UNIVERSIDADE ESTADUAL DE MARINGÁ

CENTRO DE CIÊNCIAS BIOLÓGICAS

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS

DÉBORA DOS ANJOS WEBER LUZ

TRATAMENTO DA COLITE EXPERIMENTAL E DE SUAS MANIFESTAÇÕES EXTRAINTESTINAIS COM CURCUMINA (*Curcuma longa* Linn) NANO ENCAPSULADA

MARINGÁ-PR

2021

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Tese apresentada ao Programa de Ciências Biológicas (área de concentração - Biologia Celular e Molecular) da Universidade Estadual de Maringá, para obtenção do grau de Doutor em Ciências Biológicas.

Orientadora: Prof. Dra. Maria Raquel Marçal Natali

MARINGÁ-PR

2021

Dados Internacionais de Catalogação-na-Publicação (CIP)
(Biblioteca Central - UEM, Maringá - PR, Brasil)

Luz, Débora dos Anjos Weber

L979t

Tratamento da colite experimental e de suas manifestações extraintestinais com curcumina (*Curcuma longa* Linn) nano / Débora dos Anjos Weber Luz. -- Maringá, PR, 2021.

110 f.: il. color., figs., tabs.

Orientadora: Profa. Dra. Maria Raquel Marçal Natali.

Tese (Doutorado) - Universidade Estadual de Maringá, Centro de Ciências Biológicas, Departamento de Ciencias Morfologicas, Programa de Pós-Graduação em Ciências Biológicas (Biologia Celular), 2021.

1. Nanoencapsulação. 2. Neurodegeneração. 3. Inflamação. 4. Colite experimental. 5. Estresse oxidativo. I. Natali, Maria Raquel Marçal, orient. II. Universidade Estadual de Maringá. Centro de Ciências Biológicas. Departamento de Ciencias Morfologicas. Programa de Pós-Graduação em Ciências Biológicas (Biologia Celular). III. Título.

CDD 23.ed. 615.32439

Márcia Regina Paiva de Brito - CRB-9/1267

FOLHA DE APROVAÇÃO

DÉBORA DOS ANJOS WEBER LUZ

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Dissertação/tese apresentada ao Programa de Pós-Graduação em Ciências Biológicas do Centro de Ciências Biológicas da Universidade Estadual de Maringá, como requisito parcial para obtenção do título de Doutor em Ciências Biológicas pela Banca Examinadora composta pelos membros:

Aprovada em: 25 de março de 2021.

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BIOGRAFIA

Débora dos Anjos Weber Luz nasceu em Machado/MG em 21/03/1980. Possui graduação em Fisioterapia pela Pontifícia Universidade Católica de MG - *Campus* Poços de Caldas (2004), Pós graduação em Bioquímica e Fisiologia do Exercício pela UNIFAL-MG (2006) e licenciatura em Ciências Biológicas pelo Centro Claretiano de Batatais-SP, sistema EAD (2007). Atou como professora de nível fundamental e médio nas áreas de Ciências e Biologia, em escola particular de sua cidade natal, no período de 2007 a 2012. Iniciou estágio no laboratório de Histologia da Universidade Estadual de Maringá-PR no ano de 2013. Em 2014, ingressou no curso de Pós graduação em Ciências Biológicas (área de concentração – Biologia Celular e Molecular), em nível de mestrado, na Universidade Estadual de Maringá-PR, realizando estudos na área de diabetes e neurônios entéricos, concluindo o curso em julho de 2016. No mesmo ano ingressou no curso de doutorado aprofundando seus estudos em neurônios entéricos, com enfoque nas doenças inflamatórias intestinais. Membro do grupo de pesquisa em Neurônios Entéricos: Filogenia, ontogênese e doenças carenciais e linha de pesquisa: Morfologia do trato gastrointestinal.

AGRADECIMENTO

Agradeço a Deus por me provar a cada dia que possuo mais força e coragem que eu imaginava ter algum dia, por me fazer superar cada desafio que encontro pelo caminho.

À professora Dra. Maria Raquel Marçal Natali que me deu a oportunidade de estágio no laboratório de histologia e depois me aceitou como aluna. Agradeço pela confiança em meu trabalho!

Às técnicas do laboratório de Histologia, Maria dos Anjos e Maria Ângela, Sílvia e Ana Paula, a grande ajuda e esforço que desempenham em nossas coletas e no dia-a-dia das práticas laboratoriais. Sem elas tudo seria muito mais complicado e menos alegre!

Especial agradecimento à Dra. Ana Paula de Santi-Rampazzo, que me ensinou cada passo da pesquisa, como organizar e desempenhar cada técnica e sempre está disponível a ajudar! Além de ter se tornado grande amiga, confidente e companheira!

Aos técnicos e colegas dos Laboratórios de Fisiologia e Bioquímica e do Complexo de Centrais de Apoio à Pesquisa (COMCAP), pelo apoio durante os experimentos. Em especial a Elizete Santos (*in memoriam*), que teve o grande sorriso e boa risada silenciados pela Covid-19.

As colegas de Laboratório de Histotécnica, Camila Quaglio Neves, Stephanie Carvalho Borges, Beatriz Petroncine, Nayra Branquinho, Lucimara Rodrigues e Francielle Ramalho, Pedro Zonta e a todos que estiveram presentes durante as coletas.

Especial agradecimento a Stephanie e Camila, pela grande ajuda com a execução das técnicas experimentais ao final do meu período gestacional.

Ao professor Odinei Hess Gonçalves e sua aluna Priscila Freitas, pela ajuda na produção das nanopartículas.

A professora Ciomar Aparecida Bersani Amado pela ajuda na estruturação do trabalho, sua receptividade e ajuda técnica.

As professoras Nilza Cristina Buttow e Larissa Carla Schneider pelos ensinamentos nas horas vagas.

Aos meus pais que me apoiam incondicionalmente! Sem eles nada seria possível...

Ao meu marido, amigo e companheiro de vida, Gilberto, que me fez ter a coragem de largar um emprego e uma vida já organizada para vir para uma cidade desconhecida, longe de todos os amigos e família, para dar esse passo engrandecedor no conhecimento.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) e à Fundação Araucária, pela concessão das bolsas de estudos.

À Universidade Estadual de Maringá, ao Departamento de Ciências Morfológicas e ao Programa de Pós-Graduação em Ciências Biológicas, área de Concentração em Biologia Celular e Molecular, por fornecer as condições necessárias à elaboração desta tese.

APRESENTAÇÃO

Esta tese é composta por dois artigos científicos.

O primeiro artigo intitulado: "Nanoparticles containing curcumin prevent inflammatory activity and oxidative imbalance in the kidney, liver and lung, resulting from experimental colitis induced by 2,4,6-trinitrobenzenossulfonic acid.", teve como objetivo formular e caracterizar as curcumina nanoencapsulada e avaliar os efeitos do tratamento com estas nanopartículas nas manifestações extraintestinais no rim, fígado e pulmão, decorrentes da colite experimental em ratos Wistar. Em consonância com as regras do Programa de Pós-graduação em Ciências Biológicas, este artigo foi redigido de acordo com as normas do periódico (Naunyn-Schmiedeberg's Archives of Pharmacology) (ISSN 1432-1912; Fator de impacto: 2.050) e Qualis (Ciências Biológicas I): B1

O segundo artigo intitulado: "Nanoencapsulated curcumin (Curcuma longa L.) prevents oxidative, inflammatory and neurodegenerative changes in the myenteric plexus resulting from experimental colitis." teve o objetivo de avaliar os efeitos do tratamento com nanopartículas de curcumina sobre o estado inflamatório, oxidativo e dos neurônios entéricos do cólon distal de ratos com colite experimental. Em consonância com as regras do Programa de Pós-graduação em Ciências Biológicas, este artigo foi redigido de acordo com as normas do periódico (Food and Chemical Toxicology) (ISSN, 0278-6915; fator de impacto: 4.679) e Qualis (Ciências Biológicas I): A2.

RESUMO GERAL

INTRODUÇÃO: As doenças inflamatórias intestinais (DIIs) são desordens imunológicas crônicas de etiologia desconhecida tendo como principais formas de expressão a Doença de Crohn e a Retocolite ulcerativa. Manifestações extraintestinais da colite resultam do rompimento da barreira intestinal decorrente da inflamação tecidual. O modelo experimental de colite, em ratos Wistar foi induzido por ácido 2,4,6 trinitrobenzeno sulfônico (TNBS) via enema em dose única. A nanoencapsulação da curcumina pelo polímero poli(vinilpirrolidona) (PVP) objetivou melhorar a resposta dos efeitos biológicos deste composto.

OBJETIVOS: Este trabalho objetivou produzir e caracterizar a curcumina nanoencapsulada e realizar testes para detectar a dose mínima efetiva (DME) no tratamento da colite experimental. A DME foi utilizada para tratar os ratos com colite experimental nos períodos de 7 e 28 dias. Foram avaliados os efeitos do tratamento sobre os aspectos biométricos, histológicos, atividade enzimática inflamatória, estado oxidativo e na densidade e no perfil dos neurônios mioentéricos de população geral (HuC/D+) e subpopulação nitrérgica (nNOS+) do cólon distal. Também foram avaliadas as manifestações extraintestinais (MEI) decorrentes da colite experimental no rim, fígado e pulmão bem como a eficácia do tratamento com curcumina nanoencapsulada nestes órgãos.

METODOLOGIA: A produção da curcumina nanoencapsulada foi realizada por extração da curcumina dos rizomas de *Curcuma longa* L. e a encapsulação no polímero PVP. Após a produção das nanopartículas, foi determinada a Dose Mínima Efetiva (DME) da curcumina encapsulada para o tratamento da colite experimental. Os ratos foram distribuídos em 3 grupos (n=5) tratados com as doses de 10, 25 e 50 mg/kg de curcumina nanoencapsulada (TN_{10}, TN_{25} e TN_{50}), para controles foram usados um grupo colítico foi tratado com água (TA), um com enema de solução salina, tratado com água (SA) e outro sem enema e sem tratamento (GC). O tratamento diário, via gavagem, ocorreu durante 7 dias. Foram realizadas avaliações diárias que geraram um Índice de Atividade da Doença (IAD) e após a eutanásia avaliações macroscópicas das lesões da mucosa do cólon distal e da inflamação tecidual por meio de análise da atividade enzimática da mieloperoxidase (MPO) e da N-acetilglucosaminidase (NAG). Na segunda etapa, os ratos com colite experimental foram tratados com a DME determinada na primeira etapa. Os ratos foram distribuídos em grupos (n=6) com colite induzida por TNBS ou enema com solução

salina para controle e tratados com 25 mg/kg de curcumina nanoencapsulada (TN e SN) ou água (TA e SA) e um grupo sem enema e sem tratamento (GC). O tratamento por gavagem e avaliações diárias para gerar o IAD, foram realizados por 7 ou 28 dias. Após o tratamento foi coletada uma amostra sanguínea para análise das enzimas hepáticas transaminase glutâmico oxalacético (TGO) e transaminase glutâmico pirúvica (TGP) prosseguindo a eutanásia e laparotomia mediana para coleta do o cólon distal, rim, fígado e pulmão. O cólon foi avaliado quanto aos parâmetros biométricos e as lesões macroscópicas da mucosa colônica. Amostras deste tecido foram destinadas a análise histológica e do *score* inflamatório. Outras amostras foram destinadas para análises da atividade das enzimas MPO, NAG e dos níveis de nitrito (NO_2^-). O estresse oxidativo tecidual foi avaliado por meio das análises da atividade enzimática da superóxido dismutase (SOD) e da glutationa S-transferase (GST) e dos níveis de glutationa reduzida (GSH) e de hidroperóxidos lipídicos (LOOH). Também foram avaliadas a densidade e perfil dos neurônios HuC/D^+ e $nNOS^+$ do plexo mioentérico por imunomarcação. O rim, o fígado e o pulmão foram pesados e amostras de cada órgão foram destinadas para análise das enzimas indicadoras de estresse oxidativo SOD, GST e catalase (CAT), dos níveis de GSH e LOOH, e das enzimas inflamatórias MPO e NAG, para avaliar as manifestações extraintestinais.

RESULTADOS: Análises térmicas e espectroscópicas indicaram a eficiente encapsulação da curcumina na matriz do polímero. Os testes com as diferentes doses indicaram que 25 mg/kg de curcumina nanoencapsulada reduziu o IAD, a atividade da MPO e da NAG comparado ao grupo TA, sendo a DME. Na segunda etapa, o grupo TA apresentou alto IAD e *score* macroscópico, aumento das túnicas intestinais e do *score* inflamatório, após 7 e 28 dias, comparado ao grupo GC. Após 7 dias a atividade da TGO e TGP foram maiores no grupo TA comparado ao grupo GC. Aos 28 dias, a atividade da TGO foi maior no grupo TA comparado ao GC. A atividade da MPO foi maior após 7 dias, enquanto a atividade da NAG e os níveis de NO_2^- foram maiores após 7 e 28 dias no grupo TA comparado ao GC. A atividade da SOD, foi menor após 7 dias, e a redução dos níveis de GSH e do aumento dos níveis de LOOH ocorreram após 7 e 28 dias, no grupo TA comparado ao GC. A avaliação dos neurônios mioentéricos indicou redução da densidade e do perfil neuronal em ambas as populações no grupo TA comparado ao grupo GC após 7 e 28 dias. Com o tratamento, o grupo TN apresentou menor IAD após 7 e 28 dias, e redução das túnicas do cólon comparado ao grupo TA. A atividade das transaminases

hepáticas aumentadas pela colite foram menores no grupo TN comparado ao grupo TA, após 7 e 28 dias. A atividade da MPO e NAG e os níveis de NO_2^- foi menor no grupo TN comparado ao grupo TA, após 7 e 28 dias. Após 7 dias a atividade de SOD foi maior e aos 7 e 28 dias os níveis de LOOH foram menores no grupo TN comparado ao grupo TA. Os níveis de GSH do grupo TN foram menores apenas após 28 dias, comparado ao TA. A densidade neuronal de ambas as populações foi maior no grupo TN comparado ao grupo TA após 7 e 28 dias, embora, aos 28 dias, a densidade $nNOS^+$ tenha permanecido menor comparado ao grupo GC. O perfil neuronal de ambas as populações foi maior no grupo TN comparado ao TA após 7 e 28 dias, no entanto, após 7 dias, o perfil neuronal HuC/D^+ permaneceu menor comparado ao grupo controle. Como MEI, houve redução do peso dos órgãos aos 7 dias e apenas do rim e pulmão aos 28 dias no grupo TA em comparação com o grupo GC. Após 7 dias, as defesas antioxidantes foram reduzidas nos rins e pulmões no grupo TA em comparação com o grupo GC. No rim, houve redução da atividade da CAT, SOD e dos níveis de GSH e aumento dos níveis de LOOH. No pulmão, houve redução da atividade de SOD e dos níveis de GSH e aumento dos níveis de LOOH. Após 28 dias, o grupo TA mostrou uma redução nas defesas antioxidantes no rim e no fígado. No rim, houve redução da atividade de CAT e GST e aumento dos níveis de LOOH. No fígado, houve redução dos níveis de GSH e aumento dos níveis de LOOH. A atividade de MPO dos três órgãos foi maior no grupo TA em relação ao grupo GC, após 7 dias. Após 28 dias, apenas o fígado apresentou maior atividade de MPO no grupo TA em relação ao grupo GC. A atividade do NAG foi maior no fígado e no pulmão, no grupo TA em relação ao grupo GC, após 7 dias. Exceto para o peso do rim aos 7 dias, o peso dos órgãos foi maior no grupo TN em comparação ao grupo TA. O grupo TN teve desequilíbrios oxidativos prevenidos no rim após 7 e 28 dias e no fígado aos 28 dias, mas no pulmão, os níveis de GSH e LOOH após 7 dias, não diferiram no grupo TN do grupo TA. No entanto, o tratamento foi capaz de prevenir o aumento da atividade inflamatória de MPO e NAG após 7 e 28 dias, no grupo TN em relação ao TA.

CONCLUSÃO: A dose de 25 mg/kg de curcumina nanoencapsulada evitou as alterações oxidativas e inflamatórias teciduais e preveniram a neurodegeneração mioentérica, mostrando-se eficaz na prevenção de alterações locais e MEI da colite experimental.

Palavras-Chaves: nanoencapsulação, inflamação, estresse oxidativo, neurodegeneração, colite experimental

ABSTRACT

INTRODUCTION: Inflammatory bowel diseases (IBDs) are chronic immunological disorders of unknown etiology, the main forms of expression of which are Crohn's disease and ulcerative colitis Extraintestinal manifestations of colitis result from the rupture of the intestinal barrier due to tissue inflammation. The experimental model of colitis in Wistar rats was induced by 2,4,6 trinitrobenzene sulfonic acid (TNBS) via enema in a single dose. The nano encapsulation of curcumin by the polymer poly(vinylpyrrolidone) (PVP) aimed improving the biological effects of this compound.

AIMS: This work aimed to produce and characterize nano encapsulated curcumin and to perform tests to detect the minimum effective dose (MED) for the treatment of experimental colitis. The MED was used to treat rats with experimental colitis for 7 or 28 days. It was assessed the effects of this treatment on biometric and histological parameters, inflammatory enzymes activity, oxidative state, and density and profile of myenteric neurons in the general population (HuC/D+) and in the nitrergic subpopulation (nNOS+) of the distal colon. Extraintestinal manifestations (EIM) resulting from experimental colitis in the kidneys, liver, and lungs were also evaluated, as well as the effectiveness of the treatment with nano encapsulated curcumin in these organs.

METHODOLOGY: The production of nano encapsulated curcumin was carried out through the extraction of curcumin from the rhizomes of Curcuma longa L. and encapsulation in PVP polymer. After the production of the nanoparticles, the Minimum Effective Dose (MED) of the encapsulated curcumin was determined for the treatment of experimental colitis. The rats were divided into three groups (n=5/group) treated with doses of 10, 25, and 50 mg/kg of nano encapsulated curcumin (TN10, TN25, and TN50); as controls, one colitic group was treated with water (TA), another with saline enema and treated with water (SA) and a third without enema or treatment (GC). The treatment occurred daily, through gavage, for 7 days. Daily evaluations were performed that generated a Disease Activity Index (DAI); after euthanasia, macroscopic evaluations of the lesions of the distal colon mucosa and tissue inflammation through enzyme activity of myeloperoxidase (MPO) and N-acetylglucosaminidase (NAG) were made. In the second stage, the rats with experimental colitis were treated with the MED determined in the first stage. The rats were divided into groups (n=6/group) with TNBS-induced colitis or saline solution enema as controls and treated with 25 mg/kg of nano encapsulated curcumin (TN and SN, respectively) or

water (TA and SA, respectively) and a group without enema or treatment (GC). The treatment by gavage and daily assessments to generate the DAI were carried out for 7 or 28 days. After treatment, a blood sample was collected for analysis of the liver enzymes glutamic oxalacetic transaminase (TGO) and pyruvic glutamic transaminase (TGP) followed by euthanasia and median laparotomy to collect the distal colon, kidneys, liver, and lungs. The biometric parameters of the colon and the macroscopic lesions of the colonic mucosa were recorded. Samples of this segment were used for histological analyses and inflammatory score. Other samples were used to analyze MPO and NAG activities and nitrite levels ($NO2^{-}$). Tissue oxidative stress was assessed through superoxide dismutase (SOD) and glutathione S-transferase (GST) enzyme activities and levels of reduced glutathione (GSH) and lipid hydroperoxides (LOOH). The density and profile of the HuC/D+ and nNOS+ neurons of the myenteric plexus were also evaluated by immunostaining. The kidneys, liver, and lungs were weighed and samples from each organ were sent for analysis of the oxidative stress enzymes SOD, GST and catalase (CAT); GSH and LOOH levels; and of the inflammatory enzymes MPO and NAG, in order to assess extraintestinal manifestations.

RESULTS: Thermal and spectroscopic analyses indicated the efficient encapsulation of curcumin in the polymer matrix. Tests with different doses indicated that 25 mg/kg of nano encapsulated curcumin reduced DAI, MPO and NAG activity compared with the TA group, being considered as the MED. In the second stage, induction of colitis in the TA group generated high DAI and macroscopic score, increased intestinal tunics, and inflammatory score, after 7or 28 days, compared with the CG group. After 7 days, the activity of TGO and TGP was higher in the TA group compared with the GC group. After 28 days, TGO activity was higher in the TA group compared with CG. MPO activity was higher after 7 days, while NAG activity and $NO2^{-}$ levels were higher after 7 and 28 days, in the TA group compared with CG. SOD activity was lower after 7 days, and the reduction in GSH levels and increase in LOOH levels occurred after 7 and 28 days in the TA group compared with CG. The evaluation of myenteric neurons indicated a reduction in density and neuronal profile in both populations in the TA group compared with the CG group after 7 and 28 days. After curcumin treatment, the TN group had lower DAI after 7 and 28 days, and a reduction in colon tunics compared with the TA group. The activity of liver transaminases increased by colitis was lower in the TN group compared with the TA group after

7 and 28 days. MPO and NAG activity and $NO2^{-}$ levels were lower in the TN group compared with the TA group after 7 and 28 days. After 7 days, the SOD activity was higher and at 7 and 28 days the levels of LOOH were lower in the TN group compared with the TA group. The GSH levels of the TN group were lower only after 28 days compared with TA. The neuronal density of both populations was higher in the TN group compared with the TA group after 7 and 28 days, although, at 28 days, the nNOS+ density remained lower compared with the CG group. The neuronal profile of both populations was higher in the TN group compared with TA after 7 and 28 days; however, after 7 days, the HuC/D+ neuronal profile remained lower compared with the control group. As extraintestinal manifestations, reduced organ weight was detected after 7 days and only kidneys and lungs were reduced after 28 days in the TA group compared with the CG group. After 7 days the anti-oxidants defences was reduced in kidney and lung in TW group compared with CG group. In the kidney, there was a reduction in CAT and SOD activities and GSH levels, and an increase in LOOH levels. In the lung, there was a reduction in the SOD activity and in the levels of GSH and an increased in the LOOH levels. After 28 days, the TW group showed a reduction in antioxidants defences in the kidney and liver. In the kidney, there was a reduction in CAT and GST activity and an increase in LOOH levels. In the liver, there was a reduction in GSH levels, and an increase in LOOH levels. The MPO activity of the three organs was greater in the TA group compared with CG after 7 days. After 28 days, only the liver showed greater MPO activity in the TA group compared with the GC group. NAG activity was higher in the liver and lungs in the TA group compared with the CG group after 7 days. Except for kidney weight at 7 days, the weight of the organs was higher in the TN group compared with the TA group. The TN group had prevented oxidative imbalances in the kidney after 7 and 28 days and in the liver at 28 days, but in the lung, the levels of GSH and LOOH after 7 days, did not differ in the TN group of the TA group. However, the treatment was able to prevent the increase in the inflammatory activity of MPO and NAG after 7 or 28 days in the TN group compared with TA.

CONCLUSION: The dose of 25 mg/kg of nano encapsulated curcumin prevented oxidative and inflammatory tissue changes and prevented myenteric neurodegeneration, proving to be effective in preventing local changes and extraintestinal manifestations of experimental colitis.

Keywords: nanoencapsulation, inflammation, oxidative stress, neurodegeneration, experimental colitis

Sumário

ARTIGO I: ... 1

Nanoparticles containing curcumin prevent inflammatory activity and oxidative imbalance in the kidney, liver and lung, resulting from experimental colitis induced by 2,4,6-trinitrobenzene sulfonic acid. ... 2

ABSTRACT ... 3

DECLARATIONS ... 4

INTRODUCTION ... 5

MATERIALS AND METHODS ... 7

Production and physical characterization of nano encapsulated curcumin ... 7

Differential Scanning Calorimetry - DSC (Perkin Elmer DSC4000) ... 7

Fourier Transform Infrared Spectroscopy – FTIR (Shimadzu Affinity-1) ... 7

Animals ... 8

Induction of the experimental model of colitis and treatment with nano encapsulated curcumin ... 8

***In vivo* evaluation** ... 9

Tissue collection ... 9

Biochemical analyses ... 9

Determination of CAT activity ... 10

Determination of SOD activity ... 10

Determination of GST activity ... 10

Quantification of GSH levels ... 11

Quantification of LOOH levels ... 11

Determination of MPO and NAG activity ... 11

Statistical Analysis ... 12

RESULTS ... 12

Characterization of nanoparticles containing curcumin ... 12

Treatment of EIM with nanoparticles containing curcumin ... 13

***In vivo* evaluations** ... 13

Organ weight ... 13

Oxidative State ... 14

The activity of MPO and NAG enzymes ... 14

DISCUSSION ... 15

REFERENCES ... 19

TABLES ... 25

FIGURES ... 30

ARTIGO II 1

Curcumina (*Curcuma longa* L.) nano encapsulada previne alterações oxidativas, inflamatórias e neurodegenerativas na colite experimental. 1

Curcumina (*Curcuma longa* L.) nano encapsulada previne alterações oxidativas, inflamatórias e neurodegenerativas do plexo mioentéricos decorrentes da colite experimental. 2

Nano encapsulated curcumin (Curcuma longa L.) prevents oxidative, inflammatory and neurodegenerative changes in the myenteric plexus resulting from experimental colitis. 2

RESUMO 4

ABSTRACT 5

Destaques 6

1 INTRODUÇÃO 7

2. MATERIAIS E MÉTODOS 8

2.1 Produção das nanopartículas 8

2.2 Delineamento experimental 9

2.3 Animais 10

2. 3.1 Indução da Colite Experimental 10

2.3.2 Tratamento e Avaliação *in vivo* 10

2.3.3 Coleta dos tecidos 11

2.4 Transaminases Hepáticas 12

2.5 Análise Histológica 12

2.6 Estado Oxidativo Tecidual 13

2.6.1 Determinação da atividade da superóxido dismutase (SOD) e glutationa S-transferase (GST) 13

2.6.2 Quantificação dos níveis de glutationa reduzida (GSH) e hidroperóxidos lipídicos (LOOH) 13

2.7 Atividade das enzimas inflamatórias mieloperoxidase (MPO) e N-acetilglucosaminidase (NAG) 14

2.8 Quantificação dos níveis de nitrito (NO_2^-) 14

2.9 Plexo Mioentérico do Cólon Distal - População neuronal geral (HuC/D^+) e subpopulação nitrérgica ($nNOS^+$) 15

2.9.1 Análise morfoquantitativa neuronal 15

2.10 Análises Estatísticas 16

3 RESULTADOS 16

3.1 Etapa 1: Determinação da Dose Mínima Efetiva (DME) 16

3.2 Etapa 2: Tratamento da colite experimental com a DME de 25 mg/kg 17

3.2.1 Transaminases Hepáticas 18

3.2.2 Análise Histológica 20

3.2.3 Estado Oxidativo 21

3.2.4 Estado Inflamatório 22

3.2.5 Análise morfoquantitativa da inervação intrínseca intestinal 23

4. DISCUSSÃO 23

REFERÊNCIAS 28

Legendas das Figuras: 37

FIGURAS 40

ANEXO I: PARECER DA COMISSÃO DE ÉTICA EM USO DE ANIMAIS DA UNIVERSIDADE ESTADUAL DE MARINGÁ (CEUA/UEM)...43

ANEXO II: SUBMISSION GUIDELINES - NAUNYN-SCHMIEDEBERG'S ARCHIVES OF PHARMACOLOGY 46

ANEXO III: SUBMISSION GUIDELINES - FOOD AND CHEMICAL TOXICOLOGY 56

ARTIGO I:

Nanoparticles containing curcumin prevent inflammatory activity and oxidative imbalance in the kidney, liver and lung, resulting from experimental colitis induced by 2,4,6-trinitrobenzenossulfonic acid.

Nanoparticles containing curcumin prevent inflammatory activity and oxidative imbalance in the kidney, liver and lung, resulting from experimental colitis induced by 2,4,6-trinitrobenzene sulfonic acid.

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ABSTRACT

Objective: To evaluate the extraintestinal manifestations in the kidney, liver, and lung in Wistar rats, and the effect of treatment with nano encapsulated curcumin on these changes.

Methods: The sonication technique was performed for simultaneous extraction and encapsulation of the curcumin powder in the poly(vinylpyrrolidone) polymer. Enema with saline or 2,4,6-trinitrobenzene sulfonic acid was performed for the control and colitis induction group, respectively, and nano encapsulated curcumin or water was used for the treatment. After 7 and 28 days of treatment, euthanasia was performed to remove the kidneys, liver, and lungs for oxidative evaluation by analyzing the enzymes catalase, superoxide dismutase, and glutathione S-transferase and levels of reduced glutathione and lipid hydroperoxides and the inflammatory activity of the myeloperoxidase and N-acetylglucosaminidase enzymes.

Results: Curcumin was efficiently extracted from the plant matrix and encapsulated by poly(vinylpyrrolidone). In colitic rats treated for 7 days, there was an imbalance in antioxidant defenses with increased lipid peroxidation in the kidneys and lungs. Myeloperoxidase activity was higher in all organs and N-acetylglucosaminidase activity was higher in the liver and lung. At 28 days, there was an imbalance in antioxidant defenses in the kidneys and liver with greater lipid peroxidation in these organs, and myeloperoxidase activity was greater in the liver. The treatment prevented these changes in both periods; however, in the lungs, lipid peroxidation was not influenced by the treatment.

Conclusion: Nano encapsulated curcumin prevented oxidative stress and inflammatory changes in tissues, proving to be effective in preventing extra-intestinal manifestations of experimental colitis.

Keywords: inflammatory bowel disease, oxidative stress, inflammation, nanoparticles, polyvinylpyrrolidone.

DECLARATIONS

Funding

This work was supported by Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) Financing Code 001 and Fundação Araucária (472223).

Conflict of interests

The authors declare that they have no competing financial interests or personal relationships that may have influenced the work reported in this article.

Availability of data and material

The data underlying this article will be shared at reasonable request to the corresponding author. Code availability Not applicable.

Authors' contributions

MRMN, OHG, NCB, CABA conceived and designed research. NCB, OHG contributed with reagents. DAWL, PDFS, SCB, CQN, NCB contributed to methodology and data collection. DAWL, PDFS, SCB, CQN, OHG contributed to data analysis. DAWL, MRMN, OHG, CABA contributed to the article writing.

Ethics approval

All the procedures with the animals were carried out only after approval by the Ethics Commission on Animal Experimentation of the Institution

Consent to participate Not applicable

Consent for publication

All authors critically revised the manuscript for important intellectual content and have approved the final version of the manuscript.

Acknowledgment

The author would like to thank the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) Financing Code and Fundação Araucária, financial development agencies of the state of Paraná, for financial support. Department of Morphological Sciences, the State University of Maringá-UEM for the technical support, the techniques of the Histology laboratory and the Graduate Program in Food Technology at the Federal Technological University of Paraná- UTFPR for the production of nano encapsulated curcumin.

INTRODUCTION

Inflammatory bowel diseases (IBD) are mainly expressed by the forms of Crohn's disease (CD) or ulcerative colitis (UC), both of which are characterized by chronic and recurrent intestinal ulcerations that affect the gastrointestinal tract. These diseases have a global increase in incidence, including in countries with recent industrialization, such as the countries of Africa, Asia, and South America (Ng et al. 2017; Mak et al. 2020). Of multifactorial etiology, IBD has an association of genetic components, increased oxidative stress, uncontrolled immune responses that involve the intestinal microbiota, and environmental factors, culminating in a local inflammatory response (Isene et al. 2014; Gul et al. 2015; Moura et al. 2015).

The experimental model of colitis induction using 2,4,6-trinitrobenzene sulfonic acid (TNBS) by Morris et al. (1989) is one of the most used in preclinical studies (Antoniou et al. 2016), presenting characteristics compatible with those of Chron's disease in humans (Wirtz et al. 2017; Saade et al. 2019). The intrarectal application of TNBS associated with ethanol, which leads to the rupture of the epithelial barrier, triggers transmural inflammation with infiltration of neutrophils, macrophages, and lymphocytes (Wirtz et al. 2017). The impairment of the intestinal barrier allows the extravasation of bacteria and antigens to the organism, thus, in addition to local inflammation, inflammation can occur in other organs besides the intestine (Aydin et al. 2016), characterizing the extra-intestinal manifestations (EIM).

EIM affects up to 50% of patients with IBD, being an inflammatory event outside the intestine dependent on the immunological events that occur in the intestine, or even, an independent inflammatory event that is perpetuated by IBD or by shared genetic and environmental events (Hedin et al. 2019). The rupture of the intestinal barrier-forming junctions due to the deleterious effects of the inflammatory process, together with the increase in local oxidative stress, are the factors that allow the leakage of inflammatory infiltrates and intestinal bacteria that lodge in other organs, triggering inflammation (Moura et al. 2015; Vavricka et al. 2015). The organs and structures most affected by EIM are the skin, joints, eyes, hepatobiliary tract, kidneys, lungs, and vascular system (Algaba et al. 2020).

The main substances currently used in the treatment of IBD are corticosteroids, non-steroidal anti-inflammatory drugs, and immunosuppressants that cause side effects, especially if

used for prolonged periods as occurs in IBDs (Gajendran et al. 2018; Dorrington et al. 2020). Therapies with biological agents have also been used in the treatment of IBDs, however, failures in response to treatment often occur (Arora and Shen 2015). Thus, the search for alternative therapies with anti-inflammatory and anti-oxidant action, highlights the importance of research with natural products, as has been happening in this group understudy with propolis (Gonçalves et al. 2013), copaiba oil (Barbosa et al. 2018), a fraction of ethyl acetate from Trichilia catiguá (Catuaba) (Vicentini et al. 2018), with promising results.

Curcumin is a natural, phenolic compound, extracted from the *Curcuma longa* Linn (*C.longa* L.) rhizome, originally from India, cultivated in other countries in Asia, and also in Africa, Central, and South America, with good adaptation in tropical climates (Velayudhan et al. 2012). It has an anti-inflammatory effect (Chai et al. 2020), antioxidant (Priyadarsini et al. 2003), antimicrobial (Ohno et al. 2017), among others. However, curcumin is a hydrophobic compound, with low solubility, photosensitivity and degrades under conditions of basic and neutral pH, which makes its handling difficult (Shaikh et al. 2009). Its low solubility results in low absorption and bioavailability, in addition to being quickly metabolized and eliminated from the body (Matos et al. 2019).

Several formulations have been developed to improve the bioavailability of curcumin, including nanoparticles, liposomes, complexes with phospholipids, and cyclodextrins (Shaikh et al. 2009; De Almeida et al. 2018; Rocha et al. 2020). Polymeric nanoparticles are drug carrier systems with a diameter of less than 1 μm, developed for different application routes, increasing the bioavailability of substances (He et al. 2019). Poly(vinylpyrrolidone) (PVP) is a synthetic, amorphous, highly hygroscopic polymer with amphiphilic characteristics and low toxicity (Koczkur et al. 2016). PVP encapsulated nano curcumin has been studied by this group and has already been evaluated for its physical characteristics, cytotoxicity, and anti-inflammatory and antioxidant activities. Although promising, it is necessary to evaluate how these nanoparticles behave in an in vivo system, in addition to evaluating their real effectiveness in the treatment of IBD.

This study aimed to investigate the effect of nano encapsulated curcumin on EIM resulting from the experimental model of TNBS-induced colitis. To this end, changes in the kidneys, liver, and lungs of rats about organ weight, oxidative stress, and inflammatory tissue activity were evaluated.

MATERIALS AND METHODS

Production and physical characterization of nano encapsulated curcumin

The extraction of curcumin from the rhizomes of turmeric and the simultaneous encapsulation by PVP was performed by combining the extraction techniques by ultrasound-assisted extraction and solid dispersion described by Dos Santos et al. (2019). Rhizomes of *C. longa* L. were peeled, frozen at -80 ° C and lyophilized (Liotop, model L101), then the particles were crushed and sieved, using particles with diameters between 100 and 35 *mesh Tyler*, to obtain the nanoparticles. The sonication technique was used for the simultaneous extraction and encapsulation of curcumin. A Falcon tube containing curcumin was kept in a bath at 30 °C, with the ultrasound probe positioned inside. Separately, PVP was added to ethanol under gentle agitation. This solution was poured into the Falcon tube with curcumin and the solution was sonicated (Fisher Scientific 60 kHz, 120W, 1/8 "tip) for 3 min in pulse mode (30 s of sonication and 10 s of pause) to avoid the sample was overheated. At the end of this time, the solution was filtered (0.45 μm porosity) and taken to an oven at 60 °C for ethanol evaporation. The nanoparticles were stored protected from light at 10 °C.

Differential Scanning Calorimetry - DSC (Perkin Elmer DSC4000)

In aluminum sample holders, 3 to 5 mg of each material (pure curcumin, PVP, nanoparticles, and a manual mixture of PVP-curcumin for comparison) were weighed and subjected to heating from 0 to 300 ° C, with a heating rate of 20 °C/min and nitrogen gas flow of 50 mL/min.

Fourier Transform Infrared Spectroscopy – FTIR (Shimadzu Affinity-1)

The analysis allows the identification of chemical interactions between curcumin and PVP, indicating the encapsulation of the compounds in the polymeric matrix. It was carried out in the range of 4000 to 600 waves/cm, with a resolution of 2 waves/cm and 32 cumulative scans. About

10 mg of each sample (pure curcumin, PVP, nanoparticles, and a manual mixture of PVP-curcumin for comparison) were dispersed in 100 mg of KBr and compressed to form tablets.

The physical characterization and electron microscopy analysis of the transmission of nano encapsulated curcumin obtained by the same encapsulation method is described by Santos et al. (2020).

Animals

Fifty male Wistar rats (*Rattus novergicus*, 90 days old) from the Central Vivarium of the State University of Maringá, were housed in the Sector Vivarium of the Department of Morphological Sciences and maintained in light / dark cycles (12 h) and stable temperature (22 ± 2 °C). They received standard rodent feed (Nuvital® - Nuvilab, Brazil) and water *ad libitum*. Experimental treatments took place for 7 and 28 days. All procedures in this study were carried out following the National Council for Animal Experimentation Control - CONCEA and approved by the Animal Use Ethics Committee of the State University of Maringá - CEUA / UEM (CEUA 2342171116).

Induction of the experimental model of colitis and treatment with nano encapsulated curcumin

For each treatment period, the rats were randomly assigned to 5 groups (n = 5):

1) Control group (CG) = without enema and without gavage;

2) Saline / water group (SW) = saline enema, water gavage;

3) Saline / nanoparticles group (SN) = saline enema, gavage with nano encapsulated curcumin;

4) TNBS / water group (TW) = enema with TBNS, gavage with water;

5) TNBS / nanoparticles group (TN) = enema with TNBS and gavage with nano encapsulated curcumin.

Before induction of colitis, the rats were fasted for 12 h (Da Silva et al. 2010) with free access to water, then were anesthetized with doses of Thionembutal® Sodium (45 mg/kg, Cristália Produtos Farmacêuticos e Químicos, Brazil). The induction occurred via a single dose enema of 0.6 mL of the solution containing 0.3 mL of 5% TNBS (15 mg - Sigma-Aldrich, USA)

dissolved in 0.3 mL of 30% ethanol. The control group received a single dose of 0.6 mL of 0.9% saline. To perform the enema, the rats were positioned upside down, suspended by the tail, and held in this position for 2 min (Da Silva et al. 2010) after application, to avoid refluxing the solution. The nano encapsulated curcumin (25 mg/kg) was diluted in drinking water and to control the treatment 0.5 ml of drinking water was administered, both orally (gavage).

In vivo **evaluation**

The in vivo evaluations took place daily, with the initial body weight referring to the weighing performed before the induction and the feed consumption evaluated from the first day after the induction of the experimental model. Feed consumption was calculated by subtracting the excess feed at the end of the 24 hours from the quantity offered.

The Disease Activity Index (DAI) was adapted from Cooper et al. (1993) (Table 1). The consistency and occurrence of visible blood in the stool, and the assessment of body weight, were scored individually from 0 to 4, generating a daily average, referring to the DAI (Barros et al. 2010). In 7 days, the DAI of the 1st, 3rd and 6th days after the induction was considered and for the 28 days, from the 1st, 3rd, 6th, 13th, 20th, and 27th days after the induction to obtain the results.

Tissue collection

At the end of each experimental period, euthanasia occurred with a lethal dose of Sodium-Thionembutal® (120 mg/kg) intraperitoneally, followed by median laparotomy to collect the kidney, liver, and lung, which were weighed, and the samples were sent for analysis oxidative state and inflammatory activity of the tissue.

Biochemical analyses

For analysis of oxidative and inflammatory parameters, kidney, liver, and lung samples were washed in 0.9% saline, frozen in liquid nitrogen, and kept in a freezer at -80 °C.

Subsequently, the samples were weighed and homogenized in potassium phosphate buffer (200 mM, pH 6.5). An aliquot was used to quantify the levels of reduced glutathione (GSH) and the remaining homogenate was centrifuged for 20 min at 9000 g. The supernatant was used to determine the enzyme activity of catalase (CAT), superoxide dismutase (SOD), and glutathione S-transferase (GST), as well as the levels of lipid hydroperoxide (LOOH) and protein quantification. The precipitate was used to determine the enzymatic activity of myeloperoxidase (MPO) and N-acetylglucosaminidase (NAG). All the material remained refrigerated, during the analyzes, to avoid the degradation of the proteins.

Determination of CAT activity

The assay used to determine the enzymatic activity of CAT was based on the method of Aebi (1984), which assesses the degradation of hydrogen peroxide (H_2O_2) by CAT. From the initial supernatant, an aliquot was diluted in potassium phosphate buffer (0.2 M, pH 6.5) and this solution was added to the mixing solution (TRIS-HCl buffer, 1 mM, and ethylenediamine tetra-acetic acid, 5 mM) pH 8.5, distilled water, and 30% H_2O_2. The readings were performed on a spectrophotometer (240 nm) and the results expressed in µmol/min/mg of protein.

Determination of SOD activity

To determine the enzymatic activity of SOD, the method of Marklund and Marklund (1974) was used. TRIS-HCl buffer and ethylenediamine tetra-acetic acid were mixed with the sample and stirred, then pyrogallol (1 mM) was added and the reaction was incubated for 20 min. The reaction was stopped with HCl (1 N) and centrifuged (4 min, 14000 g). The supernatant was subjected to a spectrophotometer (405 nm). The results were expressed in U of SOD/mg of protein.

Determination of GST activity

The activity of the GST enzyme was determined by adapted the method described by Warholm et al. (1985) by adding potassium phosphate buffer (0.1 M, pH 6.5) to the supernatant. An aliquot of the diluted sample was added to a reaction solution (potassium phosphate buffer, 1-chloro-2,4-dinitrobenzene, and GSH). The readings were performed on a spectrophotometer (340 nm). The results were expressed in mmol/min/mg of protein, using the extinction coefficient of 9.6 mM/cm.

Quantification of GSH levels

The levels of GSH were determined by the method of Sedlak and Lindsay (1968) adding 12% trichloroacetic acid to the initial homogenate followed by centrifugation (15 min, 9700 g). Aliquots of the supernatant were added to the TRIS-HCl buffer (0.4 M, pH 8.9). The reaction started with the addition of 5,5'-dithiobis 2-nitrobenzoic acid (1 mM) and was interrupted after 5 min for immediate reading on a spectrophotometer (412 nm). The individual values were interpolated in a standard GSH curve, expressed in μg of GSH/g of tissue.

Quantification of LOOH levels

The quantification of LOOH was carried out through the oxidation test of iron II in the presence of orange xylenol (Jiang et al. 1991). The supernatant samples were homogenized in P.A methanol and centrifuged (30 min. 10,000 g, 4 ºC). After centrifugation, the supernatant was added to the reaction medium and incubated for 30 min, in the dark, and at room temperature. The readings were performed on a spectrophotometer (560 nm). The results were expressed in mmol/g of tissue, using the extinction coefficient of 4.3 mm/cm.

Determination of MPO and NAG activity

To evaluate the enzymatic activity of MPO and NAG, the precipitate was suspended in potassium phosphate buffer (80 mM) with 0.5% hexadecyltrimethylammonium (pH 5.4) and centrifuged (20 min, 11000 g, 4 °C). To determine the MPO activity the reaction was started by

diluting tetramethylbenzidine (18.4 mM), after 3 min at 37 ºC it was stopped by the addition of sodium acetate (1.46 M, pH 3.0). The readings were performed on a spectrophotometer at 620 nm (Smiderle et al. 2014).

NAG activity was evaluated in the presence of citrate buffer (50 mM, pH 4.5) and 4-nitrophenyl N-acetyl-β-D-glucosaminide solution (2.24 mM). After incubation for 60 min at 37ºC, the reaction was stopped with glycine buffer (200 mM, pH 10.4), and readings were performed on a spectrophotometer at 405 nm (Bailey 1988).

The results were expressed as optical density (O.D.) /min/mg of protein, for both analyzes.

Statistical Analysis

Data were assessed for normality using the Kolmogorov-Sminov test. Nonparametric data were analyzed by the Kruskal-Wallis test and Dunn's post-test, while parametric data were analyzed by Analysis of Variance (ANOVA) and Tukey's post-test. The results were expressed as mean ± standard error and the level of significance was set at $p < 0.05$. Graph Pad Prism® 5.0 software was used for these analyzes.

RESULTS

Characterization of nanoparticles containing curcumin

The thermal properties of free and encapsulated curcumin were analyzed by DSC (Fig. 1a). The thermogram of pure curcumin showed an endothermic peak at 179.5 °C (ΔH = 129.4 J/g), the curve of pure PVP, peak between 50 and 130 °C while the physical mixture showed an attenuated curve and the nanoparticles, absence of peak.

The FTIR spectra of pure curcumin, PVP, the physical mixture of compounds and nanoparticles are shown in figure 1b. The spectrum of pure curcumin reveals typical absorption peaks of this compound at 3513/cm, 1630/cm, 1601/cm, 1423 and 1285/cm, 1206/cm, 1151 / cm, and 1026 / cm. The PVP showed absorption peaks of 2800-3000/cm and 1530-1800/cm.

Treatment of EIM with nanoparticles containing curcumin

In vivo evaluations

In both experimental periods, there was a reduction in feed intake on the 1st day after induction in the TW and TN groups compared with the GC, SW, and SN groups. The feed intake in SW and SN was also lower than the CG group on the first day after induction (Fig. 2a and Fig. 2d). There was a progressive increase in feed intake in the TW and TN groups, but at the end of the 7-day treatment, these groups had lower consumption compared with the CG, SW, and SN groups (Fig.2a). At the end of the 28-day treatment, there was no statistical difference between the groups (Fig. 2d).

In the 7-day period, there was initially a reduction in body weight after the induction of colitis, remaining reduced in the TW and TN groups compared with the GC, SW, and SN groups at end of treatment (Fig. 2b). In the 28-day period, the reduction in the body weight was observed until the 7th day in the TN group and until the 14th day in the TW group when the beginning of an increase in body weight was observed. After treatment for 28 days, the TN group showed greater weight gain compared with the TW group (Fig. 2e).

The induction of experimental colitis generated high DAI until the 3rd day after the induction of the model, in both experimental periods. At the end of the 7-day treatment, the TN group reached a lower score than the TW group and a higher score than the CG, SW, and SN groups (Fig 2c). After 28 days of treatment, the score in the TN group was lower than the TW group and was equal to the groups CG, SW and SN (Fig. 2f).

Organ weight

Kidney, liver and lung weight were lower at 7 days in the TW group compared with the CG. At 28 days, only kidney and lung weight were lower in the TW group compared with the CG group. In the TN group, at 7 days, only the weight of the liver and lung were higher compared with

the TW group. At 28 days the weight of the kidney and lung were higher in the TN group compared with the TW group (Table 2).

Oxidative State

After the 7 days, there was a reduction in CAT and SOD activity and in GSH levels, and an increase in LOOH levels in the kidney in TW group compared with the CG group (Table 3). In liver, there was no alteration at 7 days (Table 4). In the lung, there was reduction in the SOD activity and GSH levels, and an increase in LOOH levels (Table 5) in the TW group compared with the CG group. The treatment with nanoparticles containing curcumin (TN) was able to prevent the changes detected in the kidney but in the lung prevented the reduction in the SOD activity without interfering in the GSH and LOOH levels.

In the 28 days, there was a reduction in the CAT and GST activity and an increase in the LOOH levels in the kidney (Table 3). In the liver, there was a reduction in the GSH levels, and an increase in the LOOH levels in TN group compared with CG group (Table 4). In the lung, there was no alteration at 28 days. The treatment prevented these changes in the TN group compared with the TW group.

The activity of MPO and NAG enzymes

The analysis of the MPO activity of the kidney (Fig. 3a), liver (Fig. 3b), and lung (Fig. 3c) of the TW group indicated an increase in enzymatic activity in of 7-day period compared with the CG group. In 28 days the MPO activity in group TN was higher only in the liver compared with the CG group (Fig 3d, 3e, 3f). The treatment prevented the increase in the activity of these enzymes in both experimental periods in the TN group compared with the TW group.

The NAG activity increased in the liver and the lung in the TW group compared with CG only in the experimental period of 7 days and the treatment, prevented increased enzyme activity in the group TN compared with group TW (Fig.4c).

DISCUSSION

The use of nanoparticles to encapsulate curcumin is one of the strategies to control the release of curcumin in the body and increase its bioavailability. Ultrasound-assisted extraction is a simple and fast method with high extraction yield, which uses a low amount of organic solvents compared with conventional techniques, depending on the operating conditions used. The efficiency of this encapsulation technique is due to the acoustic cavitation mechanism which, when generating ultrasonic waves that radiate in a liquid medium, lead to the formation of cavitation bubbles. This mechanism increases the solid and solvent contact and accelerates the extraction process (Chemat et al. 2017). Simultaneous encapsulation extraction reduces production time and the cost of the encapsulated final product. Other works in this group (Dos Santos et al. 2019; Santos et al. 2020) have already studied this technique extensively, so this work aims to apply how these nanoparticles can be used in a new field.

DSC analysis is an indirect assessment of the encapsulation of curcumin in the PVP matrix, indicating the physical state of curcumin, PVP and nano encapsulated curcumin. The high endothermic peak of curcumin verified in this analysis (179.5 °C) is attributed to the melting temperature of this compound (Pan et al. 2013; Dos Santos et al. 2019). The wide thermal transition observed in the pure PVP curve (50-130 ° C), is due to the evaporation of water present in the material (Turner and Schwartz 1985). Both the melting peak of curcumin, attenuated by the dilution effect, and the transition from PVP are observed in the curve of the physical mixture, confirming the maintenance of the crystalline state of the bioactive compound. On the other hand, the absence of the peak associated with curcumin in the nanoparticle thermogram indicates the change in its physical state from crystalline to amorphous, due to the interactions between the chains of the compounds after the encapsulation process (Li et al. 2015; Dos Santos et al. 2019).

The absorption peaks revealed in the spectrum of pure curcumin are typical of this compound. The peaks indicate the stretching of hydroxyls (3513/cm), carbonyls (1630/cm), C = C bonds of benzene rings (1601/cm), C - phenolic and enolic (1423/cm and 1285/cm), and bonds Aromatic C - H and C - O - C (1151/cm and 1026/cm) (Pan et al. 2013; Homayouni et al. 2019;

Mahmud et al. 2019). The PVP, on the other hand, has bands related to the stretching vibrations of the C - H bonds (2800-3000/cm) and carbonyl groups (1530-1800/cm) (Dos Santos et al. 2019). Although the peaks associated with the pure compounds are observed in the spectrum of the physical mixture, some of them are absent or displaced in the spectrum of the nanoparticles, as is the case of the vibration peaks of the hydroxyl and carbonyl groups, respectively. This indicates the existence of bonds, such as hydrogen bonds, between curcumin and PVP molecules resulting from the encapsulation of the bioactive compound in the polymeric matrix (Khan and Rathod 2014; Sadeghi et al. 2016; Matos et al. 2019). These analyzes showed that curcumin was efficiently encapsulated by PVP.

After the production and characterization of the nanoparticles, they were used to treat the EIM of rats with experimental colitis. The signs of the establishment of the experimental model were verified in the in vivo evaluations, which indicated the occurrence of diarrhea with the presence of rectal blood and loss of body weight resulting in high DAI in rats with colitis. These results are in line with those found in the literature (Chen et al. 2017; Chamanara et al. 2019).

The weight of the kidney, liver, and lung was lower in the TA group, indicating organ atrophy. Rtibi et al. (2016) found a reduction in liver and lung weight in an experimental model of colitis with Dextran Sodium Sulfate (DSS). In colitis experimental model, naive T cells are converted to T helper (TH) 1 and 17. TH17 cells secrete interleukins (IL) 17, among others, capable of exerting inflammatory effects on intestinal, endothelial, fibroblast, macrophage epithelial cells among others (Brand 2009). The IL17 family is related to neutrophil infiltration into inflamed tissues and appears to be related to fibrosis in the kidney (Mehrotra et al. 2017), liver (Wree et al. 2018), and lung (Wilson et al. 2010). Curcumin can inhibit T cell differentiation into TH17 and also reduce IL17 expression (Xie et al. 2009; Zhao et al. 2017).

The EIM was confirmed by the imbalance in oxidative defenses in Organs analyzed organs. Previous studies of EIM resulting from different models of experimental colitis also point to oxidative changes in the kidney (Rtibi et al. 2016; Kayhan et al. 2018), liver (Gul et al. 2015; Farombi et al. 2016; Rtibi et al. 2016), and lung (Ozyilmaz et al. 2011).

In the intestinal mucosa of experimental models of IBD, reactive oxygen species (ROS) are related to the severity and progression of the disease and also to the EIM (Moura et al. 2015). However, the imbalance generated by oxidative stress is not restricted to the intestinal mucosa

but can extrapolate to the innermost layers and reach the systemic circulation (Moura et al. 2015; Bourgonje et al. 2020). Among the ROS, the superoxide anion (O_2^-), H_2O_2, and hydroxyl radical (OH•) stand out, highly reactive molecules, capable of inducing cellular damage, promoting lipid peroxidation (Balmus et al. 2016; Wang et al. 2016). The antioxidant enzymes SOD and CAT, as well as non-enzymatic defenses, such as GSH, are part of the body's antioxidant system. SOD catalyzes the dismutation of O_2^- into O_2 and H_2O_2 which can be eliminated by other enzymes, such as glutathione peroxidase (GPX) or by CAT (Bhattacharyya et al. 2014). In this study, the enzymatic activity of SOD and CAT was altered, indicating a change in the ability to neutralize ROS.

GSH is a non-enzymatic antioxidant defense and acts as an electron donor, neutralizing some reactive species. Together with the enzymes GST, GPX, and GR make up the glutathione system (Bhattacharyya et al. 2014). GSH levels were altered in the kidney and liver, indicating an imbalance in the glutathione system, in the rats with EIM.

LOOH are secondary products to the action of ROS, which act directly on the structure and function of the cell membrane, which can lead to cell death (Wang et al. 2016). The increase in the levels of LOOH, resulting from the induction of colitis in group TW in rats with EIM, indicates serious cellular damage generated by oxidative stress.

The increase in the inflammatory activity of the MPO and NAG enzymes, observed in this study is another indication of the EIM in the organs. Other studies have also found increased MPO activity in the liver, kidney, and lung in several models of experimental colitis (Farombi et al. 2016; Rtibi et al. 2016; Grandi et al. 2019).

The MPO is present in neutrophil granules and the traffic of these cells in the inflammatory process is regulated by interleukin (IL)-6 (Fielding et al. 2008; Chami et al. 2018). The MPO is released by degranulation catalyzes the reaction of H_2O_2 with oxidizable compounds present in the medium, forming substances capable of oxidizing various cell structures and damaging them (Rtibi et al. 2016), is considered a marker of inflammatory tissue damage (Farombi et al. 2016). NAG is a macrophage lysosomal enzyme released in the presence of lipopolysaccharides that come into contact with this cell in an inflammatory process (Kalaiselvan and Rasool 2016).

The increase in the oxidative imbalances and inflammatory activity observed in these organs may be related to increased permeability in the intestinal barrier, with possible onset 2 h after the induction of the experimental colitis induced by TNBS (Yamada et al. 1992).

The paracellular permeability of the intestinal barrier is controlled by specialized adhesion structures such as tight junctions (TJ) and adhesion junctions (AJ). Both TJ and AJ are associated with a cortical actin cytoskeleton (Bruewer et al. 2003). The remodeling of this cytoskeleton unleashes the force that drives the breakdown of adhesion structures in the face of stressors (Wang et al. 2012) and pro-inflammatory agents like cytokines (Bruewer et al. 2003). As a consequence of the increased permeability of the intestinal barrier, the translocation of bacteria and their products occurs (Palma et al. 2007; Akcan et al. 2008; Dönder et al. 2018), resulting in secondary infections in organs beyond the intestine. Translocated bacteria and their products have a pro-inflammatory activity that can be associated with renal (Nymark et al. 2009), liver (Palma et al. 2007), and pulmonary (Liu et al. 2020) disorders.

When treating rats with nanoparticles containing encapsulated curcumin, we found that both inflammatory tissue activity and oxidative damage were avoided.

According to Priyadarsini et al. (2003), the ability to neutralize free radicals in curcumin is linked to the phenolic groups OH or CH_2 of the diketone portion. Free radicals can be transferred to either of these H atoms, however, the phenolic group is more labile and thus plays a greater role in antioxidant activity and kinetics of free radicals. In this way, curcumin can sequester various types of free radicals, such as O_2^- (Ak and Gülçin 2008), OH• (Barzegar and Moosavi-Movahedi 2011), H_2O_2 (Ak and Gülçin 2008; Barzegar and Moosavi-Movahedi 2011), and singlet oxygen (Das and Das 2002) amongst others.

In addition to the direct action on free radicals, curcumin can act indirectly on the expression of antioxidant enzymes such as SOD (Panchal et al. 2008; Yarru et al. 2009), CAT (Panchal et al. 2008), glutathione reductase, GPX (Yarru et al. 2009) and GST (Ye et al. 2007). These properties of curcumin may have allowed the treated rats with nanoparticles containing curcumin not to develop the oxidative imbalances observed in the group of no treated colitic rats. Mouzaoui et al. (2012), in a paper on acute colitis and liver toxicity induced by TNF- α, demonstrated that curcumin was able to reduce the influx of neutrophils in the colon and also reduced the activity of MPO in the liver of mice. Curcumin can modulate several cytokines, such

as TNF-α, IFN-γ, IL-1 IL-6 interacting with transcription and signaling molecules (Ghandadi and Sahebkar 2016; Kahkhaie et al. 2019). In this way, the inflammatory responses mediated by these molecules are modulated by the action of curcumin.

The integrity of the intestinal barrier also can be directly influenced by the action of curcumin. In vitro studies by (Wang et al. 2017) demonstrate that curcumin was able to regulate the release of IL-1b by macrophages and intestinal epithelial cells and also its intracellular signaling pathways, which lead to rupture of the intestinal paracellular barrier. Curcumin can also regulate the expression of junction proteins of the TJ family such as occludin proteins (Wang et al. 2012), ZO-1 (Wang et al. 2012, 2017), claudins-1 and 7, and actin filaments, also acting in the cellular organization (Wang et al. 2017).

Thus, the action of curcumin in the intestine, acting to inhibit inflammatory activity and reduce oxidative stress, led to the prevention of oxidative and inflammatory changes observed in the organs of the untreated group.

With the results obtained, we conclude that the kidney was the organ most affected by the damages resulting from the experimental colitis and that nano encapsulated curcumin prevented oxidative changes and migration of inflammatory cells in organs analyzed, proving to be effective in preventing EIM resulting from the experimental colitis.

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TABLES

Table 1. Score of the Disease Activity Index (DAI), adapted from Cooper et al. (1993).

Score	Weight loss %	Stool Consistency	Visible Blood in Stool
0	0	Normal	Absent
1	1-5		
2	5-10	Pasty	
3	10-20		
4	>20	Diarrhoea	Present

Table 2. Weight of kidney, liver and lung of rats with experimental colitis after 7 and 28 days of treatment with nanoparticles containing curcumin in the control (CG), saline/water (SW) and saline groups/nanoparticles (SN), TNBS/water (TW) and TNBS/nanoparticles (TN).

		Kidney (g)	Liver (g)	Lung (g)
7 days	CG	1.49±0.05	12.71±0.62	0.99±0.09
	SW	1.48±0.06	12.12±0.64	0.67±0.05^{a}
	SN	1.40±0.05	12.89±0.66	0.99±0.08^{b}
	TW	1.06±0.03^{abc}	8.51±0.61^{abc}	0.72±0.05^{ac}
	TN	1.11±0.04^{abc}	11.21±0.33^{acd}	0.98±0.11^{bd}
28 days	CG	1.49±0.08	12.98±0.80	1.20±0.06
	SW	1.50±0.12	12.75±0.77	1.17±0.06
	SN	1.52±0.07	12.51±0.17	1.22±0.08
	TW	1.29±0.03^{abc}	12.33±0.19	1.04±0.01^{abc}
	TN	1.48±0.07^{d}	12.11±0.46	1.19±0.04^{d}

One-way Anova analyses and Tukey post-test, n = 5. Results expressed as mean ± SE. ^{a} $p < 0.05$ *vs.* CG group; ^{b} $p < 0.05$ *vs.* SW group; ^{c} $p < 0.05$ *vs.* SN group; ^{d} $p < 0.05$ *vs.* TW group.

Table 3. Activity of the antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), glutathione S-transferase (GST) and levels of reduced glutathione (GSH) and lipid peroxides (LOOH) analysed in the kidney of rats of the control (CG), saline-water (SW), saline-nanoparticles (SN), TNBS-water (TW) and TNBS-nanoparticles (TN) groups, after treatment for 7 and 28 days.

		CAT μmol/min/mg of protein	SOD U SOD/mg of protein	GST mmol/min/mg of tissue	GSH μg GSH/mg of tissue	LOOH mol/g of tissue
7 DAYS	CG	0.24±0.03	0.27±0.016	4.16±0.035	345.5±9.73	75.34±1.41
	SW	0.22±0.01	0.24±0.007	4.73±0.51	437.9±14.73[a]	77.86±0.95
	SN	0.21±0.01	0.25±0.006	4.71±0.42	316.6±5.98	76.32±1.29
	TW	0.13±0.01[abc]	0.17±0.016[abc]	3.86±0.30	273.1±15.82[ab]	82.05±0.93[a]
	TN	0.26±0.01[d]	0.28±0.022[d]	4.26±0.34	331.5±15.69[bd]	74.29±0.98[d]
28 DAYS	CG	0.080±0.001	1.31±0.09	7.23±0.52	1499±35.58	71.88±1.96
	SW	0.069±0.001	1.25±0.09	7.21±0.47	1498±45.79	72.93±1.26
	SN	0.080±0.004	1.54±0.04	8.14±0.20	1619±25.54	71.56±1.25
	TW	0.054±0.004[abc]	1.10±0.02[c]	4.39±0.24[abc]	1445±64.97	77.56±0.34[ac]
	TN	0.068±0.003[d]	1.25±0.07	6.73±0.30[d]	1556±28.16	71.15±0.72[d]

One-way Anova and Tukey's post-test, n = 5. Results expressed as mean ± SE. [a] $p < 0.05$ *vs.* CG group; [b] $p < 0.05$ *vs.* SW group; [c] $p < 0.05$ *vs.* SN group; [d] $p < 0.05$ *vs.* TW group.

Table 4. Activity of the antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), glutathione S-transferase (GST) and levels of reduced glutathione (GSH) and lipid peroxides (LOOH) analysed in the liver rats of the control (CG), saline-water (SW), saline-nanoparticles (SN), TNBS-water (TW) and TNBS-nanoparticles (TN) groups, after 7 and 28 days of treatment. One-way Anova and Tukey post-test, n = 5. Results expressed as mean ± SE.

		CAT µmol/min/mg protein	SOD U SOD/mg protein	GST mmol/min/ mg tissue	GSH µg GSH/mg tissue	LOOH mol/g tissue
7 DAYS	CG	0.17±0.007	0.17±0.011	12.81±1.01	252.7±11.13	45.69±0.76
	SW	0.15±0.014	0.17±0.017	11.40±0.68	243.5±7.18	46.95±1.01
	SN	0.15±0.010	0.17±0.008	11.07±0.54	245.0±3.84	45.87±1.40
	TW	0.13±0.008	0.16±0.011	9.41±1.44	244.7±6.71	48.26±0.80
	TN	0.18±0.013	0.19±0.007	12.89±0.92	245.6±11.8	44.13±1.27
28 DAYS	CG	0.089±0.002	0.51±0.02	6.66±0.38	613.3±10.89	81.64±1.29
	SW	0.082±0.004	0.53±0.02	6.68±0.51	590.9±20.04	82.86±0.74
	SN	0.089±0.002	0.56±0.03	6.08±0.19	593.3±27.00	81.18±0.46
	TW	0.086±0.001	0.51±0.02	6.25±0.13	507.3±20.21[abc]	86.64±0.37[abc]
	TN	0.080±0.001	0.54±0.03	6.66±0.27	609.4±12.60[d]	82.49±1.81[d]

[a] $p < 0.05$ *vs.* CG group; [b] $p < 0.05$ *vs.* SW group; [c] $p < 0.05$ *vs.* SN group; [d] $p < 0.05$ *vs.* TW group.

Table 5. Activity of the antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), glutathione S-transferase (GST) and levels of reduced glutathione (GSH) and lipid peroxides (LOOH) analysed in the lungs of rats of the control (CG), saline-water (SW), saline-nanoparticles (SN), TNBS-water (TW) and TNBS-nanoparticles (TN) groups, after 7 and 28 days of treatment.

		CAT	SOD	GST	GSH	LOOH
		μmol/min/mg de protein	U SOD/mg protein	mmol/min/mg tissue	μg GSH/mg tissue	mol/g tissue
7 DAYS	CG	0.09±0.004	0.76±0.09	1.44±0.22	245.4±21.12	24.93±0.68
	SW	0.08±0.008	0.64±0.06	1.35±0.07	210.3±8.09	25.41±0.57
	SN	0.09±0.006	0.56±0.03	1.48±0.06	200.6±6.06	24.82±0.75
	TW	0.08±0.008	0.34±0.04[ab]	1.29±0.08	186.0±4.75[a]	28.87±0.21[ac]
	TN	0.10±0.004	0.66±0.02[d]	1.53±0.07	188.8±3.26[a]	25.87±0.42
28 DAYS	CG	0.10±0.010	1.16±0.08	1.62±0.05	243.4±8.80	19.76±0.90
	SW	0.09±0.009	1.45±0.02	1.42±0.08	234.8±6.37	21.62±0.96
	SN	0.10±0.010	0.15±0.14	1.51±0.15	244.6±16.66	17.95±2.14
	TW	0.09±0.005	1.21±0.05	1.46±0.04	233.2±9.86	21.76±0.95
	TN	0.11±0.005	1.18±0.04	1.43±0.02	240.8±5.40	21.31±0.51

GST 7 day-treatment - Kruskal-Wallis and Dunns post-test, others One-way Anova analyses and Tukey post-test, n = 5. Results expressed as mean ± SE. [a] $p < 0.05$ *vs.*; [b] $p < 0.05$ *vs.* SW group; [c] $p < 0.05$ *vs.* SN group; [d] $p < 0.05$ *vs.* TW group.

FIGURES

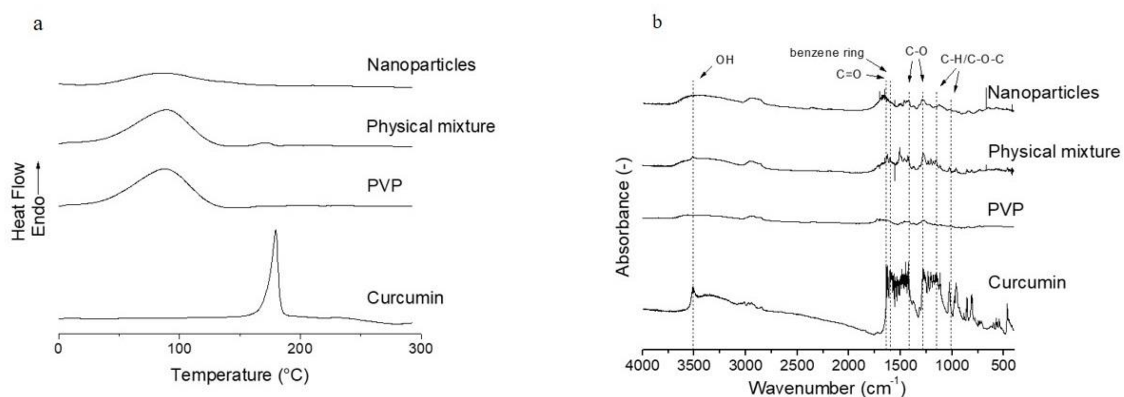

Fig. 1 Differential Scanning Calorimetry (DSC) thermograms of curcumin, PVP, physical mixture (curcumin: PVP, 1:10 m / m) and nanoparticules (a). Fourier Transform Infrared Spectroscopy **(**FTIR) spectra of curcumin, PVP, physical mix (curcumin: PVP, 1:10 m / m) and nanoparticles (b)

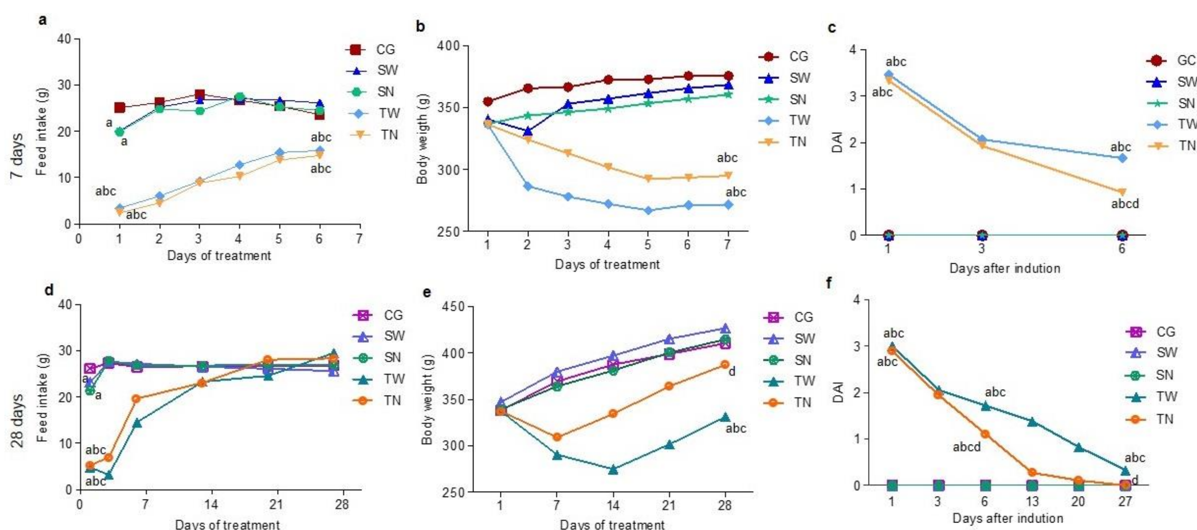

Fig. 2 Initial and final feed intake (a,d); body weight (b,e) and Disease Activity Index – DAI (c,f) of rats in 7 and 28-day experimental periods in the control group (GC), saline/water group (SW), saline/nanoparticles group (SN), TNBS/water (TW) and TNBS/nanoparticles group (TN). One-way Anova and Tukey post-test, n = 5. Results expressed as mean ± SE. [a] $p < 0.05$ *vs.* CG group; [b] $p < 0.05$ *vs.*; [c] $p < 0.05$ *vs.* SN group; [d] $p < 0.05$ *vs.* TW group

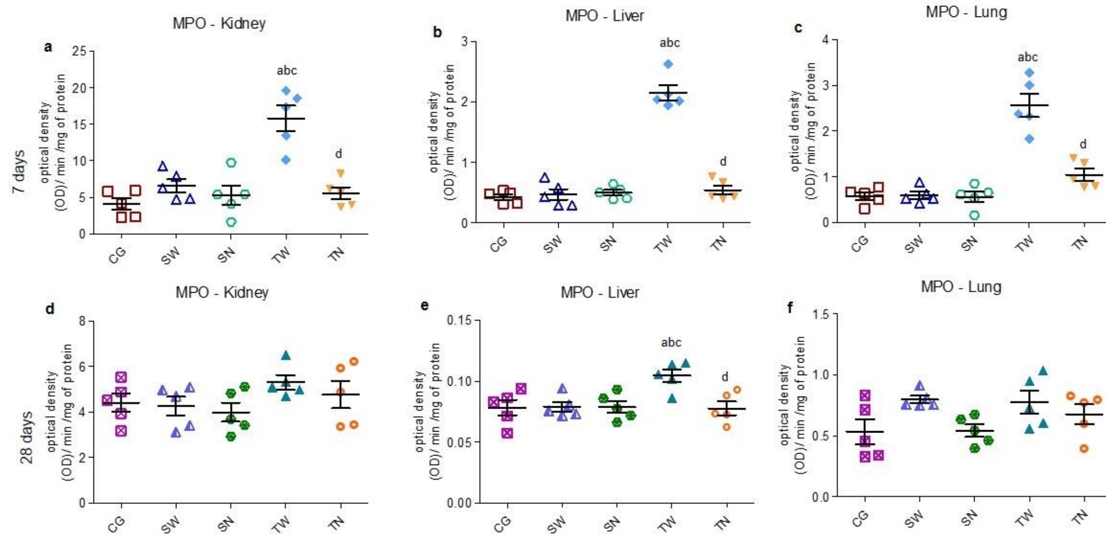

Fig. 3 Mieloperoxidase (MPO) activity in kidney (a,d) liver (b,e) and lung(c,f) of rats in 7 and 28-day experimental periods in the control group in the control group (GC) saline/water group (SW), saline/nanoparticles group (SN), TNBS/water (TW) and TNBS/nanoparticles group (TN). One-Way Anova and Tukey post-test, n = 5. Results expressed as mean ± SE. ^{a} $p < 0.05$ *vs.* CG group; ^{b} $p < 0.05$ *vs.* SW group; ^{c} $p < 0.05$ *vs.* SN group; ^{d} $p < 0.05$ *vs.* TW group

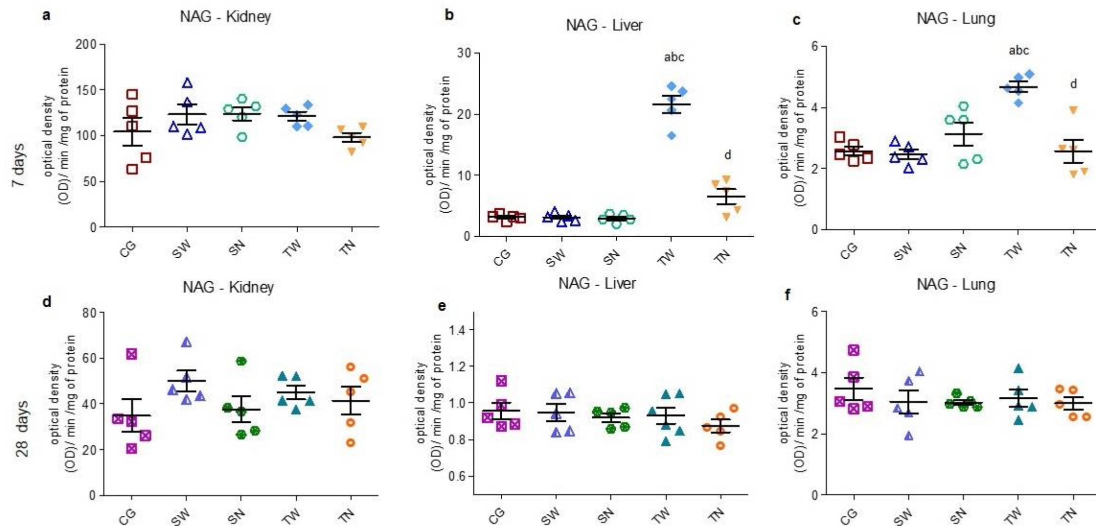

Fig. 4 N-acetylglucosaminidase (NAG) activity in kidney (a,d) liver (b,e) and lung(c,f) of rats in 7 and 28-day experimental periods in the control group (GC), saline/water group (SW), saline/nanoparticles group (SN), TNBS/water (TW) and TNBS/nanoparticles group (TN). One-Way Anova and Tukey post-test, n = 5. Results expressed as mean ± SE. ^{a} $p < 0.05$ *vs.* CG group; ^{b} $p < 0.05$ *vs.* SW group; ^{c} $p < 0.05$ *vs.* SN group; ^{d} $p < 0.05$ *vs.* TW group

ARTIGO II

Curcumina (*Curcuma longa* L.) nano encapsulada previne alterações oxidativas, inflamatórias e neurodegenerativas na colite experimental.

Curcumina (*Curcuma longa* L.) nano encapsulada previne alterações oxidativas, inflamatórias e neurodegenerativas do plexo mioentéricos decorrentes da colite experimental.

Nano encapsulated curcumin (Curcuma longa L.) prevents oxidative, inflammatory and neurodegenerative changes in the myenteric plexus resulting from experimental colitis.

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Legendas:

DC – doença de Crohn; DII – doença inflamatória intestinal; DME – dose mínima efetiva; DSS – sulfato de sódio dextrana; ERO– espécies reativas de oxigênio; GC – grupo controle; GSH, glutationa reduzida; GST – glutationa S-transferase; H_2O_2 – peróxido de hidrogênio; HuC/D^+ - proteína para estudo dos neurônio mioentéricos de população geral; IAD- índice de atividade da doença; IFN-γ - interferon gama; IL – interleucina; IMC – índice de massa do cólon distal; LOOH, - hidroperóxido lipídico; MPO – mieloperoxidase; NAG – N-acetilglucosaminidase; NO - óxido nítrico; NO_2^- - nitrito; $nNOS^+$ - óxido nítrico sintase neuronal; P2X7R – receptores P2X7; PBS – tampão fosfato salino; PVP – poli(vinilpirrolidona); RCU- retocolite ulceratica; SA – grupo salina/água; SN – grupo salina/nanopartículas; SNE – sistema nervoso entérico; TA – grupo TNBS/água; TGO – transaminase glutâmica oxalacética; TGP – transaminase glutâmica pirúvica; TN – grupo TNBS/nanopartículas; TN_{10} - grupo TNBS tratado com 10 mg/kg de nanopartículas; TN_{25} - grupo TNBS tratado com 25 mg/kg de nanopartículas; TN_{50} – grupo TNBS tratado com 50 mg/kg de nanopartículas; TNBS - ácido 2,4,6- trinitrobenzenossulfônico; TNF-α – fator de necrose tumoral alfa.

RESUMO

Determinamos a dose mínima efetiva de curcumina nanoencapsulada e avaliamos seus efeitos sobre alterações geradas pela colite experimental em ratos Wistar. A colite foi induzida por ácido 2,4,6-trinitrobenzeno sulfônico via enema. Foram testadas 3 doses (10, 25 e 50 mg/kg) e a dose de 25 mg/kg foi utilizada no tratamento por 7 e 28 dias. Utilizamos um Índice de Atividade da Doença (IAD) para avaliar os ratos. Após eutanásia amostras do cólon distal foram destinadas para análise histológica, da atividade da mieloperoxidase (MPO), N-acetilglucosaminidase (NAG) e níveis de nitrito (NO_2^-), de estresse oxidativo e para imunomarcação da população neuronal geral (HuC/D$^+$) e subpopulação nitrérgica (nNOS$^+$) do plexo mioentérico. Os ratos colíticos não tratados apresentaram elevados IAD, aumento do *score* inflamatório, da atividade da MPO, NAG e dos níveis de NO_2^-; alterações oxidativas e redução da densidade e do perfil neuronal em ambas populações analisadas aos 7 e 28 dias. O tratamento reduziu o IAD, a atividade da MPO, NAG e níveis de NO_2^-, as alterações oxidativas e a neurodegeneração em ambos períodos. Concluímos que a dose de 25 mg/kg de curcumina nanoencapsulada foi evitou as alterações inflamatórias, oxidativas e preveniu a neurodegeneração do plexo mioentérico em ratos com colite experimental.

Palavras chave: Estresse oxidativo; inflamação; neurônios entéricos; TNBS, polivinilpirrolidona.

ABSTRACT

We determined the minimum effective dose of nanoparticles containing curcumin and evaluated its effects on changes caused by experimental colitis in Wistar rats. Colitis was induced by 2,4,6-trinitrobenzene sulfonic acid via enema. Three doses were tested (10, 25 and 50 mg/kg) and the dose of 25 mg/kg was used in the treatment for 7 and 28 days. We used a Disease Activity Index (DAI) to evaluate the rats. After euthanasia, samples of the distal colon were used for histological analysis, myeloperoxidase (MPO), N-acetylglucosaminidase (NAG) and nitrite levels (NO_2^-), oxidative stress and immunostaining of the general neuronal population (HuC/D$^+$) and nitrergic subpopulation (nNOS$^+$) of the myenteric plexus. Untreated colitic rats showed high IAD, increased inflammatory score, MPO, NAG activity and NO_2^- levels; oxidative changes and reduced density and neuronal profile in both populations analyzed at 7 and 28 days. The treatment reduced IAD, MPO activity, NAG and NO_2^- levels, oxidative changes and neurodegeneration in both periods. We concluded that the dose of 25 mg/kg of nano encapsulated curcumin prevented inflammatory and oxidative changes and myoenteric plexus neurodegeneration in rats with experimental colitis.
Keywords: Oxidative stress; inflammation; enteric neurons; TNBS, polyvinylpyrrolidone.

Destaques

Ácido 2,4,6-trinitrobenzenosulfônico (TNBS) induz resposta inflamatória em ratos

Inflamação, estresse oxidativo e neurodegeneração entérica caracterizam a colite experimental

Curcumina nanoencapsulada ameniza as alterações da colite induzida por TNBS.

1 INTRODUÇÃO

As doenças inflamatórias intestinais (DII) se caracterizam por alternância entre períodos ativos e de remissão dos sintomas compreendendo entre outras a Doença de Crohn (DC) e a Retocolite ulcerativa (RCU). Estas patologias apresentam alta incidência e prevalência em países desenvolvidos como os da América do Norte e norte da Europa. No entanto há um aumento na incidência nos países em desenvolvimento, onde a industrialização vem crescendo, como os países da África, da Ásia e da América do Sul.[1]

As manifestações clínicas que compreendem as disfunções intestinais envolvem alterações do Sistema Nervoso Entérico (SNE) [2] sendo que os danos gerados na fase ativa da doença persistem na fase quiescente.[3] O SNE é acometido por ação dos mediadores do processo inflamatório[4] e também pelo aumento dos radicais livres e estresse oxidativo decorrentes da inflamação tecidual.[2,5]

O SNE está localizado ao longo do trato gastrointestinal e sua integridade funcional é responsável pelo desempenho das funções fisiológicas deste trato.[3] A inflamação intestinal resulta na redução do número de neurônios entéricos[6] e alteração do seu código químico.[7,8]

Os tratamentos convencionais pra as DII, a base de corticoides e imunomoduladores, podem trazer diversos efeitos colaterais[9,10] e a busca de terapias alternativas complementares podem melhorar a qualidade de vida dos pacientes.

A curcumina é um diferuloilmetano [1,7-bis(4-hidroxi3-metoxifenil)-1,6- heptadieno-3,5-diona] sendo um dos pigmentos curcuminóides predominantes na raiz da *Curcuma longa* Linn (*C.longa* L.).[11] É um composto rico em polifenois, apresentando propriedades anti-inflamatórias,[12,13] antioxidantes e sequestradora de radicais livres[14,15] entre outras. No entanto sua forma *in natura* possui baixa solubilidade em água[16,17] sendo degradada em condições de pH básico e neutro além de possuir fotossensibilidade.[18,19] Quando administrada oralmente possui baixa absorção, rápida metabolização hepática e intestinal, rápida excreção e ausência de atividade farmacológica de seus metabólitos resultando em baixa biodisponibilidade.[20] Esta característica faz com sejam necessárias altas doses por via oral, a fim de atingir o efeito esperado nos tratamentos.[21]

Várias formulações têm sido desenvolvidas a fim de melhorar a biodisponibilidade da curcumina, entre elas o encapsulamento na forma de nanopartículas, lipossomas, complexos com fosfolipídios e ciclodextrinas. Os resultados da encapsulação apontam no sentido de uma melhor absorção e distribuição tecidual, permitindo assim a redução da dose utilizada. [21,22]

Muitos estudos relatam o uso da curcumina no tratamento das DIIs,[14,23–26] porém o uso da curcumina *in natura* ou curcumina encapsulada relacionado à neuroproteção nas DIIs ainda é pouco discutido. Como o processo inflamatório envolve as alterações das células do SNE, o uso deste produto natural com potente propriedade anti-inflamatória e antioxidante, na forma encapsulada potencializa sua ação, sendo uma alternativa no tratamento dos danos gerados pela colite experimental à inervação intrínseca e morfologia intestinal.

Assim este estudo teve por objetivo determinar uma dose mínima efetiva (DME) de curcumina nanoencapsulada para o tratamento da colite experimental e avaliar seus efeitos nas alterações clínicas, histológicas, inflamatórias e oxidativas bem como sobre o SNE.

2. MATERIAIS E MÉTODOS

2.1 Produção das nanopartículas

Rizomas de cúrcuma (*C.longa* L.) foram adquiridos no comércio local, na cidade de Campo Mourão-PR. Para a extração e encapsulação foram utilizados álcool etílico absoluto P.A (NEON, 99,8%) e poli(vinilpirrolodona) (PVP - Sigma-Aldrich, massa molecular média 40.000 g.mol-1). A extração da curcumina dos rizomas e a encapsulação em PVP foram realizadas conforme descrito por Dos Santo et al. [27] Um tubo Falcon contendo pó da cúrcuma (tamanhos entre 100 e 35 *mesh* Tyler) foi mantido em banho-maria a 25°C, sendo a sonda do ultrassom (Fisher Scientific, 120W, 100% potência, equipada com sonda de 1/8”) posicionada em seu interior, a cerca de 2cm do fundo. Separadamente o PVP foi adicionado ao solvente (álcool etílico) sob agitação branda por 5 min. Essa solução foi vertida no tubo Falcon e a solução foi sonicada por 3 min. Em regime de pulsos (30 seg de sonicação e 10 seg de pausa) a fim de evitar o sobreaquecimento da amostra. Ao fim deste tempo, a solução foi filtrada (porosidade de

0,45 µm) e levada à estufa de circulação de ar para evaporação do etanol a 60°C. O pó resultante foi armazenado em frasco protegido da luz a 10°C até ser utilizado.

As características físicas da curcumina nanoencapsulada e a eficácia do processo de encapsulação foram previamente relatadas por Dos Santos et al.[27]

2.2 Delineamento experimental

Este estudo foi realizado em duas etapas:

1) Teste para determinar a dose mínima efetiva (DME) das nanopartículas no tratamento da colite experimental. Os ratos foram tratados diariamente, por 7 dias.

2) Tratamento da colite experimental com as nanopartículas, com a DME determinada na primeira etapa. Tratamento diário por 7 e 28 dias.

Os grupos da etapa de determinação da DME e da etapa de tratamento estão dispostos na tabela 1.A indução da colite experimental, o tratamento e as avaliações *in vivo* ocorreram da mesma forma em ambas as etapas.

Tabela 1. Grupos experimentais, tipo de enema e tratamento da primeira e segunda etapa

	Grupos	Solução do enema	Tratamento via oral (gavagem)
Primeira etapa (n=5) (7 dias)	**GC**	X	X
	SA	0,6 mL Solução salina 0,9%	0,5mL de água
	TA	0,6mL TNBS/etanol 30% (v/v)	0,5mL de água
	TN_{10}	0,6mL TNBS/etanol 30% (v/v)	10 mg/kg de nanopartículas
	TN_{25}	0,6mL TNBS/etanol 30% (v/v)	25 mg/kg de nanopartículas
	TN_{50}	0,6mL TNBS/etanol 30% (v/v)	50 mg/kg de nanopartículas
Segunda etapa (n=6) (7 e 28 dias)	**GC**	X	X
	SA	0,6 mL de solução salina 0,9%	0,5 mL de água
	SN	0,6 mL de solução salina 0,9%	25 mg/kg de nanopartículas
	TA	0,6mL 15 mg TNBS/etanol 30% (v/v)	0,5 mL de água
	TN	0,6mL 15 mg TNBS/etanol 30% (v/v)	25 mg/kg de nanopartículas

GC = Grupo Controle

2.3 Animais

Foram utilizados 90 ratos Wistar (*Rattus novergicus*), machos com 90 dias de idade (355.3 ± 35.68 g), provenientes do Biotério Central da Universidade Estadual de Maringá. Os animais foram alocados no Biotério Setorial do Departamento de Ciências Morfológicas, mantidos em ciclos de 12 h de claro/escuro com temperatura controlada (22 ± 2°C), recebendo água *ad libitum* e ração padrão para roedores (Nuvital® – Nuvilab, Brasil). Os procedimentos deste estudo seguiram as orientações do Conselho Nacional de Controle de Experimentação Animal – CONCEA e foram aprovados pelo Comitê de Ética no Uso de Animal da Universidade Estadual de Maringá – CEUA/UEM (CEUA 2342171116).

2. 3.1 Indução da Colite Experimental

O enema foi induzido com ácido 2,4,6-trinitrobenzeno sulfônico (TNBS) ou solução salina 0,9%, após jejum *overnight* e anestesia com 45 mg/kg de Tiopental Sódico-Thionembutal® (Cristália Produtos Químicos Farmacêuticos, Brasil) e ocorreram como descrito por Vicentini et al.[28], sendo realizado por meio de cânula de polietileno n°4 inserida 8 cm a partir do ânus. O procedimento ocorreu com os ratos de ponta cabeça e mantidos na mesma posição durante 2 min após o enema, a fim de evitar o refluxo da solução.[29]

2.3.2 Tratamento e Avaliação *in vivo*

O tratamento foi realizado diariamente, por 7 ou 28 dias, iniciando no mesmo dia da indução do enema. A água, para os grupo controles, e as nanopartículas diluídas em água, foram administradas via oral (gavagem).

A avaliação *in vivo* foi realizada diariamente e teve início no primeiro dia após o enema, sendo que o peso inicial foi referente à pesagem ocorrida antes da indução da colite. A avaliação dos ratos, para gerar o índice de Atividade da Doença (IAD), foi adaptada dos critérios de Cooper et al. [30] Foram pontuados: peso corporal (0 – sem perda de peso, 1 – até 5% de perda do peso corporal, 2 - entre 5,1-10% de perda do peso corporal, 3 – 10,1-20% de

perda do peso corporal, 4 - acima de 20% de perda do peso corporal), consistência das fezes (0 – normal, 2 – perda de consistência, 4 – diarreia) e ocorrência de sangue retal (0 – ausente 4 – presente). Cada critério foi pontuado individualmente e uma média diária foi gerada correspondendo ao IAD.[31] Para concluir a avaliação clínica também foi verificado o consumo de ração (peso em g), obtido pela subtração das sobras de ração do montante oferecido, após 24 h.

2.3.3 Coleta dos tecidos

Na primeira etapa, após os 7 dias de tratamento com as doses testes, os ratos foram anestesiados com 120 mg/kg de Tiopental Sódico-Thionembutal® (Cristália Produtos Químicos Farmacêuticos, Brasil), sendo realizada a laparotomia mediana para a retirada do cólon distal. O segmento foi aberto na borda mesocólica e, após lavagem com solução salina 0,9%, foi realizada a avaliação macroscópica para pontuação do nível da lesão baseado no escore de Appleyard and Wallace[32] : (0) ausência de danos, (1) hiperemia localizada sem ulcerações, (2) ulcerações com pouca hiperemia e sem espessamento de parede, (3) ulcerações com inflamação em apenas um local, (4) dois ou mais sítios de ulceração e inflamação, (5) dois ou mais sítios maiores de inflamação e ulceração, ou um sítio maior de inflamação e ulceração estendendo-se por mais que 1cm de comprimento, (6-10) Se o dano for maior que 2cm de comprimento, adiciona-se 1 ponto a cada cm envolvido além da medida inicial, ainda:(0) ausência de aderências intestinais, (1) com aderências intestinais .

Em seguida amostras do segmento foram destinadas para análises bioquímicas de avaliação dos níveis teciduais da mieloperoxidase (MPO) e N-acetilglucosaminidase (NAG).

Na segunda etapa, após o tratamento com a DME por 7 ou 28 dias, seguiu-se a eutanásia para coleta dos tecidos. Os ratos foram anestesiados com 45 mg/kg de Tiopental Sódico-Thionembutal® e coletados 4 mL de sangue por meio de punção cardíaca, para análise da hepatotoxidade. Em seguida receberam uma overdose do mesmo anestésico (80 mg/kg) sendo realizada a laparotomia mediana para a retirada do cólon distal.

Foi mensurado o comprimento do intestino grosso e posteriormente o comprimento e largura do cólon distal, seguido de pesagem deste segmento. Foi calculado o índice de massa

do cólon distal (IMC) (peso cólon distal/peso corporal X 100%)[23]. Amostras do cólon distal foram destinadas para as análises imuno-histoquímicas dos neurônios do plexo mioentérico, em seguida o segmento foi aberto pela borda mesocólica, lavado com solução salina 0,9% e realizada a avaliação macroscópica do nível da lesão baseado no *score* de Appleyard e Wallace[32] descrito acima (item 2.3.3). O restante do tecido teve amostras destinadas à análise histológica, análise do estado oxidativo, dosagem das enzimas inflamatórias MPO e NAG e dos níveis de nitrito (NO_2^-).

2.4 Transaminases Hepáticas

As amostras sanguíneas foram centrifugadas 15 min a 3000 g, e o soro armazenado à -80°C. As análises bioquímicas das enzimas hepáticas transaminase glutâmico oxalacético (TGO) e transaminase glutâmico pirúvica (TGP) foram realizadas usando Kits de determinações bioquímicas Gold Analisa®.

2.5 Análise Histológica

Amostras do cólon distal foram lavadas em tampão fosfato salino (PBS 0,1M, pH 7.4), fixadas em paraformaldeído 4%, emblocadas em parafina e realizados cortes histológicos semisseriados (5 µm de espessura) em micrótomo rotativo (Leica® RM2145) sendo dispostos 5 cortes/lâmina. Os cortes foram corados em Hematoxilina-Eosina para a caracterização morfométrica das túnicas intestinais (parede total, mucosa, submucosa, muscular) e caracterização qualitativa dos danos inflamatórios. Os danos inflamatórios foram graduados semi-quantitativamente, utilizando-se os critérios modificados de Boyer et al.[3] pontuando: a perda da arquitetura da mucosa (0-ausente, 1-leve, 2-médio, 3-severo); a extensão do infiltrado inflamatório celular (0 a 3 - ausente para transmural); a formação de abcessos na cripta (0-ausente, 1- presente); a intensidade e dilatação de vasos sanguíneos (0-normal, 1- elevada).

As imagens foram capturadas em microscópio óptico Olympus com kit para captura de imagens Axioscop 2 Plus Zeiss® e as análises estimadas pela mensuração de 100 pontos

aleatórios/animal através do sistema de análises de imagens (Image Pró Plus® 4.5 – Media Cybernetics, Inc.), com resultados expressos em µm.

2.6 Estado Oxidativo Tecidual

As metodologias utilizadas para análise do estado oxidativo tecidual foram descritas por Rissato et al.[33] Após lavagem em PBS as amostras foram mantidas em freezer -80°C. Para proceder as análises as amostras foram homogeneizadas em tampão fosfato de potássio e uma alíquota separada para quantificação dos níveis de glutationa reduzida (GSH) sendo o restante centrifugado. O sobrenadante foi destinado às análises das atividades das enzimas superóxido dismutase (SOD) e glutationa S-transferase (GST), para os níveis de hidroperóxidos lipídicos (LOOH) e quantificação de proteínas.

2.6.1 Determinação da atividade da superóxido dismutase (SOD) e glutationa S-transferase (GST)

Para determinar a atividade da SOD, tampão TRIS-HCl EDTA foi adicionado as amostras e misturado ao pirogalol. A reação foi interrompida com ácido clorídrico e levada a centrifugação. A absorbância utilizada para leitura em 405nm e os resultados foram expressos em U SOD/mg de proteína.[34]

A atividade da GST foi determinada adicionando tampão fosfato de potássio ao sobrenadante e em uma alíquota desta solução foi misturada a solução de reação. A absorbância utilizada para leitura em 340 nm, usando o coeficiente de extinção de 9,6 mM/cm. Os resultados foram expressos em mmol/min/mg de proteína.[35]

2.6.2 Quantificação dos níveis de glutationa reduzida (GSH) e hidroperóxidos lipídicos (LOOH)

Para determinar os níveis de GSH, acrescentou-se ácido tricloroacético 12% ao homogenato, seguido por centrifugação. Após centrifugação a reação foi iniciada com 5.5-

ditiobis 2-ácido nitrobenzóico sendo a leitura realizada com absorbância de 412nm. Os valores individuais foram interpolados em uma curva padrão de GSH, expressos em µg GSH/g tecido.[36]

Para a quantificação de LOOH foi utilizado o ensaio de oxidação de ferro II na presença de xilenol laranja.[37] Amostras do sobrenadante foram homogeneizadas em metanol e centrifugadas. O sobrenadante foi adicionado ao meio reacional e incubado, no escuro, em TA. A absorbância utilizada para leitura foi de 560nm, usando o coeficiente de extinção de $4.3 mM^{-1} cm^{-1}$ e os resultados expressos em mmol/mg de tecido.

2.7 Atividade das enzimas inflamatórias mieloperoxidase (MPO) e N-acetilglucosaminidase (NAG)

Para a avaliação da atividade enzimática da MPO e da NAG, foi utilizado o precipitado resultante das amostras centrifugadas para as análises do estado oxidativo tecidual. O precipitado foi ressuspendido em tampão fosfato de potássio 0,08 M com hexadeciltrimetilamônio (pH 5.4). As amostras foram novamente centrifugadas (20 min, 11000 g).

A reação para MPO ocorreu na presença de tetrametilbenzidina (18.4 mM) e peróxido de hidrogênio (H_2O_2) sendo interrompida com acetato de sódio (1.46 M, pH 3.0). A leitura foi realizada em 620 nm.[38]

A atividade da NAG foi avaliada na presença de tampão citrato (50 mM pH 4,5) e solução de 4-nitrophenyl N-acetyl-β-D-glucosaminide (2,24 mM). Após incubação, por 60 min a 37º C, a reação foi interrompida com tampão glicina (200 mM pH 10,4) e a realizada a leitura em espectrofotômetro em 405 nm.[39] Os resultados foram expressos em densidade óptica (D.O)/min/mg de proteína para ambas as análises.

2.8 Quantificação dos níveis de nitrito (NO_2^-)

Amostras do cólon distal foram homogeneizadas em PBS e centrifugadas a 3000 g por 1 min. O sobrenadante foi destinado para a quantificação dos níveis de NO_2^-, indicador da produção de óxido nítrico (NO), utilizando a reação de Griess adaptado Tiwari et al.[40] Foram

utilizadas a solução A (ácido fosfórico, sulfanilamida, H_2O destilada) e a solução B (N-1-naphitiletilenodiamida, H_2O destilada) misturadas vol/vol, sendo então adicionadas as alíquotas das amostras em placa de 96 poços. A leitura foi realizada em espectrofotômetro (570 nm). A curva padrão foi feita utilizando diluições de $NaNO_2$ de 100 mM a 1.56 mM. Os resultados foram expressos em µg/µL.

2.9 Plexo Mioentérico do Cólon Distal - População neuronal geral (HuC/D$^+$) e subpopulação nitrérgica (nNOS$^+$)

A imunomarcação do plexo mientérico do cólon distal foi realizada de acordo com Vicentini et al.[28] As amostras foram lavadas em PBS preenchidas com paraformaldeído 4% e, com as extremidades amarradas, deixadas imersas no mesmo fixador. Para a imunomarcação neuronal os preparados totais da túnica muscular foram lavados em solução de PBS+Triton X-100 e deixados em uma solução de bloqueio. Foram então incubados em solução contendo os anticorpos primários anti-HuC/D (mouse, 1:800; n°. catálogo A21271 Invitrogen, USA) e anti-nNOS (rabbit 1:500; nº do catálogo 8309, Santa Cruz Biotec., USA). Após a incubação, os preparados totais foram lavados em PBS+Triton X-100 e incubados em nova solução com os anticorpos secundários Alexa Fluor 546 anti-mouse (1:500; n°. catálogo A10036, Invitrogen, USA) e Alexa Fluor 488 anti-rabbit (1:500; n°. catálogo A21206, Invitrogen, USA) e por fim realizadas lavagens com PBS. Os preparados totais foram montados entre lâmina e lamínula com ProLong® Gold antifade reagent (Invitrogen, USA). Um controle negativo foi realizado em todas as reações.

2.9.1 Análise morfoquantitativa neuronal

Para a análise morfoquantitativa dos neurônios HuC/D$^+$ e nNOS$^+$ foi utilizado microscópio de fluorescência Olympus FSX-100. As imagens foram capturadas em objetiva de 20x por câmera de alta resolução, sendo transmitidas para um microcomputador. As quantificações foram realizadas por amostragem em 40 imagens/animal, capturadas

aleatoriamente na circunferência intestinal, sendo que a área de cada imagem foi de $0.0147cm^2$ e a área total por animal foi de $0,588\ cm^2$. Os resultados foram expressos em $neurônios/cm^2$.

Para a análise morfométrica foram mensuradas aleatoriamente as áreas de 100 corpos celulares de neurônios/animal. Os resultados foram expressos em μm^2. Estas análises foram realizadas utilizando o sistema de análises de imagens Image Pró Plus® 4.5 – Media Cybernetics, Inc.

2.10 Análises Estatísticas

A normalidade dos dados foi avaliada pelo teste de Kolmogorov-Sminov. Dados não paramétricos foram analisados pelo teste de Kruskal-Wallis e pós-teste de Dunn's enquanto os dados paramétricos foram submetidos à Análise de Variância (One-way ANOVA) e pós-teste de Tukey sendo os resultados expressos como média±desvio padrão com o nível de significância de 5%. Para estas análises foi utilizado o software Graph Pad Prism® 5.0.

3 RESULTADOS

3.1 Etapa 1: Determinação da Dose Mínima Efetiva (DME)

Não houve diferença no consumo inicial de ração entre os grupos TA (10,10±3,45) TN_{10} (9,30 ±3,83), TN_{25} (5,78±1,33) e TN_{50} (9,49±2,67), porém estes apresentaram menor consumo em relação aos grupos GC (28,98±3,00) e SA (20,35±4,17) ($p<0,001$). O consumo final de ração foi menor no grupo TN_{50} (22,98±5,26) comparado ao grupos GC (28,78±2.15) ($p<0,05$) e os demais grupos não diferiram entre si.

No início da etapa de teste da DME não houve diferença de peso corporal entre os grupos. Ao final da etapa de teste, o peso corporal dos grupos TA (347,5 g ±14,29, $p<0,01$), TN_{10} (343,7 g ±15,68, $p<0,001$), TN_{25} (347,8 g ±14,19, $p<0,01$) e TN_{50} (348,8 g ±20,97, $p<0,01$) foram menores comparado ao grupo GC (387,0 g ±10,86). Porém não houve diferença no peso corporal dos grupos tratados com as diferentes doses em relação ao grupo TA.

Ao final da primeira etapa, o IAD do grupo TA (1,19±0,16) foi maior que do GC (0,0±0,0, p<0,001). Os grupos TN_{25} (0,46±0,08, p<0,001) e TN_{50} (0,59±0,12, p<0,01) apresentaram menor IAD comparado ao grupo TA (1,19±0,12), no entanto o grupo TN_{10} (0,93±0,12) não diferiu do grupo TA (1,19±0,12, p<0,05).

A avaliação macroscópica do cólon distal do grupo TA apontou regiões hiperêmicas, inflamadas e com grandes ulcerações, resultando em alto *score* macroscópico (8,00±0,27) comparado ao GC (0,0±0,0, p<0,001). Os grupos TN_{10} (8,66±0,43), TN_{25} (7,10±0,40) e TN_{50} (7,00±0,31) não apresentaram redução significativa comparado ao grupo TA.

A atividade da enzima MPO estava aumentada no grupo TA (14,11±2,76) comparado ao GC (3,55±0,71, p<0,001). Os grupos TN_{25} (6,62±2,44) e TN_{50} (7,23±1,21) apresentaram menor atividade enzimática comparado ao grupo TA (14,11±2,76, p<0,001), no entanto o grupo TN_{50} ainda apresentou atividade enzimática maior em relação ao grupo GC (3,55±0,71, p<0,05). No grupo TN_{10} (13,93±2,44) a atividade da MPO não diferiu do grupo TA (14,11±2,76). A atividade da NAG também foi maior no grupo TA (108,3±17,77) comparado ao GC (79,50±7,77, p<0,01). Os grupos TN_{25} (71,29±5,04, p<0,001) e TN_{50} (78,93±3,89, p<0,01) tiveram menor atividade enzimática comparado ao grupo TA (108,3±17,77), e o grupo TN_{10} (94,82±12,13) não diferiu em comparação ao grupo TA.

Concluiu-se que a DME indicada para o tratamento da colite experimental com as nanopartículas foi de 25 mg/kg por ter reduzido o IAD e as atividade enzimáticas da MPO e NAG comparado ao grupo não tratado.

3.2 Etapa 2: Tratamento da colite experimental com a DME de 25 mg/kg

A colite experimental levou a ocorrência de diarreia, presença de sangue retal, redução do consumo de alimentos e peso corporal. No primeiro dia após a indução o consumo de ração foi menor no grupo nos grupos TA e TN comparado ao GC, aos 7 e 28 dias de tratamento. Ao final dos 7 dias o consumo de ração no grupo TA permaneceu menor em relação aos grupos GC, SA e SN e no grupo TN o consumo não diferiu do grupo TA e foi menor em relação ao grupo GC (Fig. 1A). Ao final dos 28 dias de tratamento o consumo de ração foi maior nos grupos TA e TN comparado aos grupos GC e SA (Fig. 1B). A partir do primeiro dia após a indução da colite houve redução do peso corporal nos grupos TA e TN em ambos os períodos

experimentais. Após 7 dias de tratamento o peso corporal permaneceu menor nos grupos TA e TN em relação aos grupos GC, SA e SN (Fig. 1C). Após 28 dias de tratamento o grupo TN apresentou maior peso corporal comparado a TA (Fig. 1D). O IAD nos grupos TA e TN foram maiores no primeiro dia após a indução em ambos os períodos experimentais, comparado aos grupos GC, SA e SN. Houve redução do IAD ao longo do tratamento sendo que a partir do 7º dia, em ambos períodos experimentais, o IAD do grupo TN foi menor que o do grupo TA, ainda permanecendo maior que o IAD dos grupos GC, SA e SN (Figs. 1E, 1F). No período experimental de 28 dias foi possível observar que o IAD do grupo TN igualou-se aos grupos CG, SA e SN no 14º dia de tratamento. O IAD do grupo TA permaneceu maior que o dos grupos CG, SA e SN até o final do tratamento de 28 dias(Fig. 1F).

A avaliação dos parâmetros biométricos do intestino apontou que houve redução do comprimento total do intestino grosso no grupo TA comparado ao grupo GC após 7 dias. Após 7 e 28 dias foram observadas a redução do comprimento, aumento do peso, da largura do cólon distal e do IMC, no grupo TA comparado ao GC. Com o tratamento de 7 dias, o comprimento total do intestino, o comprimento, o peso e a largura do cólon distal não diferiram entre os grupos TN e TA, no entanto o IMC foi menor no grupo TN comparado ao grupo TA. Após o tratamento por 28 dias, o peso do cólon distal e o IMC foram menores no grupo TN comparado ao TA (Tabela 2).

A avaliação macroscópica do cólon distal após 7 dias de tratamento apontou lesões severas no grupo TA e TN (Fig. 2A) com ulcerações de tamanhos variados (*score* 8-9.5) gerando alto *score* macroscópico comparado ao grupo GC (Fig. 2B), sem influência do tratamento neste parâmetro. Após 28 dias foi observado uma cicatrização nas feridas e ulcerações de tamanhos reduzidos (*score* 3-5) do grupo TA (Fig. 2C), que apresentou maior *score* macroscópico comparado ao GC (Fig. 2D). O grupo TN apresentou melhor cicatrização (Fig. 2C.), reduzindo o *score* macroscópico (*score* 1-3) porém sem diferenciar do grupo TA (Fig. 2D).

3.2.1 Transaminases Hepáticas

Após 7 dias experimentais os níveis das enzimas TGO (59,67±5,95) e TGP (22,67±1,32) no grupo TA, foram maiores comparado ao GC (50,00±4,20, e 19,25±1,60) (*p*<0,05). A atividade da TGO (47,00±3,31) e da TGP (17,42±1,53) no grupo TN foi menor que no grupo TA (59,67±5,95 e 22,67±1,32) *(p*<0,001, *p*<0,01). No período experimental de 28 dias apenas a atividade da TGO estava maior no grupo TA (48,83±2,27) comparado ao GC (39,17±4,20, *p*<0,05). Ao final do tratamento no grupo TN (39,50±5,45) os níveis desta enzima foram menores comparado ao grupo TA (48,83±2,27) (*p*<0,05).

Tabela 2. Parâmetros biométricos: comprimento total do intestino grosso (CI), comprimento do cólon distal (CC), peso do cólon distal (PC), largura do cólon distal (LC), Índice de Massa do Cólon (IMC), relação peso/comprimento do cólon distal (P/C) dos ratos dos grupos controle (GC), salina água (SA), salina/nanopartículas (SN), TNBS/água (TA) e TNBS/ nanopartículas (TN) tratados por 7 e 28 dias.

		GC	SA	SN	TA	TN
7 dias	**CI (cm)[a]**	14,33±0,81	14,42±0,58	14,33±1,16	12,42±0,73[«^$]	13,23±0,68
	CC(cm)[a]	9,16±0,60	8,70±0,40	8,85±0,58	7,35±0,69[¢^$]	7,71±0,63
	PC(g)[b]	1,22±0,13	1,14±0,07	1,24±0,07	3,72±0,53[*^#]	2,79±0,76
	LC(cm)[b]	1,01±0,04	0,96±0,05	0,95±0,05	2,85±0,13[*^€]	2,56±0,27[¥$]
	IMC(%)[a]	0,34±0,05	0,33±0,04	0,36±0,03	1,27±0,28[¢¤€]	0,92±,020[¢¤€£]
28 dias	**CI (cm)[b]**	12,75±1,03	13,00±0,70	12,92±0,49	11,75±0,27	11,67±0,51
	CC(cm)[b]	8,91±0,80	7,75±0,41	8,25±0,27	6,16±0,25[*]	5,41±0,37[*$]
	PC(g)[a]	1,33±0,12	1,23±0,23	1,19±0,16	2,15±0,34[¢¤€]	1,40±0,13[&]
	LC(cm)[b]	0,95±0,05	1,00±0,08	0,93±0,05	1,78±0,07[«¥$]	1,50±0,16[$]
	IMC(%)[a]	0,34±0,01	0,33±0,04	0,34±0,05	0,68±0,08[¢¤€]	0,36±0,03[&]

[a]One-way ANOVA, e pós-teste de Tukey. b Kruskal-Wallis e pós teste de Dunn's. Resultados expressos como média±desvio padrão (n=6).

[*] *p*<0,05 *vs* GC; [«] *p*<0,01 *vs* GC; [¢] *p*<0,001 *vs* GC; [¥] *p*<0,05 *vs* SA; [^] *p*<0,01 *vs* SA; [¤] *p*<0,001 *vs* SA; [#] *p*<0,05 *vs* SN; [$] *p*<0,01 *vs* SN; [€] *p*<0,001 *vs* SN; [§] *p*<0,05 *vs* TA; [£] *p*<0,01 *vs* TA; [&] *p*<0,001 *vs* TA.

3.2.2 Análise Histológica

Os cortes histológicos dos grupos GC, SA, SN após 7 (Figs.3A, 3C, 3E) e 28 dias (Figs. 3B, 3D, 3F) revelam características morfológicas típicas nas túnicas do cólon distal.

Após 7 dias, o cólon distal dos animais do grupo TA (Fig. 3G) apresentou perda da arquitetura da mucosa com ulcerações em diversos pontos, extensa área de infiltrados inflamatórios na túnica submucosa, vasodilatação na túnica muscular. O tratamento com as nanopartículas durante 7 dias gerou um efeito positivo no grupo TN o qual apresentou redução na destruição da arquitetura e das ulcerações na túnica mucosa, porém ainda permaneceu com extensas áreas de infiltrado inflamatório (Fig. 3I). Após 28 dias, no grupo TA (Fig. 3H) a arquitetura da mucosa permaneceu desestruturada, porém com redução dos pontos de ulcerações e infiltrados inflamatórios. O tratamento por 28 dias, levou a melhor reestruturação da túnica mucosa, com evidente redução de infiltrado inflamatório (Fig. 3J).

Houve alteração na espessura das túnicas tanto aos 7 quanto 28 dias (Tabela 3). O grupo SA apresentou menor espessura das túnicas mucosa, muscular e parede total enquanto a túnica submucosa teve maior espessura comparado ao grupo GC em ambos os períodos de tratamento. O uso das nanopartículas por 7 dias fez o grupo SN apresentar menor espessura da túnica submucosa e maior espessura da túnica muscular, e aos 28 dias todas as alterações foram prevenidas em relação ao grupo SA. O grupo TA apresentou maior espessura em todas as túnicas comparado ao grupo GC aos 7 e 28 dias. No grupo TN a espessura das túnicas foi menor em relação ao grupo TA, porém permaneceu maior comparado aos grupos GC, SA, SN em ambos os períodos.

Constatou-se que as alterações histológicas, aos 7 dias,n o grupo TA levou ao maior *score* inflamatório comparado ao grupo GC e SN e aos 28 dias o *score* foi maior comparado aos grupos GC, SA e SN (Figs. 4A, 4B). Após o tratamento em ambos os períodos, o grupo TN apresentou redução não significativa do *score* em relação ao grupo TA.

As figuras 5A e 5B evidenciam gânglios mientéricos dos grupos GC de 7 e 28 dias, respectivamente. Presença constante de polimorfonucleares no interior dos gânglios nos grupos TA após os períodos experimentais de 7 (Fig. 5C) e 28 dias (Fig. 5D) e a redução qualitativa destas células após o tratamento nos diferentes períodos experimentais (Fig. 5E e 5F).

Tabela 3. Morfometria das túnicas mucosa (T.M), submucosa (T.SM), muscular (T.MU) e parede total (P.T) do cólon distal dos grupos controle (GC), salina/água (SA), salina/nanopartículas (SN), TNBS/água (TA), e TNBS/nanopartículas (TN) com a dose de 25 mg/Kg no período de 7 e 28 dias. Resultados expressos em µm.

		GC	SA	SN	TA	TN
7 dias	T.M	250,9±37,31	240,4±34,66$^{«}$	248,4±41,12	374,4±77,39$^{¢¤€}$	342,70±71,64$^{¢¤€\&}$
	T.SM	29,2±7,80	36,3±10,57$^{¢}$	31,2±6.14$^{¢¤}$	86,9±47,67$^{¢¤€}$	55,9±27,95 $^{¢¤€\&}$
	T. MU	71,0±13,58	66,8±14,20$^{¢}$	71,3±12,92$^{¤}$	92,3±15,43$^{¢¤€}$	85,0±18,54$^{¢¤€\&}$
	P.T	354,9±61,33	341,3±45,05*	350,7±52,23	543,2±86,7$^{¢¤€}$	487,4±94,74$^{¢¤€\&}$
28 dias	T.M	304.5±72,82	270,7±45,29$^{¢}$	309,5±48,70$^{*¤}$	392,2±59,41$^{¢¤€}$	314,0±44,05$^{¢¤\&}$
	T.SM	35,5±9,30	37,96±10,44$^{«}$	34,6±8,69$^{¤}$	74,0±18,42$^{¢¤€}$	41,9±7,98$^{¢¤€\&}$
	T. MU	83,3±16,64	77,0±16,48$^{¢}$	83,1±9,39$^{¤}$	178,6±56,34$^{¢¤€}$	120,1±55,04$^{¢¤€\&}$
	P.T	421,6±45,41	392,6±59,09$^{¢}$	423,7±52,56$^{¤}$	639,5±108,1$^{¢¤€}$	481,0±81,28$^{¢¤€\&}$

Kruskal-Wallis e pós teste de Dunn's. Resultados expressos como media±desvio padrão (n=5).
* *p*<0,05 vs GC; $^{«}$ *p*<0,01 *vs* GC; $^{¢}$ *p*<0,001 *vs* GC; $^{¥}$ *p*<0,05 vs SA; $^{\wedge}$ *p*<0,01 *vs* SA; $^{¤}$ *p*<0,001 *vs* SA; $^{\#}$ *p*<0,05 vs SN; $^{\$}$ *p*<0,01 *vs* SN; $^{€}$ *p*<0,001 *vs* SN; § *p*<0,05 vs TA; $^{£}$ *p*<0,01 *vs* TA; $^{\&}$ *p*<0,001 *vs* TA.

3.2.3 Estado Oxidativo

A colite experimental gerou desequilíbrio oxidativo tecidual aos 7 e 28 dias (tabela 4). Aos 7 dias a atividade da SOD no grupo TA foi menor comprado aos grupo GC e SN. A atividade da GST foi maior no grupo SA comparado ao grupo GC, TA e TN. Os níveis de GSH foram menores no grupo TA comparado ao grupo GC. Como consequência das alterações oxidativas os níveis de LOOH foram maiores no grupos TA comparado ao grupo GC. Aos 28 dias os níveis de GSH foram menores no grupo TA comparado ao grupo GC e os níveis de LOOH foram maiores no grupo TA em relação ao grupo GC.

Após o tratamento por 7 dias no grupo TN a atividade da SOD foi maior comparado aos grupos TA e SA. Houve um aumento não significativo dos níveis de GSH e a redução significativa do níveis de LOOH no grupo TN comparado ao grupo GC. O tratamento por 28

dias aumentou significativamente os níveis de GSH e reduziu significativamente os níveis de LOOH comparado ao grupo TA. Estes resultados indicam a ação antioxidante do tratamento com as nanopartículas.

Tabela 4. Atividade das enzimas superóxido dismutase (SOD – U SOD/mg de proteína) e glutationa S-transferase (GST –mmol/min/mg de tecido) e dos níveis de glutationa reduzida (GSH –mg GSH/mg de tecido) e hidroperóxidos lipídicos (LOOH –mol/g de tecido). Ratos dos grupos controle (GC), salina/água (SA) salina/nanopartículas (SN), TNBS/água (TA), e

		GC	SA	SN	TA	TN
7 Dias	**SOD**	0,40±0,045	0,35±0,007	0,37±0,016	0,31±0,010¢#	0,41±0,029^&
	GST	3,99±1,09	5,60±1,05*	4,54±0,73	3,09±0,69^	3,74±0,39¥
	GSH	432,9±86,0	469,7±77,9	422,2±35,8	310,8±32,5*^#	403,8±36,3
	LOOH	30,16±1,6	31,01±1,7	31,53±2,1	35,80±2,4«¥#	30,76±1,3£
28 dias	**SOD**	0,64 ±0,05	0,62±0,05	0,58±0,02	0,63±0,04	0,58±0,06
	GST	7,55±1,1	9,56±1,0	7,48±1,6	8,64±0,6	7.97±0,7
	GSH	1674±121,8	1669±77,9	1598±114,7	1454±36,42*^	1645±104,7§
	LOOH	71,21±5,5	74,85±1,9	72,26±2,3	78,29±2,1*#	72,32±1,6§

TNBS/nanopartículas (TN) com a dose de 25 mg/Kg no período de 7 e 28 dias.

One-way ANOVA, e pós-teste de Tukey. Resultados expressos como media±desvio padrão (n=5).
* *p*<0,05 *vs* GC; « *p*<0,01 *vs* GC; ¢ *p*<0,001 *vs* GC; ¥ *p*<0,05 *vs* SA; ^ *p*<0,01 *vs* SA; ¤ *p*<0,001 *vs* SA;
\# *p*<0,05 *vs* SN; $ *p*<0,01 *vs* SN; € *p*<0,001 *vs* SN; § *p*<0,05 *vs* TA; £ *p*<0,01 *vs* TA; & *p*<0,001 *vs* TA.

3.2.4 Estado Inflamatório

Após 7 dias, a atividade da MPO (Fig. 6A), da NAG (Fig. 6B) e os níveis de NO_2^- (Fig. 6C) foram maiores no grupo TA comparado aos grupos GC, SA e SN. Após 28 dias, o grupo TA apresentou maior atividade enzimática e da NAG (Fig. 6E) e maiores níveis de NO_2^- (Fig. 6F) comparado aos grupos GC, SA e SN.

O tratamento por 7 dias, levou a menor atividade enzimática da MPO e da NAG e reduziu os níveis de NO_2^- no grupo TN comparado ao grupo TA. Após 28 dias de tratamento o

grupo TN apresentou menor atividade da NAG e menores níveis de NO_2^- comparado ao grupo TA.

3.2.5 Análise morfoquantitativa da inervação intrínseca intestinal

A imunomarcação dos corpos neuronais de população total (HuC/D^+) do plexo mientérico do cólon distal dos ratos está apresentada na figura 7A e dos corpos neuronais da subpopulação nitrérgica ($nNOS^+$), na figura 7B. A sobreposição ganglionar das imunomarcações está apresentada na figura 7C.

Houve redução da densidade neuronal HuC/D^+ (Fig.7D, 7G) e dos neurônios $nNOS^+$ (Fig 7E, 7H) no grupo TA comparado ao grupo GC, SA e SN aos 7 e 28 dias. Após 7 dias o perfil neuronal no grupo TA foi menor, em ambas as populações analisadas, comparado aos grupos GC, SA e SN (Fig. 7F). Aos 28 dias o perfil dos neurônios HuC/D^+ do grupo TA foi menor que dos grupos GC, SA e SN (Fig.7I) e o perfil dos neurônios $nNOS^+$ foi menor que o perfil dos grupos GC e SN (Fig. 7I).

O tratamento após 7 dias resultou em maior densidade e perfil dos neurônios HuC/D^+ e $nNOS^+$ no grupo TN comparado ao grupo TA, embora o perfil neuronal HuC/D^+ tenha permanecido menor em relação ao grupo GC. Após o tratamento por 28 dias a densidade neuronal em ambas as populações foi maior comparado ao grupo TA, no entanto a densidade HuC/D^+ permaneceu menor em relação ao grupo GC. O perfil neuronal HuC/D^+ do grupo TN foi maior comparado ao grupo TA e o perfil dos neurônios $nNOS^+$ foi maior que o do grupo TA e também que dos grupos SA e SN.

4. DISCUSSÃO

Os benefícios da curcumina livre nas doenças inflamatórias intestinais são descritas na literatura,[14,23–25] no entanto a sua baixa solubilidade e biodisponibilidade interferem na sua utilização. Assim, objetivamos verificar se nanopartículas sintetizadas com o encapsulante PVP, por aumentar a sua biodisponibilidade[41] com dose reduzida, seriam capazes de proporcionar os mesmos benefícios das altas doses do composto natural. Trabalhos anteriores deste grupo de pesquisa demostraram que a técnica utilizada na obtenção das nanopartículas, a dispersão

sólida, é capaz de promover a interação entre PVP e a curcumina presente na *C.longa L.,* produzindo nanopartículas com as características necessárias. Estes trabalhos mostraram que essa técnica é eficiente em melhorar a dispersibilidade em água da curcumina e em potencializar a sua bioatividade em testes *in vitro* e *in vivo*. [27,42] A interação curcumina-PVP ao aumentar a sua solubilidade, apresenta um mecanismo de dissolução controlada do composto e a curcumina é liberada para a células por difusão.[43]

O modelo experimental de colite induzida por TNBS em ratos reproduz aspectos semelhantes aos da DII em humanos tais como ocorrência de diarreia, sangramento retal, redução do peso corporal, lesões transmurais no cólon, alterações oxidativas teciduais e intensa resposta inflamatória,[44,45] parâmetros observados neste estudo.

As avaliações dos parâmetros biométricos do cólon distal indicaram retração tecidual e aumento do peso e da largura do cólon distal, decorrentes da colite experimental. Houve também um aumento no IMC decorrente da colite no grupo TA, indicando edema no segmento, sendo este índice um marcador da severidade da inflamação. [46]

Ao realizarmos o tratamento da colite experimental com a dose de 25 mg/kg de nanopartículas, determinada por meio de testes entre três diferentes doses, foi possível verificar redução do IAD, dos parâmetros biométricos do intestino e do edema no segmento. Houve prevenção do aumento das túnicas intestinais. A atividade das enzimas MPO e NAG e dos níveis de NO_2^- foram menores com o tratamento, assim como o desequilíbrio oxidativo no tecido. Houve um efeito protetor nos neurônios mientéricos do cólon distal nos ratos tratados comparado aos não tratados. Estes achados corroboram com os achados de estudos prévios.[14,23,26,47]

O uso do TNBS para a indução do modelo experimental levou a alteração nas transaminases hepáticas, indicando hepatotoxicidade deste composto. Nossos resultados indicaram um papel hepatoprotetor da curcumina, demonstrado pela redução significativa da atividade das transaminases hepáticas.

No cólon distal, a indução da colite experimental com TNBS desencadeia uma resposta imune celular exacerbada. Esta resposta é caracterizada por denso infiltrado de células $TCD4^+$ que levam a resposta inflamatória Th1, com secreção predominante das citocinas fator de necrose tumoral alfa (TNFα), interferon gama (IFNγ) e interleucina-2 (IL-2).[48] No entanto,

citocinas pro-inflamatórias também são responsáveis pela liberação de espécies reativas de oxigênio (ERO),[49] o que faz com que o processo inflamatório e de dano oxidativo tecidual estejam intimamente ligados.

As alterações histológicas decorrentes da colite experimental observadas neste estudo incluem destruição da arquitetura da túnica mucosa, com pontos de abcessos e ulcerações. No período de 28 dias foram observadas alterações decorrentes do processo de cicatrização como distorções de criptas. Extensas áreas de infiltrados polimorfonucleares, principalmente na túnica submucosa mas também nas túnicas mucosa e muscular. Infiltrados polimorfonucleares também foram encontrados no interior de gânglios mientéricos, corroborando com os achados de Vicentini et al.[28]

Podemos relacionar o intenso infiltrado de células inflamatórias ao aumento da atividade tecidual das enzimas inflamatórias MPO,[26,28] liberada por neutrófilos ativos[50] e NAG, presente em macrófagos ativos,[51] verificados por meio de ensaios bioquímicos.

Os neutrófilos liberam substâncias com potencial bactericida, como as ERO, além da enzima MPO.[52,53] A ação bactericida da MPO está relacionada a capacidade de reagir o H_2O_2 com compostos oxidáveis, entre eles o cloreto, gerando o ácido cloroso, uma molécula altamente reativa que interage com proteínas e compostos nitrogenados, gerando danos nas células e tecidos.[54] A MPO também pode utilizar o NO_2^- como substrato, oxidando-o a dióxido de nitrogênio, que promove a nitratação de proteínas[55] e atua na peroxidação lipídica,[56] contribuindo para os danos celulares que ocorrem durante o processo inflamatório.

A NAG é uma enzima lisossomal liberada na presença de lipopolissacarídeos que entram em contato com os macrófagos em um processo inflamatório.[57] A atividade da NAG encontra-se aumentada em modelo experimental de colite como demonstrado por Andrade et al.,[58] utilizando indução da colite por meio de dextran sulfato de sódio (DSS) em camundongos.

Os níveis de NO_2^-, subproduto NO, produzido pela óxido nítrico sintase (NOS), presente em várias células incluído macrófagos,[59] estavam aumentados no grupo TA, corroborando com os achados de Machado et al.[60] Mediadores inflamatórios[54,61] e lipopolissacarídeos presentes na membrana de bactérias gram-negativas[62] são estimuladores da produção de NO durante a patogênese das DII. Em situação de inflamação, quando a

produção de NO está aumentada, seu excesso reage com o ânion superóxido (O_2^-) presente no meio, produzindo peroxinitrito ($ONOO^-$), outro potente agente oxidante.[63]

A curcumina é capaz de atuar em várias vias e mecanismos da inflamação, modulando o processo inflamatório. Larmonier et al.[12] em ensaio *in vitro* verificaram que a curcumina foi capaz de inibir a secreção de substâncias quimiotáxicas para os neutrófilos. Seus ensaios foram confirmados por modelo de peritonite em camundongos pré-tratados com curcumina onde os ratos tratados apresentaram menor quantidade de neutrófilos recrutados. A curcumina também é capaz de agir sobre a produção de NO, diretamente na proteína e na expressão da iNOS além de vias de ativação genética desta enzima.[13]

Estudos em humanos[64,65] e em modelos experimentais[60,66] indicam a relação do aumento do estresse oxidativo, a depleção das defesas antioxidantes e a ocorrência das DII. Desta maneira o aumento do estresse oxidativo é um indicador indireto da ativação destas doenças[67]. O acúmulo das ERO, decorrente da instalação do processo inflamatório também está intimamente ligado ao desenvolvimento e agravamento da patologia[68]. No presente estudo constatamos, no grupo TA, o aumento da peroxidação lipídica tecidual, sugerindo danos celulares gerados pelo estresse oxidativo e pelo processo inflamatório, juntamente com redução das defesas antioxidantes representadas pela redução da atividade da SOD após 7 dias e dos níveis de GSH teciduais após 7 e 28 dias, estando de acordo com os achados de Machado et al [60].

A curcumina possui grande atividade antioxidante. Estudos apontam sua capacidade de captar radicais livres como H_2O_2, O_2^- [15], NO[69] e agentes oxidantes como $ONOO^-$,[63] reduzindo os danos gerados por estes reagentes às estruturas celulares. Mouzaoui et al.[14] utilizando mitocôndrias isoladas de cólon de camundongos, demonstraram que a curcumina (5 μM) aumentou os níveis de GSH, reduziu a geração de O_2^-, a peroxidação lipídica e níveis de NO mitocondriais comparado a mitocôndrias expostas a TNBS (100 e 50 μM) sem o tratamento preventivo com a curcumina. No mesmo estudo os autores comprovaram a ação da curcumina *in vivo* demonstrando que o tratamento com curcumina intraperitoneal (25 mg/Kg) aplicado logo após a indução da colite com TNBS, reduziu os níveis de NO e peroxidação lipídica e ainda minimizou a redução do níveis de GSH no cólon dos camundongos tratados.

Finalmente foram analisadas as densidades e o perfil celular da população neuronal geral e subpopulação nitrérgica mioentérica por meio de imunomarcação HuC/D$^+$ e nNOS$^+$, respectivamente. Nossos resultados apontaram redução na densidade da população neuronal geral e subpopulação nitrérgica assim como a redução do perfil neuronal em ambas populações nos ratos com colite experimental. Estes resultados estão de acordo com estudos prévios. [6,28]

Winston et al.[7] verificaram que a colite induzida por TNBS foi capaz de reduzir a expressão das proteínas NOS, ChAT e PGP9.5, marcador válido para população neuronal geral. Assim a redução de atividade celular pode estar associada a redução do perfil neuronal.

A morte neuronal durante o processo inflamatório pode ser decorrente de vários mecanismos, entre eles o aumento da concentração de purinas, como a adenosina trifosfato (ATP) liberadas por células endoteliais,[70] inflamatórias[71] entre outras, ativando receptores P2X7 (P2X7R). Da Silva et al.[6] demonstraram que subpopulações neuronais , entre elas a nitrérgica, possuem estes receptores. Gulbransen et al[72], utilizando modelo experimental de colite induzida por DSS em camundongos C57BL/6, demostraram que a morte neuronal ocorre devido ao aumento do ATP extracelular que ativa os P2X7R neuronais e levam, preferencialmente, a morte de neurônios nitrérgicos.

A curcumina é capaz de inibir a expressão dos P2X7R como demonstrado por Zhang et al.[73] utilizando modelo experimental de estresse leve e crônico em ratos Sprague-Dawley, sendo que o grupo de ratos que recebeu curcumina via gavagem (100 mg/Kg) apresentou menor expressão do P2X7R no hipocampo, comparado ao grupo não tratado. Assim a curcumina poderia impedir um dos mecanismos que levam a morte de neurônios, durante os processos inflamatórios, resultando a maior densidade neuronal observada neste estudo no grupo TN.

Desta maneira concluímos que a DME da curcumina nanoencapsulada foi 25mg/kg. Esta dose foi capaz de reduzir o IAD, evitar o aumento das túnicas do cólon, inibindo a inflamação e estresse oxidativo teciduais além de prevenir a morte e hipertrofia neuronal, causadas pela colite induzida por TNBS em ratos Wistar.

Contribuições do autor

Débora dos Anjos Weber Luz: conceito do trabalho, metodologia, coleta e análise de dados, escrita original. Camila Quaglio Neves: metodologia, análise de dados. Stephanie Carvalho Borges: metodologia, análise de dados, revisão da escrita. Nilza Cristina Buttow: metodologia, recursos. Ciomar Apareceida Bressani: conceito do trabalho, revisão da escrita. Odinei Hess Gonçalves: metodologia, recursos, análise de dados, revisão da escrita. Maria Raquel Marçal Natali: conceito do trabalho, recursos, supervisão, administração do trabalho, revisão da escrita, aquisição de fundos.

Conflitos de interesse

Os autores declaram que não possuem interesses financeiros concorrentes ou relações pessoais que possam ter influenciado o trabalho relatado neste artigo.

Financiamento

Este estudo foi apoiado pela da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) Código de Financiamento 001 e Fundação Araucária (472223).

Declaração de disponibilidade de dados

Os dados que suportam os resultados deste estudo estão disponíveis mediante solicitação ao autor correspondente.

Agradecimento

Os autores agradecem ao Programa de Pós-graduação de Ciências Biológicas da Universidade Estadual de Maringá, as técnicas do laboratório de Histologia da Universidade Estadual de Maringá-UEM e ao Programa de Pós-Graduação em Tecnologia de Alimentos (PPGTA) da Universidade Tecnológica Federal do Paraná - UTFPR pela produção das curcumina nanoencapsulada.

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Legendas das Figuras:

Figura 1 Avaliação *in vivo* dos ratos com colite experimental da etapa de tratamento por 7 e 28. Consumo de ração **(A, B)** peso corporal **(C, D)** e Índice da Atividade da Doença (IAD) **(E, F)** nos períodos experimentais de 7 e 28 dias, respectivamente. Peso corporal e IAD dos dias 6 (7 e 28 dias) e dia 14 - One-way ANOVA e pós-teste de Tukey. Demais análises - Kruskal-Wallis e pós teste de Dunn's; Resultados expressos como média ± desvio padrão (n=6).

* p<0,05 *vs* grupo controle (GC); « p<0,01vs GC; ¢ p<0,001vc GC;

¥ p<0,05 *vs* grupo salina/água (SA); ^ p<0,01 vs SA; ¤ p<0,001 vs SA;

# p<0,05 *vs* grupo salina/nanopartículas (SN); $ p<0,01 vs SN; € p<0,001 vs SN;

§ p<0,05 *vs* grupo TNBS/água (TA); £ p<0,01 vs TA; & p<0,001 vs TA.

TN- grupo TNBS/nanopartículas.

Figura 2 Avaliação macroscópica do cólon distal dos grupos experimentais de 7 **(A)** e 28 dias **(C)**. *Score* macroscópico das lesões **(B,D)** baseado nos critérios de Appleyard e Wallace (Appleyard and Wallace 1995) após os períodos de 7 e 28 dias, respectivamente. Kruskal-Wallis e pós teste de Dunns. Resultados expresso como média ± desvio padrão (n=6).

* p<0,05 *vs* grupo controle (GC); « p<0,01vs GC; ¢ p<0,001vc GC;

¥ p<0,05 *vs* grupo salina/água (SA); ^ p<0,01 vs SA; ¤ p<0,001 vs SA;

# p<0,05 *vs* grupo salina/nanopartículas (SN); $ p<0,01 vs SN; € p<0,001 vs SN;

§ p<0,05 *vs* grupo TNBS/água (TA); £ p<0,01 vs TA; & p<0,001 vs TA.

TN- grupo TNBS/nanopartículas.

Figura 3. Avaliação histológica do cólon distal de ratos após os períodos experimentais de 7 e 28 dias. Ratos dos grupos GC **(A, B)**, SA **(C,D)**, SN **(E, F)** aos 7 e 28 dias respectivamente, com aspecto morfológico normal. Cólon do grupo TA aos 7 dias **(G)** apresentando grande infiltrado inflamatório, abcessos (seta preta) e destruição da arquitetura da túnica mucosa, com área de ulceração. Cólon do grupo TA aos 28 dias **(H)** com infiltrado inflamatório restrito a submucosa com alteração estrutural da túnica mucosa, como distorção de criptas (seta vermelha). Cólon do grupo TN aos 7 dias **(I)** e TN ao 28 dias **(J)** de tratamento com 25 mg/kg de curcumina nanoencapsulada indicando redução de infiltrado. Aumento 10X. Barra = 100µm. GC - grupo

controle; SA - grupo salina/água; SN - grupo salina/ nanopartículas; TA - grupo TNBS/água; TN – grupo TNBS/nanopartículas.

Figura 4. *Score* inflamatório após período experimental de 7 **(A)** e 28 **(B)** dias. One-way Anova e pós teste de Tukey. Resultados expressos como média ± desvio padrão (n=5).

* *p*<0,05 *vs* grupo controle (GC); « *p*<0,01 *vs* GC; ¢ *p*<0,001 *vs* GC;

¥ *p*<0,05 *vs* grupo salina/água (SA); ^ *p*<0,01 *vs* SA; ¤ *p*<0,001 *vs* SA;

# *p*<0,05 *vs* grupo salina/nanopartículas (SN); $ *p*<0,01 *vs* SN; € *p*<0,001 *vs* SN;

§ *p*<0,05 *vs* grupo TNBS/água (TA); £ *p*<0,01 *vs* TA; & *p*<0,001 *vs* TA.

TN- grupo TNBS/nanopartículas.

Figura 5. Gânglios mientéricos do cólon distal de ratos após 7 **(A,C,E)** e 28 dias **(B,D,F)**. Grupos controle **(A,B)**, grupo TNBS/água **(C, D)** com seta indicando células polimorfonucleadas e do grupo tratado com 25 mg/kg de nanopartículas, indicando redução qualitativa destas células **(E, F).** Aumento 40X. Barra= 100µm.

Figure 6 Atividade das enzimas mieloperoxidase - MPO **(A,D)** e N-acetilglucasaminidase - NAG **(B,E)** e níveis de nitrito - NO_2^- **(C,F)** em ratos com colite experimental após 7 e 28 dias. One-way ANOVA, e pós-teste de Tukey. Resultados expressos como media±desvio padrão (n=5).

* *p*<0,05 *vs* grupo controle (GC); « *p*<0,01 *vs* GC; ¢ *p*<0,001 *vs* GC;

¥ *p*<0,05 *vs* grupo salina/água (SA); ^ *p*<0,01 *vs* SA; ¤ *p*<0,001 *vs* SA;

# *p*<0,05 *vs* grupo salina/nanopartículas (SN); $ *p*<0,01 *vs* SN; € *p*<0,001 *vs* SN;

§ *p*<0,05 *vs* grupo TNBS/água (TA); £ *p*<0,01 *vs* TA; & *p*<0,001 *vs* TA.

TN- grupo TNBS/nanopartículas.

Figura 6 Fotomicrografia de neurônios imunorreativos da população geral - HuC/D$^+$ **(A)** e subpopulação nitrérgica nNOS$^+$ **(B)** do cólon distal de ratos do GC. Sobreposição ganglionar das marcações **(C)**. Barra=50µm. Densidade neuronal HuC/D$^+$ **(D,G)** e nNOS$^+$ **(E,H)**. One-Way ANOVA e pós teste de Tukey. Perfil neuronal **(F,I)** Kruskal-Wallis e pós test de Dunn's. Resultados expresso como média±desvio padrão *p*<0.05).

* $p<0,05$ *vs* grupo controle (GC); « $p<0,01$ *vs* GC; ¢ $p<0,001$ *vs* GC;

¥ $p<0,05$ *vs* grupo salina/água (SA); ^ $p<0,01$ *vs* SA; ¤ $p<0,001$ *vs* SA;

# $p<0,05$ *vs* grupo salina/nanopartículas (SN); $ $p<0,01$ *vs* SN; € $p<0,001$ *vs* SN;

§ $p<0,05$ *vs* grupo TNBS/água (TA); £ $p<0,01$ *vs* TA; & $p<0,001$ *vs* TA.

TN- grupo TNBS/nanopartículas.

FIGURAS

Figura 1.

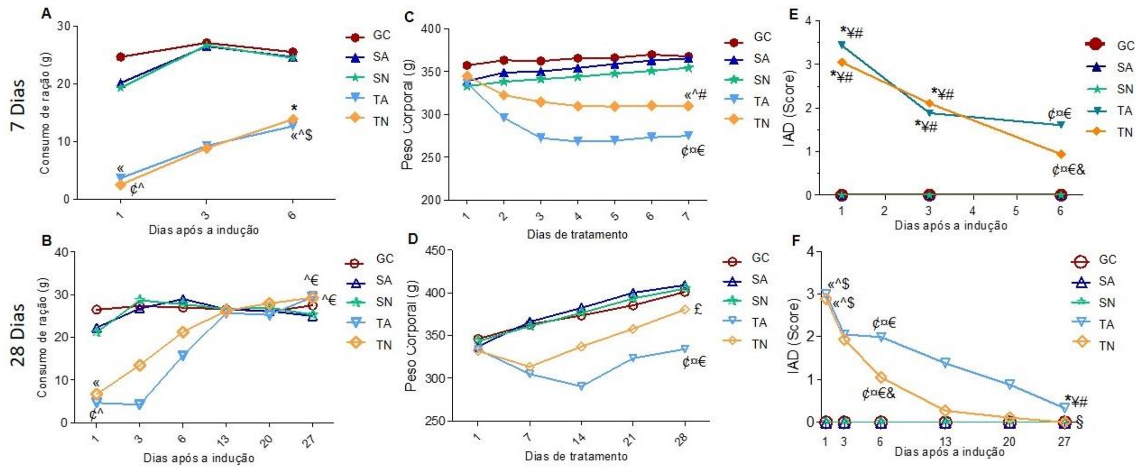
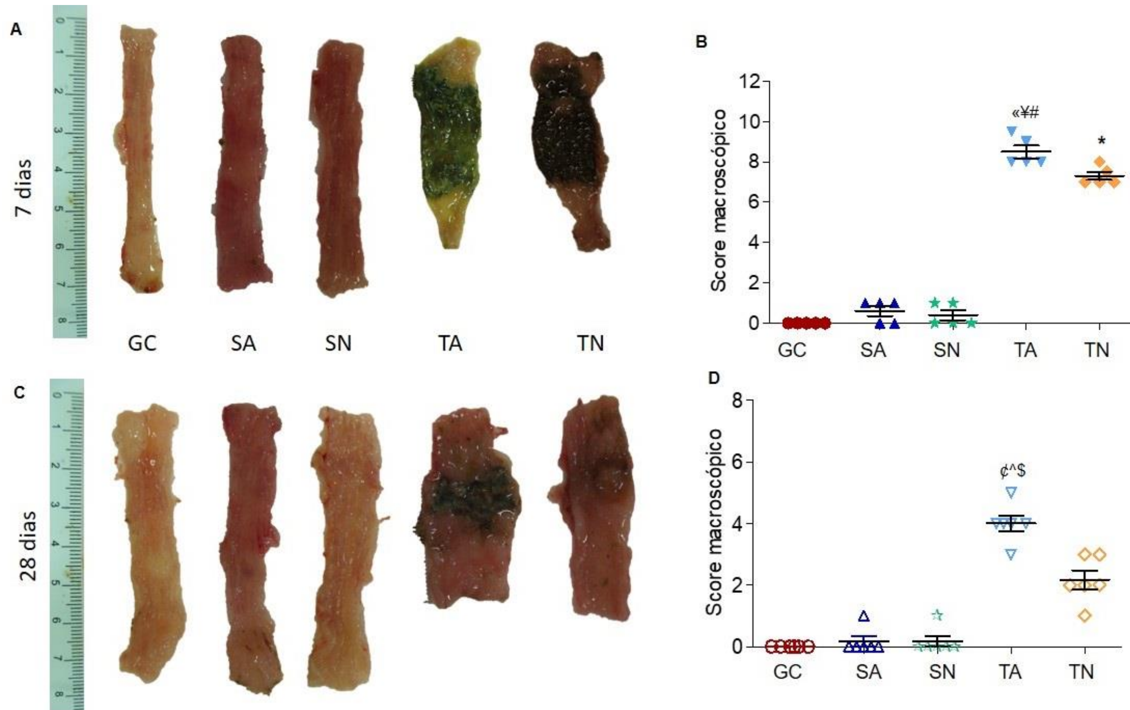


Figura 2.



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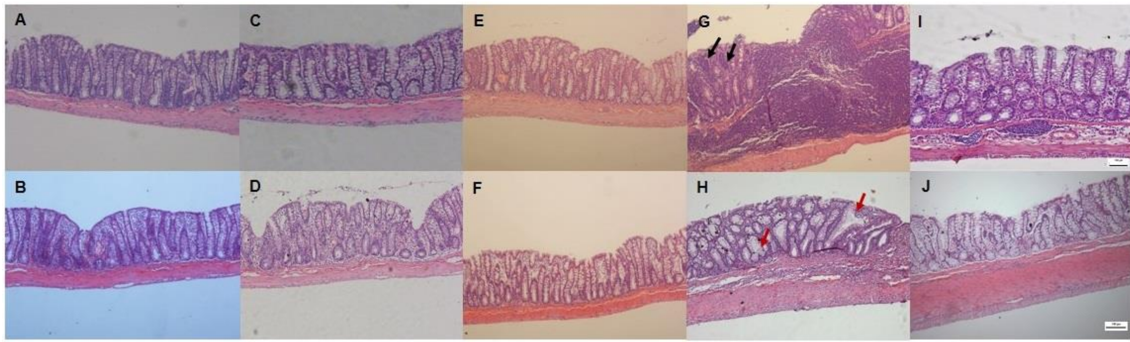
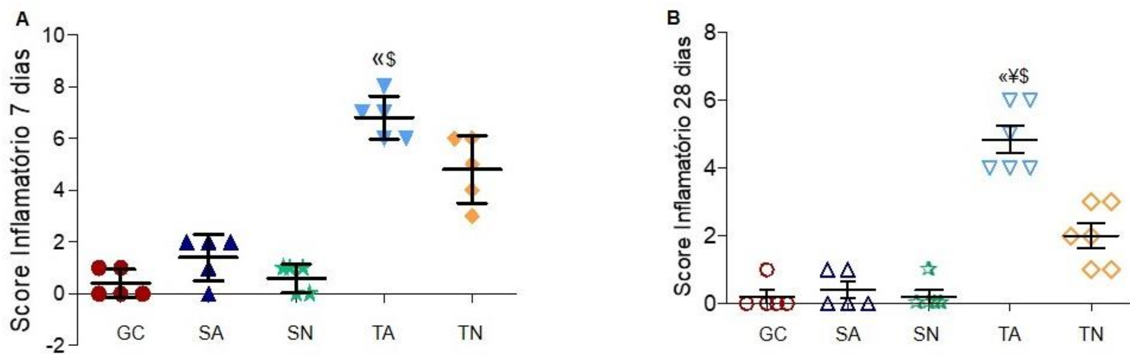


Figura 4



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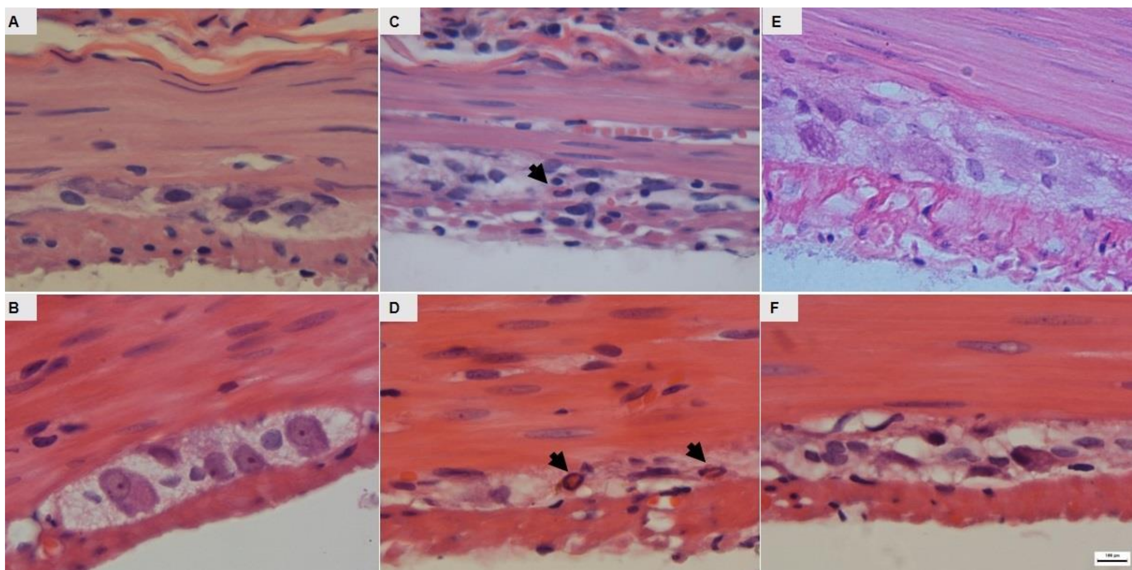

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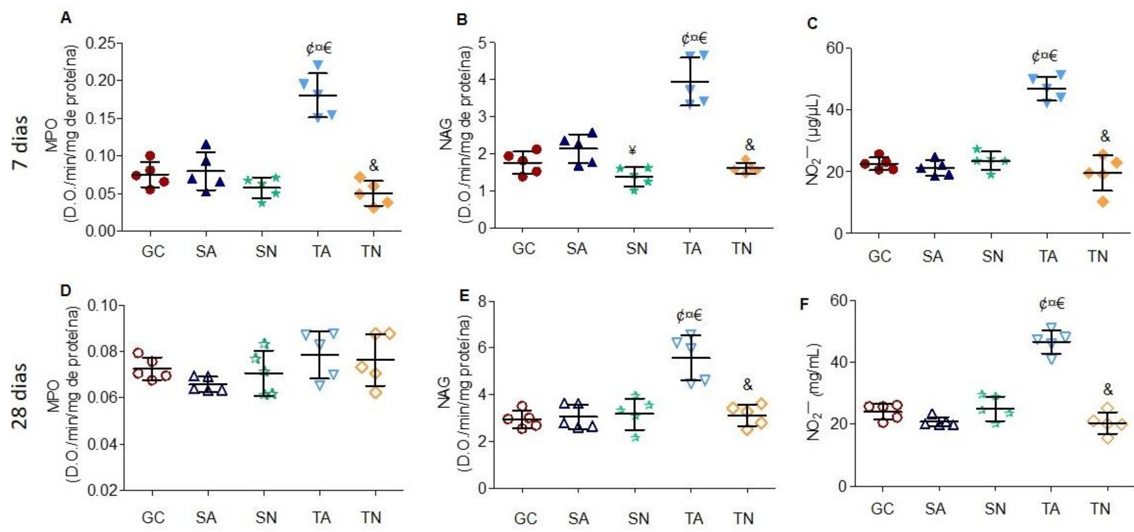

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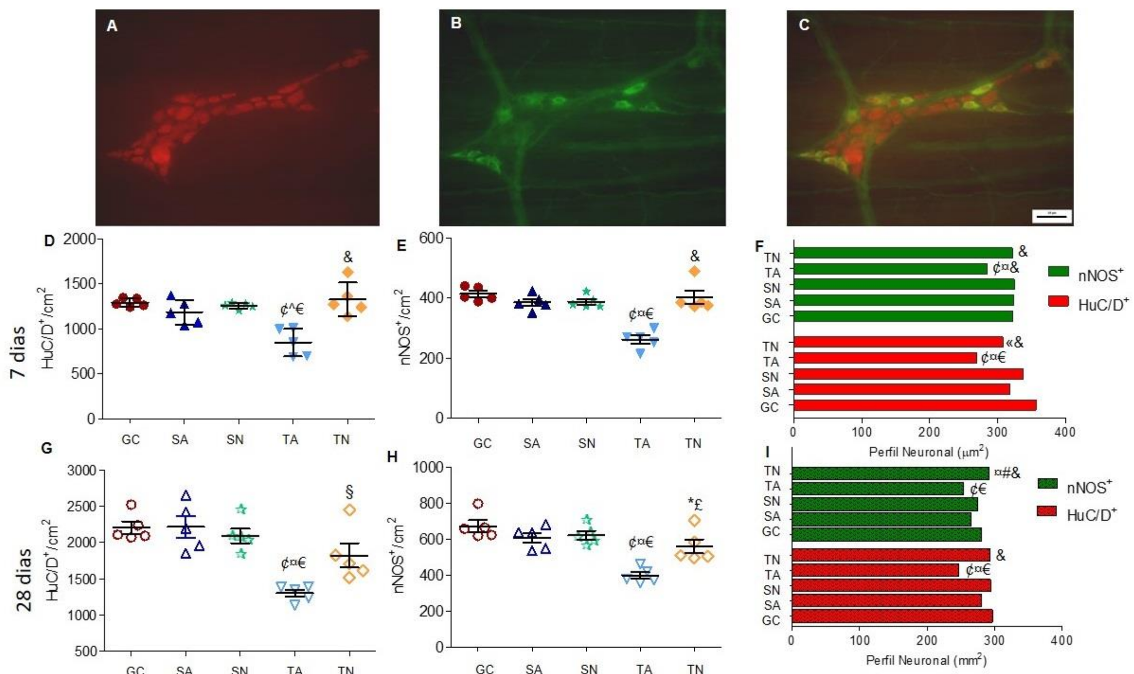

ANEXO I: PARECER DA COMISSÃO DE ÉTICA EM USO DE ANIMAIS DA UNIVERSIDADE ESTADUAL DE MARINGÁ (CEUA/UEM)

Comissão de Ética no Uso de Animais da Universidade Estadual de Maringá

CERTIFICADO

Certificamos que a proposta intitulada "CURCUMINA ENCAPSULADA EM NANOPARTÍCULAS: UMA PERSPECTIVA PARA O TRATAMENTO DA COLITE EXPERIMENTAL", protocolada sob o CEUA nº 2342171116 (ID 001081), sob a responsabilidade de **Maria Raquel Marçal Natali** *e equipe; Ciomar Aparecida Bersani Amado; Ana Paula de Santi Rampazzo; Débora dos Anjos Weber Luz; Bruno Ambrosio da Rocha* - 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 Estadual de Maringá (CEUA/UEM) na reunião de 13/02/2017.

We certify that the proposal "ENCAPSULATED CURCUMIN IN NANOPARTICLES: A PERSPECTIVE FOR TREATMENT OF EXPERIMENTAL COLITIS", utilizing 186 Isogenic rats (186 males), protocol number CEUA 2342171116 (ID 001081), under the responsibility of **Maria Raquel Marçal Natali** *and team; Ciomar Aparecida Bersani Amado; Ana Paula de Santi Rampazzo; Débora dos Anjos Weber Luz; Bruno Ambrosio da Rocha* - 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 State University of Maringá (CEUA/UEM) in the meeting of 02/13/2017.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 02/2017 a 08/2019 | Área: Dcm-Ciências Morfológicas

Origem: Biotério Central da UEM
Espécie: Ratos isogênicos | sexo: Machos | idade: 80 a 90 dias | N: 186
Linhagem: Wistar | Peso: 250 a 350 g

Local do experimento: Laboratório de Histotécnica Animal - Depto de Ciências Morfológicas -Bloco H79 UEM

Maringá, 27 de fevereiro de 2021

Profa. Dra. Tatiana Carlesso dos Santos
Coordenadora da CEUA/U
Universidade Estadual de Maringá

Profa. Dra. Erika Seki Kioshima Cótica
Coordenadora Adjunta da CEUA/UEM
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whatsapp 3011-4597. - tel: 55 (44) 3011-4444 Horário de
atendimento: 2ª a 6ª, das 8h às 11h40 e 14h às 17h30:
e-mail: ceea@uem.br
CEUA N 2342171116

Maringá, 15 de setembro de 2017
CEUA N 2342171116

Ilmo(a). Sr(a).Responsável: Maria Raquel Marçal Natali Área: Dcm-ciências Morfológicas

Título da proposta: "CURCUMINA ENCAPSULADA EM NANOPARTÍCULAS: UMA PERSPECTIVA PARA O TRATAMENTO DA COLITE EXPERIMENTAL".

Parecer Consubstanciado da Comissão de Ética no Uso de Animais UEM (ID 000203)

A Comissão de Ética no Uso de Animais da Universidade Estadual de Maringá, no cumprimento das suas atribuições, analisou e
APROVOU a Emenda (versão de 21/julho/2017) da proposta acima referenciada.

Resumo apresentado pelo pesquisador: "Este projeto de pesquisa consta de duas fases dependentes. A primeira fase é para determinar a dose com a melhor resposta que será utilizada no tratamento da colite experimental. A segunda fase é de tratamento de 7 e 28 dias dos ratos com colite experimental, com a dose determinada na primeira fase. Realizamos a primeira fase de teste de dose efetiva com 3 doses diferentes (20, 10 e 5 mg/Kg de curcumina encapsulada em nanopartículas)com ausência de resultado significativo, como descrito na emenda. Desta maneira, para garantirmos a efetividade do tratamento, é necessário testar 3 novas doses (50, 25 e 10 mg/Kg de curcumina encapsulada em nanopartículas), com os animais que já estavam previstos. Como no trabalho foi determinado que esta fase é necessária pra obter a dose que será utilizada no tratamento da colite experimental, temos que procurar a dose efetiva que reduzirá, significativamente, a inflamação gerada pela colite experimental. Para tal estamos realizando testes bioquímicos para quantificar a atividade de mieloperoxidase (MPO), N-acetilglucosaminidase (NAG) e quantidade de óxido nítrico (NO) teciduais. Estes novos testes estão em fase de análise. Desta forma temos a necessidade de solicitar mais animais para dar continuidade a segunda fase deste projeto. Para que possamos realizar as análises propostas nos 7 grupos (CC, SA, TA, TCurNano, SCurNano, TCurNanovazia, SCurNanovazia) com 7 e 28 dias de tratamento (n=10), perfazendo um total de 140 animais. Também estamos solicitando a inclusão de análise bioquímicas de estresse oxidativo (metodologias já descritas no projeto inicial) para fígado, rim e pulmão, para verificar se o tratamento por 7 e 28 dias gera toxicidade sistêmica. ".

Comentário da CEUA: "A Comissão de Ética no Uso de Animais (CEUA-UEM), na sua reunião de 14/09/2017, APROVOU os procedimentos éticos apresentados neste Protocolo, visto que a metodologia proposta é compatível com a legislação pertinente à ética no uso de animais na experimentação, na forma da Lei no 11.794/08, Decreto 6.899/09, Resolução Normativa nº 01/2010 – CONCEA e complementares, Lei Estadual no 14.037/03, Diretriz Brasileira para o cuidado e a utilização de animais para fins científicos e didáticos – DBCA (portaria nº 596 – CONCEA - de 25/junho/2013, disponível no endereço http://www.ppg.uem.br/index.php/etica-biosseguranca/ceua) e Resolução UEM nº 004/2016-CEP, vez que não se constatam óbices legais para o desenvolvimento dos procedimentos experimentais nos moldes propostos pelo(a) pesquisador(a).".

Profa. Dra. Tatiana Carlesso dos Santos
Coordenadora da CEUA/UEM
Universidade Estadual de Maringá

Profa. Dra. Erika Seki Kioshima Cótica
Coordenadora Adjunta da CEUA/UEM
Universidade Estadual de Maringá

ANEXO II: SUBMISSION GUIDELINES - NAUNYN-SCHMIEDEBERG'S ARCHIVES OF PHARMACOLOGY

Important Submission Policy

Since the journal has experienced a rather extensive wave of fraud submissions of papers from paper mills and papers including fake data from external service laboratories we had to extend the submission prerequisites to the following requests:

• Request of institutional email addresses. At the minimum, at least the corresponding author should provide an institutional email address.

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otherwise it may be regarded as a new manuscript. When a revised manuscript is submitted, all changes should be clearly marked in color and the original version should also be returned.

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Book

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Book chapter

Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

Online document

Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

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