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**ATIVIDADE ANALGÉSICA E ANTI-INFLAMATÓRIA DO
AMBROXOL NO TRATAMENTO DA ENDOMETRIOSE:
ESTUDO EXPERIMENTAL COM RATAS WISTAR**

GUSTAVO MEDEIROS FROTA

São Luís

2026



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Defesa de tese apresentada ao Programa de Pós-Graduação em Biotecnologia da Rede Nordeste de Biotecnologia (RENORBIO), ponto focal Maranhão, como requisito para obtenção do título de Doutor em Biotecnologia.

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Gustavo Medeiros Frota

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*“Não podemos acrescentar dias à nossa vida,
mas podemos acrescentar vida aos nossos dias.”*

Sanghamitra Ganguly

RESUMO

A endometriose é uma doença ginecológica inflamatória crônica, frequentemente associada à dor pélvica, infertilidade e impacto significativo na qualidade de vida das pacientes. Apesar dos avanços terapêuticos, os tratamentos atualmente disponíveis apresentam limitações relacionadas à resposta clínica, recorrência dos sintomas e efeitos adversos, reforçando a necessidade de novas abordagens farmacológicas. Estruturada em dois capítulos complementares, esta tese teve como objetivo investigar o potencial terapêutico do ambroxol na endometriose, integrando evidências provenientes de uma revisão bibliométrica e de um estudo experimental original. Inicialmente, realizou-se uma análise bibliométrica da literatura acerca das atividades farmacológicas do ambroxol em modelos experimentais, utilizando as bases Web of Science e Scopus. Foram incluídos 24 estudos publicados entre 1994 e 2023, com maior representação nas áreas de Medicina (33,3%), Farmacologia (30,6%), Bioquímica (16,7%) e Neurociências (11,1%). Em seguida, conduziu-se um estudo experimental em modelo animal de endometriose, no qual o ambroxol foi administrado por via oral nas doses de 10, 50 e 100 mg/kg/dia, durante 21 dias. Foram avaliados parâmetros comportamentais de dor espontânea, sensibilidade mecânica e desempenho motor por meio da Rat Grimace Scale, teste de von Frey e teste rotarod, respectivamente. Também foram avaliados os níveis séricos de IL-1 β , a resposta inflamatória peritoneal, o volume dos implantes endometrióticos e a integridade epitelial. O ambroxol reduziu as manifestações de dor espontânea e aumentou o limiar de retirada ao estímulo mecânico durante o tratamento, enquanto o desempenho motor foi preservado. No 21º dia, os níveis séricos de IL-1 β foram de 56,48, 5,01 e 47,65 pg/mL nos grupos tratados com ambroxol nas doses de 10, 50 e 100 mg/kg, respectivamente, em comparação a 178,96 pg/mL no grupo controle negativo, com reduções significativas em todos os grupos tratados. Observou-se também redução da resposta inflamatória peritoneal. As doses de 50 e 100 mg/kg reduziram o volume dos implantes endometrióticos e promoveram alterações histopatológicas caracterizadas por redução da integridade epitelial. Em conjunto, os achados desta tese demonstram efeitos analgésicos, anti-inflamatórios e estruturais do ambroxol em modelo experimental de endometriose e fornecem evidências pré-clínicas que sustentam a continuidade das investigações sobre seu potencial terapêutico nessa condição.

Palavras-chave: Endometriose; Inflamação; Dor; Ambroxol; Ratos Wistar.

ABSTRACT

Endometriosis is a chronic inflammatory gynecological disease frequently associated with pelvic pain, infertility, and a significant impact on patients' quality of life. Despite therapeutic advances, currently available treatments have limitations related to clinical response, symptom recurrence, and adverse effects, highlighting the need for new pharmacological approaches. Structured into two complementary chapters, this thesis aimed to investigate the therapeutic potential of ambroxol in endometriosis by integrating evidence from a bibliometric review and an original experimental study. Initially, a bibliometric analysis of the literature on the pharmacological activities of ambroxol in experimental models was conducted using the Web of Science and Scopus databases. Twenty-four studies published between 1994 and 2023 were included, with the main research areas comprising Medicine (33.3%), Pharmacology (30.6%), Biochemistry (16.7%), and Neuroscience (11.1%). Subsequently, an experimental study was conducted using an animal model of endometriosis, in which ambroxol was orally administered at doses of 10, 50, and 100 mg/kg/day for 21 days. Behavioral parameters of spontaneous pain, mechanical sensitivity, and motor performance were assessed using the Rat Grimace Scale, von Frey test, and rotarod test, respectively. Serum IL-1 β levels, peritoneal inflammatory response, endometriotic implant volume, and epithelial integrity were also evaluated. Ambroxol reduced spontaneous pain manifestations and increased the mechanical withdrawal threshold during treatment, while motor performance was preserved. On day 21, serum IL-1 β levels were 56.48, 5.01, and 47.65 pg/mL in the groups treated with ambroxol at 10, 50, and 100 mg/kg, respectively, compared with 178.96 pg/mL in the negative control group, with significant reductions in all treated groups. A reduction in the peritoneal inflammatory response was also observed. The 50 and 100 mg/kg doses reduced endometriotic implant volume and promoted histopathological changes characterized by decreased epithelial integrity. Taken together, the findings of this thesis demonstrate analgesic, anti-inflammatory, and structural effects of ambroxol in an experimental model of endometriosis and provide preclinical evidence supporting further investigation of its therapeutic potential in this condition.

Keywords: Endometriosis; Inflammation; Pain; Ambroxol; Wistar rats.

LISTA DE FIGURAS

REFERENCIAL TEÓRICO 16

Figura 1. Vista lateral esquemática da pelve ilustrando as 4 formas de endometriose: 1: endometrioma (OMA); 2: endometriose peritoneal superficial (SUP); 3: endometriose subperitoneal infiltrante profunda (DIE); 4: adenomioma (Ad) 18

Figura 2. Representação esquemática da atividade imunoinflamatória mediada por células Th17 e IL-17 no microambiente peritoneal da endometriose 22

Figura 3. Estrutura química do cloridrato de ambroxol 28

CAPÍTULO 1 32

Figure 1. Temporal trend of publications on the antioxidant, analgesic, and anti-inflammatory actions of Ambroxol in experimental models, 1994–2023 42

Figure 2. Distribution by country of publications on the antioxidant, analgesic, and anti-inflammatory actions of Ambroxol in experimental models, 1994–2023 43

Figure 3. Distribution of thematic areas of publications on the antioxidant, analgesic, and anti-inflammatory actions of Ambroxol in experimental models, 1994–2023 43

Figure 4. Distribution by authors of publications on the antioxidant, analgesic, and anti-inflammatory actions of Ambroxol in experimental models, 1994–2023 44

Figure 5. Indicators of publications on the analgesic, anti-inflammatory, and antioxidant activities of Ambroxol in experimental models, 1994–2023. (A) CiteScore, (B) SJR, and (C) SNIP 46

Figure 6. Citations of publications related to the antioxidant, analgesic, and anti-inflammatory effects of Ambroxol in experimental models, 1994–2023 48

Figure 7. H-index of publications related to the antioxidant, analgesic, and anti-inflammatory effects of Ambroxol in experimental models, 1994–2023 48

Figure 8. Co-occurrence of keywords used in publications on the antioxidant, analgesic, and anti-inflammatory effects of Ambroxol in experimental models, 1994–2023 49

CAPÍTULO 2 58

Figure 1. Assessment of spontaneous pain using the RGS. Lines represent the mean $\pm$ standard deviation (score range: 0–2). Statistical comparisons were performed between Abx 10, Abx 50, Abx 100, Progesterone, and Sham groups vs. Control(–). Significance levels are indicated as follows: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. Two-way ANOVA with Tukey's post hoc test. The dashed line represents the baseline ($y = 0$), offset for visualization purposes 61

- Figure 2.** Tactile sensory assessment using the von Frey test. Lines represent the mean $\pm$ standard deviation (range: 0.008–300 g of force). Statistical comparisons were performed between Abx 10, Abx 50, Abx 100, Progesterone, and Sham groups vs. Control(–). Significance levels are indicated as follows: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. Two-way ANOVA with Tukey’s post hoc test. The dashed line represents the baseline ($y = 0$), offset for visualization purposes 62
- Figure 3.** Assessment of motor activity/forced ambulation using the rotarod test. Lines represent the mean $\pm$ standard deviation (score range: 1–5). Statistical comparisons were performed between Abx 10, Abx 50, Abx 100, Progesterone, and Sham groups vs. Control(–). Significance levels are indicated as follows: ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. Two-way ANOVA followed by Tukey’s post hoc test. The dashed line represents the baseline ($y = 0$), offset for visualization purposes 62
- Figure 4.** Assessment of IL-1 β concentration at D21. Data are presented as mean $\pm$ standard deviation. ** $p < 0.01$ for Control(–) vs. Sham, Progesterone, Abx 50, and Abx 100; *** $p < 0.001$ for Control(–) vs. Abx 50 mg. One-way ANOVA followed by Tukey’s post hoc test 63
- Figure 5.** Cell count/mL in peritoneal lavage at D21. Data are presented as mean $\pm$ standard deviation. * $p < 0.05$, between Control(–) vs. Abx 100 mg, Progesterone and Sham; ** $p < 0.01$, between Control(–) vs. Abx 10 mg and Abx 50 mg. One-way ANOVA followed by Tukey’s post hoc test 63
- Figure 6.** Representative ultrasonographic images illustrating cyst size and volume measurements across experimental groups. Panels (A1–E1) correspond to D0, and panels (A2–E2) correspond to D21. Experimental groups are defined as follows: (A1,A2) Control(–), (B1,B2) Abx 10, (C1,C2) Abx 50, (D1,D2) Abx 100, and (E1,E2) Progesterone. Cyst dimensions were assessed using three orthogonal diameters (d_1 , d_2 , and d_3), and cyst volume (Vol) was estimated based on these measurements.. 64
- Figure 7.** Representative photomicrographs of histological sections of endometriotic implants and internal abdominal wall (Sham group) excised at day 21. Each row corresponds to an experimental group (A–F), and each column represents a magnification (4 $\times$, 10 $\times$, 40 $\times$). (A) Control(–); (B) Abx 10; (C) Abx 50; (D) Abx 100; (E) Progesterone; (F) Sham. Sections (5 μ m) were stained with hematoxylin and eosin (H&E). Colored arrows indicate: red, endometriotic epithelium; blue, fibrosis; green, inflammatory infiltrate; yellow, vascular congestion; white, Nabothian cyst. Scale bars: 500 μ m (4 $\times$), 200 μ m (10 $\times$), and 50 μ m (40 $\times$) 67
- Figure 8.** Uterine autotransplant fragment fixed to the right parietal peritoneum of a Wistar rat 72

LISTA DE TABELAS E QUADROS

CAPÍTULO 1 32

Table 1. Experimental studies on the analgesic, anti-inflammatory, and antioxidant actions of Ambroxol in experimental models, 1994–2023 40

Table 2. Number of citations for the top 5 publications on the analgesic, anti-inflammatory, and antioxidant activities of Ambroxol in experimental models, 1994–2023 44

CAPÍTULO 2 58

Tabela 1. Variação do tamanho dos focos endometrióticos entre os grupos tratados com solução fisiológica 0,9%, 1,0 mL/dia, v.o.; ambroxol 10 mg/kg/dia, v.o.; ambroxol 50 mg/kg/dia, v.o.; ambroxol 100 mg/kg/dia, v.o.; e acetato de medroxiprogesterona em dose única intramuscular de 15 mg/kg 65

Tabela 2. Comparação dos escores semiquantitativos de persistência epitelial entre os grupos tratados com solução fisiológica 0,9%, 1,0 mL/dia, v.o.; ambroxol 10 mg/kg/dia, v.o.; ambroxol 50 mg/kg/dia, v.o.; ambroxol 100 mg/kg/dia, v.o.; e acetato de medroxiprogesterona em dose única intramuscular de 15 mg/kg 65

Table 3. Classification of endometrial implant growth 73

Table 4. Semi-quantitative scoring system for evaluating epithelial cell persistence in endometrial autotransplants 76

LISTA DE SIGLAS E ABREVIATURAS

ABX	Ambroxol
AINEs	Anti-inflamatórios Não Esteroidais
ANOVA	Análise de Variância
ARDS	Acute Respiratory Distress Syndrome
COX-2	Ciclooxigenase-2
CYP3A4	Citocromo P450 3A4
DPC	Dor Pélvica Crônica
DPOC	Doença Pulmonar Obstrutiva Crônica
EDTA	Ethylenediamine Tetraacetic Acid
H&E	Hematoxilina e Eosina
IL	Interleucina
IL-1 β	Interleucina 1 beta
JAK	Janus Kinase
JCR	Journal Citation Reports
MAC	Minimum Alveolar Concentration
MCP-1	Monocyte Chemoattractant Protein-1
MPA	Acetato de Medroxiprogesterona
NF- κ B	Fator Nuclear kappa B
NaV	Canais de Sódio Dependentes de Voltagem
NLRP3	NLR family pyrin domain containing 3
RGS	Rat Grimace Scale
SD	Standard Deviation
SNIP	Source Normalized Impact per Paper
SJR	SCImago Journal Rank
SOCS3	Suppressor of Cytokine Signaling 3
STAT3	Signal Transducer and Activator of Transcription 3
TGF- β	Fator de Crescimento Transformador Beta
Th17	T helper 17
Tregs	Linfócitos T reguladores
TNF- α	Fator de Necrose Tumoral Alfa
TTX	Tetrodotoxina
VEGF	Fator de Crescimento Endotelial Vascular

LISTA DE SÍMBOLOS

α	alpha
β	beta
cm	centímetro
g	gramas
γ	gamma
κ	kappa
kg	quilograma
L	Litro
μL	microlitro
μm	micrômetro
mg	miligrama
mL	mililitro
mm^3	milímetro cúbico
n	número amostral
p	valor de probabilidade
pg	picograma
%	porcentagem
®	marca registrada
vs.	versus
°C	grau Celsius

SUMÁRIO

1 INTRODUÇÃO	14
2 REFERENCIAL TEÓRICO	16
2.1 Endometriose	16
2.1.1 Etiopatogenia	16
2.1.2 Classificação	17
2.1.3 Dor, inflamação e modulação imune na endometriose	19
2.1.4 Diagnóstico	23
2.2 Modelos Experimentais para Endometriose	24
2.3 Tratamento	25
2.4 Ambroxol: bases farmacológicas e potencial terapêutico	27
3 OBJETIVOS	31
3.1 Objetivo Geral	31
3.2 Objetivos Específicos	31
CAPÍTULO 1	32
ARTIGO 1: New perspectives on the use of ambroxol hydrochloride: a recent bibliometric analysis in experimental models	33
CAPÍTULO 2	58
ARTIGO 2: Analgesic and Anti-inflammatory Activity of Ambroxol in The Treatment of Endometriosis: An Experimental Study in Wistar Rats	59
CONSIDERAÇÕES FINAIS	81
REFERÊNCIAS	82
Anexo A – Declaração da Publicação do Artigo de Revisão	95
Anexo B – Certificado da Publicação do Artigo Original da Tese	96
Anexo C – Parecer do CEUA	97
Anexo D – Uso de Inteligência Artificial	98

1 INTRODUÇÃO

A endometriose, uma das condições ginecológicas mais comuns, é uma doença crônica, inflamatória e estrogênio-dependente, caracterizada pela presença de tecido semelhante ao endométrio fora da cavidade uterina (Giudice; Kao, 2004). Clinicamente, manifesta-se principalmente por dor pélvica crônica, dismenorreia e infertilidade, impactando significativamente a qualidade de vida das mulheres acometidas (Hickey; Ballard; Farquhar, 2014). Estima-se que a doença afete aproximadamente 10% das mulheres em idade reprodutiva, podendo atingir prevalências de até 50% entre aquelas com infertilidade (Eisenberg et al., 2018).

O crescente corpo de evidências reforça o caráter multifatorial da endometriose, resultante da interação complexa entre fatores anatômicos, hormonais, imunológicos, genéticos, epigenéticos, ambientais e relacionados ao estresse oxidativo. Além dos aspectos biológicos, destaca-se também o impacto psicossocial da doença, frequentemente associado a sentimentos de vergonha, baixa autoestima, alterações na sexualidade e sofrimento emocional (Aerts et al., 2018; Bergeron; Likes; Steben, 2014; Desrochers et al., 2008).

Apesar dos avanços no entendimento de sua fisiopatologia, a etiologia da endometriose permanece parcialmente elucidada (Zubrzycka et al., 2015). A doença impõe um ônus econômico expressivo, comparável ao de outras condições crônicas, como diabetes, doença de Crohn e artrite reumatoide (Simoens et al., 2012, 2011), especialmente em razão da ausência de terapias curativas eficazes e pela limitação dos tratamentos disponíveis a longo prazo (Nothnick; Alali, 2016).

Estratégias terapêuticas consagradas incluem abordagens farmacológicas e cirúrgicas, com o objetivo de controle da dor, regulação hormonal e preservação da fertilidade (Dunselman et al., 2014; Kuznetsov et al., 2017). Apesar dessas intervenções, a recorrência da endometriose permanece elevada, podendo atingir taxas de até 40-50% em cinco anos, o que reflete o caráter crônico da doença (Zondervan et al., 2018).

Essas limitações evidenciam a necessidade de terapias alternativas, especialmente abordagens não hormonais que apresentem melhor perfil de segurança e eficácia sustentada, sem comprometer a fertilidade ou demandar intervenções cirúrgicas repetidas (Dunselman et al., 2014).

Novas estratégias terapêuticas vêm sendo investigadas, incluindo substâncias com propriedades anti-inflamatórias e moduladoras da resposta imune (Neto et al., 2011; Nogueira Neto et al., 2007). Os modelos experimentais desempenham papel fundamental na investigação da fisiopatologia da endometriose e na avaliação dessas novas abordagens, contribuindo para o avanço do conhecimento científico na área (Chantalat et al., 2020).

Entre as alternativas terapêuticas em investigação, destaca-se o crescente interesse por fármacos com propriedades analgésicas e anti-inflamatórias multimodais, capazes de atuar simultaneamente em vias inflamatórias, imunológicas e nociceptivas (Russo et al., 2023; Weiser, 2008). Nesse contexto as propriedades anti-inflamatórias, analgésicas e imunomoduladoras descritas para o ambroxol em diferentes modelos experimentais sustentam o interesse na investigação de seu potencial terapêutico em condições associadas à dor e à inflamação (Frota et al., 2025).

Assim sendo, formulou-se a hipótese de que esse fármaco seja capaz de reduzir a resposta inflamatória, atenuar a dor e promover alterações estruturais nos implantes endometrióticos em modelo experimental.

Para testar essa hipótese, a presente tese foi estruturada em dois capítulos complementares. O Capítulo 1 apresenta uma revisão bibliométrica da literatura acerca das atividades farmacológicas do ambroxol em modelos experimentais, permitindo identificar lacunas do conhecimento e fundamentar o estudo subsequente. O Capítulo 2 apresenta o estudo experimental original, desenvolvido em modelo animal de endometriose, destinado à avaliação dos efeitos analgésicos, anti-inflamatórios e estruturais do ambroxol.

2 REFERENCIAL TEÓRICO

2.1 Endometriose

2.1.1 Etiopatogenia

Estudiosos da endometriose identificaram relatos consistentes com suas apresentações clínicas modernas que datam do século XVII (Knapp, 1999). A primeira descrição histopatológica da doença foi realizada por von Rokitansky em 1860 (Rokitansky, 1980) e, posteriormente, em 1896, o termo “adenomiomas” foi proposto em razão da semelhança das lesões com a mucosa uterina (Cullen, 1896).

A etiopatogenia da endometriose permanece complexa e ainda não completamente elucidada, sendo reconhecida como multifatorial e resultante da interação entre fatores hormonais, imunológicos, genéticos, epigenéticos e ambientais (Zondervan et al., 2018; Zubrzycka et al., 2015).

Historicamente, três principais teorias foram propostas para explicar a origem da endometriose: a menstruação retrógrada, a metaplasia celômica e a disseminação linfática e hematogênica. Entretanto, nenhuma dessas hipóteses é capaz, isoladamente, de explicar a totalidade dos fenótipos da doença, o que reforça seu caráter heterogêneo e multifatorial (Patel et al., 2018; Vercellini et al., 2014).

A teoria da menstruação retrógrada, originalmente descrita por Sampson (1927), permanece como a hipótese mais amplamente aceita. Segundo essa teoria, células endometriais viáveis refluxam através das tubas uterinas durante a menstruação, implantando-se na cavidade peritoneal (Sampson, 1927). Entretanto, como esse fenômeno ocorre em grande parte das mulheres sem desenvolvimento da doença, mecanismos adicionais são necessários para explicar a adesão, sobrevivência e progressão das lesões ectópicas.

Alterações na resposta imune assumem papel central na patogênese da endometriose. A redução da capacidade de depuração de células endometriais ectópicas, associada a um ambiente inflamatório peritoneal favorável, contribui para a

sua sobrevivência, implantação e crescimento (Patel et al., 2018; Vinatier et al., 2001). Evidências recentes também indicam disfunções na imunidade inata e adaptativa, com ativação inflamatória persistente e possível participação de mecanismos autoimunes, contribuindo para a manutenção da doença (Garmendia et al., 2025).

Além disso, fatores hormonais, especialmente a dependência estrogênica e a resistência à progesterona, favorecem a proliferação, sobrevivência e manutenção das lesões endometrióticas. Paralelamente, mecanismos genéticos e epigenéticos modulam a expressão gênica e a resposta celular, contribuindo para a heterogeneidade clínica observada entre as pacientes. Alterações na metilação do DNA, na expressão de microRNAs e na regulação de vias inflamatórias figuram entre os principais mecanismos envolvidos na gênese e progressão da doença (Bedrick et al., 2024; Ducreux et al., 2025).

Adicionalmente, processos como estresse oxidativo, angiogênese e neurogênese participam da fisiopatologia da doença, estabelecendo um microambiente favorável à progressão das lesões e ao desenvolvimento da dor crônica, sustentado por interações entre fatores inflamatórios, imunológicos e moleculares (Bo; Wang, 2024; Zondervan et al., 2018) .

Dessa forma, a endometriose deve ser compreendida como uma condição sistêmica, multifatorial e dinâmica, na qual a interação entre fatores locais e sistêmicos determina sua instalação, progressão e manifestações clínicas.

2.1.2 Classificação

As lesões endometrióticas podem ocorrer em diferentes localizações anatômicas, incluindo ovários, trompas de falópio, ligamentos uterinos, região cervicovaginal, parede abdominal, trato urinário e intestino (Laganà et al., 2017; Vetvicka et al., 2016).

Classicamente, a endometriose é subdividida em três principais fenótipos clínico-anatômicos: endometrioma ovariano, lesões peritoneais superficiais e endometriose infiltrativa profunda (Chantalat et al., 2020). A adenomiose, tradicionalmente denominada como endometriose interna, caracteriza-se pela presença de tecido endometrial no miométrio, podendo manifestar-se de forma difusa

ou focal (adenomioma), sendo considerada uma condição associada, embora com fisiopatologia distinta (Figura 1).

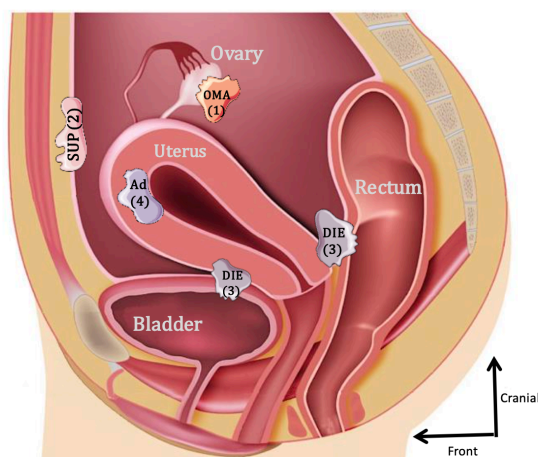


Figura 1. Vista lateral esquemática da pelve ilustrando 4 formas de endometriose: 1: endometrioma (OMA); 2: endometriose peritoneal superficial (SUP); 3: endometriose infiltrante profunda (DIE); 4: adenomioma (Ad). Fonte: Adaptada de Chantalat et al., 2020.

O sistema de classificação da endometriose mais conhecido e utilizado foi lançado pela American Society for Reproductive Medicine em 1996, classificando a gravidade da endometriose em quatro diferentes estágios (I - IV), de mínimo a grave, de acordo com a pontuação obtida a partir de achados cirúrgicos, como o tamanho dos implantes, estruturas pélvicas envolvidas e disseminação das lesões (Zondervan et al., 2018). Entretanto, esse sistema apresenta limitações importantes, especialmente por não correlacionar de forma consistente os achados anatômicos com a intensidade dos sintomas clínicos, como a dor, nem com o potencial reprodutivo das pacientes.

Outros sistemas, como a classificação Enzian (Keckstein et al., 2021), foram desenvolvidos para melhor caracterização anatômica da endometriose profunda, descrevendo compartimentos específicos da pelve. Apesar de sua utilidade na prática cirúrgica, esses modelos também permanecem centrados em parâmetros anatômicos, sem incorporar de forma consistente aspectos clínicos relevantes.

De modo geral, observa-se que os sistemas de classificação existentes priorizam a caracterização morfológica da doença, frequentemente sem adequada correlação com suas manifestações clínicas. Essa dissociação, particularmente quanto à correlação entre extensão anatômica e intensidade da dor, uma das

principais manifestações da endometriose, representa limitação relevante tanto para a prática clínica quanto para a padronização de estudos científicos, além de evidenciar a necessidade de aprimoramento dos modelos classificatórios atuais (Andres; Borrelli; Abrão, 2018; Haas et al., 2011).

2.1.3 Dor, inflamação e modulação imune na endometriose

Diversos mecanismos de dor são descritos na endometriose, incluindo níveis elevados de fatores de crescimento neural no líquido peritoneal, promovendo neurogênese; alterações na proporção entre fibras nervosas simpáticas e sensoriais; aumento da densidade neural em nódulos endometrióticos, com possível encarceramento de fibras; além da ativação de fibras nervosas por citocinas e prostaglandinas produzidas por mastócitos e outras células inflamatórias recrutadas, o que pode induzir a liberação adicional de mediadores inflamatórios por células adjacentes (Anaf, 2000; Asally; Markham; Manconi, 2019; Nezhat et al., 2017).

A endometriose constitui uma das principais causas de dor pélvica crônica (DPC) na população feminina. A DPC é definida como dor não cíclica localizada na região umbilical ou infraumbilical, com duração mínima de três a seis meses e impacto nas atividades diárias (Powell, 2014). Aproximadamente 70% das pacientes com endometriose apresentam DPC (Laufer et al., 1997; Mowers et al., 2016; Powell, 2014).

A fisiopatologia da DPC está frequentemente relacionada à sensibilização central, caracterizada pela redução do limiar doloroso decorrente da persistência do estímulo nociceptivo (Grace; Zondervan, 2004; Mathias, 1996; Zondervan et al., 1999), fenômeno que pode ser parcialmente reproduzido em modelos experimentais por meio da avaliação de hipersensibilidade mecânica e alterações comportamentais relacionadas à dor. Esse processo pode levar à despolarização excessiva de neurônios do corno dorsal da medula, resultando em excitotoxicidade, disfunção celular e perda de mecanismos inibitórios (Dubner; Ren, 2004).

Evidências moleculares e celulares relacionadas à neuropatia periférica têm-se tornado progressivamente mais consistentes. A invasão direta de fibras nervosas por lesões endometrióticas e a neuroangiogênese contribuem para a sensibilização

periférica, o desenvolvimento de memória nociceptiva e a redução da modulação descendente da dor (Anaf et al., 2002; Asante; Taylor, 2011; Malykhina, 2007; Wang et al., 2009).

A dor neuropática periférica pode manifestar-se como dor espontânea, independente de estímulo, ou como hipersensibilidade induzida por estímulos, decorrente de alterações nos neurônios sensoriais. A função neuronal depende não apenas do próprio neurônio, mas também das células gliais de suporte e do microambiente tecidual (Woolf; Mannion, 1999).

A dor inflamatória é desencadeada por dano ou inflamação tecidual, enquanto a dor neuropática decorre de lesões no sistema nervoso. Ambas podem cursar com hipersensibilidade no local da lesão e em tecidos adjacentes. Entretanto, enquanto a dor inflamatória tende a regredir com o controle do processo patológico, a dor neuropática pode persistir mesmo após a resolução do estímulo inicial, caracterizando uma alteração funcional duradoura do sistema nervoso (Woolf; Salter, 2000).

Clinicamente, pacientes podem desenvolver hiperalgesia, caracterizada pela intensificação da dor previamente leve ou moderada, e alodinia, na qual estímulos normalmente não dolorosos são percebidos como dolorosos, frequentemente associadas à disfunção do assoalho pélvico (Engeler et al., 2013; Potts; Payne, 2012).

Em mulheres com endometriose e dor pélvica, alterações neuroplásticas em regiões cerebrais relacionadas à dor têm sido descritas, sugerindo que a estimulação periférica contínua, associada ao ambiente inflamatório, pode contribuir para a cronificação da dor, tornando-a parcialmente independente da presença ou extensão das lesões (As-Sanie et al., 2012).

A inflamação desempenha papel central no desenvolvimento e progressão da endometriose, estando diretamente associada à dor e à infertilidade (Donnez, 2012; Laganà et al., 2016; Santulli et al., 2012). As cascatas inflamatórias envolvem a produção de diversos mediadores, incluindo metaloproteinases, prostaglandinas, quimiocinas e citocinas (Vetvicka et al., 2016).

Níveis aumentados de ciclooxygenase-2 (COX-2), fator de crescimento endotelial vascular (VEGF), IL-6, TNF- α , IL-8, MCP-1 e IL-10 foram descritos no líquido peritoneal de pacientes com endometriose (Sikora et al., 2017; Sikora; Mielczarek-Palacz; Kondera-Anasz, 2016).

A IL-1 β destaca-se como uma citocina pró-inflamatória central, produzida principalmente por monócitos e macrófagos ativados via inflamassoma, envolvendo o

eixo NF- κ B/NLRP3/caspase-1. Essa citocina amplifica a resposta inflamatória ao induzir a produção de outras citocinas e quimiocinas, como a IL-6, por meio da ativação da via NF- κ B (Rada et al., 2014).

A ativação da via NF- κ B por estímulos como IL-1 β e TNF- α promove a transcrição de genes pró-inflamatórios, incluindo IL-6, que, por sua vez, ativa a via JAK/STAT3, contribuindo para a manutenção de processos inflamatórios, angiogênicos e de sobrevivência celular (Brasier, 2010; Grivennikov; Karin, 2010).

A interação entre IL-6 e TGF- β também pode induzir a diferenciação de linfócitos T CD4⁺ em células Th17, com produção subsequente de IL-17, estabelecendo um ciclo de retroalimentação inflamatória (Hu et al., 2025; Kimura; Naka; Kishimoto, 2007; Ogura et al., 2008).

Mecanismos regulatórios, como a ação de proteínas SOCS3, podem modular essa resposta. Entretanto, em estados crônicos de ativação imune, tais mecanismos podem ser insuficientes, favorecendo a manutenção da sinalização pró-inflamatória (Ageeva; Rizvanov; Mukhamedshina, 2024; Gu et al., 2011; Yang et al., 2025).

Revisão sistemática de Machairiotis et al. aponta a IL-1 β como um dos principais gatilhos da cascata inflamatória associada à dor e à neuroangiogênese na endometriose, enquanto a IL-6 participa da amplificação dessa resposta. A IL-10 apresenta papel ambíguo, podendo reduzir a inflamação, mas também favorecer a tolerância imunológica e a persistência das lesões (Machairiotis; Vasilakaki; Thomakos, 2021).

A endometriose também apresenta características compatíveis com disfunção imunológica, incluindo alterações na resposta imune humoral e celular, com envolvimento de diferentes subpopulações de linfócitos T CD4⁺, como Th1, Th2 e Th17 (Abramiuk et al., 2022; Dai et al., 2025; Szukiewicz, 2022).

De forma geral, observa-se desequilíbrio entre respostas Th1/Th2 e hiperativação do eixo IL-23/Th17, associado à disfunção de células T reguladoras (Tregs), contribuindo para um microambiente permissivo à progressão da doença. A predominância de respostas Th2 pode favorecer angiogênese e imunoescape, enquanto o aumento de células Th17 está associado à maior gravidade da doença (Chang et al., 2023; Delbandi et al., 2025; Khan et al., 2022; Pashizeh et al., 2020; Xia et al., 2023).

A IL-17A, produzida por células Th17, promove a secreção de citocinas pró-inflamatórias, estimula a proliferação celular e contribui para angiogênese e evasão

imune, favorecendo a sobrevivência e crescimento das lesões endometrióticas (Figura 2) (Hirata et al., 2008; Kang et al., 2023).

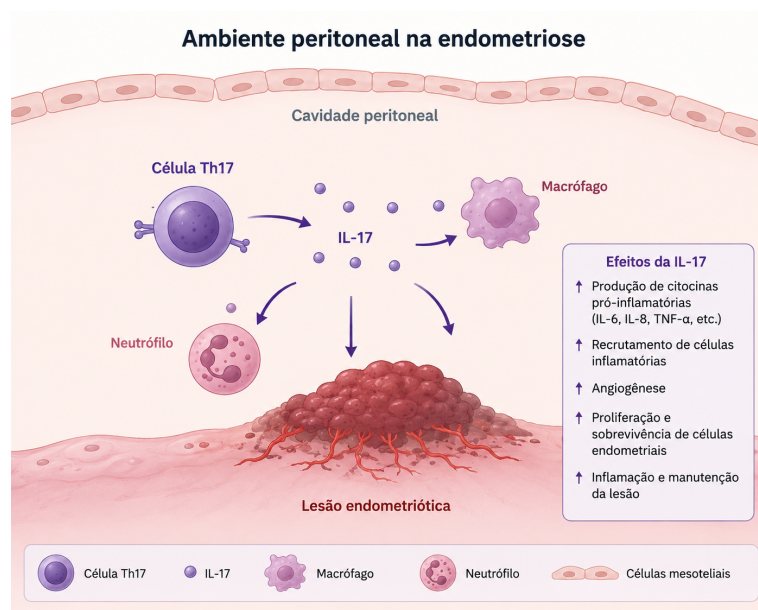


Figura 2. Representação esquemática da atividade imunoinflamatória mediada por células Th17 e IL-17 no microambiente peritoneal da endometriose. Fonte: Elaborada pelo autor com auxílio do ChatGPT (OpenAI), 2026.

Diante desses mecanismos, mediadores inflamatórios como IL-1 β , IL-6, IL-8, TNF- α , IL-17A e IL-10 destacam-se como componentes centrais do microambiente inflamatório e imunológico da endometriose, estando diretamente envolvidos na modulação da resposta inflamatória, na angiogênese e na sensibilização nociceptiva. A avaliação desses mediadores, em conjunto com parâmetros celulares inflamatórios, constitui uma abordagem relevante para a investigação da atividade inflamatória da doença e da resposta a intervenções terapêuticas em modelos experimentais (Abramiuk et al., 2022; Ageeva; Rizvanov; Mukhamedshina, 2024; Delbandi et al., 2025).

Além disso, a hiperexcitabilidade neuronal associada à dor na endometriose envolve alterações na atividade de canais iônicos, especialmente canais de sódio dependentes de voltagem (NaV), que desempenham papel central na geração e propagação do impulso nociceptivo. Evidências mais recentes reforçam o papel dos subtipos NaV1.7 e NaV1.8 na modulação da dor neuropática e inflamatória, consolidando esses canais como alvos terapêuticos relevantes (Bennett et al., 2019; Cummins; Sheets; Waxman, 2007; Dib-Hajj et al., 2013).

Em síntese, a dor na endometriose resulta da interação entre inflamação, modulação imune, nocicepção e fatores centrais, não sendo linearmente proporcional à extensão das lesões. Essa complexidade explica a dificuldade no controle completo da dor quando abordagens terapêuticas se concentram exclusivamente no local da doença (Tracey, 2016).

2.1.4 Diagnóstico

Apesar dos avanços diagnósticos, o intervalo médio estimado entre o início dos sintomas relatados pelas pacientes e o diagnóstico definitivo da endometriose ainda é de aproximadamente sete anos (Becker et al., 2022a).

Os testes biológicos atualmente apresentam utilidade clínica limitada no diagnóstico da endometriose, não havendo, até o momento, biomarcadores com acurácia suficiente para aplicação clínica rotineira (Falcone; Flyckt, 2018). Por outro lado, os métodos de imagem têm proporcionado avanços significativos na abordagem diagnóstica da doença.

A ultrassonografia transvaginal e a ressonância magnética constituem importantes ferramentas diagnósticas para a endometriose. Esses métodos apresentam elevada acurácia para a identificação de fenótipos específicos da doença, como o endometrioma ovariano (OMA) e a endometriose infiltrativa profunda (DIE), além de permitirem a avaliação da adenomiose, condição frequentemente associada à endometriose (Guerriero et al., 2016, 2018; Nisenblat et al., 2016; Van Den Bosch et al., 2019).

Além da sua ampla aplicação clínica, a ultrassonografia tem sido empregada em modelos experimentais de endometriose para monitoramento não invasivo da evolução das lesões, permitindo avaliações seriadas do volume dos implantes e da resposta a intervenções terapêuticas. Essa abordagem reduz a necessidade de procedimentos invasivos repetidos e amplia a confiabilidade das análises longitudinais em estudos pré-clínicos (D'Amico et al., 2022; Interdonato et al., 2023).

2.2 Modelos Experimentais Para Endometriose

O desenvolvimento de modelos *in vitro* e *in vivo* capazes de reproduzir as principais características da endometriose permanece desafiador em razão de múltiplos fatores. Primeiramente, os mecanismos relacionados ao início e à persistência da doença ainda não são completamente compreendidos. Além disso, a endometriose apresenta diferentes fenótipos, incluindo endometrioma ovariano, lesões peritoneais superficiais e endometriose infiltrativa profunda. Por fim, trata-se de uma condição multifatorial, sem um único processo fisiopatológico que possa ser integralmente reproduzido em modelos experimentais (Malvezzi et al., 2020).

Diante das limitações e das considerações éticas inerentes à experimentação em humanos, a investigação da endometriose depende predominantemente de modelos experimentais em primatas não humanos e roedores, utilizados para elucidar aspectos da fisiopatologia da doença (Bruner-Tran et al., 2018).

Modelos experimentais têm sido desenvolvidos cirurgicamente em pequenos animais, como coelhos, ratos e camundongos (Bergqvist et al., 1985; Jones, 1984; Schenken; Asch, 1980; Zamah et al., 1984). Entre estes, destaca-se o modelo cirúrgico por autotransplante uterino descrito por Vernon e Wilson (1985), amplamente empregado em ratas devido à sua elevada reprodutibilidade, simplicidade técnica e capacidade de reproduzir importantes aspectos histológicos e inflamatórios da endometriose. Diversas adaptações desse modelo têm sido utilizadas em estudos pré-clínicos, consolidando-o como uma das principais abordagens experimentais para investigação da doença (Schor et al., 1999; Vernon; Wilson, 1985).

As ratas destacam-se como modelos experimentais adequados, especialmente devido ao curto ciclo estral, com duração aproximada de 4 a 5 dias, o que permite a avaliações repetidas de fenômenos hormonais e inflamatórios em períodos reduzidos (Sharpe-Timms, 2002).

No presente estudo, a escolha do modelo experimental com ratas fundamenta-se no baixo custo, ampla utilização na literatura e relativa resistência desses animais a infecções. No entanto, a extrapolação direta para a doença humana permanece limitada em razão da diversidade de técnicas experimentais empregadas e da distância filogenética entre roedores e humanos (Zhou; Zhou; Wang, 2025). Uma

limitação dos modelos em roedores é a predominância de lesões superficiais induzidas, que podem não reproduzir integralmente as formas mais complexas da doença observadas em humanos, como a endometriose infiltrativa profunda (Malvezzi et al., 2020).

Diante disso, o presente estudo empregou um modelo experimental de endometriose em ratas induzido cirurgicamente, com o objetivo de investigar parâmetros inflamatórios e comportamentais associados à dor, bem como avaliar o efeito de intervenções farmacológicas sobre esses desfechos. Considerando as limitações inerentes aos modelos em roedores, a abordagem metodológica adotada busca equilibrar reprodutibilidade experimental e relevância biológica, permitindo a análise integrada de aspectos inflamatórios e nociceptivos da doença.

2.3 Tratamento

A evolução da cirurgia aberta para a laparoscopia trouxe benefícios importantes para as pacientes, com vantagens estabelecidas de uma técnica minimamente invasiva, incluindo menores incisões abdominais, melhor visualização, menor necessidade de analgesia pós-operatória, recuperação mais rápida, menores taxas de complicações, menor tempo de internação hospitalar e melhores resultados cosméticos (Cohn et al., 2016; De Paula Andres et al., 2017; Fanfani et al., 2016).

Atualmente, a laparoscopia é considerada o padrão-ouro para o tratamento da endometriose, permitindo a remoção de implantes peritoneais, nódulos profundos e cistos ovarianos (Greene et al., 2016; Wasson, 2020). Além disso, constitui o método mais eficaz para avaliação do grau de aderências. Apesar dos bons resultados no controle da doença sintomática, apresenta taxas relevantes de recorrência, podendo atingir valores mais elevados em períodos prolongados, o que frequentemente demanda novas intervenções cirúrgicas (Giudice; Kao, 2004; Guo, 2009).

Mais recentemente, a cirurgia laparoscópica assistida por robô tornou-se disponível (Berlanda et al., 2017) e vem sendo utilizada em diversos procedimentos ginecológicos, incluindo o tratamento da endometriose (Advincula et al., 2004; Bush; Apte, 2015; Dubin et al., 2017; Elliott et al., 2004; Manchana et al., 2015; O'Malley; Smith; Fowler, 2015). Entre suas potenciais vantagens estão maior precisão cirúrgica,

ausência de tremor, melhor percepção de profundidade e maior amplitude de movimento, características que podem contribuir para melhores resultados em casos selecionados (García; Olvera; Montoya, 2008; Luu; Uy-Kroh, 2017).

Como alternativa ou adjuvante ao tratamento cirúrgico, utilizam-se terapias hormonais de longo prazo, que atuam principalmente por meio da supressão da função ovariana e da redução da estimulação estrogênica das lesões endometrióticas (Dunselman et al., 2014; Millochau et al., 2016; Tafi et al., 2015).

Entre os progestagênios, o acetato de medroxiprogesterona (MPA) destaca-se como uma das opções terapêuticas amplamente utilizadas no manejo da endometriose, atuando por meio da supressão da ovulação, indução de atrofia endometrial e modulação da resposta inflamatória, com consequente redução da atividade das lesões ectópicas (Clemenza et al., 2023; Dunselman et al., 2014). Estudos demonstram sua eficácia na redução da dor associada à doença, incluindo dismenorreia, dispareunia e dor pélvica crônica, sendo considerado uma opção de primeira linha em diretrizes clínicas (Crosignani et al., 2006; BRASIL, 2025).

O MPA tem sido amplamente utilizado como referência em modelos experimentais de endometriose, em virtude de seu uso consolidado na prática clínica. No presente estudo, foi utilizado como grupo comparador, permitindo a avaliação do efeito de novas intervenções terapêuticas em relação a um tratamento consolidado.

De modo geral, anti-inflamatórios não esteroidais (AINEs), contraceptivos orais combinados e progestagênios, como dienogeste, são utilizados como terapias de primeira linha para o controle da dor. As opções de segunda linha incluem agonistas do hormônio liberador de gonadotropina (GnRH), inibidores de aromatase e, em casos selecionados, opioides (Becker et al., 2022b). Entretanto, essas abordagens apresentam limitações relevantes, como eficácia variável, efeitos adversos importantes e restrições ao uso prolongado, incluindo sintomas hipoestrogênicos, alterações metabólicas e risco de dependência (Dunselman et al., 2014; Giudice, 2010; Johnson et al., 2013; “Practice Bulletin No. 114”, 2010).

Além disso, abordagens complementares, como fisioterapia do assoalho pélvico, acupuntura, psicoterapia e o uso de moduladores da dor, como gabapentina e amitriptilina, podem ser empregadas como estratégias adjuvantes no manejo da dor crônica associada à endometriose (Montenegro et al., 2007; “Treatment of pelvic pain associated with endometriosis”, 2014; Xu et al., 2017).

Mesmo com múltiplas opções terapêuticas disponíveis, a maioria das abordagens apresenta caráter predominantemente sintomático, com eficácia limitada na modificação do curso da doença e taxas relevantes de recorrência após a suspensão do tratamento. Esse cenário evidencia a necessidade de desenvolvimento de novas estratégias terapêuticas capazes de atuar de forma mais abrangente sobre os mecanismos fisiopatológicos da endometriose, incluindo inflamação, modulação imune e excitabilidade neuronal.

2.4 Ambroxol: bases farmacológicas e potencial terapêutico

O ambroxol, também conhecido como Trans-4-(2-amino-3,5-dibromobenzilamino)-ciclohexanol, é um fármaco multifacetado de ações mucoativas e secretolíticas, com uma longa história de uso no tratamento de várias doenças do trato respiratório, como bronquite e DPOC, e demonstrou ainda ser eficaz no alívio da dor de garganta (Renovanz, 1975; Russo et al., 2023). Devido aos seus múltiplos mecanismos de ação, o fármaco tem sido considerado potencial candidato ao reposicionamento para condições de dor crônica multifatorial, como síndrome dolorosa regional complexa e fibromialgia, além de apresentar racional farmacológico para investigação em outras doenças inflamatórias e dolorosas, incluindo a endometriose.

Tendo seu primeiro registro por um membro da União Europeia em 1978 (EUROPEAN MEDICINES AGENCY, 2015), o ambroxol possui perfil de segurança bem estabelecido, com ampla avaliação pré-clínica, boa tolerabilidade clínica, dados de toxicologia e interação medicamentosa disponíveis, além de um conjunto consistente de propriedades farmacodinâmicas e farmacocinéticas descritas (Malerba; Ragnoli, 2008; Weiser, 2008).

Derivado da bromexina, o agente apresenta um centro quiral, tornando a molécula assimétrica (Figura 3), o que permite sua existência na forma de dois enantiômeros. Teoricamente, a quiralidade pode influenciar propriedades farmacocinéticas e farmacodinâmicas, como absorção, distribuição, metabolismo e interação com alvos moleculares. No entanto, o fármaco é utilizado clinicamente na

forma racêmica, não havendo evidências consistentes de diferenças terapêuticas relevantes entre os enantiômeros (Malerba; Ragnoli, 2008; Weiser, 2008).

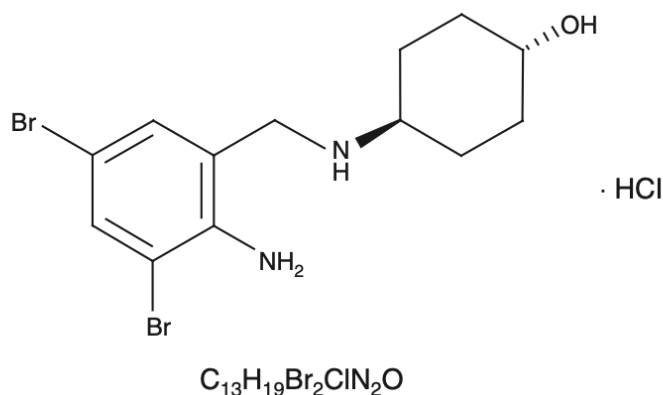


Figura 3. Estrutura química do Cloridrato de Ambroxol. Fonte: Adaptada de Malerba; Ragnoli, 2008.

O ambroxol apresenta rápida absorção pelo trato gastrointestinal, biodisponibilidade entre 70% e 80%, concentração plasmática máxima entre 1 e 2 horas e meia-vida terminal em torno de 10 horas, permitindo administração em intervalos de 12 horas. Seu metabolismo é predominantemente hepático, via CYP3A4, e aproximadamente 90% da dose é eliminada pela urina em até cinco dias, parcialmente na forma inalterada (Kantar et al., 2020; Weiser, 2008).

Considerando seus potenciais efeitos analgésicos, antioxidantes e anti-inflamatórios, o ambroxol tem recebido atenção especial, sendo avaliado em modelos experimentais, incluindo estudos in vivo e in vitro, bem como em investigações clínicas voltadas ao manejo da dor. Sua atividade analgésica sugere potencial alívio em condições crônicas, neuropáticas e inflamatórias (Frota et al., 2025).

As propriedades anestésicas locais do ambroxol foram descritas ainda na década de 1970, quando soluções tópicas demonstraram supressão de reflexos sensoriais de forma comparável ou superior a anestésicos locais clássicos (Klier; Papendick, 1977; Püschmann; Engelhorn, 1978). Posteriormente, estudos demonstraram que esse efeito está relacionado ao bloqueio de canais de sódio dependentes de voltagem (NaV) em neurônios sensoriais, com potência relevante em comparação a anestésicos locais estabelecidos (Weiser, 2006; Weiser; Wilson, 2002). O ambroxol destaca-se como um dos poucos inibidores disponíveis com atividade sobre NaV1.8, canal diretamente envolvido na fisiopatologia da dor neuropática (Russo et al., 2023; Weiser; Wilson, 2002).

Estudos subsequentes demonstraram o papel dos canais tetrodotoxina-resistentes, atualmente classificados como NaV1.8, nos mecanismos de nociceção mecânica, térmica e inflamatória, especialmente em neurônios sensitivos de pequeno diâmetro (Akopian et al., 1999). Evidências mais recentes indicam que a dor neuropática resulta da interação entre diferentes subtipos de canais de sódio, incluindo NaV1.7, NaV1.8 e NaV1.9, contribuindo para estados de hiperexcitabilidade neuronal (Liu; Wood, 2011).

A potência do ambroxol sobre esses canais apresenta caráter uso-dependente, com maior afinidade por neurônios hiperexcitáveis, como observado em condições inflamatórias e neuropáticas, o que reforça seu potencial como modulador da dor (Russo et al., 2023).

Em modelos experimentais, o ambroxol demonstrou reduzir níveis de citocinas pró-inflamatórias, como TNF- α , IL-6 e IL-1 β , além de modular o estresse oxidativo em diferentes tecidos (Ge et al., 2016; Mitini-Nkhoma et al., 2021; Su et al., 2004). Esses efeitos estão associados à redução da hiperalgesia e da alodinia mecânica em modelos de inflamação periférica crônica (Belkouch et al., 2014; Gaida et al., 2005).

Na prática clínica, o ambroxol tem sido explorado em condições como fibromialgia e síndromes dolorosas complexas, com relatos de redução de sintomas como hiperalgesia, alodinia e dor difusa, além de efeitos sobre marcadores inflamatórios e estresse oxidativo (Kern; Schwickert, 2017; Martínez-Martínez et al., 2017; Meng et al., 2020).

Além de sua ação periférica, evidências farmacológicas sugerem que o ambroxol pode alcançar o sistema nervoso central, o que amplia seu potencial de modulação de mecanismos relacionados à hiperexcitabilidade neuronal e à sensibilização central. Entretanto, a extensão e a relevância clínica dessa penetração permanecem dependentes da dose, da formulação e do modelo avaliado (Weiser, 2008).

Nos últimos anos, o ambroxol também vem sendo investigado em outras condições, incluindo doenças neurodegenerativas associadas a mecanismos celulares complexos, reforçando seu potencial de reposicionamento farmacológico (Bouscary et al., 2019; Mohamed; Al-Jasmi, 2024; Silveira et al., 2025). Esse conjunto de dados contribui para consolidar seu perfil de segurança e sua aplicabilidade em diferentes condições clínicas, particularmente aquelas associadas à dor e à inflamação.

Ainda assim, as evidências clínicas são limitadas em número e rigor metodológico, de modo que o uso do ambroxol para dor crônica permanece em caráter experimental, demandando validação por estudos controlados.

Em conjunto, os achados pré-clínicos sugerem que o ambroxol apresenta um perfil farmacológico compatível com os principais mecanismos envolvidos na inflamação e modulação da dor, com potencial relevância para o microambiente peritoneal. Esse conjunto de ações sustenta seu potencial efeito multimodal sobre os componentes inflamatórios e nociceptivos da doença, justificando sua investigação em modelo experimental de endometriose.

3 OBJETIVOS

3.1 Objetivo Geral

Investigar o potencial terapêutico do ambroxol na endometriose, integrando evidências provenientes de uma análise bibliométrica sobre suas atividades farmacológicas em modelos experimentais e de um estudo experimental de seus efeitos analgésicos, anti-inflamatórios e estruturais em ratas Wistar com endometriose.

3.2 Objetivos Específicos

- Caracterizar, por meio de revisão bibliométrica, a produção científica relacionada às atividades analgésica, anti-inflamatória e antioxidante do ambroxol em modelos experimentais, identificando tendências de pesquisa e lacunas do conhecimento;
- Determinar os efeitos do ambroxol sobre a manifestação de dor espontânea em ratas com endometriose;
- Avaliar os efeitos do ambroxol sobre a alodinia mecânica e o desempenho motor em ratas com endometriose;
- Analisar a ação anti-inflamatória do ambroxol, por meio da dosagem sérica de IL-1 β .
- Determinar o escore de integridade epitelial dos implantes endometrióticos após tratamento.
- Quantificar a redução volumétrica dos implantes após tratamento com o ambroxol.
- Comparar os efeitos do ambroxol e do acetato de medroxiprogesterona em ratas com endometriose experimental.

CAPÍTULO 1

Este capítulo apresenta uma revisão bibliométrica publicada em 2025 (Anexo A), cujo objetivo foi identificar tendências de pesquisa e lacunas relacionadas às atividades farmacológicas do ambroxol em modelos experimentais, fornecendo subsídios para a formulação da hipótese e para o delineamento metodológico do estudo experimental apresentado no Capítulo 2.

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O conteúdo apresentado a seguir corresponde integralmente ao artigo publicado, sendo reproduzido em sua forma original.

New perspectives on the use of ambroxol hydrochloride: a recent bibliometric analysis in experimental models

Novas perspectivas de uso do cloridrato de ambroxol: uma recente análise bibliométrica em modelos experimentais

Nuevas perspectivas para el uso del clorhidrato de ambroxol: um reciente análisis bibliométrico en modelos experimentales

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ABSTRACT

Ambroxol, also known as Trans-4-(2-amino-3,5-dibromobenzylamino)-cyclohexanol (ABX), is a chemical compound with mucolytic properties used to treat pulmonary diseases such as bronchitis and cystic fibrosis. It enhances mucus clearance in the respiratory tract. Recent studies have suggested its broad therapeutic potential, including analgesic, antioxidant, and anti-inflammatory effects in various clinical contexts. This study aimed to systematically identify research trends and critical points in the pharmacological activities of ambroxol hydrochloride in experimental models. A bibliometric review was conducted, retrieving 24 publications from the Web of Science and Scopus databases without temporal restrictions, using appropriate English keywords. The quality of the publications was assessed using impact metrics, productivity, term density mapping, and author network analysis. The average publication rate was 0.7 articles per year, with significant growth starting in the 2000s, a notable increase between 2016 and 2020, and a peak in 2021 with 5 articles published. China was the country with the highest number of publications. The primary field of publication was medicine (33.3%), and the journals with the best metrics were Intensive Care and Clinical and Experimental Medicine. Keyword co-occurrence analysis identified a complex network of 77 terms, divided into 4 clusters. Co-authorship analysis revealed 139 authors, divided into 21 clusters, with the largest cluster connecting 24 authors. A scarcity of publications addressing the pleiotropic effects of ambroxol in experimental models was observed. This bibliometric review highlights the need for further research in this field to explore new therapeutic approaches and possibilities, leveraging the potential effects of this drug.

Keywords: Animal. Ambroxol. Treatment. Analgesic. Anti-Inflammatory. Antioxidant.

RESUMO

O Ambroxol, também conhecido como Trans-4-(2-amino-3,5-dibromobenzylamino)-ciclohexanol (ABX), é um composto químico com propriedades mucolíticas para tratamento de doenças pulmonares, como bronquite e fibrose cística, tendo a capacidade de estimular a eliminação de muco nas vias respiratórias. Estudos têm sugerido um amplo potencial terapêutico deste fármaco, com ações analgésicas, antioxidantes e anti-inflamatórias, em diferentes contextos clínicos. Diante disso, este estudo teve como objetivos fornecer sistematicamente tendências de pesquisa e pontos críticos no campo das atividades farmacológicas do cloridrato de ambroxol em modelos experimentais. Trata-se de uma revisão bibliométrica, onde foram recuperadas 24 publicações relacionadas a esse tema, sem delineamento temporal, das bases de dados Web of Science e Scopus, usando palavras-chave apropriadas em inglês. A qualidade das publicações foi avaliada por meio de métricas de impacto, produtividade, mapeamento de densidade de termos e rede de autores. A taxa média de publicação foi de 0,7 artigos por ano, com maior crescimento a partir dos anos 2000, tendo aumento significativo entre 2016 e 2020, e atingindo o pico em 2021 com 05 artigos por ano. A China foi o país com o maior número de publicações. A principal área de publicação foi medicina (33,3%), os periódicos com melhores métricas foram

Intensive Care e Clinical and Experimental Medicine. A relação de ocorrência entre palavras chaves identificou uma rede complexa de 77 termos, divididos em 4 clusters. E a ocorrência de co-autores identificou 139 nomes, divididos em 21 clusters, sendo que o maior cluster conectou 24 nomes. Observou-se escassez de publicações que abordassem o pleiotropismo de ação do Ambroxol em modelos experimentais. Após realizar esta revisão bibliométrica, pode-se inferir que mais pesquisas são necessárias neste campo, para desenvolver novas abordagens e possibilidades terapêuticas, explorando os potenciais efeitos deste fármaco.

Palavras-chave: Animal. Ambroxol. Tratamento. Analgésico. Anti-inflamatório. Antioxidante.

RESUMEN

El ambroxol, también conocido como Trans-4-(2-amino-3,5-dibromobencilamino)-ciclohexanol (ABX), es un compuesto químico con propiedades mucolíticas para el tratamiento de enfermedades pulmonares, como la bronquitis y la fibrosis quística, teniendo la capacidad de estimular la eliminación de moco en el tracto respiratorio. Estudios han sugerido un amplio potencial terapéutico de este fármaco, con acciones analgésicas, antioxidantes y antiinflamatorias, en diferentes contextos clínicos. Por lo tanto, este estudio tuvo como objetivo proporcionar sistemáticamente tendencias de investigación y puntos críticos en el campo de las actividades farmacológicas del clorhidrato de ambroxol en modelos experimentales. Se trata de una revisión bibliométrica, donde se recuperaron 24 publicaciones relacionadas con este tema, sin delineación temporal, de las bases de datos Web of Science y Scopus, utilizando palabras clave apropiadas en inglés. La calidad de las publicaciones se evaluó utilizando métricas de impacto, productividad, mapeo de densidad de términos y red de autores. La tasa promedio de publicación fue de 0,7 artículos por año, con mayor crecimiento a partir de la década del 2000, con un aumento significativo entre 2016 y 2020, y alcanzando su pico en 2021 con 05 artículos por año. China fue el país con mayor número de publicaciones. El principal área de publicación fue medicina (33,3%), las revistas con mejores métricas fueron Intensive Care y Clinical and Experimental Medicine. La relación de ocurrencia entre palabras clave identificó una red compleja de 77 términos, divididos en 4 grupos. Y la aparición de coautores identificó 139 nombres, divididos en 21 grupos, y el grupo más grande conectó 24 nombres. Hubo escasez de publicaciones que abordaran la pleiotropía de la acción del Ambroxol en modelos experimentales. Luego de realizar esta revisión bibliométrica, se puede inferir que se necesita más investigación en este campo, para desarrollar nuevos enfoques y posibilidades terapéuticas, explorando los efectos potenciales de este fármaco.

Palabras clave: Animal. Ambroxol. Tratamiento. Analgésico. Antiinflamatorio. Antioxidante.

1 INTRODUCTION

The investigation of the pharmacological properties of natural and synthetic compounds is a highly significant area in biomedical research, aimed at developing effective and safe therapies for various health conditions.

Ambroxol (ABX), also known as Trans-4-(2-amino-3,5-dibromobenzylamino)-cyclohexanol, has a long history of use as a mucolytic agent over several decades. It enhances ciliary mucus clearance and exhibits specific activity in the respiratory epithelium (Cazan *et al.*, 2018). As an active metabolite of bromhexine (2-amino-3,5-dibromo-N-methylbenzylamine), ABX demonstrates anti-inflammatory and antioxidant properties (Pfeifer *et al.*, 1997), in addition to its anesthetic effects (Ratjen *et al.*, 1985).

Given its potential analgesic, antioxidant, and anti-inflammatory effects, ABX has garnered particular attention and has been the subject of study and evaluation in various clinical and experimental contexts. Its analgesic activity has been explored in preclinical and clinical studies, suggesting potential pain relief in conditions such as chronic, neuropathic, and inflammatory diseases. Understanding the scope and mechanisms underlying this activity is crucial for improving pain management strategies, especially in light of the growing concern over adequate symptom control (Furgala *et al.*, 2018; Cao; Hou; Zhang, 2019).

The investigation of the pharmacological properties of substances with pleiotropic action profiles, such as ABX, has become an area of increasing interest in the scientific community. In the face of a scarcity of new effective therapeutic agents, researchers have turned their attention to the potential secondary therapeutic uses of these compounds in diverse contexts. This bibliometric review aims to systematically identify research trends and critical points in the pharmacological activities of ABX in experimental models.

2 MATERIALS AND METHODS

2.1 ETHICAL CONSIDERATIONS

As this study involved the analysis of published research, ethical approval was not required. The data used were publicly available and did not involve direct interaction with human subjects or their personal information. Furthermore, no authors were contacted for additional information about their publications, as the analysis focused solely on the data available within the publications themselves.

2.2 DATA SOURCES AND SEARCH STRATEGY

The focus of this review was to investigate the analgesic, antioxidant, and anti-inflammatory actions of ABX in *in vivo* experimental models. The studies included in the review were published in indexed journals, with no temporal restrictions. The search was conducted in the following databases: Web of Science and Scopus.

These databases were chosen due to their recognition and reputation as sources of studies focused on clinical practice. Combining these databases helps identify a greater number of studies on the topic across diverse countries and is frequently used in bibliometric analyses.

The search strategy was conducted during April and May 2024, with data collected independently by peers to ensure the inclusion of all relevant articles. The following descriptors, combined with Boolean operators, were used: [(Ambroxol) OR Ambroxol Hydrochloride] AND [(Anti-Inflammatory Agents OR Analgesics OR Antioxidants)] AND [(Animals OR *in vivo* OR Rattus)].

2.3 ELIGIBILITY CRITERIA

To select studies aligned with the review's focus, the following inclusion criteria were applied: original articles published in indexed databases, in English, with experimental designs (studies using animal models), and without year

restrictions. Excluded were duplicate articles, dissertations, theses, case studies, clinical protocols, editorials, book chapters, conference abstracts, event proceedings, and articles accepted for publication but not yet published. In cases of disagreement regarding study selection, a third author resolved the discrepancy. All study information was extracted from the Scopus database, ensuring a comprehensive data collection for bibliometric analysis.

The articles were evaluated based on bibliometric indicators, including citation frequency by author and journal, CiteScore, Journal Citation Report (JCR), and Source Normalized Impact per Paper (SNIP). Additionally, the following factors were considered: countries of publication, journal types, study areas, keyword co-occurrence, and co-authorship relationships among the authors of the selected studies.

2.4 BIBLIOMETRIC ANALYSIS

For the bibliometric analysis, evaluative techniques were employed, including productivity metrics (number of articles per year and number of documents per author) and impact metrics (number of citations per journal and number of citations per author). Relational techniques were also utilized, such as bibliographic coupling and co-word analysis.

All data were analyzed using VOSviewer® version 1.6.20 (Leiden University, Netherlands), which was used for bibliometric mapping and cluster analysis. VOSviewer® is a specialized software designed to construct and visualize bibliometric networks, enabling the visualization of academic output in terms of publications and citation information within a specific field (Van Eck; Waltman, 2023). Based on similarity, a series of cluster analysis algorithms were applied to identify natural divisions, or clusters, within the research network. This facilitates the visualization of co-authorship networks among researchers, institutions, and countries (Zhao *et al.*, 2021).

The frequency of words in the abstracts of the articles was graphically represented using the online platform Word Cloud Art. Finally, the data generated

from these quantitative descriptive analyses are presented in graphs, tables, and images, displaying the results in absolute numbers and percentages.

3 RESULTS

3.1 CHARACTERISTICS OF THE INCLUDED STUDIES

Using the descriptors in the databases, 107 studies were initially identified (39 in Web of Science and 68 in Scopus). After applying the eligibility criteria, 52 studies remained (22 from Web of Science and 30 from Scopus). However, 28 duplicates were excluded, resulting in a final total of 24 studies included in the bibliometric data analysis, with an average of 0.7 publications per year during the studied period.

Table 1 provides a descriptive synthesis of the 24 studies, including the year, authors, study title, journal, and impact factor according to the Journal Citation Report. It was observed that the highest number of publications on the topic began in the 2000s, with a significant increase between 2016 and 2020 (Figure 1).

Table 1. Experimental studies on the analgesic, anti-inflammatory, and antioxidant actions of Ambroxol in experimental models, 1994–2023.

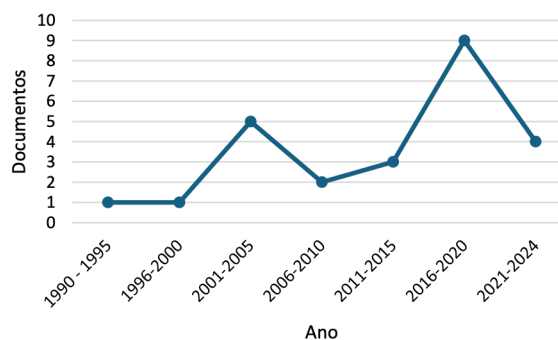
Authors, year	Title	Journal	JCR*
Lapenna <i>et al.</i> , 1994	Ambroxol is a scavenger of hypochlorous acid and monochloramine	Pharmacology	3.1
Strapková; Nosál'ová; Franová, 1999	Mucolytics and antioxidant activity	Life Sciences	6.1
Stetinová; Herout; Kvetina, 2004	In vitro and in vivo antioxidant activity of ambroxol	Clinical and Experimental Medicine	4.6
Su <i>et al.</i> , 2004	Inhibition of inflammatory responses by ambroxol, a mucolytic agent, in a murine model of acute lung injury induced by lipopolysaccharide	Intensive Care Medicine	38.9
Gaida <i>et al.</i> , 2005	Ambroxol, a Nav1.8-preferring Na ⁺ channel blocker, effectively suppresses pain symptoms in animal models of chronic, neuropathic and inflammatory pain	Neuropharmacology	5.3
Haque; Gilani, 2005	Effect of ambroxol, spirulina and vitamin-E in naphthalene induced cataract in female rats	Indian Journal of Physiology and Pharmacology	ausent e

Miyazaki <i>et al.</i> , 2005	The effect of taste masking agents on in situ gelling pectin formulations for oral sustained delivery of paracetamol and ambroxol	International Journal of Pharmaceutics	5.8
Weiser, 2006	Comparison of the effects of four Na ⁺ channel analgesics on TTX-resistant Na ⁺ currents in rat sensory neurons and recombinant Nav1.2 channels	Neuroscience Letters	2.5
Hama; Plum; Sagen, 2010	Antinociceptive effect of ambroxol in rats with neuropathic spinal cord injury pain	Pharmacology Biochemistry and Behavior	3.6
Li <i>et al.</i> , 2011	Effect of Ambroxol on Pneumonia Caused by <i>Pseudomonas aeruginosa</i> with Biofilm Formation in an Endotracheal Intubation Rat Model	Chemotherapy	3.3
Jiang <i>et al.</i> , 2013	Ambroxol Alleviates Hepatic Ischemia Reperfusion Injury by Antioxidant and Antiapoptotic Pathways	Transplantation Proceedings	0.9
Huang <i>et al.</i> , 2014	A thioredoxin reductase and/or thioredoxin system-based mechanism for antioxidant effects of ambroxol	Biochimie	3.9
Takeda <i>et al.</i> , 2016	Immunomodulatory effects of ambroxol on airway hyperresponsiveness and inflammation	Immune Network	6.0
Furgala <i>et al.</i> , 2018	Time-shifted co-administration of sub-analgesic doses of ambroxol and pregabalin attenuates oxaliplatin-induced cold allodynia in mice	Biomedicine & Pharmacotherapy	7.5
Yoshida <i>et al.</i> , 2018	Effects of the expectorant drug ambroxol hydrochloride on chemically induced lung inflammatory and neoplastic lesions in rodents	Journal of Toxicologic Pathology	1.2
Barut <i>et al.</i> , 2019	Uroprotective effect of ambroxol in cyclophosphamide-induced cystitis in mice	International Urology and Nephrology	2.0
Cao; Hou; Zhang, 2019	Ambroxol alleviates ventilator-induced lung injury by inhibiting c-Jun expression	European Review for Medical and Pharmacological Sciences	3.3
Sunkari <i>et al.</i> , 2019	Protective effects of ambroxol in psoriasis like skin inflammation: Exploration of possible mechanisms	International Immunopharmacology	5.6
Furgala-Wojas <i>et al.</i> , 2020	Comparison of bromhexine and its active metabolite-ambroxol as potential analgesics reducing oxaliplatin-induced neuropathic pain-pharmacodynamic and molecular docking studies	Current Drug Metabolism	2.3
Khoury <i>et al.</i> , 2020	A synergistic effect of Ambroxol and Beta-Glucosylceramide in alleviating immune-mediated hepatitis: A novel immunomodulatory non-immunosuppressive formulation for treatment of immune-mediated disorders	Biomedicine and Pharmacotherapy	7.2
Er <i>et al.</i> , 2021	The impact of ambroxol on the anti-inflammatory effect of azithromycin in lung tissue.	Veterinarski Arhiv	0.5

Cavalu <i>et al.</i> , 2022	Ambroxol, a mucolytic agent, boosts HO-1, suppresses NF- κ B, and decreases the susceptibility of the inflamed rat colon to apoptosis: A new treatment option for treating ulcerative colitis	Faseb Journal	4.8
Gultekin <i>et al.</i> , 2022	Could Ambroxol reduce cytokines in hepatic ischemia-reperfusion injury in rats?	Bratislava Medical Journal- Bratislavske Lekarske Listy	1.5
Patzwaldt <i>et al.</i> , 2023	Repurposing the mucolytic agent ambroxol for treatment of sub-acute and chronic ischaemic stroke	Brain Communications	4.8

Source: Prepared by the authors themselves

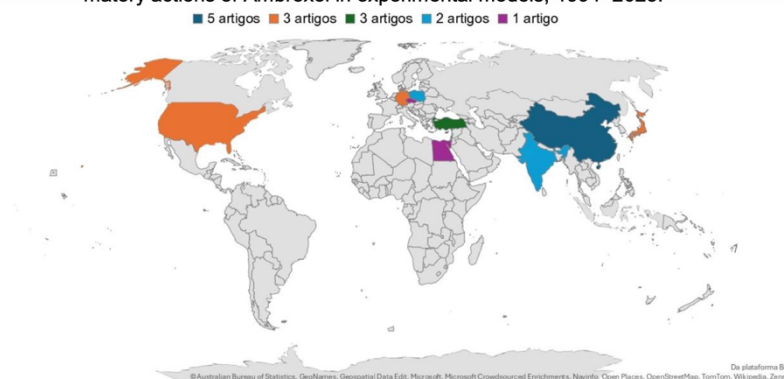
Figure 1. Temporal trend of publications on the antioxidant, analgesic, and anti-inflammatory actions of Ambroxol in experimental models, 1994–2023.



Source: Prepared by the authors themselves

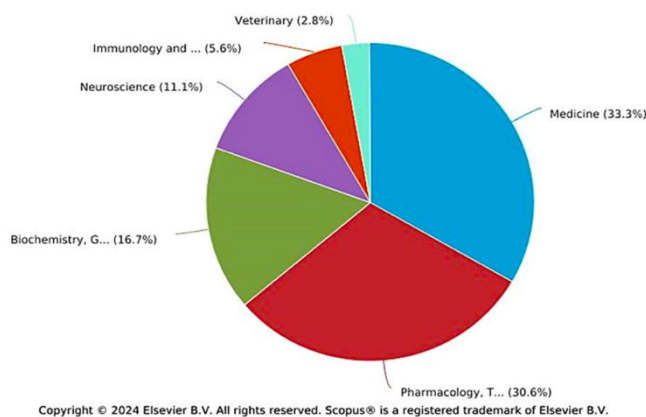
A total of 10 countries were identified through VOSviewer analysis. China had the highest number of publications (5), followed by Germany, Japan, Turkey, and the United States (3 publications each), India and Poland (2 publications each), and Cyprus, the Czech Republic, and Egypt (1 publication each) (Figure 2).

Figure 2. Distribution by country of publications on the antioxidant, analgesic, and anti-inflammatory actions of Ambroxol in experimental models, 1994–2023.



According to Scopus, ten research areas were identified. Medicine had the highest number of publications (33.3% – 8 publications), followed by Pharmacology (30.6% – 7 publications), Biochemistry (16.7% – 4 publications), and Neuroscience (11.1% – 3 publications) (Figure 3).

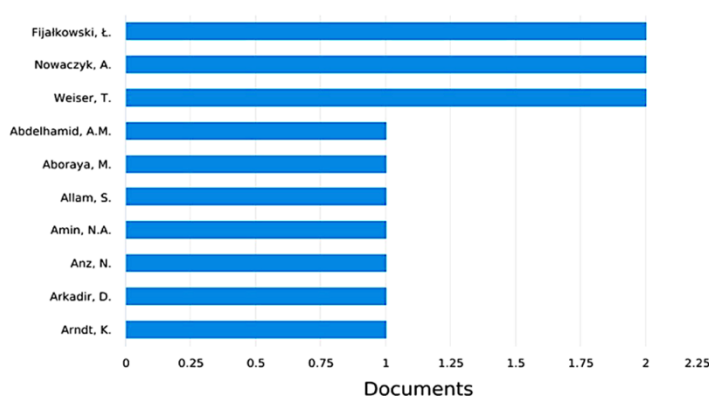
Figure 3. Distribution of thematic areas of publications on the antioxidant, analgesic, and anti-inflammatory actions of Ambroxol in experimental models, 1994–2023.



The analysis of documents by authors revealed that Fijalkowski, Nowaczyk, and Weiser had the highest number of published documents, with

multiple articles per author. The remaining authors had only one publication each (Figure 4).

Figure 4. Distribution by authors of publications on the antioxidant, analgesic, and anti-inflammatory actions of Ambroxol in experimental models, 1994–2023.



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Source: Prepared by the authors themselves

3.2 CITATION ANALYSIS

Table 2 shows the citation counts for the period before 2020 and from 2020 to 2024. The five studies with the highest citation counts are highlighted, along with the journals that published them: *Intensive Care Medicine* (112 citations, CiteScore 34.5, SJR 6.229, SNIP 5.381), *Neuropharmacology* (82 citations, CiteScore 9.8, SJR 1.307, SNIP 1.120), *Current Drug Metabolism* (64 citations, CiteScore 6.3, SJR 0.574, SNIP 0.818), *European review for medical and pharmacological sciences* (43 citations, CiteScore 6.3, SJR 0.634, SNIP 0.666), *Life Sciences* (41 citations, CiteScore 10.8, SJR 1.207, SNIP 1.121).

Table 2. Number of citations for the top 5 publications on the analgesic, anti-inflammatory, and antioxidant activities of Ambroxol in experimental models, 1994–2023.

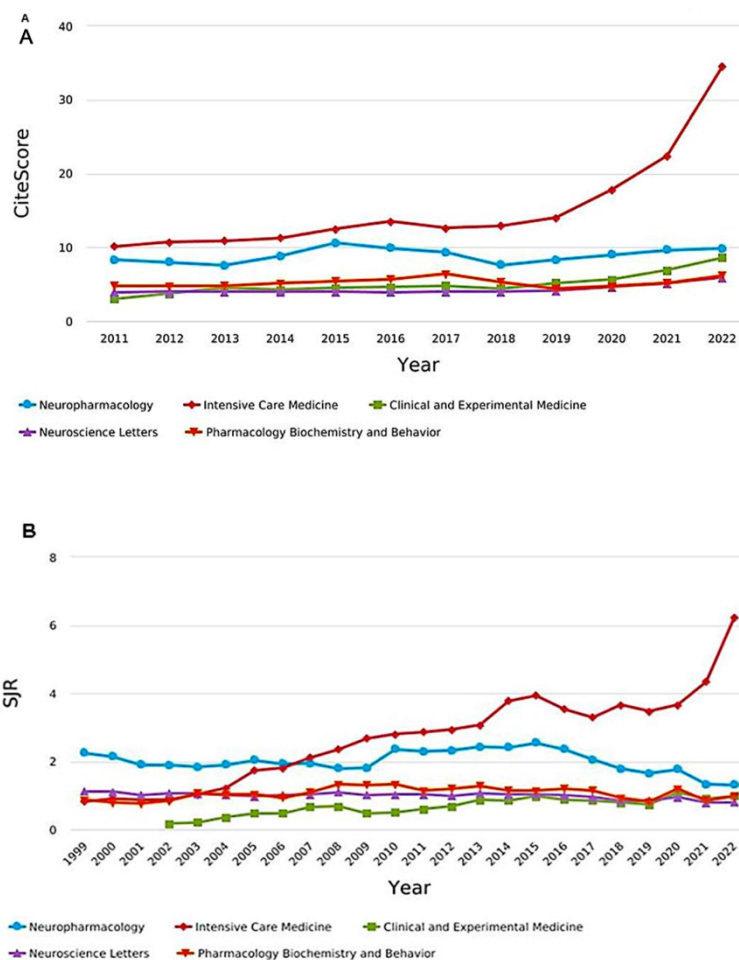
Title	Journal	< 2020	2020	2021	2022	2023	2024	Total
Inhibition of inflammatory responses by ambroxol, a mucolytic agent, in a murine model of acute lung injury	Intensive Care Medicine	79	9	11	7	4	2	112

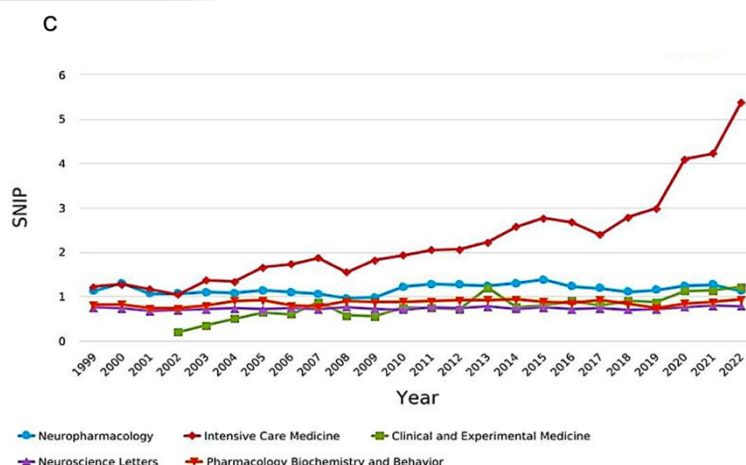
induced by lipopolysaccharide									
Ambroxol, a Nav1.8- preferring Na ⁺ channel blocker, effectively suppresses pain symptoms in animal models of chronic, neuropathic and inflammatory pain	Neuropharmacology	67	5	4	4	2	0	82	
Comparison of the effects of four Na ⁺ channel analgesics on TTX-resistant Na ⁺ currents in rat sensory neurons and recombinant Nav1.2 channels	Current Drug Metabolism	52	3	4	2	3	0	64	
Antinociceptive effect of ambroxol in rats with neuropathic spinal cord injury pain	European review for medical and pharmacological sciences	30	4	4	2	2	1	43	
In vitro and in vivo antioxidant activity of ambroxol	Life Sciences	30	4	0	4	2	1	41	

Source: Prepared by the authors themselves

The journals with the most publications over the years were also evaluated based on their CiteScore (A), SJR (B), and SNIP (C) indices. As shown in Figure 5, Intensive Care Medicine saw an increase in its scores over the years, while Clinical and Experimental Medicine showed a slight, albeit less significant, increase in its indices.

Figure 5. Indicators of publications on the analgesic, anti-inflammatory, and antioxidant activities of Ambroxol in experimental models, 1994–2023. (A) CiteScore, (B) SJR, and (C) SNIP.





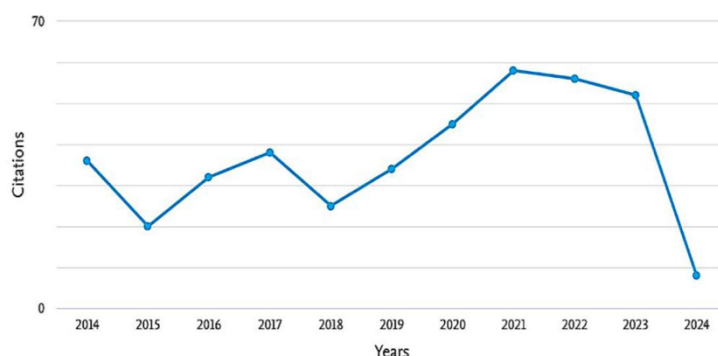
CiteScore, similar to the impact factor, is a metric that evaluates the citation impact of journals. Launched in 2016 by the Scopus database, it is calculated based on the number of citations received by a journal in a given year for documents published in the three preceding years, divided by the number of documents published in those same three years (James *et al.*, 2019).

CiteScore calculations are based on data compiled from journals indexed in a specific bibliographic database, making them influenced by the editorial decisions of that database. Since Scopus has broader coverage than Web of Science, it has the advantage of encompassing a larger number of journals (Caregnato; Vanz, 2020).

The SJR graph represents the weighted average of citations received in the selected year by documents published in the journal in the three preceding years. The SNIP graph shows a corrective metric to account for differences in citation potential across different fields (Oliveira *et al.*, 2023).

A general evaluation of the selected studies regarding citation counts over the last 10 years revealed an increase in citations from 2018 to 2021, with a decline in 2024 (Figure 6). This trend may be attributed to the pandemic period, as studies published on ABX during this time were often related to COVID-19, a theme not included in this bibliometric review.

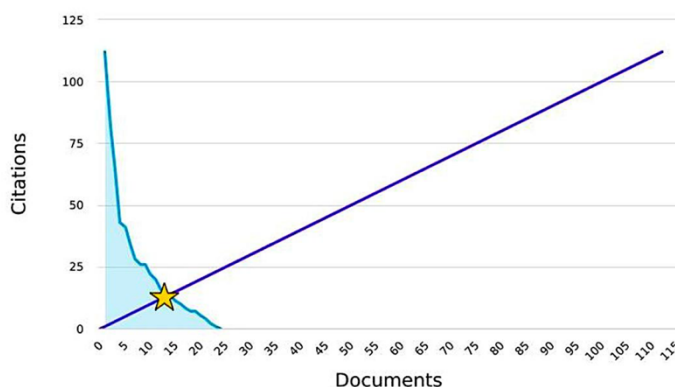
Figure 6. Citations of publications related to the antioxidant, analgesic, and anti-inflammatory effects of Ambroxol in experimental models, 1994–2023.



Source: Prepared by the authors themselves

Figure 7 presents the H-index of the published studies, illustrated by a 45° line. The H-index is based on the largest number of articles included that have received at least the same number of citations. The graph models a 1:1 relationship between publishing and being cited. An author's publication history is mapped on this graph, starting with the most cited publication and moving to the least cited (Hirsch, 2005). In this review, of the 24 studies included, 13 were cited at least 13 times according to the H-index.

Figure 7. H-index of publications related to the antioxidant, analgesic, and anti-inflammatory effects of Ambroxol in experimental models, 1994–2023.



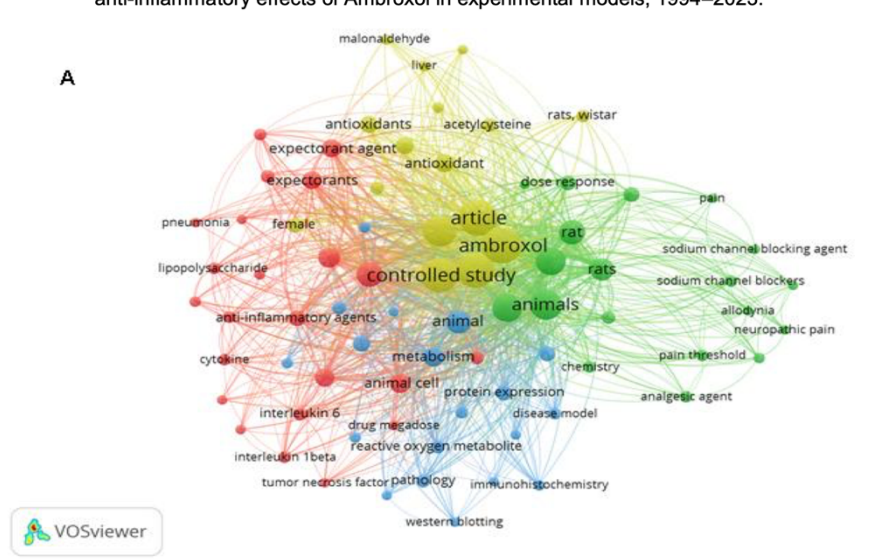
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Source: Prepared by the authors themselves

3.3 KEYWORD CO-OCCURRENCE ANALYSIS

In this section, the keywords used by authors in their articles were analyzed. The co-occurrence analysis identified a complex network of connections among 77 terms, divided into 4 clusters (Figure 8).

Figure 8. Co-occurrence of keywords used in publications on the antioxidant, analgesic, and anti-inflammatory effects of Ambroxol in experimental models, 1994–2023.



Source: Prepared by the authors themselves

The most frequently occurring keywords formed the most important nodes in the network. These included: ambroxol, article, controlled study, animals, and rat. Four clusters of terms were identified. The size of the node and item indicates the frequency of a keyword's occurrence, and the relationship between terms is stronger the closer they are to each other.

The largest cluster, represented in red, consists of 23 items and is related to the anti-inflammatory activities of ambroxol. The second cluster, represented in green, consists of 19 items and is associated with the antinociceptive and analgesic effects of ambroxol. The blue cluster includes 19 terms and relates to metabolic activity, protein expression, and histopathological effects of ABX. The

fourth cluster, in yellow, consists of 16 terms primarily related to the antioxidant activity of ABX.

4 DISCUSSION

This bibliometric review aimed to highlight the analgesic, antioxidant, and anti-inflammatory actions of ABX based on the analysis of studies in experimental rat models. Despite the extensive literature on the classic mucolytic use of ambroxol, when focusing on its unconventional applications, a scarcity of studies exploring its use for other purposes became evident. Only 24 studies were found in indexed databases, prompting the decision to avoid temporal restrictions in the review.

Given this limited body of evidence, there is a pressing need to investigate the efficacy and safety of ABX on a broader scale through controlled clinical trials in diverse experimental contexts. The lack of robust data on the actions of this drug may influence medical recommendations and treatment guidelines (Su *et al.*, 2004; Cavalu *et al.*, 2022; Patzwaldt *et al.*, 2023).

The main findings of the included studies revealed potential additional actions of ABX, such as its synergistic effect when combined with another drug in immune hepatitis, suggesting a non-immunosuppressive immunomodulatory formulation for immune-mediated disorders (Miyazaki *et al.*, 2005; Khoury *et al.*, 2020). Furthermore, another study highlighted this immunomodulatory effect in the context of airway hyperreactivity and inflammation (Takeda *et al.*, 2016).

Several studies explored the anti-inflammatory and protective actions of ABX in pathologies such as cataracts, cystitis, and lung injuries (Haque; Gilani, 2005; Baruti *et al.*, 2019; Er *et al.*, 2021), as well as its ability to suppress pain symptoms in animal models of chronic, neuropathic, and inflammatory pain (Gaida *et al.*, 2005). The antioxidant action of ABX was also demonstrated through a mechanism involving thioredoxin (Huang *et al.*, 2014).

When analyzing the geographical distribution of the studies, it is noteworthy that China leads in the number of publications on ABX (Cao; Hou; Zhang, 2019; Su *et al.*, 2004; Li *et al.*, 2011; Jiang *et al.*, 2013; Huang *et al.*,

2014). This may reflect the country's growing interest in scientific research, likely driven by government policies promoting research and technological development over recent decades (Vianini, 2017).

However, it is also important to highlight that countries such as Germany (Gaida *et al.*, 2005; Weiser, 2006; Patzwaldt *et al.*, 2023), Japan (Miyazaki *et al.*, 2005; Takeda *et al.*, 2016; Yoshida *et al.*, 2018), and the United States (Hama; Plum; Sagen, 2010), traditionally known for their strength in research and innovation, have also made significant contributions to the literature on ABX. This suggests a global interest and a diversity of scientific approaches related to this drug.

However, it is important to note that the number of publications does not necessarily reflect the quality or relevance of the studies. Variations in methodology, study objectives, and the quality of the journals where the studies were published can impact the interpretation of results (Burihan, 2002).

Another consideration is that the analysis by country provides a general overview of the geographical distribution of publications but may not fully capture international collaboration and knowledge exchange. This review reveals a broad global distribution of author groups, ranging from developed countries to developing regions, yet with weak interconnections. Notably, during the co-authorship analysis, the largest collaboration network resides in a major cluster of predominantly Egyptian authors, with citations in the same thematic area but no connections to other clusters. This observation may reflect regional isolation among researchers and limited opportunities for knowledge exchange, as well as a lack of interinstitutional support (Doğan; Karaca, 2020).

One way to assess the impact of these studies is to evaluate the citation counts of authors and the journals in which they were published. The concentration of citations for certain authors can be influenced by various factors, such as the quality and relevance of their work, their research area, collaboration with other researchers, and the visibility of their publications in high-impact journals. In this review, the authors with the highest citation counts were Weiser and Bai, with Weiser having two articles (Su *et al.*, 2004; Gaida *et al.*, 2005). This suggests that an author with multiple publications and a high citation count may

indicate a significant contribution to the field but could also imply a concentration of research around that specific author.

Another notable result of this bibliometric review is the limited impact of the publications, as evidenced by the h-index. However, when other citation metrics and impact scores such as CiteScore, SJR, and SNIP are considered, the focus on specific aspects of the analgesic, antioxidant, and anti-inflammatory actions of ABX becomes evident.

Regarding citations by journal, the most cited were Intensive Care Medicine, Neuropharmacology, Current Drug Metabolism, European Review for Medical and Pharmacological Sciences, and Life Sciences. These journals are characterized by their high CiteScore, SJR, and SNIP metrics, suggesting rigorous editorial processes, peer review, and high-quality standards, publishing only research of significant relevance (Colledge *et al.*, 2010; Moed, 2010; Vieira, 2013). Interestingly, the most significant publications were predominantly found in journals dedicated to general medicine and neuroscience, with only two of the top five articles published in pharmacology-specific journals.

The review also analyzed keyword density, a crucial tool for understanding the evolution of knowledge, identifying trends and emerging themes, visualizing concept networks, and uncovering research gaps and opportunities (Nascimento *et al.*, 2013). During the keyword density analysis, the terms "ambroxol," "animals," and "rat" stood out, which were primarily used in the search strategy. However, despite the studies addressing the anti-inflammatory, antioxidant, and analgesic actions of ABX, these specific terms were not as recurrent, perhaps indicating a lack of emphasis on exploring new therapeutic approaches and possibilities for ABX.

5 CONCLUSION

In conclusion, this review highlights the quality and quantity of publications on the use of ABX for purposes beyond its conventional applications in experimental models. It is noteworthy that no bibliometric review on this topic has been conducted to date, making this research innovative.

Bibliometric reviews provide a comprehensive overview of accumulated knowledge, identify gaps, trends, and emerging themes in research, and offer a solid foundation for contextualizing and guiding new studies and research projects.

This review significantly contributes to clinical practice by systematically gathering and analyzing the existing scientific literature on ABX. It provides an updated and comprehensive perspective on the pharmacological properties of this compound, which is crucial for healthcare professionals in making informed, evidence-based clinical decisions.

However, this review has some limitations, primarily related to the limited number of studies found. The scarcity of publications on this topic, combined with the restriction to experimental research and English-language studies, further reduced the sample size. While there is extensive literature on ambroxol hydrochloride, most studies focus on its conventional applications, particularly in respiratory tract conditions, which are already well-established in the literature.

Therefore, it is crucial to conduct further investigations into the potential effects of ambroxol in different scenarios. The field of drug repositioning is growing, and due to the relatively low cost of ambroxol, it represents an interesting alternative for developing innovative therapeutic strategies. This is especially important in less developed regions, where affordable and effective treatments for pain and inflammation are urgently needed.

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O conteúdo apresentado neste capítulo corresponde integralmente ao artigo publicado, sendo mantido em sua forma original.

CAPÍTULO 2

Este capítulo apresenta o artigo original publicado na revista *Pharmaceuticals* (Anexo B), cujo objetivo foi investigar os efeitos analgésicos e anti-inflamatórios do ambroxol em modelo experimental de endometriose, buscando contribuir para o desenvolvimento de estratégias terapêuticas que integrem os componentes inflamatórios e nociceptivos da doença.

O estudo foi aprovado pelo Comissão de Ética no Uso de Animais (CEUA) e conduzida em modelo experimental com ratas Wistar (Anexo C). Foram avaliados desfechos comportamentais, inflamatórios, ultrassonográficos e histológicos, com o objetivo de avaliar, de forma integrada, os efeitos farmacológicos do ambroxol. Dessa forma, este capítulo corresponde à etapa experimental da tese, contribuindo para o avanço do conhecimento sobre estratégias terapêuticas não hormonais no tratamento da endometriose.

Referência do artigo:

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O conteúdo a seguir corresponde à versão integral da publicação, reproduzida sem alterações em relação ao manuscrito original.



Article

Analgesic and Anti-Inflammatory Activity of Ambroxol in the Treatment of Endometriosis: An Experimental Study in Wistar Rats

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Abstract

Background/Objective: This study evaluated the analgesic and anti-inflammatory effects of ambroxol in an experimental model of endometriosis. **Methods:** Ambroxol was administered at doses of 10, 50, and 100 mg/kg (Abx 10, Abx 50, and Abx 100) by daily gavage for 21 days. A medroxyprogesterone-treated group (Progesterone) was included as a positive control. Pain was assessed using validated behavioral tests, including the Rat Grimace Scale (RGS), the von Frey test, and the rotarod test. Additionally, interleukin-1 β (IL-1 β) levels and total leukocyte counts were measured in peritoneal lavage fluid. The volumetric reduction in endometriotic implants was evaluated by ultrasonography, while histopathological analysis characterized inflammatory infiltrate and epithelial layer integrity using a standardized scoring system. **Results:** All ambroxol doses reduced spontaneous pain manifestations throughout the treatment. The mechanical withdrawal threshold significantly increased from the second week onward, and motor quality improved over the course of the study. A significant reduction in IL-1 β levels compared with the negative control (Control(-)) was observed on day 21. Abx 50 and Abx 100 significantly reduced implant volumes (48.2% and 56.2%, respectively) and promoted marked disruption of the endometriotic epithelial layer. When compared with Progesterone, higher doses—particularly 100 mg/kg—demonstrated comparable efficacy. **Conclusions:** Taken together, these pleiotropic effects support the potential for drug repurposing in endometriosis.

Keywords: endometriosis; inflammation; pain; ambroxol; rats

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1. Introduction

Endometriosis is a chronic, estrogen-dependent inflammatory condition characterized by the presence of ectopic endometrial tissue. Affecting approximately 10% of women of reproductive age, it represents a major cause of chronic pelvic pain and infertility. Recent advances in reproductive biology and immunology indicate that the disease is not solely defined by the anatomical presence of lesions. Instead, it involves a profound dysregulation of inflammatory, neuroimmune, and neuroplastic pathways that sustain persistent pain and lesion progression [1,2].

The pathophysiology of endometriosis arises from a complex interplay between immune dysregulation and sustained activation of pro-inflammatory pathways—including NF- κ B, JAK/STAT3, and the NLRP3 inflammasome—as well as hormonal and epigenetic alterations that maintain a state of chronic inflammation. Cytokines such as IL-1 β , IL-6, and IL-17 play a central role in amplifying these processes, promoting stromal proliferation, angiogenesis, apoptosis resistance, and neural remodeling—processes directly linked to pelvic pain and the dissemination of endometriotic lesions [3–5].

Pain associated with endometriosis is multifactorial in origin. It integrates peripheral sensitization mediated by cytokines and neuropeptides; neuroangiogenesis induced by nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF); neuroimmune responses involving mast cells, macrophages, and T cells; and central sensitization phenomena that perpetuate hyperalgesia. Approximately half of all patients exhibit neuropathic characteristics, reinforcing the need for therapeutic approaches capable of modulating both inflammation and neuronal excitability [6,7].

Currently available therapies, primarily hormonal and surgical, offer only partial and transient relief. They are associated with significant recurrence rates and limited utility for women who wish to conceive or have contraindications to ovarian suppression. This scenario has driven the search for non-hormonal strategies with improved safety profiles that can target both peripheral and central inflammatory and nociceptive pathways [8].

In this context, drug repurposing with established anti-inflammatory and antinociceptive properties emerges as a promising alternative. Among potential candidates, ambroxol, a mucolytic with well-established clinical use, has gained increasing attention due to pharmacological effects extending beyond its traditional respiratory indications.

Experimental evidence indicates that ambroxol exerts analgesic activity by blocking voltage-gated sodium channels, particularly Nav1.7 and Nav1.8, which are associated with neuropathic and inflammatory pain. Furthermore, the drug reduces the release of pro-inflammatory mediators such as IL-1 β , IL-6, and TNF- α , attenuates oxidative stress, and modulates glial activation. These findings suggest a multimodal action profile that could be potentially beneficial for chronic pelvic pain associated with endometriosis [9,10].

Despite these promising findings, the potential role of ambroxol in the modulation of pain and inflammatory pathways associated with endometriosis remains poorly explored. Given the significant pain burden associated with this condition and the therapeutic gap for non-hormonal alternatives, investigating whether ambroxol can modulate both inflammatory activity and nociceptive circuits in endometriosis is highly relevant. Therefore, the present study aimed to evaluate the impact of ambroxol on spontaneous pain, mechanical allodynia, motor performance, inflammatory markers, and the integrity of endometriotic lesions in a validated experimental model. Additionally, its efficacy was compared to medroxyprogesterone acetate, a current reference hormonal therapy.

This work provides robust preclinical evidence supporting the potential of ambroxol as a non-hormonal therapeutic alternative for a condition that still lacks safe and effective long-term treatment options.

2. Results

2.1. Assessment of Spontaneous Pain

The Abx 100 and Progesterone groups exhibited similar behavioral profiles throughout the treatment period. During the first week, both groups showed a significant reduction in RGS scores compared with Control(−) (0.714 vs. 1.714; $p = 0.0017$), indicating a relevant analgesic effect. The remaining treated groups did not show a significant reduction in facial pain expression at this stage. By day 14 (D14), a more consistent effect of ambroxol was observed, with convergent reductions in pain scores across all Abx groups (mean values ranging from 0.285 to 0.428) compared with Control(−) (1.428; p values ranging from 0.0039 to 0.0177). Similarly, the Progesterone group remained significantly different from the Control(−) group (0.285 vs. 1.428; $p = 0.0039$). By day 21 (D21), scores in all treated groups converged to the lowest value observed (0.142), remaining significantly lower than those of Control(−) (1.285; $p = 0.0039$). No significant differences were observed among ambroxol doses over time (Figure 1).

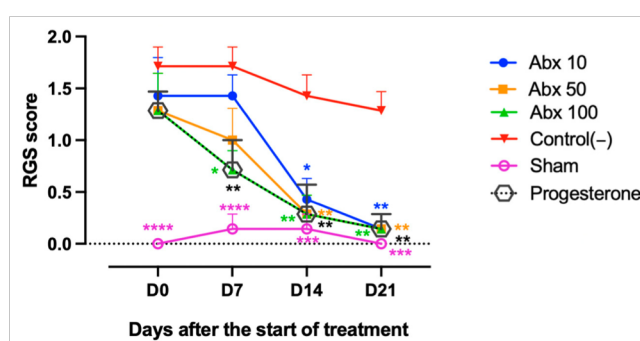


Figure 1. Assessment of spontaneous pain using the RGS. Lines represent the mean $\pm$ standard deviation (score range: 0–2). Statistical comparisons were performed between Abx 10, Abx 50, Abx 100, Progesterone, and Sham groups vs. Control(−). Significance levels are indicated as follows: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. Two-way ANOVA with Tukey’s post hoc test. The dashed line represents the baseline ($y = 0$), offset for visualization purposes.

2.2. Assessment of Mechanical Allodynia

On day 7 (D7), the progesterone group showed a significant increase in mechanical pain threshold compared with Control(−) (22.65 vs. 13.43; $p = 0.018$). Moreover, this group differed significantly from the Abx 50 and Abx 100 groups (22.65 vs. 12.78 and 13.68; $p = 0.0134$ and 0.0231 , respectively). Antiallodynic effects of ambroxol became evident on D14, particularly at the 100 mg/kg dose, which reached the highest threshold value (49.42), and differed significantly from Control(−), Abx 10, Abx 50, and Progesterone ($p < 0.0001$). At this time point, the Abx 10 and Abx 50 groups showed increased threshold, approaching the Progesterone group and remaining significantly higher than Control(−) (27.79; 32.79 and 29.39 vs. 19.24; $p = 0.0492$; 0.0001 and 0.0063 , respectively). By D21, all treated groups exhibited elevated nociceptive thresholds (ranging from 43.72 to 46.47), reaching values comparable to the Sham group (46.78) and significantly higher than those of the Control(−) (28.91; $p < 0.0001$) (Figure 2). Two-way ANOVA revealed a highly significant time $\times$ treatment interaction ($p < 0.0001$), indicating that treatment effects were time-dependent.

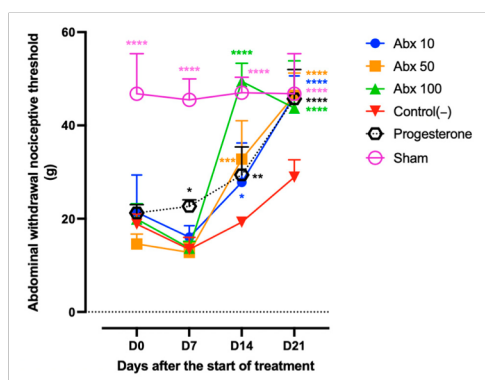


Figure 2. Tactile sensory assessment using the von Frey test. Lines represent the mean $\pm$ standard deviation (range: 0.008–300 g of force). Statistical comparisons were performed between Abx 10, Abx 50, Abx 100, Progesterone, and Sham groups vs. Control(–). Significance levels are indicated as follows: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. Two-way ANOVA with Tukey's post hoc test. The dashed line represents the baseline ($y = 0$), offset for visualization purposes.

2.3. Assessment of Motor Activity (Forced Ambulation)

Groups subjected to pharmacological treatment exhibited performance comparable to the Sham group, maintaining stable performance and high scores throughout most of the study, indicating preserved motor function with normal or near-normal ambulation. The differences between Abx 10, Abx 50, Abx 100, and Progesterone compared with Control(–) were statistically significant at D7 ($p < 0.0019$), D14 ($p < 0.0001$), and D21 ($p = 0.0094$ for Abx 10; $p < 0.0001$ for the other groups). However, a late decline in motor performance was observed in the Abx 10 group at D21. Although this did not result in a statistically significant reduction compared with Control(–), it was associated with significantly lower performance compared with the Abx 100, Progesterone, and Sham groups ($p = 0.0378$, 0.0044, and 0.0270, respectively) (Figure 3).

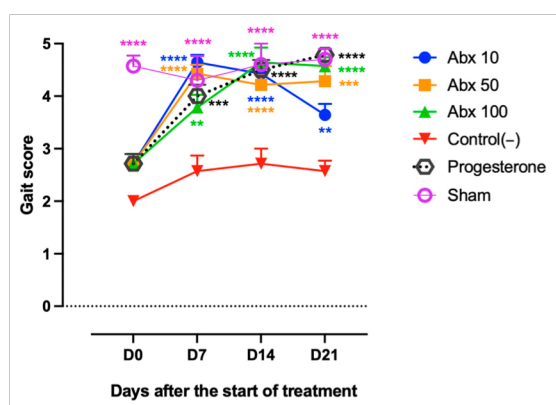


Figure 3. Assessment of motor activity/forced ambulation using the rotarod test. Lines represent the mean $\pm$ standard deviation (score range: 1–5). Statistical comparisons were performed between Abx 10, Abx 50, Abx 100, Progesterone, and Sham groups vs. Control(–). Significance levels are indicated as follows: ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. Two-way ANOVA followed by Tukey's post hoc test. The dashed line represents the baseline ($y = 0$), offset for visualization purposes.

2.4. Determination of IL-1 β Concentration

All treated groups (Progesterone and Abx 10, Abx 50, and Abx 100) demonstrated a significant reduction in IL-1 β levels compared with Control(–), which exhibited an intense inflammatory response at D21 (mean: 178.96 pg/mL). Pairwise comparisons showed that doses of 10 mg/kg and 100 mg/kg led to statistically significant reductions (56.48 and 47.65 vs. 178.96; $p = 0.0052$ and $p = 0.0029$). Progesterone and Abx 50 also showed strong anti-inflammatory effects (37.23 and 5.01 vs. 178.96; $p = 0.0015$ and 0.0002, respectively), with the latter showing the lowest mean IL-1 β levels among the treated groups (Figure 4).

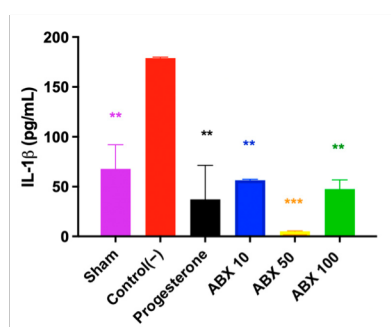


Figure 4. Assessment of IL-1 β concentration at D21. Data are presented as mean $\pm$ standard deviation. ** $p < 0.01$ for Control(–) vs. Sham, Progesterone, Abx 50, and Abx 100; *** $p < 0.001$ for Control(–) vs. Abx 50 mg. One-way ANOVA followed by Tukey's post hoc test.

2.5. Total Inflammatory Cell Count in Peritoneal Lavage

The Control(–) group exhibited a higher leukocyte count (232,800), indicating intense peritoneal inflammatory activity. Progesterone demonstrated an anti-inflammatory effect, with a cell count similar to the Sham group and significantly lower than that of Control(–) (113,200 vs. 232,800; $p = 0.03$). All ambroxol doses exhibited a response comparable to the progesterone group, with significantly lower cell counts than Control(–) (64,400, 78,000, and 106,720 vs. 232,800; $p = 0.0013$, 0.0032, and 0.0210, respectively), indicating a potent anti-inflammatory effect (Figure 5).

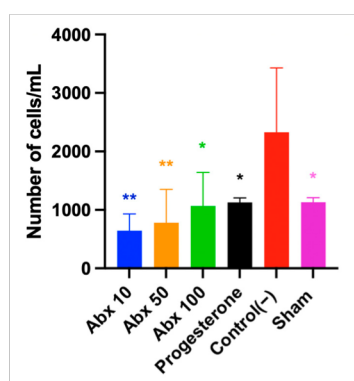


Figure 5. Cell count/mL in peritoneal lavage at D21. Data are presented as mean $\pm$ standard deviation. * $p < 0.05$, between Control(–) vs. Abx 100 mg, Progesterone and Sham; ** $p < 0.01$, between Control(–) vs. Abx 10 mg and Abx 50 mg. One-way ANOVA followed by Tukey's post hoc test.

2.6. Volumetric Analysis of Implants

At D21, ultrasonographic analysis (Figure 6) revealed that the Control(−) group maintained endometriotic volumes similar to baseline (47.85 vs. 40). The Progesterone group showed the greatest reduction, both compared with its baseline volume (D0: 45.71 vs. D21: 14.28; $p < 0.001$) and relative to Control(−) at D21 ($p = 0.0006$). In the ambroxol-treated groups, cystic volumes in Abx 50 and Abx 100 decreased significantly between D0 and D21 ($p = 0.0111$ and 0.0006 , respectively) and were significantly lower than those of Control(−) at D21 ($p = 0.0220$ and 0.0111 , respectively). The Abx 10 group showed a slight volumetric reduction without statistical significance ($p = 0.0797$) (Table 1).

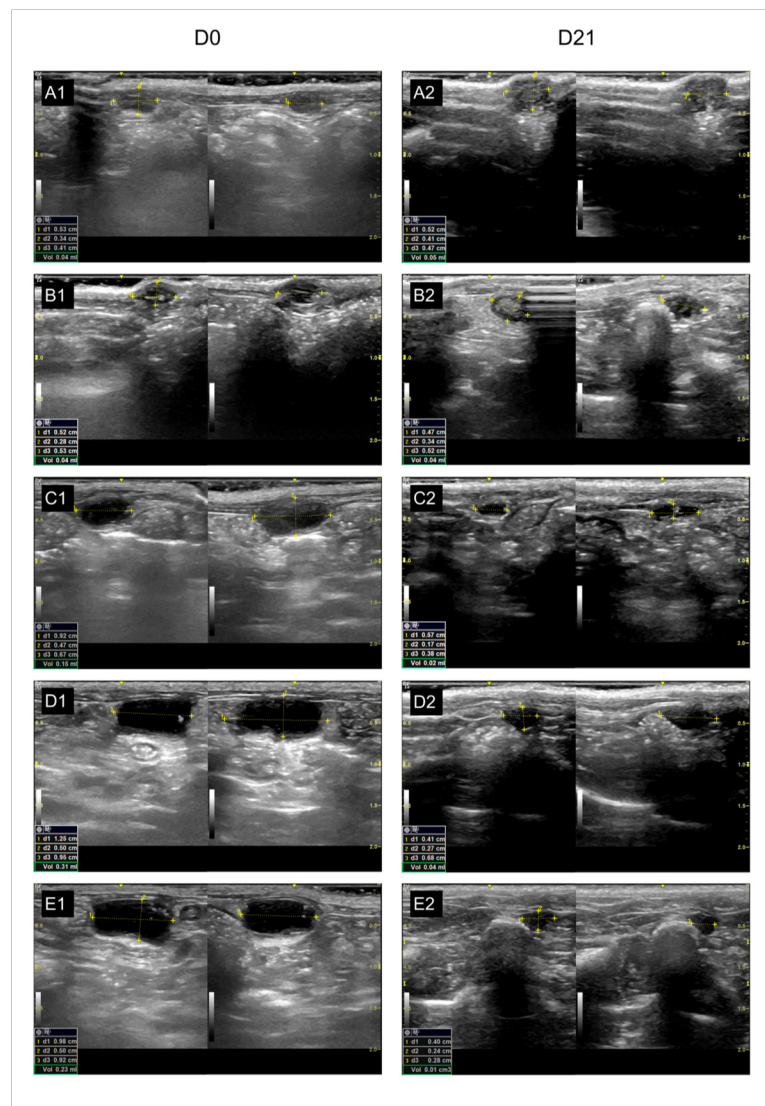


Figure 6. Representative ultrasonographic images illustrating cyst size and volume measurements across experimental groups. Panels (A1–E1) correspond to D0, and panels (A2–E2) correspond to

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D21. Experimental groups are defined as follows: (A1,A2) Control(−), (B1,B2) Abx 10, (C1,C2) Abx 50, (D1,D2) Abx 100, and (E1,E2) Progesterone. Cyst dimensions were assessed using three orthogonal diameters (d1, d2, and d3), and cyst volume (Vol) was estimated based on these measurements.

Table 1. Variation in the size of endometriotic foci after 21 days of treatment with oral 0.9% saline solution (1.0 mL/day), oral ambroxol (10, 50, and 100 mg/kg/day), or a single intramuscular dose of medroxyprogesterone (15 mg/kg).

	Initial Volume (mm ³) ± SD	Final Volume (mm ³) ± SD	Absolute Var. (mm ³) ± SD (Var. %)	<i>p</i> *
Control(−) (A)	47.85 ± 9.94	40 ± 5.77	7.85 ± 4.17 (16.4%)	0.8225
Abx 10 (B)	54.28 ± 7.86	38.57 ± 6.90	15.71 ± 0.96 (28.9%)	0.0797
Abx 50 (C)	41.42 ± 8.99	21.42 ± 6.90	20 ± 2.09 (48.2%)	0.0111
Abx 100 (D)	45.71 ± 9.75	20 ± 16.03	25.71 ± 6.28 (56.2%)	0.0006
Progesterone (E)	45.71 ± 12.72	14.28 ± 5.34	31.43 ± 7.38 (68.7%)	<0.0001
<i>p</i> **	0.9360 (A vs. B)	>0.9999 (A vs. B)		
	0.9360 (A vs. C)	0.0220 (A vs. C)		
	>0.9999 (A vs. D)	0.0111 (A vs. D)		
	>0.9999 (A vs. E)	0.0006 (A vs. E)		
	0.2435 (B vs. C)	0.0425 (B vs. C)		
	0.7434 (B vs. D)	0.0220 (B vs. D)		
	0.7434 (B vs. E)	0.0013 (B vs. E)		
	0.9955 (C vs. D)	>0.9999 (C vs. D)		
	0.9955 (C vs. E)	0.8875 (C vs. E)		
	>0.9999 (D vs. E)	0.9681 (D vs. E)		

* Two-way ANOVA followed by Tukey's multiple comparisons test was used to compare initial and final volumes within each group (D0 vs. D21). ** The same analysis was used to compare mean volumes between groups at each time point (D0 and D21). *p* < 0.05 was considered statistically significant.

2.7. Histological Evaluation

Histological analysis assessed endometrial epithelium, inflammatory infiltrate, stromal cellularity, vascularization, and the extent of implant infiltration into adjacent tissue (Figure 7).

Regarding preservation of the cystic epithelial layer (assessed at D21) using a validated semi-quantitative score, treatment with higher doses of ambroxol (50 and 100 mg/kg) and Progesterone promoted significant reductions in scores compared with Control(−) (1.57, 1.28, and 1.16 vs. 2.83; *p* = 0.0366, 0.0064, and 0.0033, respectively), with no significant differences among these groups. The Abx 10 group (2.57) showed no significant difference compared with Control(−) (*p* = 0.9885) (Table 2).

Table 2. Comparison of semi-quantitative epithelial persistence scores among groups treated with oral 0.9% saline solution (0.5 mL/day), oral ambroxol (10, 50, and 100 mg/kg/day), or a single intramuscular dose of medroxyprogesterone (15 mg/kg).

	Mean ± SD
Control(−) (A)	2.83 ± 0.40
Abx 10 (B)	2.57 ± 0.53
Abx 50 (C)	1.57 ± 0.78
Abx 100 (D)	1.28 ± 0.75
Progesterone (E)	1.16 ± 0.98
<i>p</i> *	0.9885 (A vs. B)
	0.0366 (A vs. C)
	0.0064 (A vs. D)
	0.0033 (A vs. E)
	0.1013 (B vs. C)
	0.0182 (B vs. D)
	0.0116 (B vs. E)

0.9990 (C vs. D)
0.8703 (C vs. E)
0.9990 (D vs. E)

* Two-way ANOVA followed by Tukey’s multiple comparisons test was used to compare mean epithelial persistence scores between groups. $p < 0.05$ was considered statistically significant.

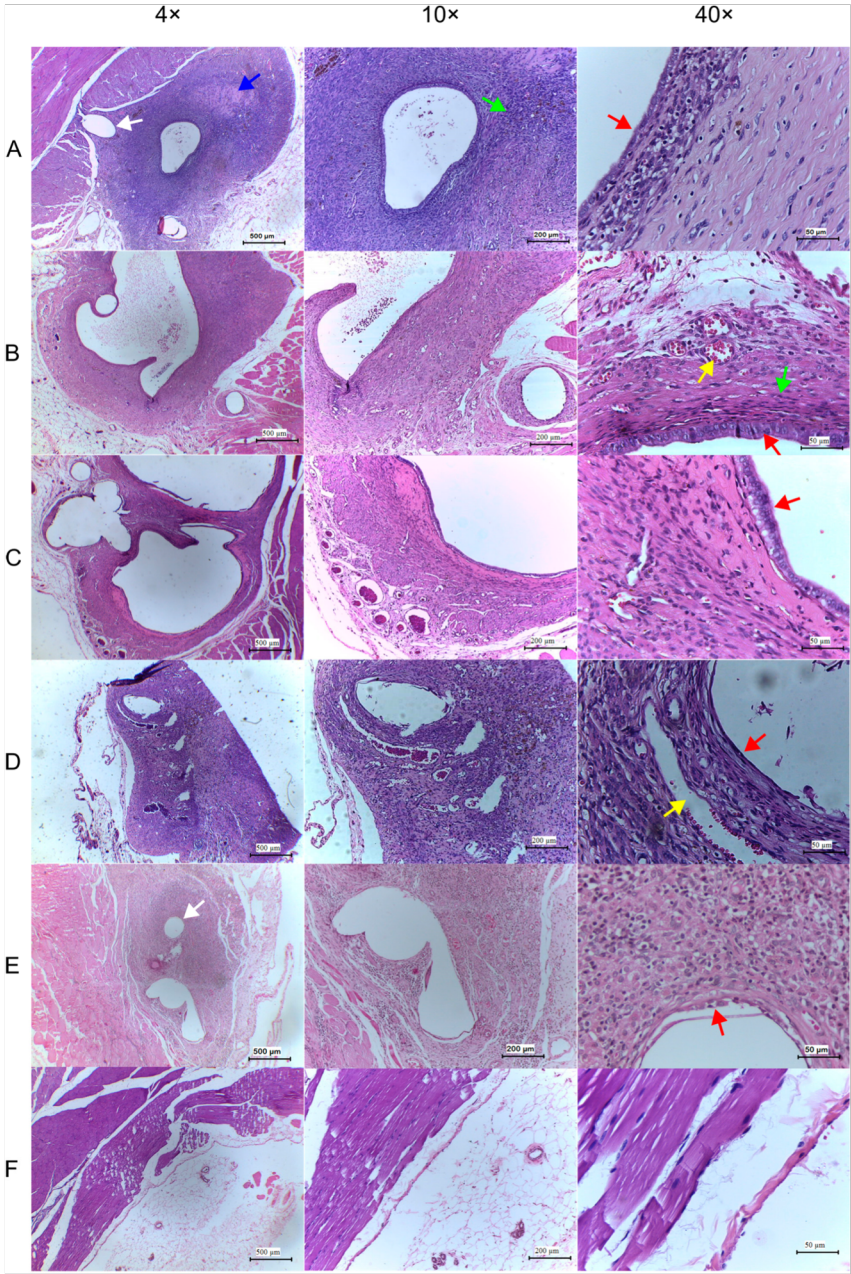


Figure 7. Representative photomicrographs of histological sections of endometriotic implants and internal abdominal wall (Sham group) excised at day 21. Each row corresponds to an experimental group (A–F), and each column represents a magnification (4×, 10×, 40×). (A) Control(–); (B) Abx 10; (C) Abx 50; (D) Abx 100; (E) Progesterone; (F) Sham. Sections (5 µm) were stained with hematoxylin and eosin (H&E). Colored arrows indicate: red, endometriotic epithelium; blue, fibrosis; green, inflammatory infiltrate; yellow, vascular congestion; white, Nabothian cyst. Scale bars: 500 µm (4×), 200 µm (10×), and 50 µm (40×).

3. Discussion

In the present study, treatment with different doses of ambroxol was associated with a significant reduction in pain and inflammatory activity related to endometriosis. Behavioral assessments demonstrated a progressive reduction in pain expression on the RGS and a substantial attenuation of hyperalgesia from the first week of treatment, as evidenced by improved mobility in the rotarod test. In addition, increased nociceptive thresholds in the von Frey test were observed by the end of the second week. These analgesic effects were sustained until the conclusion of the experiment.

A temporal variation was also observed in the Control(–) group, with a reduction in mechanical sensitivity at later time points. This may reflect intrinsic dynamics of the experimental model, including partial attenuation of inflammation and behavioral adaptation to repeated testing. To account for these time-dependent changes, a two-way ANOVA was applied, enabling evaluation of both time and treatment effects.

Notably, a statistically significant time × treatment interaction ($p < 0.0001$) was identified, indicating that treatment effects were time-dependent and varied throughout the experimental period. Treated groups consistently showed greater and sustained increases in nociceptive thresholds, supporting a true treatment effect.

Therefore, although temporal changes in the Control(–) group may have influenced the interpretation of differences across time points, they do not account for the magnitude or persistence of the effects observed in the treated groups, which reflect a genuine treatment-related response.

Voltage-gated sodium channels are strongly associated with mechanical, thermal, and inflammatory nociception [11]. Patch-clamp studies have demonstrated that ambroxol blocks these channels with 12–21% greater efficacy than classical sodium channel blockers such as lidocaine, benzocaine, and mexiletine [12]. Based on this mechanism, several experimental studies have investigated the role of ambroxol in models of chronic and neuropathic pain.

In chronic pain models, ambroxol (1000 mg/kg) has been reported to produce greater reductions in allodynia and hyperalgesia than gabapentin (100 mg/kg), achieving improvements of up to 70% in inflammatory models such as Freund's complete adjuvant-induced monoarthritis [13]. Similarly, in spinal cord injury models induced by thoracic compression, oral doses ranging from 30 to 1000 mg/kg significantly reduced mechanical and thermal hypersensitivity without impairing motor performance [14].

These findings suggest that ambroxol exerts analgesic activity predominantly through peripheral blockade of Nav channels, particularly Nav1.8. Consistent with this mechanism, the present study demonstrated effective analgesic activity of orally administered ambroxol in an experimental model of endometriosis-associated chronic pain.

The highest dose tested (100 mg/kg) initially showed the greatest analgesic effect during the first two weeks of treatment, followed by the intermediate dose (50 mg/kg), as observed in the von Frey and RGS assessments. From day 14 onward, however, all treated groups exhibited stable analgesic responses that persisted until the end of the experiment. The absence of statistically significant differences among doses at the final time point

suggests that even the lowest dose tested was sufficient to maintain meaningful analgesic activity.

Nevertheless, this observation should be interpreted in light of these limitations. The relatively small number of animals per group may have limited the statistical power to detect subtle dose–response differences. Taken together, the results do not support a strictly dose-dependent effect; rather, they indicate a partially dose- and time-dependent response, with greater effects observed at higher doses in the early phase, likely associated with more pronounced cystic volume reduction and epithelial regression, followed by a convergence of responses at later time points.

Additional experimental evidence supports the analgesic potential of ambroxol in neuropathic pain models. In a murine model of oxaliplatin-induced peripheral neuropathy (10 mg/kg, intraperitoneal), intravenous administration of ambroxol at doses of 12.5 and 37.5 mg/kg significantly reduced cold allodynia in the cold-plate test. In that study, the lower dose required combination with pregabalin to achieve significant analgesia, whereas the higher dose was effective during the early phase of allodynia [15].

In the present study, ambroxol treatment also produced clear anti-inflammatory effects. A reduction in the inflammatory response was evidenced by decreased IL-1 β levels at day 21, reduced inflammatory infiltrate within the lesions, and decreased cellularity in the peritoneal lavage. These findings suggest that ambroxol may be associated with the modulation of inflammatory signaling pathways.

One possible anti-inflammatory mechanism underlying these effects involves modulation of the NF- κ B/NLRP3/caspase-1/IL-1 β inflammasome axis, as suggested by previous studies. Through modulation of these signaling pathways, ambroxol may, at least in part, attenuate the release of pro-inflammatory cytokines and reduce the intensity of the inflammatory cascade associated with endometriosis.

Among the treated groups, the 50 mg/kg dose exhibited the lowest mean IL-1 β levels. However, this finding should be interpreted with care. Cytokine concentrations in inflammatory models often display substantial inter-individual variability and do not necessarily follow a strictly linear dose–response pattern. Importantly, all samples were collected, processed, and analyzed under identical experimental conditions, minimizing methodological variability.

Therefore, the markedly low values observed in the Abx 50 group may reflect biological variability rather than a consistent pharmacological superiority of this dose. Notably, all treated groups demonstrated a consistent reduction in inflammatory markers compared with controls, supporting the overall anti-inflammatory effect of ambroxol.

The anti-inflammatory activity of ambroxol has also been reported in other experimental models. In a chronic obstructive pulmonary disease model induced by lipopolysaccharide and cigarette smoke exposure, oral ambroxol significantly reduced serum IL-1 β , PGE $_2$, and COX-2 levels, while decreasing pulmonary expression of MUC5AC and TLR4 [16].

Similarly, in an acetic acid-induced ulcerative colitis model, ambroxol administration reduced both macroscopic and microscopic tissue damage and restored the colon weight-to-length ratio. These effects were accompanied by increased antioxidant activity (Nrf2, HO-1, and catalase) and reduced levels of inflammatory mediators, including NF- κ B, MPO, IL-6, and TNF- α , along with increased IL-10 levels [17].

The histological findings of the present study were consistent with this anti-inflammatory profile. Progressive reductions in inflammatory cell infiltration were observed across treated groups, with stromal cellularity in the Abx 100 group approaching that observed in progesterone-treated animals.

Cytological analysis of the peritoneal lavage similarly demonstrated a significant reduction in total leukocyte counts across all treated groups. These observations reinforce

the anti-inflammatory potential of ambroxol in endometriosis-associated inflammatory activity.

Regarding epithelial preservation and volumetric changes in excised endometriotic cysts, antiproliferative and volume-reducing effects were observed only with the intermediate (50 mg/kg) and high (100 mg/kg) doses of ambroxol, as well as with progesterone. These treatments resulted in significant atrophy of both the endometriotic epithelial layer, as reflected by lower Keenan scores [18], and cyst volumes at D21.

Over the past decade, several studies have suggested potential antitumor and antiproliferative effects of ambroxol hydrochloride, with promising results. Preclinical *in vitro* studies have demonstrated significant effects of ambroxol in lung carcinoma (A549) cell cultures, including increased activation of pro-apoptotic pathways, downregulation of anti-apoptotic pathways, and enhanced cytotoxicity [19–21].

In a mouse model bearing subcutaneous xenografts of multiple myeloma, treatment with ambroxol (100 mg/kg, intraperitoneally) for 21 days resulted in a significant reduction in tumor growth ($p < 0.01$) [22].

These current lines of evidence suggest potential tissue-protective and antiproliferative properties of ambroxol, particularly with sustained administration at higher doses, and support its possible role as an adjuvant agent in the control of tumor progression.

The significant reductions in cystic volume and epithelial integrity observed with higher doses of ambroxol appear to play a key role in enhancing analgesic responses in behavioral pain assessments from the early weeks of treatment. The 100 mg/kg dose achieved the highest nociceptive thresholds (von Frey test) and the lowest pain expression scores (RGS), with effects comparable to or even greater than those observed with progesterone, followed by the intermediate dose (50 mg/kg), albeit with lower magnitude.

Notably, cystic involution was not required for the initial analgesic effects of ambroxol, as evidenced by the Abx 10 group, which showed early reductions in mechanical hyperalgesia despite the absence of significant effects on lesion volume. This finding suggests that the early analgesic response may be mediated by intrinsic anti-inflammatory or neuromodulatory properties of the drug. However, the decline in motor performance observed during the final week in this group indicates that, in the absence of antiproliferative and cyst-involutive effects at this dose, the analgesic effect may not be sustained over time. Conversely, higher doses were associated with more stable and sustained responses.

We propose that the observed analgesic profile may be related to a combination of mechanisms attributed to ambroxol, including its well-established action on voltage-gated Na^+ channels. Based on the findings of the present study, oral administration of ambroxol hydrochloride demonstrated a favorable pleiotropic profile, particularly at intermediate and higher doses, with progressive consolidation of its effects over time with continuous treatment.

Ambroxol appears to act on multiple pathways involved in the pathogenesis of endometriosis, promoting significant analgesic and anti-inflammatory effects, a substantial reduction in histological scores and inflammatory cytology, as well as marked cystic involution. Collectively, these findings support further investigation of ambroxol as a potential non-hormonal therapeutic option or adjuvant in the management of this condition.

In comparing outcomes across treatment groups, progesterone served as the reference treatment within our protocol, demonstrating consistent effects across all assessed domains over time. No statistically significant differences were observed when compared with higher doses of ambroxol, particularly 100 mg/kg/day, with a similar magnitude of response.

This difference in dosing regimens reflects the distinct pharmacokinetic and pharmacodynamic profiles of the treatments. Medroxyprogesterone acetate administered intramuscularly exhibits a depot-like profile, characterized by an initial rise in systemic

concentrations followed by a gradual decline and sustained exposure over time, rather than a rapid loss of activity [23–25].

Importantly, the biological effects of progestins are not determined exclusively by peak serum concentrations. Their pharmacodynamic activity is mediated through progesterone receptor signaling, involving genomic regulation of pathways related to inflammation, cell proliferation, and tissue remodeling. Consistently, current reviews and clinical guidelines describe progestins as first-line therapies acting through sustained hormonal modulation rather than immediate peak-dependent effects [2,23].

Mechanistic evidence further supports the genomic and pharmacodynamic basis of progestin activity in endometriosis. Progesterone receptor signaling has been shown to regulate the transcription of genes involved in inflammation, cell proliferation, and tissue remodeling, modulating inflammatory responses and suppressing estrogen-driven proliferation in endometriotic tissue through downstream genomic mechanisms [26].

In parallel, progestins induce decidualization and progressive atrophy of endometrial tissue, with therapeutic effects dependent on sustained receptor activation and cumulative downstream signaling rather than immediate pharmacological action [27]. Together, these mechanisms support a time-dependent pharmacodynamic response, consistent with the progressive therapeutic effects observed in the present study.

This framework supports a time-dependent pharmacodynamic response and helps explain the temporal pattern observed in the present study, in which the progesterone group showed more evident effects at later time points. Such findings are consistent with cumulative biological activity on inflammatory signaling and lesion remodeling, rather than a purely concentration-driven early peak effect.

In the clinical evaluation of pain, progesterone was the only treatment to significantly increase nociceptive thresholds (von Frey test) from the first week, indicating an early and consistent response. This initial effect may be related to the rise in systemic concentrations following intramuscular administration, whereas the more pronounced effects observed at later time points are consistent with prolonged pharmacological activity and the progressive genomic effects of progestins on inflammatory modulation and endometriotic tissue remodeling [2,23,25].

However, it is well recognized that treatment with medroxyprogesterone acetate, in addition to requiring parenteral administration, may not be sustainable in the long term due to adverse effects associated with induced hypoestrogenism, including bone loss, irregular bleeding, weight gain, and cognitive impairment [23,24,28]. In contrast, ambroxol does not directly interfere with ovarian function or its associated physiological processes, which represents a potential therapeutic advantage.

Several limitations should be acknowledged. First, the treatment duration was relatively short (three weeks). In addition, important inflammatory and angiogenic mediators, such as IL-4, IL-17, IFN- γ , TGF- β , TNF- α , NF- κ B, and VEGF, were not evaluated. Another limitation was the absence of serial measurements of the cytokines analyzed. Furthermore, oxidative stress markers, including MDA, Nrf2/HO-1, GSH, and SOD, were not assessed, and differential cell counts in the peritoneal lavage were not performed.

Although the *a priori* sample size calculation indicated a requirement of 48 animals, the final analyzed sample comprised 38 animals due to anesthetic mortality and exclusion of animals that did not develop cystic lesions according to predefined inclusion criteria. These losses are inherent to this type of experimental model and occurred before treatment allocation.

In repeated-measures designs, sample size estimation is sensitive to assumptions regarding the correlation among repeated observations, and plausible variation in this parameter may substantially influence the required sample size. Within a reasonable correlation range of 0.5–0.6, the final sample remained acceptable for evaluation of the primary

outcomes, as post hoc power analysis for the final sample of 38 animals indicated statistical power ranging from 0.73 to 0.85. Nevertheless, the reduced sample size may have limited statistical sensitivity to detect subtle differences in secondary outcomes or dose–response relationships.

Therefore, future investigations in experimental endometriosis should adopt expanded methodological approaches. These include longer treatment durations and diversified dosing regimens, such as neoadjuvant use or combination with other agents. In addition, stepped dosing or dose-tapering strategies should be evaluated to determine therapeutic equivalence and potential inclusion in pharmacological rotation protocols. Finally, future studies should incorporate a more comprehensive panel of cytokines and oxidative stress markers.

4. Materials and Methods

4.1. Study Characterization

This randomized preclinical experimental study was conducted at the Experimental Laboratory for the Study of Pain (LEED), located at the Don Delgado University Campus of the Federal University of Maranhão (UFMA), between August and September 2024.

4.2. Ethical Aspects

The study was approved by the Ethics Committee on Animal Use (CEUA-UFMA) under protocol number SEI 23115.034119/2023-99.

4.3. Study Population

Sample size was determined a priori using statistical power analysis performed with G*Power software (version 3.1.9.7; Heinrich Heine Universität Düsseldorf, Germany), based on a repeated-measures ANOVA design with intra- and intergroup interactions. The initial calculation indicated a requirement of 48 animals (8 per group) to detect biologically relevant differences, with a 95% confidence level and a significance level of 5% ($\alpha = 0.05$). Statistical power ($1 - \beta$) was set at 80% ($\beta = 0.20$), corresponding to a maximum 20% probability of a type II error. An effect size (f) of 0.25 was assumed.

The study included adult female Wistar rats (*Rattus norvegicus albinus*), aged two months, nulliparous, weighing 200–300 g, obtained from the Central Animal Facility, UFMA.

4.4. Experimental Anesthetic Procedure

Following a 12 h fast, the rats were weighed and anesthetized via intramuscular injection (posterior aspect of the right thigh) of ketamine (75 mg/kg) and xylazine (10 mg/kg). Anesthetic maintenance was performed using a DL700 Lite inhalation anesthesia system (DeltaLife®, São José dos Campos, SP, Brazil) with isoflurane at 1.5 Minimum Alveolar Concentration (MAC), ensuring adequate hypnosis, immobility, and muscle relaxation throughout the procedure. Vital signs were continuously monitored. Anesthetic depth was continuously monitored by evaluating the corneal and tail-flick reflexes, and oxygen supplementation was provided during inhalational anesthesia.

During anesthetic induction for the surgical procedure, four animals died unexpectedly and were excluded prior to further experimental procedures. Random allocation to experimental groups was performed only after surgical induction of endometriosis and subsequent ultrasonographic confirmation of endometriotic cyst implantation. The final number of animals included in each group after all exclusions is described in Section 4.7.

4.5. Surgical Induction of Experimental Endometriosis

Once anesthetized, the animals were placed in the supine position on the surgical table, with the limbs secured in abduction, and were maintained under continuous monitoring of vital signs (heart and respiratory rate) using a multiparameter monitor (ECG-DL650, DeltaLife®, São José dos Campos, SP, Brazil). The operative field in the caudoventral abdominal region (4 cm × 5 cm) underwent manual hair removal, followed by mechanical cleansing and antisepsis with a 10% povidone–iodine solution (Riodeine®, Rioquímica, São José do Rio Preto, SP, Brazil), and placement of a fenestrated sterile drape.

Autotransplantation technique was performed as follows: a 3 cm midline incision was made using a No. 15 scalpel blade, involving the skin, musculoaponeurotic layer, and peritoneum, followed by identification of intracavitary organs and exposure of the uterus, adnexa, and mesentery. Subsequently, the middle third of the left uterine horn was identified, grasped, ligated, and transected using 6-0 nylon suture (Mononylon®, Ethicon, Somerville, NJ, USA) with three simple sutures to ensure hemostasis [29,30].

Concomitantly, the uterine specimen was sectioned into longitudinal slices followed by transverse cuts to obtain a 4.5 mm × 4.5 mm flap, which was maintained in Ringer's lactate solution (Ringer Lactato®, B. Braun, Melsungen, Germany). A transfixing suture using 6-0 nylon thread was placed on the serosal surface of the flap, which was then kept tagged.

The flaps were autotransplanted onto the right parietal peritoneal surface of the abdominal wall, maintaining the serosal surface in contact with the parietal peritoneum and the endometrial surface facing the peritoneal cavity. Fixation was achieved using simple sutures on the previously tagged flap (Figure 8). Throughout the procedure, the peritoneal cavity was covered with gauze soaked in 0.9% sodium chloride solution. Abdominal wall closure was performed in two planes using continuous sutures involving the musculoaponeurotic layer and the skin, with 5-0 nylon.



Figure 8. Uterine autotransplant fragment fixed to the right parietal peritoneum of a Wistar rat.

Immediate post-anesthetic recovery occurred in a controlled environment, and no hormonal supplementation was administered before or after laparotomy. After the surgery, all animals were observed for 21 days in the animal facility without additional pharmacological interventions, except for immediate postoperative analgesia consisting of dipyrone (100 mg/kg) + morphine (2.5 mg/kg), administered as a single subcutaneous dose. Additional analgesic doses were available on demand during the postoperative period [31].

4.6. Ultrasonographic Confirmation of Experimental Endometriosis and Inclusion Criteria

Twenty-one days after the implantation, the rats were again subjected to inhalational anesthesia with isoflurane at 1.5 MAC to identify and measure the dimensions of the autotransplanted foci. A portable ultrasound device (LOGIQ E®, GE HealthCare, Chicago, IL, USA) equipped with a high-frequency linear transducer (14–22 MHz) was used. All examinations were performed by a single experienced ultrasonographer who was blinded to the experimental groups.

Implant growth was classified according to the Quereda classification (Table 3) [32]. Only implants classified as grade II or III cystic growth, defined as those with the largest diameter measuring 2.0 mm or greater, were included in the study.

Table 3. Classification of endometrial implant growth (adapted from [32]).

Growth Grade	Implant Characteristics
0	The implant disappears or, if visible, does not develop into a cyst.
I	The implant forms a vesicle whose largest diameter is <2 mm or, if larger, is solid
II	The implant forms a fluid-filled cyst, with the largest diameter ≥ 2 mm but <4.5 mm (smaller than the initial implant size).
III	The vesicle diameter is similar to or greater than the initial implant size (≥ 4.5 mm).

After the surgical induction period, ultrasonography revealed that six animals did not develop cystic lesions meeting the predefined inclusion criteria and were therefore euthanized (as described in Section 4.11) and excluded from the study. Consequently, the final analyzed sample consisted of 38 animals distributed across the experimental groups. No additional exclusions occurred after treatment initiation.

In addition to measuring implant diameters, ultrasonography also provided volumetric measurements. These values enabled comparison of implant volumes before and after treatment, which were assessed using the same ultrasound device by the same ultrasonographer.

4.7. Treatment Protocol

After ultrasonographic confirmation, the rats were randomly allocated to six groups, and treatment protocols were initiated according to group assignment: Control(−) ($n = 6$), which received oral gavage with 0.9% sodium chloride solution (0.5 mL/100 g body weight) for 21 days [30]; Progesterone ($n = 6$), which received a single intramuscular dose of medroxyprogesterone acetate (15 mg/kg) on the first day of treatment [33–35]; Sham ($n = 5$), which did not undergo endometriosis induction and received no treatment; and three ambroxol-treated groups receiving daily oral gavage for 21 days: Abx 10 (10 mg/kg; $n = 7$), Abx 50 (50 mg/kg; $n = 7$), and Abx 100 (100 mg/kg; $n = 7$).

Doses were determined using allometric scaling based on body surface area to appropriate translation from human clinical doses (ranging from standard respiratory doses to high-dose regimens used in acute respiratory distress syndrome (ARDS)) to the rat model [34,36]. A standard human body weight of 60 kg was assumed for the dose conversion, in accordance with established interspecies scaling principles.

Considering that ambroxol doses used in human clinical practice range from 20 to 75 mg/day for the treatment of mild respiratory diseases to much higher doses of 1–3 g/day used in ARDS management [37,38], application of the dose conversion formula yields approximate equivalents of 2.04–7.71 mg/kg and 102.8–308.3 mg/kg, respectively, in the rat model.

Accordingly, doses of 10, 50, and 100 mg/kg of ambroxol were selected to encompass the main therapeutic ranges used in these clinical contexts. The highest dose tested (100 mg/kg) was chosen because it lies within the upper range of the human-to-animal allometric scaling while remaining below the maximum doses previously reported as safe and

effective in experimental pain and inflammatory models, thereby allowing evaluation of potential dose-dependent effects without exceeding pharmacologically relevant exposure levels.

4.8. Blinding

All outcome assessors responsible for behavioral testing, biochemical assays, and histological analyses were blinded to group allocation throughout data collection and analysis. Treatments were administered by a separate researcher who was not involved in outcome assessment. Data analysis was performed using coded datasets to maintain blinding until completion of the statistical evaluation.

4.9. Clinical Assessment of Pain

Behavioral assessment of pain was initiated 24 h after ultrasonographic confirmation of ectopic endometrial implantation. Evaluations were performed before treatment initiation (Day 0, D0) and on D7, D14, and D21.

Pain assessments were conducted by direct observation. Prior to the evaluations, animals underwent a 7-day habituation period to minimize stress-related interference and ensure consistency in behavioral responses.

To minimize bias related to false-positive findings in behavioral tests due to routine handling (e.g., cage cleaning, drug administration, and animal manipulation) and environmental factors (temperature, noise, and lighting), all nociceptive and spontaneous pain assessments were performed before gavage. This approach ensured that handling associated with treatment administration did not interfere with the evaluations. Additionally, room temperature and lighting conditions were maintained similar to those of the animal facility, and testing was conducted in a quiet environment, with only the two evaluators present in the room [39].

4.9.1. Assessment of Spontaneous Pain

Spontaneous pain was assessed using the Rat Grimace Scale (RGS), a validated behavioral tool based on facial expression analysis in rodents [40–42]. This method does not require prior training and can be performed in the home cage, minimizing environmental interference.

Facial action units evaluated included orbital tightening, nose/cheek flattening, ear changes, and whisker position [43,44]. Each parameter was scored as follows: 0 (absence of pain), 1 (moderate pain), and 2 (marked pain). The overall RGS score was calculated as the mean of the scores assigned by three blinded evaluators [45].

4.9.2. Assessment of Mechanical Allodynia

Mechanical allodynia was assessed using the von Frey test with an electronic von Frey-type aesthesiometer (Insight®, Insight Equipamentos Científicos, Ribeirão Preto, SP, Brazil), consisting of a pressure transducer connected to a digital force counter expressed in grams (g), as previously described for mechanical nociceptive threshold assessment [46,47]. A disposable polypropylene tip (0.5 mm diameter) was used to apply stimuli to the anterior pelvic region.

Animals were individually placed in transparent acrylic boxes measuring (12 cm × 20 cm × 17 cm) on a wire mesh platform (5 mm² grid) and allowed to habituate for 15 min prior to testing. A mirror positioned 25 cm below the platform facilitated visualization of the pelvic region.

A single blinded evaluator applied a linearly increasing force through the mesh grid to the pelvic region until an abdominal withdrawal response defined as an abdominal defensive movement (retraction/withdrawal). Stimuli were repeated up to six times until

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three consistent withdrawal responses were obtained. The nociceptive threshold was defined as the mean of these three measurements. Intervals of approximately 60 s were allowed between stimuli to minimize sensitization. Nonspecific behaviors (e.g., grooming or exploratory activity) were not considered valid responses.

This approach represents an adaptation of von Frey-based mechanical stimulation for assessment of abdominal sensitivity in visceral pain models, as described in previous studies of visceral hypersensitivity [48,49].

4.9.3. Assessment of Motor Activity/Forced Ambulation

Motor performance was assessed using a rotarod apparatus (IITC Life Science, Woodland Hills, CA, USA) operating at a constant speed of 16 revolutions per minute for 300 s.

The quality of forced ambulation was graded using an adapted numerical scale ranging from 5 to 1, as follows: 5, normal limb use; 4, mild claudication; 3, severe claudication; 2, intermittent limb disuse; and 1, complete limb disuse [50].

4.10. Blood Collection

On D21, prior to euthanasia, animals were anesthetized with isoflurane at 1.5 MAC. Whole blood was then collected by aortic puncture into EDTA-containing tubes to preserve cellular and plasma components.

4.10.1. Plasma Separation

Blood samples were centrifuged at $3000\times g$ for 15 min to separate cellular components from plasma. The plasma fraction was carefully transferred to microtubes (Eppendorf®, Hamburg, Germany) and stored at $-80\text{ }^{\circ}\text{C}$ until analysis.

4.10.2. Cytokine Quantification

IL-1 β levels were quantified using an enzyme-linked immunosorbent assay (ELISA), in accordance with the manufacturers' instructions (ELK Biotechnology®, Wuhan, China and R&D Systems, Minneapolis, MN, USA). Absorbance was measured using an ELISA microplate reader (KASUAKI®, model DR-200Bc; DIATEK Instruments, Wuxi, China). All measurements were performed in triplicate for each sample to ensure analytical reliability and reproducibility.

4.11. Peritoneal Lavage and Leukocyte Counting

On D21, peritoneal lavage was performed under aseptic conditions. A volume of 10 mL of phosphate-buffered saline (PBS, pH 7.4), previously cooled to $4\text{ }^{\circ}\text{C}$, was injected into the peritoneal cavity of each animal [51,52]. This was followed by gentle abdominal massage for 1 min to facilitate cell detachment, after which the fluid was carefully aspirated using a sterile syringe and immediately transferred to microtubes kept on ice [53].

Samples were centrifuged at $400\times g$ for 10 min at $4\text{ }^{\circ}\text{C}$. The resulting cell pellet was resuspended in red blood cell lysis buffer [54]. After incubation for 5 min at room temperature, samples were centrifuged again at $400\times g$ for 10 min, and the pellet was resuspended in sterile PBS.

For cell quantification, 10 μL aliquots were loaded into a Neubauer chamber and analyzed under light microscopy [52]. Total leukocyte counts (cells/mL) were calculated by multiplying the mean number of cells per large square by the chamber correction factor (10^4) and the dilution factor introduced during the lysis procedure [51,53].

4.12. Euthanasia

On D21, animals in each group were euthanized by intramuscular injection of an anesthetic solution containing ketamine hydrochloride (300 mg/kg) and xylazine hydrochloride (30 mg/kg), in accordance with Resolution No. 1000 of 11 May 2012, of the Federal Council of Veterinary Medicine [55]. Death was confirmed by respiratory arrest and absence of reflexes [56].

After inspection of the abdominal cavity, endometrial implantation sites were identified and carefully excised. The specimens were rinsed with 0.9% sodium chloride solution and placed in labeled containers containing 10% buffered formalin.

4.13. Histological Analysis of Ectopic Endometrial Tissue

Histological analysis was performed at the Multiuser Histology Laboratory of the Graduate Program in Health Sciences, UFMA. Endometriotic implant samples from the abdominal wall of animals in the Control(–), treated, and Sham groups were collected on D21 and immediately fixed in 10% buffered formaldehyde (pH 7.2). After 48 h, samples underwent standard histological processing, including dehydration in graded ethanol, clearing in xylene, and paraffin embedding [57,58].

Tissue sections (3–5 µm) were obtained extending from one edge of the implant to the other and from the cystic epithelial surface to the underlying muscle layer, and were stained with H&E. Images were acquired at different magnifications using a camera coupled to an ICC50 light microscope (Leica Microsystems®, Wetzlar, Germany) equipped with 10× eyepieces and 4× (500 µm), 10× (200 µm), and 40× (50 µm) objectives. Histological evaluation was performed by direct observation by a single trained examiner blinded to group allocation.

Persistence of epithelial cells in the autotransplants after treatment was evaluated semi-quantitatively using a histological scoring system (Table 4) [18], in which greater impairment of epithelial layer integrity was interpreted as indicative of greater regression of endometriotic implants.

Table 4. Semi-quantitative scoring system for evaluating epithelial cell persistence in endometrial autotransplants (adapted from [18]).

Score	Epithelial Characteristic
3	Well-preserved epithelial layer
2	Moderately preserved epithelium with leukocytic infiltrate
1	Poorly preserved epithelium, with only occasional epithelial cells
0	Absence of the epithelial layer

4.14. Statistical Analysis

Data distribution was assessed for normality using the Shapiro–Wilk test, and homogeneity of variances was evaluated using Levene’s test. As the assumptions for parametric analysis were met, parametric methods were used for group comparisons.

Comparisons among experimental groups were performed using one-way analysis of variance (one-way ANOVA) or, when two sources of variability were present, two-way analysis of variance (two-way ANOVA). When appropriate, Tukey’s post hoc test was applied for multiple comparisons.

Data were analyzed using GraphPad Prism® software (version 7.0; GraphPad Software, San Diego, CA, USA). A *p*-value < 0.05 was considered statistically significant.

5. Conclusions

Oral administration of ambroxol (10, 50, and 100 mg/kg/day) in an experimental model of endometriosis resulted in the following outcomes:

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- Antinociceptive activity: Significant increases in nociceptive threshold and reductions in both primary mechanical hyperalgesia and spontaneous pain.
- Volumetric reduction: Doses of 50 and 100 mg/kg significantly reduced endometriotic implant volume, with effects comparable to medroxyprogesterone acetate.
- Anti-inflammatory activity: Marked reductions in IL-1 β levels were observed, with efficacy comparable to progesterone.
- Epithelial disruption: Ambroxol significantly disrupted the cystic epithelial lining of endometriotic implants, contributing to lesion regression, similarly to progesterone.

These findings indicate that ambroxol exerts a multimodal effect, targeting both sensory and structural components of endometriosis, and support its potential as a repurposed non-hormonal therapeutic strategy for disease management.

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Conflicts of Interest: The authors declare no conflicts of interest.

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O conteúdo apresentado neste capítulo corresponde integralmente ao artigo publicado, sendo mantido conforme a versão original.

CONSIDERAÇÕES FINAIS

A endometriose permanece como uma doença inflamatória crônica, multifatorial e frequentemente associada à dor persistente, impactando significativamente a qualidade de vida das pacientes. Apesar dos avanços no entendimento de sua fisiopatologia, as abordagens terapêuticas atualmente disponíveis ainda apresentam limitações importantes, incluindo recorrência da doença, efeitos adversos e resposta clínica variável, o que reforça a necessidade de estratégias não hormonais capazes de atuar de forma mais ampla sobre os mecanismos inflamatórios, imunológicos e nociceptivos envolvidos na doença.

Os dados apresentados nesta tese reforçam o crescente interesse científico relacionado ao ambroxol para além de sua aplicação clássica na prática respiratória. A análise bibliométrica demonstrou crescimento das investigações envolvendo as propriedades farmacológicas do ambroxol, especialmente suas atividades anti-inflamatória, antioxidante e analgésica, ao mesmo tempo em que evidenciou uma produção científica ainda limitada acerca desses efeitos pleiotrópicos em modelos experimentais., voltada à avaliação das possíveis aplicações terapêuticas do fármaco na doença.

Os resultados experimentais demonstraram que o tratamento com ambroxol exerceu efeitos analgésicos e anti-inflamatórios, reduzindo as manifestações de dor espontânea e aumentando o limiar de retirada ao estímulo mecânico, sem comprometimento do desempenho motor. O tratamento também reduziu os níveis séricos de IL-1 β e a resposta inflamatória peritoneal. Além disso, nas doses de 50 e 100 mg/kg, promoveu redução do volume dos implantes endometrióticos e da integridade epitelial, evidenciando efeitos sobre componentes inflamatórios, nociceptivos e estruturais da endometriose experimental.

Embora os resultados obtidos ainda demandem validação clínica, esta tese reforça o potencial translacional do ambroxol como candidato ao reposicionamento farmacológico para o tratamento da endometriose. Os achados apresentados fornecem bases experimentais para futuras investigações pré-clínicas e clínicas, contribuindo para o desenvolvimento de estratégias terapêuticas não hormonais, multimodais e direcionadas aos mecanismos neuroimunes e inflamatórios envolvidos na doença.

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Anexo A – Declaração da Publicação do Artigo de Revisão Derivado da Tese

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DECLARAÇÃO

Caderno Pedagógico, ISSN 1983-0882, declara para os devidos fins, que o artigo intitulado New perspectives on the use of ambroxol hydrochloride: a recent bibliometric analysis in experimental models de autoria de Gustavo Medeiros Frota, Tamira Mendes Cunha, Amanda Tissore Forwille Reis, Rafael França Lima, Priscylla Gouveia Mendonça, Anne Caroline Silva Nogueira da Cruz, Michelline Joana Tenório Albuquerque Madruga Mesquita, Isabela Bastos Jácome de Souza, Rafael de Abreu Lima, Rafael Cardoso Carvalho, Marcelo Souza de Andrade, Joao Batista Santos Garcia, Maria do Socorro de Sousa Cartágenes, foi publicado no v.22, n.7, de 2025.

A revista é on-line, e os artigos podem ser encontrados ao acessar o link:

<https://ojs.studiespublicacoes.com.br/ojs/index.php/cadped/issue/view/149>

DOI: <https://doi.org/10.54033/cadpedv22n7-222>

Por ser a expressão da verdade, firmamos a presente declaração.

Curitiba, 26 maio 2025.

Equipe Editorial



Anexo B – Certificado da Publicação do Artigo Original da Tese



Anexo C – Parecer do CEUA



COMISSÃO DE ÉTICA NO USO DE ANIMAIS
CIAEP: 02.0341.2019

UNIVERSIDADE FEDERAL DO MARANHÃO



CERTIFICADO (02/2024)

Certificamos que a proposta intitulada: "**Atividade analgésica, anti-inflamatória e antioxidante do ambroxol no tratamento da endometriose: estudo experimental com ratas Wistar**", **Processo n. 23115.034119/2023-99**, sob a responsabilidade da **Profa. Dra. MARIA DO SOCORRO DE SOUSA CARTAGENES**, que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi considerado **APROVADO** pela Comissão de Ética no Uso de Animais (CEUA - UFMA) da Universidade Federal do Maranhão, na reunião realizada em 17/11/2023.

We certify that the proposal: "**Analgesic, anti-inflammatory and antioxidant activity of ambroxol in the treatment of endometriosis: experimental study with Wistar rats**", **Process n. 23115.034119/2023-99**, under the responsibility of **Prof. Dr. MARIA DO SOCORRO DE SOUSA CARTAGENES**, which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, sub phylum Vertebrata (except humans beings) for scientific research purposes (or teaching) - is in accordance with Law No. 11,794, of October 8, 2008, Decree No. 6.899, 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 Ethics Committee on Animals Use of the Federal University of Maranhão (CEUA - UFMA), in meeting of 11/17/2023.

PROPOSTA

Finalidade: Pesquisa **Área:** Ciências da Saúde
Vigência: 01/03/2024 a 31/08/2026

ANIMAIS

Origem: Biotério Central da Universidade Federal do Maranhão

Espécie: *Rattus norvegicus* **Sexo:** Fêmeas **Idade:** 60 dias
(variedade albinus) **Peso:** 180g-250 g
da linhagem WISTAR

AMOSTRA

48

Local do experimento: Biotério Setorial da Pós-Graduação do Centro de Ciências Biológicas e da Saúde, Universidade Federal do Maranhão – CCBS/UFMA.

São Luís, 25 de janeiro de 2024.

Profa. Dra. Rachel Melo Ribeiro
Presidente da Comissão de Ética no Uso de Animais – CEUA/UFMA

Anexo D – Uso de Inteligência Artificial

DECLARAÇÃO DE ACORDO PARA UTILIZAÇÃO DE INTELIGÊNCIA ARTIFICIAL (Art. 12, inciso I, da Resolução nº 374/2025 – CONSAD)

Eu, **Gustavo Medeiros Frota**, discente regularmente matriculado no Programa de Pós-graduação RENORBIO, declaro que fui devidamente autorizada pela **Profa. Dra. Maria do Socorro de Sousa Cartágenes**, orientadora do projeto de pesquisa intitulado: “**Atividade analgésica e anti-inflamatória do ambroxol no tratamento da endometriose: estudo experimental com ratas Wistar**” a utilizar ferramentas de Inteligência Artificial, no desenvolvimento das atividades acadêmicas relacionadas a este trabalho.

O uso da Inteligência Artificial foi realizado de forma ética, responsável e transparente, com finalidade de apoio à organização de ideias, revisão textual, esclarecimento conceitual e aprimoramento da escrita, não substituindo a autoria intelectual, a análise crítica ou a responsabilidade acadêmica da discente.

Este acordo está em conformidade com o disposto no Art. 12, inciso I, da Resolução nº 374/2025 – CONSAD, que estabelece a necessidade de acordo prévio entre discente e docente, com registro da autorização para uso de IA no contexto acadêmico.

Declaro, ainda, que todas as informações, análises, interpretações e conclusões apresentadas no trabalho são de minha inteira responsabilidade, respeitando os princípios da integridade acadêmica e da originalidade.

Por ser verdade, firmamos a presente declaração.

Prompts/Comandos Utilizados (Art. 12, inciso III)

- i. *“Atue como um pesquisador especialista em fisiopatologia da endometriose, neuroinflamação e dor pélvica crônica. Desenvolva uma síntese da literatura científica sobre os mecanismos inflamatórios, imunológicos e hormonais envolvidos na endometriose, com foco em citocinas pró-inflamatórias, angiogênese, neurogênese e sensibilização nociceptiva. Utilize estudos indexados em bases como PubMed, Scopus e Web of Science, empregando linguagem acadêmica compatível com tese de doutorado em biotecnologia.”*
- ii. *“Atue como um farmacologista experimental com experiência em neuroinflamação e dor neuropática. Auxilie na busca e análise da literatura científica relacionada aos efeitos farmacológicos do ambroxol além da atividade mucolítica clássica, incluindo bloqueio de canais Nav1.7/Nav1.8, propriedades antioxidantes, anti-inflamatórias e possíveis efeitos imunomoduladores. Utilize evidências experimentais indexadas em bases como PubMed, Scopus e Web of Science relacionadas à dor crônica, inflamação e endometriose.”*
- iii. *“Atue como um pesquisador especializado em dor inflamatória e imunomodulação. Apresente uma análise da literatura científica sobre os mecanismos relacionados à IL-1 β , IL-6, IL-17A, TNF- α , TGF- β , células Th17, Tregs e vias NF- κ B, JAK/STAT3 e NLRP3/caspase-1 na endometriose. Relacione esses mecanismos à sensibilização*

periférica e central, inflamação persistente e manutenção da dor pélvica crônica, utilizando bases como PubMed, Scopus e Web of Science.”

- iv. *“Atue como um especialista em revisão bibliométrica e cientometria aplicada à saúde. Auxilie na organização e interpretação dos resultados de uma revisão bibliométrica previamente conduzida nas bases Web of Science e Scopus. Discuta indicadores como CiteScore, SJR, SNIP e Índice H, além da aplicação de redes de coocorrência de palavras-chave para identificação de tendências científicas, lacunas de pesquisa e áreas emergentes relacionadas ao ambroxol, inflamação e dor.”*
- v. *“Atue como um especialista em interpretação científica de resultados experimentais. Analise criticamente os resultados relacionados ao Rat Grimace Scale, teste de von Frey, rotarod, dosagem de IL-1 β , análise ultrassonográfica e avaliação histopatológica dos implantes endometrióticos. Sugira interpretações fisiopatológicas compatíveis com a literatura científica atual, sem modificar os dados originais do estudo.”*
- vi. *“Atue como um revisor científico experiente em periódicos biomédicos internacionais. Realize análise crítica da discussão científica da tese, avaliando coerência entre resultados, interpretação fisiopatológica, limitações metodológicas e relevância translacional dos achados. Preserve integralmente a autoria intelectual e as conclusões originais do autor, sugerindo apenas melhorias de clareza, organização argumentativa e profundidade analítica.”*
- vii. *“Atue como um especialista em redação científica e revisão acadêmica para pós-graduação stricto sensu. Realize revisão final do texto da tese com foco em clareza científica, coesão entre capítulos, fluidez argumentativa, padronização terminológica e adequação ao estilo acadêmico formal utilizado em teses biomédicas. Corrija problemas gramaticais e estruturais sem alterar o conteúdo científico, a interpretação dos resultados ou a autoria do trabalho.”*

Assinatura do Discente: _____

Assinatura do Docente: _____