recebeu, via oral, solução de carboximetilcelulose (CMC) a 0,5% e o grupo IP recebeu, via oral, solução de piperina 20mg/Kg. Na etapa de indução de dano intestinal e

tratamento o grupo I recebeu, via oral, solução de indometacina 10mg/Kg e o grupo IP recebeu, via oral, solução de indometacina 10mg/Kg mais solução de piperina 20mg/Kg. O protocolo de dosagens e duração do experimento foram de acordo com Peng et al. (2021), Shu et al. (2019) e Guo et al. (2020) com adaptações.

Os reagentes CMC (teor de pureza: 99,71), indometacina (teor de pureza: 100,90%) e piperina (teor de pureza: 98,26%), foram adquiridos por empresa comercial (respectivamente, Changshu Wealthy Science and Technology Co., Ltd., Taizhou Bona Chemical Co., Ltd. e Shaanxi Jiahe Phytochem Co., Ltd.) sendo o fornecedor responsável pelo controle de qualidade, mediante a apresentação de laudos de pureza.

Para avaliação do índice de atividade da doença (IAD) foi verificado mudanças no peso corporal, positividade de sangue nas fezes, sangramento grave e consistência das fezes, diariamente. Esses parâmetros clínicos correspondem aos sintomas observados na doença intestinal com inflamação (DII) (Jyotirmoy et al., 2008).

Após 14 dias de experimento, os animais foram eutanasiados. Para a eutanásia foram utilizados os anestésicos Cloridrato de Cetamina 10% (75mg/kg) e Cloridrato de Xilazina2% (10mg/kg), sendo administrados por via intraperitoneal. Os indicativos de morte foram a ausência de movimentos respiratórios, batimentos cardíacos e perda dos reflexos(Shu et al., 2019).

O sangue foi coletado por punção cardíaca e em seguida centrifugado para obtenção do soro. O intestino e estômago foram coletados para análise histopatológica. As fezes foram armazenadas para análise da microbiota intestinal.

2.2. Análise histopatológica

Após eutanasiados, o estômago, intestinos delgado e grosso foram removidos e mensurados os comprimentos. Os órgãos foram limpos (gorduras e tecidos aderidos à parede foram removidos), lavados em água corrente para remoção das fezes, fixados por imersão em solução de formalina tamponada a 10% por 48 horas, processadas rotineiramente e embebidas em parafina. Os cortes histológicos de 5µm de espessura foram corados com Hematoxilina-eosina (HE) para análise em microscopia de luz. A análise histomorfométrica foi realizada obtendo-se 3 segmentos sequenciais do intestino de cada animal. A altura (µm) de 10 vilosidades e 10 criptas de cada animal foi obtida medindo-se a distância vertical da extremidade superior até o limite inferior de cada uma. Os valores da altura das vilosidades e criptas para cada animal foram representados pelos valores médios dos três cortes histológicos (Jyotirmoy et al., 2008; Shu et al., 2019).

As imagens dos cortes histológicos foram capturadas em um aumento de 100x por uma câmera digital conectada a um microscópio de luz. As imagens digitais foram analisadas por meio de software apropriado (Image J® 1.50b, *National Institutes of Health*, EUA) (Gerhard et

al., 2017).

O dano tecidual foi analisado e avaliadas as alterações teciduais com seus respectivos escores conforme Pegoraro et al. (2021) (Tabela 2).

Tabela 2. Parâmetros microscópicos avaliados e seus respectivos escores.

Estômago

- Congestão: ausente (0); leve (1); moderada (2); intensa (3);
- Intensidade da inflamação: ausente (0); leve (1); moderada (2); intensa (3);
- Tipo de célula inflamatória;
- Atrofia da mucosa;
- Necrose da mucosa: ausente (0); presente e pequena (1); presente e extensa (2);
- Hiperplasia da mucosa: ausente (0); presente (1);

Intestino grosso e delgado

- Intensidade da inflamação: ausente (0); leve (1); moderada (2); intensa (3);
- Tipo de célula inflamatória
- Hiperplasia linfoide: ausente (0); presente (1);
- Ulceração do epitélio: ausente (0); presente e pequena (1); presente e extensa (2);
- Necrose da parede intestinal: ausente (0); presente (1); Atipias regenerativas do epitélio: ausente (0); presente (1);

2.3. Dosagem de marcadores bioquímicos

O sangue dos animais foi coletado por punção cardíaca sob anestesia e então centrifugado (15minutos a 1000xg em temperatura ambiente). O soro foi coletado e armazenado a -20ºC até momento das dosagens. A concentração de Aspartato aminotransferase - AST, Alanina aminotransferase - ALT, proteína, albumina, globulina, creatinina e ureia foi determinada pelo método de espectrofotometria, segundo orientações do fabricante (Roche. Diagnostics Ltd., Rotkreuz, Suíça) na plataforma de análise modular Roche Cobas 8000.

2.4. Análise metagenômica da microbiota intestinal

Foram amostradas fezes do intestino médio de 9 camundongos por tratamento. O conteúdo intestinal (fezes) de cada animal foi coletado, armazenado em microtubos livres de DNases e RNases e, imediatamente, congelado à -20°C para posterior análise metagenômica.

A extração de amostras de DNA bacteriano foi realizada usando o ZymoBIOMICSTM DNA Miniprep Kit (Zymo Research) seguindo as instruções do fabricante. As amostras de DNA foram imediatamente congeladas em freezer a -20°C até a análise molecular. A biblioteca foi preparada com primers para a região V3-V4 do 16SrRNA (~ 470 pb, amplificado com primers 341F x 806R), e os amplicons das bactérias foram sequenciados pela plataforma Illumina (Novaseq6000 PE 250). Sequência dos primers 341F 5'-CCTAYGGGRBGCASCAG-3' e 806R 5'- GGACTACNNGGTATCTAAT-3'.

Os dados da sequência foram processados e analisados com QIIME [Quantitative Insights Into Microbial Ecology, versão 2022.2.0 (https://qiime2.org/)]. Em média, um total de 153.269 leituras brutas foram sequenciadas. Inicialmente, durante as etapas de demultiplexação e trimming, as leituras de baixa qualidade foram removidas, como leituras até Q30, leituras com comprimento insatisfatório e quimeras foram removidas com o QIIME. Após esse processo, o conjunto de dados continha uma média de 106.918 leituras brutas. As leituras limpas foram usadas na definição do *ASV*(*Amplicon Sequence Variant*).

Para medir as taxas presentes nas amostras, foi utilizado um modelo preditor da região V3 e V4 (SILVA 138,99% OTUs full length sequences). Heatmaps e barplots de abundância relativa de ASVs foram gerados com Python (versão 3.7) através de códigos desenvolvidos pela empresa ByMyCell Inova Simples Ltda.

2.5. Avaliação da toxicidade aguda em larvas de *Galleria mellonella*

A toxicidade das soluções utilizadas no trabalho foram avaliadas com ensaio utilizando larvas de *Galleria mellonella*. O ensaio utilizou larvas de *G. mellonella* com peso entre 100 a 200mg, livre de melanização de acordo com as orientações de Marena et al. (2022), Spadari et al. (2019) com modificações. Cada grupo experimental foi composto por 10 larvas (n = 10). As larvas receberam injeções com seringa de insulina com agulha 13 x 0,45mm, sendo administrado 10µL/larva de cada tratamento no último proleg esquerdo de cada larva. Ao final da administração, as larvas foram transferidas para uma placa de Petri à temperatura de 30°C, restrita de alimentação. A avaliação do potencial tóxico agudo dos tratamentos foi realizada em intervalos de 24, 48, 72, 96 e 120 horas (5 dias). Considerou-se que as larvas estavam mortas quando não apresentavam resposta após estimulo físico (Marena et al. 2022; Spadari et al., 2019)

2.6. Técnica do micronúcleo

Amostras de medula óssea foram coletadas de cada fêmur de rato no momento do sacrifício. As células foram centrifugadas por 5 minutos a 2000 rpm por 2 vezes descartando-se o sobrenadante. Da suspensão resultante, uma pequena gota foi retirada e colocada na extremidade da lâmina para a realização do esfregaço. Duas lâminas por animal foram

preparadas. As lâminas foram coradas com Giemsa e secas à temperatura ambiente. As lâminas foram analisadas em teste cego. Dois mil eritrócitos policromáticos (1000 em cada lâmina) foram contados por animal usando um microscópio óptico com ampliação de 400 × para determinar o número de eritrócitos policromáticos micronucleados. Micronúcleos foram considerados ser estruturas com halos sugestivos ao redor suas membranas que mediam menos de um terço do diâmetro dos núcleos associados; os micronúcleos foram semelhantes em intensidade de coloração aos núcleos associados e foram observados no mesmo foco plano durante a microscopia (Araújo et al., 2013).

2.7.Análise estatística

Todos os dados analisados foram submetidos ao teste de normalidade usando o teste Kolmogorov-Smirnov. A comparação entre dois grupos foi realizada através do teste t de Student para dados não pareados ou pelo teste de Mann-Whitney. Os resultados foram considerados significativos para $p<0,05$. O programa de estatística usado foi o GraphPadPrism 3.0.

3 Resultados

Todos os animais sobreviveram ao período de experimentação. O peso médio dos animais do grupo I foi 22,3 ±1,51g e para o grupo IP foi 21,8 ± 0,50g. O sangue oculto nas fezes esteve presente nas fezes de ambos os grupos no período de indução, indicando presença de dano intestinal.

A análise macroscópica do intestino demonstrou um comprimento de 31,4 ± 2,4cm para o grupo I e 36,5 ± 2,1cm para o grupo IP conforme demonstrado na figura 1.

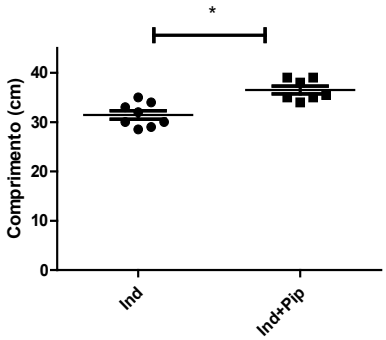

Figura 1. Avaliação do comprimento do intestino delgado em experimentação *in vivo* para avaliação da indução de dano intestinal com indometacina (10mg/kg) sozinha (Grupo I) e em presença de piperina (20mg/kg) (Grupo IP).

A análise histopatológica demonstrou uma redução de 62,5% na inflamação do intestino delgado ($p<0,05$) e 100% da necrose da parede intestinal do intestino grosso. As medições das criptas e vilosidades demonstraram aumento em mais de 100% dos valores no grupo tratado com piperina se comparado com o grupo tratado com indomedacina isolada ($p<0,05$) (Figura 2 e Tabela 3).

A analise macroscópica e microscópica do estômago não demonstraram indícios de dano para ambos os grupos.

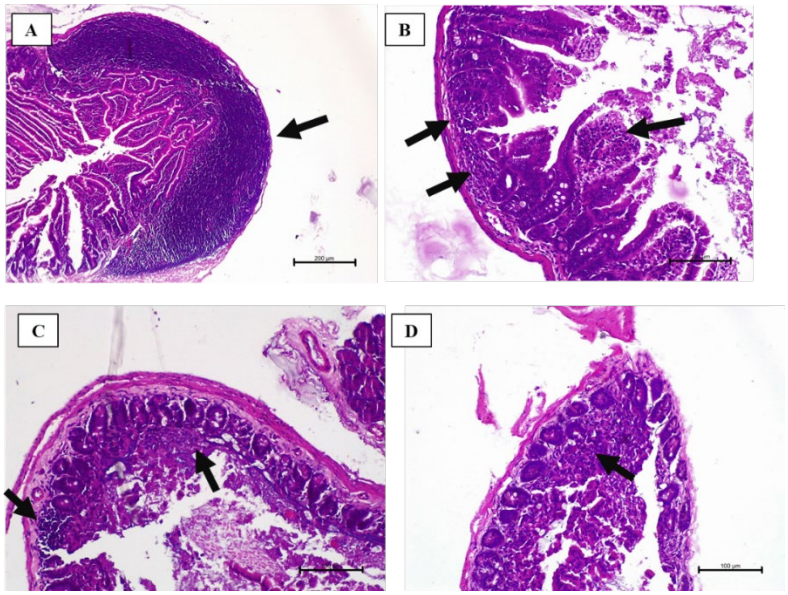

Figura 2. Avaliação microscópica do intestino delgado e intestino grosso de camundongos em experimentação *in vivo* para avaliação de dano intestinal sob os seguintes tratamentos: I- indometacina 10mg/kg IP - indometacina 10mg/kg + piperina 20mg/kg. (A) seta aponta hiperplasia linfoide em intestino delgado em grupo I – 100x; (B) inflamação leve do intestino delgado, seta aponta acumulo de linfócitos em grupo IP – 200x.(C) setas apontam infiltrados linfocitários em grupo I – 200x; (D) inflamação leve do intestino grosso em grupo IP – 200x.

Tabela 3. Avaliação histopatológica do intestino delgado e intestino grosso de camundongos em experimentação *in vivo* para avaliação da indução de dano intestinal com indometacina (grupo I) e com aplicação de piperina 20mg/kg (grupo IP)

	Intestino Delgado			Intestino Grosso		
Grupos	Intensidade de inflamação n/N (%)	Hiperplasia linfoide	Medidas Vilosidades (µm)	Intensidade de inflamação n/N (%)	Necrose em parede intestinal n/N (%)	Medidas Criptas (µm)
I	8/9 (89)	1/9 (11,1)	193,66±51	7/9 (78)	3/9 (33,3)	18,1± 3
IP	3/9 (33,3)[a]	1/9 (11,1)	450,6±89[a]	6/9 (66,7)	0/9 (0,00)[a]	48,0 ±7 [a]

[a] Diferença estatística em relação ao grupo indometacina-I ($p<0,05$)

Foram realizadas análises bioquímicas do sangue dos animais e conforme tabela 4, foi observado um valor de AST cerca de seis vezes maior no grupo I ($p<0,05$). A dosagem de ureia também apresentou valores significativos maiores nesse grupo ($p<0,05$). As demais análises séricas não apresentaram diferenças entre os grupos.

Tabela 4. Dosagens séricas de AST, ALT, creatinina, ureia, proteínas totais, albumina e globulina em camundongos de experimentação *in vivo* para avaliação da indução de dano intestinal com indometacina (10mg/kg) sozinha (grupo I) e em presença de piperina (20mg/kg) (grupo IP) * $p<0,05$

Exames laboratoriais	Grupo I	Grupo IP
Aspartato aminotransferase - AST (U/L)	646,9±69,1	94,3±1,7 *
Alanina aminotransferase - ALT (U/L)	82,1±20,1	48,9±5,25
Creatinina (mg/dl)	0,3±0,0	0,23±0,05
Uréia (mg/dl)	56,3±2,7	45,16±0,51*
Proteínas Totais (mg/dl)	4,8±0,3	4,73±0,20
Albumina (mg/dl)	3,6±0,1	3,3±0,36
Globulina (mg/dl)	1,9±0,6	1,33±0,057

O efeito da indometacina e da associação com piperina também foi avaliado na microbiota intestinal de camundongos, conforme observado na figura 3. Não foram observadas diferenças significativas entre os dois grupos, entretanto foi possivel observar presença de filos como Acidobacteriota (1%) e Myxococcota (0.6%) no grupo IP (Figura 3A). Em relação ao gênero, foi observado um aumento da diversidade no grupo IP, devido a presença de uma porcentagem maior de microrganismo classificados como “outros” que não foram incluídos nos principais grupos ($p<0,05$) (Figura 3B). As análises também demonstraram um predomínio de vias metabólicas para transportadores, funções gerais, reparo de proteínas e DNA, entretanto, sem diferença entre os grupos (Figura 3C).

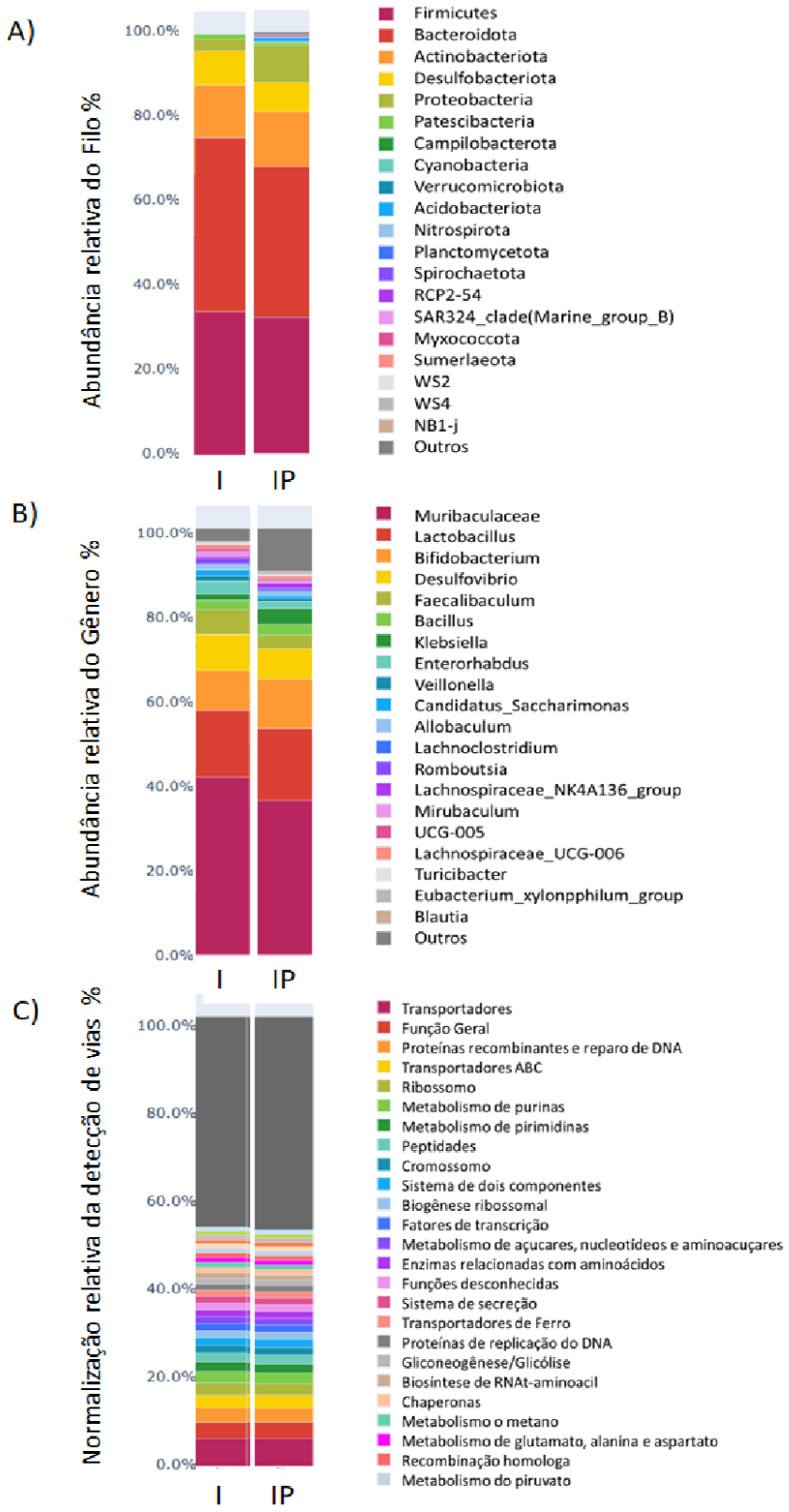

Figura 3. Avaliação da abundância relativa da microbiota intestinal obtida a partir da análise de fezes de camundongos submetidos a indução de dano intestinal com indometacina (10mg/kg)sozinha (grupo I) e na presença de piperina (20mg/kg) (Grupo IP). Considerar em A) análise referente a diversidade dos Filos dos microrganismos B) análise referente a diversidade dos Gêneros dos microrganismos C) análise referente a diversidade das vias metabólicas utilizadas pelos microrganismos

Indometacina e piperina não apresentaram toxicidade em modelo experimental utilizando *G. mellonella,* uma vez que todas as larvas sobreviveram por todo período de análise (5 dias). Entretanto, no teste do micronúcleo em eritrócitos policromáticos da medula óssea dos camundongos (Figura 4), o grupo I e IP apresentaram respectivamento índice de 0,077% e 0,038% para formação de micronúcleos, demonstrando uma redução de 50% com a administração de piperina 20mg/kg ($p<0,05$).

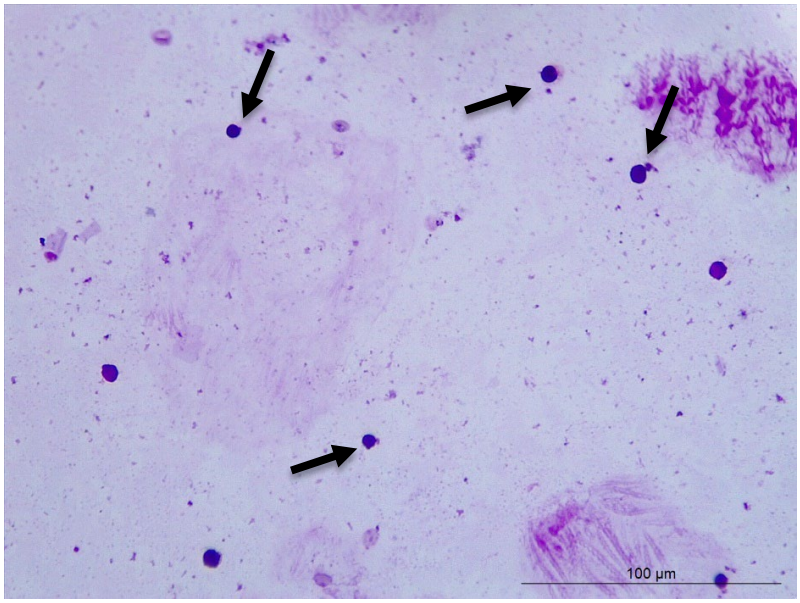

Figura 4. Fotomicrografia indicando a formação de micronúcleo em eritrócito de camundongo exposto a indometacina 10mg/kg

4. Discussão

AINES possuem muitos benefícios no tratamento da inflamação e dor, principalmente para casos de dor crônica (Bindu et al., 2020; Lázár et al., 2021). Entretanto, o uso crônico de AINES pode resultar em danos intestinais. Há relato que cerca de 76,3% de indíviduos com uso desses medicamentos apresentaram processos erosivos ou lesões ulcerativas na mucosa intestinal (Jia et al., 2023). Fato esse confirmado no presente estudo, uma vez que todos os animais que receberam indometacina 10mg/Kg, apresentaram indícios de dano intestinal na mucosa.

As análises histopatológicas apresentadas no presente estudo demonstaram redução da inflamação, desaparecimento total da necrose da parede intestinal e aumento na altura das criptas e vilosidades com o tratamento com piperina, reforçando o seu potencial efeito protetor, e sua contribuição para melhora da função de barreira e aumento da absorção de nutrientes no trato digestório.

Outros trabalhos corroboram com os dados encontrados nesse estudo como Guo et al. (2020) que avaliaram o potencial efeito da piperina, administrada durante 14 dias

em três dosagens diferentes (10, 20 e 40mg/Kg), em presença de colite ulcerativa induzida por ácido trinitrobenzenosufônico (TNBS). Os autores observaram que os tratamentos com piperina nas dosagens de 20 e 40mg/Kg foram capazes de aumentar o peso corporal dos animais, diminuir significativamente a relação peso/comprimento do colon, área ulcerada e índice de úlcera em comparação ao grupo controle.

No estudo de Shi et al. (2020) os parâmetros de dosagens foram diferentes do utilizado neste trabalho, porém, com resultados promissores. Foi avaliado o efeito do uso de curcumina associada a piperina nas concentrações de 200mg/Kg e 50mg/Kg respectivamente quando administradas durante 21 dias. Naquele estudo, foram observados aumento da altura das vilosidades e relação altura de vilosidades/profundidade das criptas significativamente maiores na mucosa do jejuno e do íleo (Shi et al., 2020).

Ainda em relação as alterações histopatológicas, Li et al. (2015) verificaram em seu estudo melhora do aspecto do fígado de animais tratados com piperina 20mg/Kg. Naquele trabalho, ratos alimentados com dieta litogênica apresentaram alterações na constiuição do fígado com embotamento das bordas, toque gorduroso e degeneração vacuolar. Enquanto isso, o grupo que recebeu piperina, com ou sem curcumina associada, apresentou fígado em coloração vermelha com borda afiada e o arranjo celular normal em microscópio óptico (Li et al., 2015). Além disso, foi observada redução da expressão da proteína NPC1L1 que tem papel importante na absorção intestinal do colesterol e no gene SREBP2 que possui função de regulação da homeostase do colesterol (Li et al., 2015).

Aspartato aminotransferase (AST) e Alanina aminotransferase (ALT) são enzimas importantes no metabolismo hepático (Morsy et al., 2020). AST é uma enzima expressa pelas mitocôndrias e encontrada em diversos órgãos, enquanto ALT é encontrada principalmente nos hepatócitos; ambas são liberadas no plasma de invíduos saudáveis em taxas constantes, no entanto, em algumas condições clínicas, onde ocorre estresse oxidativo e dano mitocondrial com possível aumento de dano aos hepatócitos, essa taxas encontram-se elevadas no sangue (Turan; Baris-Dirim, 2023; Morsy et al., 2020).

As análises sorológicas do presente estudo demonstraram valor de AST cerca de seis vezes maior no grupo I em relação ao grupo IP. Esses resultados sugerem impacto positivo da piperina nos ratos com inflamação

Em trabalho realizado por Abdelhamid et al. (2021), foi avaliado o potencial e eficácia da piperina, administrada durante três semanas na dosagem de 10mg/kg, no tratamento de fibrose hepática induzida por tiocetamida, e os resultados foram promissores com a diminuição significativa dos níveis séricos de AST e ALT. Outro estudo

realizado por Mohammadi et al. (2019) avaliou o potencial efeito protetor da piperina, administrada por 10 dias nas dosagens de 10 e 20mg/Kg, contra danos hepáticos induzidos por isquemia-reperfusão em ratos; naquele estudo os níveis de AST, ALT e Fosfatase Alcalina (ALP) reduziram significativamente no grupo tratado com piperina 20mg/Kg.

A microbiota intestinal exerce função protetora, estrutural e metabólica na prevenção de processos inflamatórios e na manutenção da homeostase intestinal (Monteros et al., 2021). Em casos de disbiose, ocorre a perturbação do equilíbrio normal entre a microbiota intestinal e o hospedeiro, e essas situações tem sido associada à obesidade, desnutrição, doenças inflamatórias intestinais (DII), distúrbios neurológicos e câncer. Acredita-se que um nível mais elevado da diversidade nas bactérias intestinais é um indicador importante da saúde do seu microbioma (Lozupone et al., 2012).

Nesse contexto, os resultados obtidos neste trabalho demonstraram um aumento significativo da diversidade de gênero na microbiota dos camundongos tratados com piperina. Esse fenômeno pode ser associado aos achados de Kondapalli et al. (2022), e He et al., (2022) que evidenciaram o potencial de extratos naturais de *Piper nigrum*, bem como a piperina, em aumentar a diversidade da microbiota intestinal e alterar sua composição.

Em estudo de He et al. (2022) foi avaliado o papel da piperina na regulação da microbiota intestinal e sua correlação com a disfunção metabólica. Naquele estudo os animais tratados com altas doses de piperina (40mg/Kg) apresentaram microbiota intestinal mais diversificada, quando comparados aos animais submetidos a outros tratamentos, além de alterar sua composição. Ainda no mesmo estudo, a piperina apresentou efeito anti-obesidade atribuído parcialmente a modulação da microbiota (He et al., 2022).

É notado que a inflamação intestinal impacta negativamente a biodiversidade do microbioma intestinal (Monteros et al., 2021). Deste modo, os achados com o tratamento utilizando piperina no presente estudo são relevantes e ressaltam a importância da associação da piperina como co-terapia com os AINES.

Além dos pontos mencionados acima, a microbiota intestinal exerce atividade importante no metabolismo de medicamentos, como é o caso da Levodopa (L-Dopa), que pode ter sua biodisponibilidade afetada pela abunância da bactéria *Enterococcus faecalis (E. faecalis)* devido a codificar a enzima Tirosina Descarboxilase (TDC) que possui forte atividade em relação a L-Dopa (Hu et al., 2023). Em estudo, Hu et al. (2023) demonstraram que a piperina, administrada em ratos na dosagem de 20mg/Kg, foi capaz de regular a abundância de *E. faecalis* e TDC, aumentando o efeito antiparkinsoniano, bem como melhora no défcit motor, da L-Dopa em ratos lesionados por 6-

hidroxidopamina.

A indução de micronúcleo em eritrócitos policromáticos das células de medula óssea é o biomarcador mais confiável para genotoxicidade mutagêncicas. Achados do estudo de Hilal Ahmad et al. (2018) sugeriram que a indometacina é um potencial agente genotóxico devido ao aumento significativo de micronúcleos em eritrócitos policromáticos. Acredita-se que o seu mecanismo genotóxico seja a falta de agregação cromossômica e os efeitos clastogênicos provocados pela geração de ROS.

A piperina não apresentou atividade tóxica em modelo experimental utilizando a *G. mellonella.* As propriedades antioxidantes, antiapoptóticas e quimioprotetoras da piperina provavelmente contribuíram para proteção frente a substâncias genotóxicas como a indometacina, uma vez que o grupo tratado com piperina apresentou redução da formação de micronúcleos quando comparado ao grupo que recebeu apenas indometacina (Ghelishli et al., 2019; Haq et al., 2021). Acredita-se que os mecanismos protetores exercidos pela piperina estejam relacionados aos efeitos antioxidantes, redução de ROS intracelular, redução dos níveis de mediadores pró-inflamatórios e antiapoptóticos (Ghelishli et al., 2019).

5 Conclusão

A piperina apresentou potencial de proteção intestinal contra dano induzido por indometacina, promovendo alterações histopatológicas benéficas, como aumento das medições de criptas e vilosidades, além de contribuir para o desaparecimento completo da necrose da parede intestinal. A piperina também foi fundamental na redução de níveis de marcadores de dano hepático, como AST. A piperina se mostrou promissora quanto a modulação do microbioma devido ao aumento da diversidade de gênero da microbiota intestinal e alteração da sua composição de forma benéfica. Portanto, a suplementação e o co-tratamento com piperina tem grande potencial na remediação dos efeitos adversos causados pelos AINES.

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ANEXO

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