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Dissertação

Efeitos farmacológicos do composto 7 cloro-4-(fenilselanil) quinolina em modelos experimentais de fibromialgia em camundongos machos e fêmeas

Ana Paula Bonato Wille

Pelotas, julho de 2025.

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Com todo amor dedico essa dissertação à minha família,
e ao meu amor, Denis. Vocês são meu porto seguro.

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“Não fui eu que ordenei a você? Seja forte e corajoso! Não se apavore e nem desanime, pois o Senhor, o seu Deus, estará com você por onde você andar” ***Josué 1:9***

RESUMO

WILLE, Ana Paula Bonato. **Efeitos farmacológicos do composto 7 cloro-4-(fenilselanil) quinolina em modelos experimentais de fibromialgia em camundongos machos e fêmeas.** 2025. 150. Dissertação (Mestrado em Bioquímica e Biprospecção) – Programa de Pós-graduação em Bioquímica e Bioprospecção. Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, Pelotas, 2025.

A fibromialgia é uma síndrome crônica caracterizada por dor generalizada e comorbidades associadas, como a depressão. Os tratamentos farmacológicos atuais utilizados na clínica têm eficácia limitada e efeitos adversos, destacando a necessidade de novas terapias. O composto 7-cloro-4-(fenilselanil)quinolina (4-PSQ) emerge como um novo candidato, especialmente por suas propriedades farmacológicas promissoras associadas ao seu mecanismo de ação multialvo. Frente a isso, esse estudo avaliou os efeitos do 4-PSQ em mecanismos fisiopatológicos da fibromialgia, incluindo disfunção monoaminérgica, desregulação glutamatérgica, disfunção do eixo HPA, estresse oxidativo e disfunções eletrolíticas. Para isso, foram utilizados dois modelos animais: indução por reserpina (0,5 mg/kg, via subcutânea) e estresse ao frio intermitente (EFI), possibilitando a comparação dos efeitos do tratamento em modelos de fibromialgia com indução química e ambiental, em camundongos de ambos os sexos. Após a indução do modelo os animais receberam 4-PSQ (1 mg/kg, via intragástrica(i.g.)), ou duloxetina (DXT) (30 mg/kg, i.g.; controle positivo) ou veículo (óleo de canola, 10 mL/kg) e, após 30 minutos, foram submetidos a testes comportamentais. Os resultados apontam que o 4-PSQ reverteu comportamentos nociceptivos e depressivos em ambos os modelos, de forma semelhante à DXT. Seus efeitos foram associados à modulação dos níveis de serotonina e glutamato, à normalização das atividades da MAO-A e das ATPases e à regulação do estresse oxidativo no sistema nervoso central de camundongos, de forma dependente do sexo e do modelo. Além disso, o 4-PSQ atenuou a ativação do eixo HPA em fêmeas expostas ao EFI. Esses achados sugerem que o 4-PSQ é promissor como base para uma terapia mais eficaz para a fibromialgia.

Palavras-chave: Reserpina, Estresse ao Frio Intermitente, Glutamato, Serotonina, Corticosterona, hipotálamo-hipófise-adrenal.

ABSTRACT

WILLE, Ana Paula Bonato. **Pharmacological effects of the compound 7-chloro-4-(phenylselanyl) quinoline in experimental models of fibromyalgia in male and female mice.** 2025. 150. Dissertation (Master in Biochemistry and Bioprospecting) – Biochemistry and Bioprospecting Postgraduate Program. Federal University of Pelotas, Pelotas, 2025.

Fibromyalgia is a chronic syndrome characterized by widespread pain and associated comorbidities, such as depression. Current pharmacological treatments in clinical practice present limited efficacy and adverse effects, highlighting the need for new therapies. The compound 7-chloro-4-(phenylselanyl)quinoline (4-PSQ) emerges as a novel candidate, particularly due to its promising pharmacological properties linked to its multi-target mechanism of action. Thus, the present study evaluated the effects of 4-PSQ on pathophysiological mechanisms of fibromyalgia, including monoaminergic dysfunction, glutamatergic dysregulation, HPA axis dysfunction, oxidative stress, and electrolyte imbalances. For this purpose, two animal models were used: chemical induction with reserpine (0.5 mg/kg, subcutaneous) and environmental induction with intermittent cold stress (ICS), allowing for a comparison of treatment effects in male and female mice in both chemically and environmentally induced models of fibromyalgia. After model induction, the animals received 4-PSQ (1 mg/kg, intragastrically [i.g.]), duloxetine (30 mg/kg, i.g.; positive control), or vehicle (canola oil, 10 mL/kg). Behavioral testing was conducted thirty minutes later of treatment. The results indicate that 4-PSQ attenuated nociceptive and depressive behaviors in both models, similarly to DXT. Its effects were associated with modulation of serotonin and glutamate levels, normalization of MAO-A and ATPase activities, and regulation of oxidative stress in the central nervous system of mice, in a sex- and model-dependent manner. Furthermore, 4-PSQ attenuated HPA axis activation in female mice exposed to ICS. These findings suggest that 4-PSQ may serve as a promising foundation for the development of more effective therapies for fibromyalgia.

Keywords: Reserpine, Intermittent Cold Stress, Glutamate, Serotonin, Corticosterone, Hypothalamic-Pituitary-Adrenal Axis.

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LISTA DE ABREVIATURAS E SIGLAS

4-PSQ	7 cloro-4-(fenilselanil) quinolina
5-HT	Serotonina
5-HIIA	Ácido 5-hidroxi-indolacético
5-HTP	5-hidroxitriptofano
AC	Adenilato ciclase
ACTH	Hormônio adrenocorticotrófico
AIRS	Antagonista e inibidor da recaptação de serotonina
ATP	Adenosina trifosfato
AMPA	α-amino 3-hidroxi-metil-5-4-isoxazolpropiônico
AMPc	Monofosfato de adenosina cíclico
BDNF	Fator neurotrófico derivado do cérebro
CAT	Catalase
CoQ10	Coenzima Q10
CRH	Hormônio liberador de corticotrofina
DA	Dopamina
DNA	Ácido desoxirribonucleico
DXT	Duloxetina
EFI	Estresse ao frio intermitente
ER	Espécies reativas
GABA	Ácido gama-aminobutírico
GCs	Glicocorticoides
GMPc	Monofosfato de guanosia cíclico
GPx	Glutationa peroxidase
GSH	Glutationa reduzida
HPA	Hipotálamo-hipófise-adrenal
IASP	*International Association for the Study of Pain*
Iκ-B	Subunidade Inibidor kappa B
IL-1β	Interleucina-1β
IRSN	Inibidor da recaptação de serotonina e noradrenalina
MAO	Monoamina oxidase

MDA	Malondialdeído
NE	Noradrenalina
NF-κB	Fator nuclear kappa B
NMDA	N-metil D-aspartato
nNOS	Óxido nítrico sintetase neuronal
$O_2^{\bullet -}$	Radical ânion superóxido
$^{\bullet}OH$	Radical hidroxila
ON	Óxido nítrico
$ONOO^-$	Peroxinitrito
SOD	Superóxido dismutase
SERTs	Transportadores de serotonina
SNC	Sistema nervoso central
SNP	Sistema nervoso periférico
TCAs	Antidepressivo tricíclico
TCC	Terapia Cognitivo-Comportamental
TNF-α	Fator de necrose tumoral alfa
VMAT	Transportador vesicular de monoaminas

SUMÁRIO

1. Introdução

A fibromialgia é a terceira condição musculoesquelética dolorosa crônica mais prevalente no mundo, afetando cerca de 2,7% da população global (Jurado-Priego et al., 2024). Sua incidência é particularmente elevada em mulheres entre 30 e 50 anos. Apesar da alta prevalência, seu diagnóstico e tratamento permanecem complexos devido à sua fisiopatologia multifatorial, que envolve interações entre fatores biológicos, psicológicos e sociais (Antonelli et al., 2025).

Além da dor generalizada, a fibromialgia é caracterizada por uma ampla gama de sintomas associados, incluindo distúrbios do sono e transtornos de humor. Essa sintomatologia complexa e heterogênea torna o manejo clínico da fibromialgia particularmente desafiador (Antonelli et al., 2025). Atualmente, o diagnóstico baseia-se na presença de dor generalizada e na presença de comorbidades, especialmente fadiga e distúrbios cognitivos (Filipovic et al. 2025). No entanto, esses critérios clínicos não são suficientes para explicar toda a complexidade da doença, demandando uma análise mais aprofundada de seus mecanismos subjacentes (Singh et al., 2019).

Nesse contexto, evidências crescentes indicam que a fibromialgia está associada a alterações nos sistemas nervoso central, periférico e autônomo, que comprometem a modulação da dor (Antonelli et al., 2025). Entre essas alterações, destacam-se distúrbios na neurotransmissão monoaminérgica, incluindo a diminuição nos níveis de serotonina (5-HT), dopamina (DA) e noradrenalina (NE), os quais estão diretamente relacionados à amplificação da sensação dolorosa (Alfaro-Rodríguez et al., 2024). Além disso, pacientes com fibromialgia frequentemente apresentam níveis reduzidos de triptofano, precursor da 5-HT, bem como alterações na expressão gênica da monoamina oxidase (MAO). A alteração desses fatores contribui para a hiperexcitabilidade nociceptiva e intensificação da percepção dolorosa (Berends et al., 2019; Singh et al., 2019; Vianello; Repič; Mavri, 2012).

Paralelamente, disfunções no sistema glutamatérgico também têm sido implicadas na sensibilização central, fenômeno caracterizado pela ativação sustentada dos neurônios do corno dorsal da medula espinhal, o que perpetua a dor crônica (Siracusa et al., 2021). Estudos por espectroscopia por ressonância magnética revelaram concentrações elevadas de glutamato no córtex insular de pacientes com fibromialgia, as quais se correlacionam com maior intensidade da dor e sintomas

depressivos (Harris et al., 2008). Essas alterações neuroquímicas afetam tanto os mecanismos facilitadores quanto os inibitórios da dor, além de influenciar outros processos fisiológicos em que esses neurotransmissores estão envolvidos (Siracusa et al., 2021).

Outro aspecto relevante na fisiopatologia da fibromialgia é a desregulação do eixo hipotálamo-hipófise-adrenal (HPA), frequentemente associada a alterações nos níveis de cortisol. Essa disfunção interfere na regulação de neurotransmissores, exacerbando a dor e aumentando o risco de transtornos psiquiátricos, como depressão e ansiedade (Antonelli et al., 2025). O estresse crônico também se mostra um fator agravante, especialmente em indivíduos com predisposição genética (Nishiyori; Ueda, 2008; Sarzi-Puttini et al., 2020)

As alterações nos sistemas monoaminérgico, glutamatérgico e do eixo HPA podem ainda levar a desequilíbrios redox e iônicos (Singh et al., 2019). O acúmulo de espécies reativas (ER) e a redução da capacidade antioxidante contribuem para a progressão das doenças (Sarzi-Puttini et al., 2020). O estresse oxidativo prejudica enzimas essenciais, como as ATPases, responsáveis pela homeostase iônica e manutenção do potencial de membrana (Gęgotek; Skrzydlewska, 2019). Alterações na Na^+/K^+-ATPase e Ca^{2+}-ATPase estão diretamente ligadas à hiperexcitabilidade neuronal, agravando tanto a dor crônica quanto os transtornos de humor associados (Bejček et al., 2021; Mei; Barrett; Hu, 2018).

Diante dessa complexidade, o tratamento da fibromialgia requer uma abordagem multimodal, incluindo educação do paciente, exercícios, terapia psicológica e farmacoterapia (Antonelli et al., 2025). Entre as opções farmacológicas, a duloxetina (DXT), um inibidor da recaptação de serotonina e noradrenalina (IRSN), destaca-se como uma das principais escolhas (Perrot et al., 2025). No entanto, seu uso prolongado pode causar efeitos adversos como distúrbios gastrointestinais, sonolência e sedação, o que frequentemente reduz a adesão ao tratamento (Siddiqui et al., 2025; Zhu et al., 2025).

Em busca de alternativas terapêuticas mais eficazes e seguras, novos compostos vêm sendo investigados. Um deles é o 7-cloro-4-(fenilselanil) quinolina (4-PSQ), um derivado de quinolina contendo um grupamento organoselênio, que demonstrou propriedades analgésicas e do tipo antidepressivas em estudos pré-clínicos (Pinz et al.,

2016; Reis et al., 2017; Da Rocha et al., 2024; De Oliveira et al., 2022; Rodrigues et al., 2021; Paltian et al., 2020; Reis et al., 2020a). Seus efeitos parecem estar relacionados à modulação de vias glutamatérgicas e monoaminérgicas, além da redução do estresse oxidativo e da modulação do eixo HPA (Da Rocha et al., 2024; De Oliveira et al., 2022; Rodrigues et al., 2021). Adicionalmente, o 4-PSQ normaliza a atividade das ATPases, restabelecendo o equilíbrio eletrolítico (Paltian et al., 2020; Reis et al., 2020a). Estudos toxicológicos preliminares indicam um bom perfil de segurança, sem evidências de danos hepáticos ou renais (Pinz et al., 2016; Reis et al., 2017).

Diante desse cenário, este trabalho teve como objetivo avaliar os efeitos do 4-PSQ em modelos experimentais de fibromialgia induzidos por reserpina ou estresse por frio intermitente (EFI) em camundongos machos e fêmeas. Foram analisados parâmetros comportamentais e bioquímicos relacionados à neurotransmissão monoaminérgica e glutamatérgica, função do eixo HPA, estresse oxidativo e atividade das ATPases, visando elucidar os mecanismos de ação do 4-PSQ nessa condição.

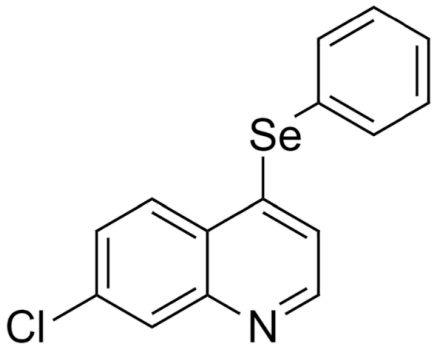

Figura 1. Estrutura química do composto 7-cloro-4-(fenilselanil) quinolina (4-PSQ).

2. Objetivos

2.1 Objetivo geral

Avaliar o potencial terapêutico do composto 4-PSQ no tratamento da fibromialgia e de suas comorbidades, investigando os mecanismos envolvidos em sua ação analgésica e validando sua eficácia por meio de diferentes abordagens experimentais.

2.2 Objetivos específicos

- Avaliar o efeito antinociceptivo do 4-PSQ frente às sensibilidades mecânica e térmica induzidas pela reserpina ou EFI em camundongos machos e fêmeas;
- Investigar o efeito do tipo-antidepressivo do 4-PSQ no fenótipo depressivo causado pela reserpina ou EFI em camundongos machos e fêmeas;
- Elucidar se a modulação da neurotransmissão glutamatérgica contribui para os efeitos farmacológicos do 4-PSQ em camundongos machos e fêmeas expostos a reserpina ou EFI;
- Analisar se a modulação dos níveis de serotonina e da atividade da MAO contribui para os efeitos farmacológicos do 4-PSQ em camundongos machos e fêmeas expostos a reserpina ou EFI;
- Verificar se os efeitos farmacológicos apresentados pelo 4-PSQ envolvem a modulação do eixo HPA em camundongos machos e fêmeas expostos a reserpina ou EFI;
- Avaliar se a modulação de parâmetros de estresse oxidativo, marcadores de dano oxidativo e a atividade de enzimas antioxidantes, contribui para os efeitos farmacológicos do 4-PSQ em camundongos de ambos os sexos expostos a reserpina ou EFI;
- Verificar se a modulação da atividade das enzimas Na^{+}/K^{+}-ATPase e Ca^{2+}-ATPase contribui para os efeitos terapêuticos do 4-PSQ em camundongos machos e fêmeas expostos a reserpina ou EFI.
- Avaliar a existência de dimorfismo sexual na resposta farmacológica ao composto 4-PSQ.

3. Revisão bibliográfica

3.1 Dor

A dor é definida como uma experiência sensorial e emocional desagradável, associada ou semelhante àquela relacionada a um dano tecidual real ou potencial (IASP, 2020). Por ser subjetiva, resulta da interação de componentes nociceptivos, emocionais, cognitivos e contextuais, tornando a experiência dolorosa única para cada indivíduo. A

dor aguda possui função protetora, atuando como mecanismo de defesa diante de estímulos nocivos, infecções, alterações da homeostase ou lesões teciduais, preservando a integridade do organismo. Já a dor crônica ultrapassa o período esperado de cicatrização, geralmente superior a três meses, perdendo seu caráter adaptativo e configurando-se como uma condição patológica (Millan, 1999).

Do estímulo nociceptivo até a percepção consciente da dor, ocorre uma sequência de eventos. A lesão tecidual ativa neurônios de primeira ordem na periferia, desencadeando a liberação de neurotransmissores e mediadores, como glutamato, prostaglandinas, serotonina e citocinas. Essa cascata sensibiliza fibras aferentes primárias, que transmitem os sinais aos neurônios de segunda ordem no corno dorsal da medula espinhal. Em seguida, os impulsos nociceptivos alcançam o tálamo e o tronco encefálico pelas vias ascendentes e, por fim, atingem o córtex cerebral, onde a dor é interpretada por diferentes áreas. Paralelamente, o organismo ativa mecanismos endógenos de antinocicepção, mediados por vias descendentes inibitórias. Interneurônios GABAérgicos e glicinérgicos no corno dorsal modulam negativamente a transmissão dos sinais, funcionando como barreira ao excesso de excitação neuronal (Siracusa et al., 2021; Millan, 1999).

Além da distinção entre dor aguda e crônica, a dor também pode ser classificada de acordo com sua origem. A forma nociceptiva decorre da ativação de nociceptores em resposta a lesões ou processos inflamatórios. A neuropática surge de danos ou disfunções no sistema nervoso somatossensorial. Já a nociplástica resulta de alterações no processamento central da dor, mesmo na ausência de lesão tecidual ou nervosa, sendo característica em síndromes como a fibromialgia (Millan, 1999).

3.2 Fibromialgia

A fibromialgia é uma condição reumatológica crônica caracterizada por dor musculoesquelética generalizada e persistente, com duração superior a três meses, acometendo ossos, músculos e tendões (Jurado-Priego et al., 2024). Essa síndrome está associada a uma amplificação das respostas do organismo a uma variedade de estímulos sensoriais, frequentemente acompanhada por sintomas somáticos como rigidez muscular, distúrbios do sono, fadiga, disfunções cognitivas e alterações de humor

(Sarzi-Puttini et al., 2020). A variabilidade e a combinação dos sintomas conferem à fibromialgia uma polissintomatologia complexa e heterogênea, dificultando o diagnóstico e o tratamento (Siracusa et al., 2021).

A identificação e avaliação da fibromialgia ainda representam um desafio, frequentemente marcado por controvérsias. Os critérios diagnósticos envolvem múltiplos domínios, como dor difusa e comorbidades emocionais, somáticas e cognitivas (Jurado-Priego et al., 2024). Para confirmar o diagnóstico, o paciente deve apresentar índice de dor generalizada ≥ 7 e gravidade dos sintomas ≥ 5 (Filipovic et al., 2025). Recentemente, a quantificação da densidade das fibras nervosas intraepidérmicas, por meio da biópsia de pele, tem sido proposta como um marcador emergente para auxiliar no processo diagnóstico (Devigili et al., 2023). No entanto, a avaliação da fibromialgia ainda se baseia majoritariamente em critérios clínicos, uma vez que não há biomarcadores específicos validados, levando muitas vezes a um diagnóstico tardio da condição (Jurado-Priego et al., 2024). Diante disso, muitas pessoas afetadas sofrem uma redução significativa na qualidade de vida, enfrentando diferentes tipos de deficiência, isolamento, estigmatização e preocupações quanto ao prognóstico a longo prazo (Ruschak et al., 2023).

Do ponto de vista epidemiológico, a fibromialgia é a terceira condição musculoesquelética mais prevalente, atrás apenas da lombalgia e da osteoartrite (Sarzi-Puttini et al., 2020; Spaeth, 2009). Estima-se que até 5% da população mundial seja afetada pela doença, com maior incidência na Europa (2,64%), seguida pela América (2,41%) e pela Ásia (1,62%) (Font Gayà et al., 2020). No Brasil estudos estimam que 2,5% da população possui fibromialgia e sua manifestação varia de acordo com aspectos sociais, psicológicos e culturais (Rezende et al., 2013; Souza e Perissinotti, 2018). De modo geral, a prevalência dessa condição aumenta com o decorrer da idade, atingindo um pico entre 50-60 anos (Sarzi-Puttini et al., 2020; White et al., 2018).

A diferença de suscetibilidade entre os sexos é um fator importante na compreensão da fibromialgia, especialmente considerando que as mulheres representam entre 80% e 96% dos casos diagnosticados (Ruschak et al., 2023). Estudos clínicos e pré-clínicos demonstram a existência de dimorfismos sexuais na percepção da dor, sendo as mulheres geralmente mais sensíveis a estímulos nocivos e mais

propensas ao desenvolvimento de sintomas depressivos (Yunus, 2001). Nelas, a dor tende a ser mais intensa, frequente, duradoura e amplamente distribuída anatomicamente quando comparada à dos homens sob condições patológicas semelhantes (Bulls et al., 2015). Além disso, sintomas característicos da fibromialgia, como dor generalizada, fadiga e depressão, são mais prevalentes e severos no sexo feminino (Yunus, 2001). Apesar dessas diferenças, o impacto negativo global da doença, incluindo a incapacidade funcional e a gravidade do quadro clínico, parece afetar homens e mulheres de forma semelhante (Wolfe, 2009).

Fatores genéticos, ambientais, endócrinos e neurológicos também podem influenciar na intensidade dos sintomas e contribuir para as diferenças entre os sexos (Sarzi-Puttini et al., 2020). Variantes genéticas associadas à dor crônica já foram identificadas, incluindo genes de canais iônicos, receptores opioides e neurotransmissores como o ácido gama-aminobutírico (GABA), a DA e o glutamato (D'Agnelli et al., 2019; Oertel e Lötsch, 2008). Estudos familiares sugerem padrão de herança autossômica dominante (Buskila e Neumann, 1997). Paralelamente, experiências adversas na infância, como abusos, negligência ou nascimento prematuro, também podem alterar circuitos da dor e reduzir o limiar nociceptivo (Haviland et al., 2010). Estressores físicos repetitivos e, especialmente, traumas psicológicos e sociais durante a infância e adolescência são considerados importantes gatilhos ambientais, com impacto mais pronunciado em mulheres (Häuser et al., 2015). Tais fatores interagem com sistemas dopaminérgico, serotoninérgico, opioidérgico e com eixos neuroendócrinos, contribuindo para a complexa fisiopatologia da síndrome (Sarzi-Puttini et al., 2020).

3.3 Mecanismos fisiopatológicos envolvidos na fibromialgia

3.3.1 Sensibilização central

Evidências científicas indicam que a fibromialgia resulta de desequilíbrios fisiológicos complexos, nos quais o sistema nervoso central (SNC) desempenha papel crucial na manutenção dos sintomas (Goebel et al., 2021; Singh et al., 2019). Um dos principais processos subjacentes a essa síndrome é a sensibilização central, um estado

caracterizado por um aumento anormal da reatividade dos neurônios centrais a diversos estímulos sensoriais, conforme definido pela *International Association for the Study of Pain* (IASP) (Loeser e Treede, 2008). A sensibilização central representa uma condição anormal, com dor persistente e desproporcional, mesmo diante de estímulos inofensivos ou ausentes (Sarchielli et al., 2007).

Essa hiperexcitabilidade central se sustenta, em parte, por mecanismos de neuroplasticidade mal adaptativa, que consolidam uma memória nociceptiva persistente (Jaffal, 2025). Mesmo após o fim do estímulo periférico, alterações funcionais e estruturais no SNC mantêm a ativação excessiva dos circuitos da dor. Estudos de neuroimagem mostram disfunções em áreas cerebrais responsáveis pelo processamento da dor, como tálamo, ínsula, córtex pré-frontal e córtex cingulado anterior (Jaffal, 2025; Martucci e Mackey, 2018). Essas alterações refletem não só a amplificação da dor, mas também sua integração disfuncional com aspectos emocionais, cognitivos e autonômicos, o que pode explicar sintomas como fadiga, distúrbios do sono e déficits de atenção frequentemente relatados na fibromialgia (Jaffal, 2025; Kim et al., 2021).

Na prática clínica, a sensibilização central se manifesta por regulação disfuncional da dor, com sintomas como alodinia (dor causada por estímulos normalmente inofensivos) e hiperalgesia (resposta aumentada à dor) (Sarzi-Puttini et al., 2020). Essa hipersensibilidade pode afetar diferentes sistemas orgânicos, intensificando a sensação de dor generalizada comumente relatada por pacientes (Fleming e Volcheck, 2015; Staud, 2010). No nível celular, esse processo envolve alterações na funcionalidade dos neurônios nociceptivos, marcadas por maior atividade de vias excitatórias, redução de mecanismos inibitórios e participação ativa das células da glia (Fleming e Volcheck, 2015; Siracusa et al., 2021).

Em condições de dor persistente, como na fibromialgia, os nociceptores sensibilizados ativam continuamente os neurônios de segunda ordem no corno dorsal da medula espinhal, promovendo reorganização sináptica e fortalecimento das conexões neurais (Staud, 2010). Isso culmina em uma maior liberação de glutamato e na ativação dos receptores α-amino 3-hidroxi-metil-5-4-isoxazolpropiônico (AMPA), desencadeando potenciais de ação em neurônios superiores. A estimulação recorrente intensifica a liberação de neurotransmissores e neuromoduladores, como a substância P, o fator neurotrófico derivado do cérebro (BDNF) e a adenosina trifosfato (ATP), que contribuem

para a despolarização neuronal e facilitam a remoção do bloqueio de magnésio (Mg^{2+}) dos receptores N-metil-d-aspartato (NMDA) (Sarchielli et al., 2007; Singh et al., 2019; Staud et al., 2001). A abertura dos canais NMDA promove um influxo significativo de íons cálcio (Ca^{2+}) para o interior celular, ativando cascatas intracelulares de sinalização dependentes de Ca^{2+} e promovendo a síntese de óxido nítrico (ON) (KAUR et al., 2019).

O influxo de íons Ca^{2+} intracelular, mediado pelo NMDA, pode estimular a ativação do fator nuclear kappa B (NF-κB), que regula genes essenciais para o funcionamento celular (Kim, 2023; Staud e Smitherman, 2002). A excitotoxicidade glutamatérgica pode promover alterações na subunidade Inibidor kappa B (Iκ-B) do NF-κB, responsável por sua inibição (Kaur et al., 2019; Kim, 2023). Tais modificações resultam na liberação e translocação do NF-κB para o núcleo celular, onde pode atuar induzindo a expressão de genes pró-apoptóticos, ativando as caspases e aumentando a produção de proteínas pró-inflamatórias e espécies oxidantes (Kaur et al., 2019; Staud e Smitherman, 2002).

Além das alterações nos parâmetros oxidativos e inflamatórios, a falha nos mecanismos inibitórios descendentes também parece desempenhar um papel importante na sensibilização central (Singh et al., 2019). No contexto da fibromialgia, o controle inibitório pode ser prejudicado por diferentes mecanismos, como a perda da homeostase do íon cloreto, a morte excitotóxica de interneurônios inibitórios após lesão nervosa, a diminuição na liberação dos neurotransmissores GABA e glicina, e/ou alterações na expressão ou funcionalidade de seus receptores. A disfunção dessas vias inibitórias favorece o desequilíbrio entre sinalização excitatória e inibitória, contribuindo para a amplificação da dor mesmo na ausência de estímulos nocivos relevantes (Basbaum et al., 2009; Jaffal, 2025).

Outro fator relevante na perpetuação da sensibilização central é a ativação crônica de células gliais, especialmente astrócitos e microglias, no corno dorsal da medula espinhal e em estruturas suprassegmentares (Findeisen; Guymer; Littlejohn, 2025). Uma vez ativadas, essas células liberam mediadores pró-inflamatórios, como interleucina-1β (IL-1β), fator de necrose tumoral alfa (TNF-α), prostaglandinas e o BDNF, que aumentam a excitabilidade neuronal e reduzem os limiares de disparo dos neurônios nociceptivos (Kamal et al., 2025). A glia também interfere na recaptação e na liberação de glutamato, contribuindo para o acúmulo extracelular do neurotransmissor e para o

agravamento da excitotoxicidade. Esses mecanismos reforçam a ruptura do equilíbrio entre sinalização excitatória e inibitória no SNC, mantendo um estado de hiperexcitabilidade típico da fibromialgia (Findeisen; Guymer; Littlejohn, 2025; Kamal et al., 2025).

A Figura 2 representa um resumo dos principais mecanismos envolvidos no processo de sensibilização central.

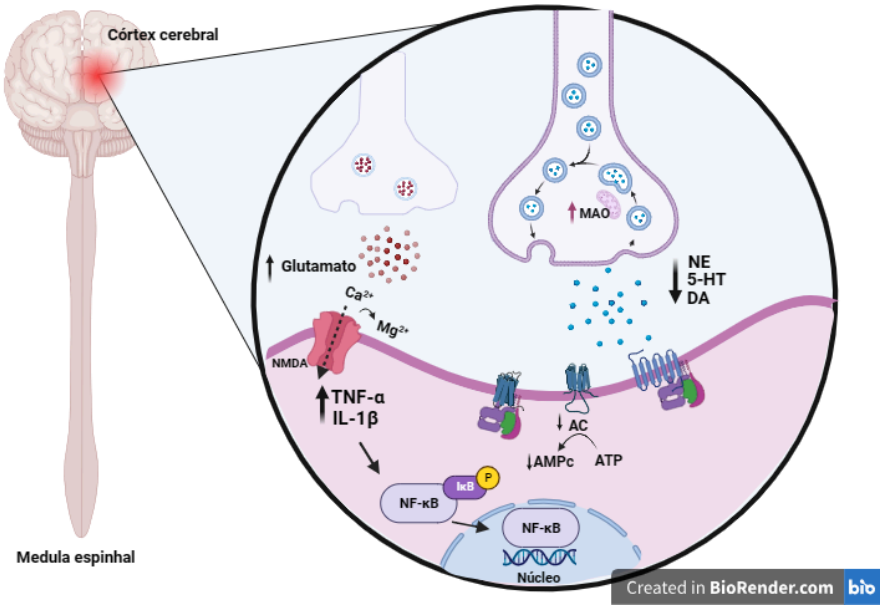

Figura 2. Representação esquemática dos mecanismos envolvidos na sensibilização central (Adaptado de KAUR et al. 2019). Na fibromialgia, a sensibilização central promove hiperexcitabilidade neuronal, com aumento da liberação de glutamato, ativação de receptores AMPA/NMDA, influxo de Ca^{2+} e ativação de cascatas intracelulares (NF-κB), por intermédio de citocinas pró-inflamatórias (TNF-α e IL-1β), culminando em estresse oxidativo, inflamação e apoptose. A redução das vias inibitórias (GABA/glicina, NE e 5-HT) e a ativação glial reforçam esse desequilíbrio, resultando em dor persistente, alodinia e hiperalgesia.

3.3.2 Distúrbios na neurotransmissão monoaminérgica

As monoaminas são neurotransmissores derivados de aminoácidos, caracterizados pela presença de um grupamento amina ligado a um anel aromático (Jiang et al., 2022). Seus principais representantes são a 5-HT, a DA e a NE, sintetizadas a partir do triptofano (no caso da 5-HT) e da tirosina (no caso da DA e da NE) (Azizi, 2022). Esses neurotransmissores são fundamentais na transmissão de sinais neuronais tanto no SNC quanto no sistema nervoso periférico (SNP) (Franco; Reyes-Resina; Navarro, 2021). A DA está envolvida no sistema de recompensa e controle motor, a NE na resposta ao estresse e alerta, e a 5-HT na modulação do humor, sono e dor (Jiang et al., 2022).

Como um componente chave do sistema monoaminérgico, a enzima MAO, nas isoformas A e B, degrada neurotransmissores monoaminérgicos, prevenindo seu acúmulo excessivo na fenda sináptica e regulando a neurotransmissão (Singh et al., 2021). A MAO-A possui maior afinidade por substratos hidroxilados, como a 5-HT e a NE, enquanto a MAO-B é seletiva por substratos não hidroxilados. Ambas as isoformas apresentam afinidade semelhante pela DA (Manzoor e Hoda, 2020). A MAO está amplamente distribuída no SNC e no SNP, com a MAO-A predominando em regiões como o córtex e o trato gastrointestinal, e a MAO-B sendo mais abundante na glia e em plaquetas (Youdim e Sandler, 1968). Alterações na atividade ou expressão da MAO têm sido associadas a diversos transtornos neuropsiquiátricos, incluindo depressão, ansiedade e doença de Parkinson (Kurian et al., 2011).

Nos últimos anos, estudos têm sugerido que a disfunção do sistema monoaminérgico pode contribuir para o desenvolvimento dos sintomas característicos da fibromialgia (Singh et al., 2019). Evidências clínicas mostram que mulheres com a doença apresentam níveis plasmáticos reduzidos de DA, 5-HT e seus metabólitos, como 5-hidroxitriptofano (5-HTP) e ácido 5-hidroxiindolacético (5-HIIA), em comparação a indivíduos saudáveis (Legangneux et al., 2001; Rus et al., 2018). Além disso, pacientes com fibromialgia exibiram concentrações mais baixas de aminoácidos essenciais, como a fenilalanina, precursora da tirosina, o que pode comprometer a síntese dos neurotransmissores catecolaminérgicos (Maes et al., 1998). Alterações no metabolismo do triptofano, precursor da serotonina, também foram observadas em pacientes com esta condição (Groven et al., 2021; Schwarz et al., 2002). Adicionalmente, verificou-se menor expressão dos transportadores de serotonina (SERTs) na membrana celular

neuronal de pacientes com fibromialgia, indicando possível redução na transcrição desses genes (Bazzichi et al., 2006). Complementando esse panorama, Meyer et al. (2006) relataram aumento da densidade da enzima MAO-A em diversas regiões cerebrais, sugerindo que sua maior atividade, especialmente em episódios depressivos, intensifica o catabolismo de 5-HT, NE e DA, agravando os sintomas.

Em estudos pré-clínicos com modelos animais, o papel central desses neurotransmissores na síndrome se correlaciona com as evidências clínicas. Singh et al. (2021) conduziram um estudo com camundongos *Swiss* que receberam repetidas administrações de reserpina, um depletor de monoaminas, revelando uma sensibilidade exacerbada à dor. Tal comportamento foi evidenciado pela presença de alodinia mecânica, associada a manifestações de comportamento tipo-depressivo e déficit cognitivo. Concomitantemente, os animais induzidos com reserpina exibiram uma redução significativa nos níveis cerebrais de 5-HT, DA e NE, juntamente com aumento da atividade da enzima MAO-A (Singh et al. 2021). Em consonância com essas descobertas, Kaur et al. (2019) observaram padrão semelhante em camundongos *Swiss*, com hipersensibilidade à dor, alterações de humor, déficits cognitivos e prejuízos motores, associados à redução nos níveis de 5-HT e DA.

De fato, em condições fisiológicas, a ativação dos neurônios serotoninérgicos e noradrenérgicos induz a liberação de NE e 5-HT no corno dorsal da medula espinhal, os quais interagem com os receptores α_2 e 5-HT_{1A}, respectivamente. A ativação desses receptores inibe a enzima adenilato ciclase (AC), reduzindo a concentração de monofosfato de adenosina cíclico (AMPc), um mensageiro secundário (Tao et al., 2019). Essa redução leva à abertura de canais de potássio (K^+) e ao fechamento dos canais de Ca^{2+}, resultando na inibição da transmissão entre neurônios primários e secundários na via ascendente da dor (Berends et al., 2019; Zhou et al., 2020). Além disso, a NE e a 5-HT podem ativar receptores α_1 e 5-$HT_{1B/1D}$ nos interneurônios inibitórios, promovendo a liberação de substâncias analgésicas como encefalinas, GABA e glicina (Pourhamzeh et al., 2022; Rus et al., 2018).

No entanto, em indivíduos com fibromialgia, há evidências de comprometimento dessas vias descendentes inibitórias, possivelmente devido à redução na disponibilidade ou na eficácia da NE e da 5-HT. Um dos mecanismos considerados, seria que a redução desses neurotransmissores levasse ao acúmulo de glutamato na fenda sináptica, que

desencadeia na liberação exacerbada de Ca^{2+} no meio intracelular. Sucessivamente, enzimas dependentes de Ca^{2+} são ativadas, incluindo a ON sintetase neuronal (nNOS), que aumenta a produção de ON. O ON atua como mediador na liberação sustentada de glutamato nos terminais pré-sinápticos, via ativação do monofosfato de guanosina cíclico (GMPc), que por sua vez estimula proteínas quinases como PKA e PKC. Essas enzimas participam da fosforilação de canais iônicos e outros alvos intracelulares, facilitando a propagação dos sinais elétricos nociceptivos (Siracusa et al., 2021; Staud e Rodriguez, 2006; Staud e Smitherman, 2002). Dessa forma, essa disfunção leva à menor ativação dos receptores inibitórios no corno dorsal e nos interneurônios locais, diminuindo a liberação de neurotransmissores analgésicos e aumentando a excitabilidade neuronal e a sensibilidade à dor (Singh et al., 2019; Tao et al., 2019). A Figura 3 indica uma representação esquemática do efeito da via das monoaminas em condições fisiológicas (Fig. 3A) e no contexto da fibromialgia (Fig. 3B).

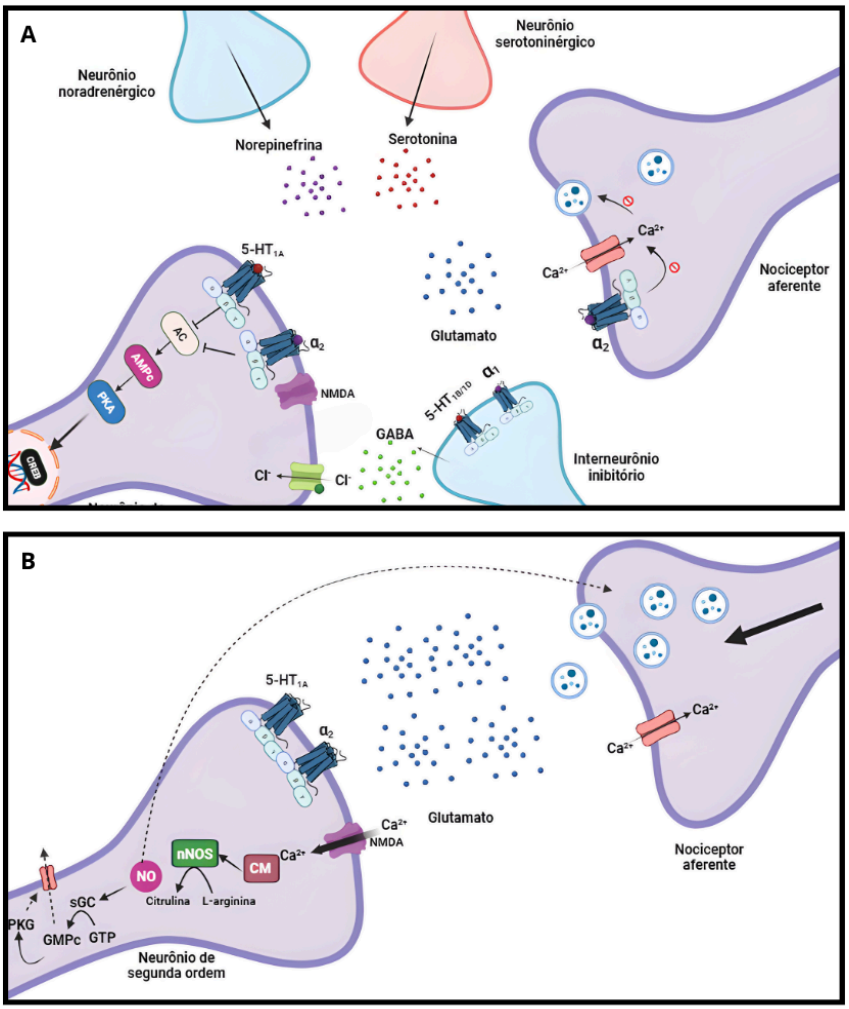

Figura 3. Representação esquemática do sistema monoaminérgico em condições fisiológicas (A), destacando o papel de 5-HT, DA e NE na modulação descendente da dor, e no contexto da fibromialgia (B), onde a redução desses neurotransmissores e o aumento da atividade da MAO resultam em falha da inibição sináptica, acúmulo de glutamato e maior excitabilidade neuronal. Fonte: Autora.

Além da NE e da 5-HT, a DA também está envolvida no processamento da dor em diferentes regiões cerebrais (Singh et al., 2019). Evidências indicam que indivíduos com fibromialgia possuem baixa disponibilidade de ligação dos receptores D_2 e D_3 corticais de DA, em comparação a controles saudáveis (Albrecht et al., 2016). Ainda, estudos com pacientes do sexo feminino demonstraram correlação positiva entre a liberação de DA em resposta à dor no estriado e a intensidade da dor percebida, sugerindo que a DA

pode ser liberada em resposta a estímulos nocivos (Wood et al., 2009). Apesar dessas evidências, o papel exato do sistema dopaminérgico na fisiopatologia da fibromialgia ainda não está totalmente elucidado.

Dessa forma, evidências clínicas e pré-clínicas indicam que as alterações na síntese, liberação, metabolismo e sinalização das monoaminas desempenham um papel crucial na fisiopatologia da fibromialgia (Alfaro-Rodríguez et al., 2024; Berends et al., 2019). Essas disfunções não apenas contribuem para a hipersensibilidade à dor, a principal característica da síndrome, mas também parecem estar envolvidas em um amplo espectro de sintomas associados, como fadiga persistente, distúrbios do sono, déficits cognitivos e humor deprimido (Alfaro-Rodríguez et al., 2024). A interconexão entre os sistemas neurotransmissores afetados e os circuitos neurais da dor, do sono e da regulação emocional sugere que a disfunção monoaminérgica pode atuar como um fator integrador desses sintomas (Berends et al., 2019).

3.3.3 Estresse oxidativo

O estresse oxidativo é caracterizado como um desequilíbrio entre a produção de espécies reativas (ER) e a capacidade do organismo de neutralizá-las por meio de sistemas antioxidantes. Esses desequilíbrios podem ocorrer pelo aumento da produção de ER ou pela diminuição da capacidade das defesas antioxidantes de combatê-las. Durante processos metabólicos fisiológicos, como a fosforilação oxidativa, pequenas quantidades de ER são geradas. Entre as principais ER estão o radical ânion superóxido ($O_2^{\bullet -}$), o peróxido de hidrogênio (H_2O_2), o radical hidroxila ($^{\bullet}OH$), o peroxinitrito ($ONOO^-$) e o NO (Olufunmilayo; Gerke-Duncan; Holsinger, 2023; Sies, 2020; Sies; Berndt; Jones, 2017).

Em condições normais, os sistemas antioxidantes são capazes de neutralizar essas ER, protegendo as células contra danos oxidativos. Dentre os principais antioxidantes enzimáticos de primeira linha destacam-se a superóxido dismutase (SOD), que catalisa a dismutação do radical $O_2^{\bullet -}$ em H_2O_2 e O_2, e a catalase (CAT), que decompõe especificamente o H_2O_2. A glutationa peroxidase (GPx), por sua vez, também atua na eliminação do H_2O_2, além de neutralizar outros hidroperóxidos. A glutationa

reduzida (GSH), além de participar diretamente da eliminação de ER, é cofator essencial para a ação da GPx, sendo, portanto, um componente central na manutenção da homeostase redox. Quando há desequilíbrio entre a produção e a depuração de ER, a homeostase redox é comprometida, tornando as macromoléculas celulares mais suscetíveis a lesões oxidativas (Olufunmilayo; Gerke-Duncan; Holsinger, 2023; Sies, 2020; Sies; Berndt; Jones, 2017).

No contexto da fibromialgia, evidências recentes sugerem uma estreita associação entre processos pró-oxidativos e a sensibilização à dor. Cordero et al., (2009) demonstraram que pacientes com fibromialgia apresentaram níveis reduzidos de coenzima Q10 (CoQ10), associados ao aumento da produção de ER em células mononucleares do sangue periférico. Esses dados indicam que alterações no metabolismo da CoQ10 podem contribuir para o desenvolvimento do estresse oxidativo. Complementarmente, Sánchez-Domínguez et al. (2015), ao analisarem biópsias de pele de pacientes com fibromialgia, observaram uma redução acentuada na atividade e na expressão de subunidades da cadeia respiratória mitocondrial, particularmente daquelas dependentes da CoQ10. Esses achados evidenciam uma disfunção mitocondrial relevante nesses indivíduos. Além disso, esses pacientes apresentaram aumento da peroxidação lipídica, refletido por níveis elevados de malondialdeído (MDA), bem como maior expressão da enzima 8-oxoguanina glicosilase, envolvida na reparação de danos oxidativos ao ácido desoxirribonucleico (DNA). Tais alterações foram associadas a níveis inflamatórios aumentados e correlacionaram-se positivamente com a intensidade da dor relatada (Sánchez-Domínguez et al., 2015). Outros estudos também apontaram uma diminuição nos níveis sanguíneos de GSH, GPx e vitaminas antioxidantes em pacientes com fibromialgia (Akkuş et al., 2009). Em contrapartida, intervenções com suplementação antioxidante têm mostrado resultados promissores. A administração de α-tocoferol elevou os níveis plasmáticos de GSH e GPx, além de reduzir a intensidade da dor muscular em indivíduos com fibromialgia (Batista et al., 2016; Nazıroğlu et al., 2010).

Estudos pré-clínicos também reforçam o papel do estresse oxidativo na fisiopatologia da fibromialgia. Martins et al. (2022) demonstraram que a exposição de camundongos machos a um modelo experimental de fibromialgia levou à inibição da atividade das enzimas GPx e Na^+/K^+-ATPase, bem como ao aumento dos níveis de ER

no córtex cerebral. Corroborando esses achados, Yüksel et al. (2017) observaram reduções nos níveis de GSH e na atividade da GPx no cérebro, músculo esquelético e gânglio da raiz dorsal, além de queda nos níveis de α-tocoferol no músculo e no plasma de animais expostos a um modelo de fibromialgia. Neste estudo, o tratamento com selênio foi capaz de restaurar os níveis de GSH, GPx e α-tocoferol nesses animais, sugerindo um efeito protetor antioxidante (Yüksel et al., 2017). Diante desse conjunto de evidências, compostos e micronutrientes com propriedades antioxidantes despontam como potenciais ferramentas terapêuticas promissoras para o manejo da fibromialgia.

3.3.4 Eixo HPA

O eixo HPA é um dos principais sistemas neuroendócrinos responsáveis pela regulação da resposta ao estresse e pela manutenção da homeostase corporal. Em situações de estresse físico ou psicológico, esse eixo é ativado, promovendo o aumento da liberação de glicocorticoides (GCs) na corrente sanguínea. O principal glicocorticoide envolvido, o cortisol em humanos e a corticosterona em roedores, exerce ampla ação reguladora em diferentes tecidos. No hipotálamo, o cortisol atua modulando a expressão e liberação de diversos neurotransmissores, incluindo a 5-HT, a NE e a DA (Menke, 2024; Tanriverdi et al., 2007). A ativação do eixo HPA tem início com a detecção de estímulos ambientais pelos neurônios do núcleo paraventricular do hipotálamo, os quais induzem a liberação do hormônio liberador de corticotrofina (CRH, *Corticotropin-Releasing Hormone*). O CRH estimula, então, a secreção do hormônio adrenocorticotrófico (ACTH, *Adrenocorticotropic hormone*) pela hipófise anterior, que age sobre o córtex da glândula adrenal, promovendo a síntese e liberação dos GCs (cortisol em humanos e a corticosterona em roedores). A regulação da liberação de GCs é exercida por meio de *feedback* negativo pelo próprio hormônio GC, agindo sobre os receptores presentes no hipotálamo e na hipófise. Entre os principais receptores envolvidos, destacam-se os mineralocorticoides, predominantemente localizados no hipocampo, responsáveis pela regulação da atividade basal do eixo HPA; e os glicocorticoides, encontrados principalmente no hipotálamo e na glândula pituitária, que respondem a níveis mais elevados desses hormônios (Menke, 2024; Papadopoulos; Cleare, 2012).

No contexto da fibromialgia, é comum a observação de alterações na resposta ao estresse, indicando uma possível disfunção do eixo HPA. De forma geral, os estudos apresentam resultados contraditórios, apontando tanto para a hipo quanto para a hiperresponsividade do eixo HPA em pacientes com essa condição (Singh et al., 2019). Riva et al. (2010) relataram que mulheres com fibromialgia apresentaram níveis plasmáticos de cortisol significativamente mais baixos ao longo do dia em comparação com controles saudáveis. De forma semelhante, Crofford (1996) observou uma hipoatividade do eixo HPA em pacientes do sexo feminino com fibromialgia, evidenciada por níveis reduzidos de cortisol urinário livre em 24 horas após a administração de hormônio liberador de corticotropina (CRH) exógeno, o que sugere uma diminuição na responsividade adrenal. Em contraste com esses achados, estudos que investigaram marcadores centrais do eixo HPA relataram padrões opostos. Griep et al. (1993) e Tsilioni et al. (2016) identificaram níveis aumentados de CRH no soro de pacientes com fibromialgia em comparação com indivíduos saudáveis, o que pode indicar uma hiperatividade hipotalâmica. Nesse contexto, Hellhammer e Wade (1993) propuseram que a hipocortisolemia ou a subativação do eixo HPA podem representar um estágio tardio de adaptação a períodos prolongados de estresse, que seriam precedidos por uma fase de hiperatividade do eixo e liberação excessiva de GCs. Esses achados aparentemente contraditórios reforçam a complexidade da regulação do eixo HPA na fibromialgia e indicam que sua disfunção pode variar conforme o estágio da doença, a duração do estresse crônico e características individuais, como traços de temperamento. Tais fatores podem modular significativamente a expressão e a intensidade dos sintomas dolorosos observados nesses pacientes (Goel et al., 2014; Hellhammer; Wade, 1993; Singh et al., 2019).

3.4 Tratamentos não-farmacológicos e farmacológicos disponíveis para a fibromialgia

3.4.1 Tratamentos não-farmacológicos

A primeira etapa do tratamento multidisciplinar da fibromialgia envolve a educação do paciente, que visa promover a compreensão da doença antes do início da farmacoterapia. Essa abordagem contribui para a reestruturação da percepção da

condição, reduzindo a ansiedade e a incerteza (Jones et al., 2015). São utilizadas estratégias como explicações sobre os mecanismos da dor, técnicas de controle do estresse e informações clínicas sobre a fibromialgia. Além disso, os pacientes são incentivados a participar ativamente do tratamento, adotando estratégias pessoais para melhorar a qualidade de vida (Jones et al., 2015; Sarzi-Puttini et al., 2020). Essa fase inicial favorece a aceitação do diagnóstico e melhora a adesão às terapias (Musekamp et al., 2019).

Entre as abordagens não farmacológicas, destaca-se a Terapia Cognitivo-Comportamental (TCC), que busca modificar padrões de pensamento disfuncionais relacionados à dor e ao sofrimento emocional. A TCC promove maior autorregulação emocional e o desenvolvimento de estratégias de enfrentamento mais eficazes, além de estimular o estabelecimento de metas voltadas à melhoria da qualidade de vida (Bennett et al., 2004). Estudos mostram que essa terapia reduz significativamente a dor e o sofrimento psicológico em pacientes com fibromialgia (Jones et al., 2015; Nicassio, 2010).

Outro componente essencial no tratamento da fibromialgia é o exercício físico, que demonstrou reduzir a dor, melhorar o sono e aliviar os sintomas depressivos (Kan et al., 2023; Serrat et al., 2020; Wang; Luo, 2024). Modalidades como exercícios aeróbicos, de alongamento, flexibilidade e fortalecimento muscular são frequentemente recomendadas, com benefícios específicos. O plano deve ser individualizado, respeitando as limitações e preferências do paciente para melhorar a adesão (Serrat et al., 2020). Os efeitos positivos do exercício podem estar relacionados à modulação do eixo HPA, ao aumento do BDNF no hipocampo e à liberação de opioides endógenos, que ativam as vias inibitórias da dor (Doerr et al., 2017).

Terapias complementares, como acupuntura, hidroterapia, eletroterapia e massoterapia, também têm se mostrado eficazes no alívio da dor e de sintomas associados, como rigidez, distúrbios do sono, ansiedade e depressão (Ahmed; Aggarwal; Lawrence, 2019; Audoux et al., 2023; Zhang et al., 2019). Tais intervenções atuam na modulação das vias nociceptivas e, quando associadas ao tratamento farmacológico, podem ter seus efeitos potencializados. No entanto, sua eficácia depende do engajamento do paciente e da continuidade do acompanhamento clínico (Ahmed; Aggarwal; Lawrence, 2019; De Ridder; Vanneste, 2017).

3.4.2 Tratamentos farmacológicos

A farmacoterapia da fibromialgia envolve diversas classes de fármacos, principalmente aqueles com ação no SNC, sendo seu principal objetivo o alívio sintomático (Filipovic et al., 2025). No entanto, entre os pacientes que fazem uso de terapia farmacológica, apenas 10% a 25% apresentam uma redução de 50% na intensidade da dor (Moore; Straube; Aldington, 2013). Ainda assim, a associação de determinados medicamentos pode promover uma melhora significativa na qualidade de vida dos pacientes (Giorgi et al., 2024).

Atualmente, três fármacos estão aprovados pela *Food and Drug Administration* (FDA) para o tratamento da fibromialgia: duloxetina e milnaciprano, ambos IRSN, e pregabalina, um anticonvulsivante. Além desses, outros medicamentos também são utilizados, incluindo antidepressivos tricíclicos, antagonistas e inibidores seletivos da recaptação de serotonina, gabapentinoides, canabinoides, antagonistas dos receptores NMDA, miorrelaxantes, entre outros. De modo geral, os opioides não são recomendados devido ao seu potencial de induzir tolerância e dependência (Filipovic et al., 2025; Giorgi et al., 2024). No entanto, alguns estudos indicam que o tramadol, um opioide fraco com baixo potencial de dependência, pode apresentar efeitos benéficos no tratamento da fibromialgia (Bennett et al., 2004; Cagle; Bay; Herndon, 2020; Rocha et al., 2020).

A escolha e o uso desses fármacos variam conforme a frequência e a intensidade dos sintomas apresentados pelos pacientes. Esses medicamentos são utilizados com o objetivo de amenizar os sintomas da fibromialgia, especialmente a dor crônica e os distúrbios associados, como fadiga, distúrbios do sono e alterações de humor. O tratamento geralmente se inicia com doses baixas, que são aumentadas de forma gradual e cautelosa, considerando que muitos pacientes não toleram os efeitos adversos associados a doses elevadas. No entanto, em alguns casos, a dose inicial prescrita já é relativamente alta, o que pode contribuir para uma maior incidência de efeitos adversos logo no início do tratamento (Filipovic et al., 2025). Estudos de meta-análise demonstram que os efeitos adversos ocorrem em 58% dos casos com o uso de milnaciprano, 50% com duloxetina, 52% com amitriptilina e até 90% com mirtazapina em contextos de dor crônica. Os principais efeitos colaterais relacionados ao uso contínuo desses

medicamentos incluem sedação, sonolência, náuseas, hipertensão, taquicardia, ganho de peso, constipação, além de outros efeitos gastrointestinais e cardiovasculares (Arnold et al., 2008; Chappell et al., 2011; Clauw et al., 2013; Goldman et al., 2010). A Tabela ¹ apresenta um resumo dos principais fármacos empregados no tratamento da fibromialgia, incluindo as doses iniciais recomendadas, bem como seus efeitos terapêuticos e adversos.

Tabela 1. Resumo dos principais fármacos empregados no manejo da fibromialgia.

Fármaco	Classe	Dose inicial	Efeitos terapêuticos	Efeitos adversos comuns
Duloxetina*	IRSN	30 mg	Efeitos sob dor e depressão	Náusea, boca seca, tontura, hipertensão, perde de apetite, sonolência e sudorese aumentada
Milnacipram*	IRSN	12,5 mg	Efeitos sob dor e na fadiga	Náusea, dor de cabeça, tontura constipação, hipertensão e taquicardia
Amitriptilina	TCAs	10-25 mg	Efeitos sob dor, fadiga e distúrbios do sono	Sonolência, ganho de peso, tontura, visão turva e retenção urinária
Ciclobenzaprina	Miorrelaxante	1-4 mg	Efeitos sob a dor, o humor e os distúrbios do sono	Hipoestesia e parestesia oral, náuseas, fraqueza, constipação e outros sintomas neurovegetativos
Mirtazapina	AIRS	15 mg	Efeitos sob a dor e distúrbios do sono	Sonolência, aumento do apetite, ganho de peso e boca seca
Pregabalina*	Gabapentinoide	25-75 mg	Efeitos sob dor, fadiga e distúrbios do sono	Tontura, sonolência, ganho de peso e edema periférico
Tramadol	Opioide	25 mg	Efeitos sob a dor	Sonolência, náuseas, tonturas, constipação e sudorese excessiva

Dronabinol	Canabinoide	5 mg	Efeitos sob a dor e depressão	Tontura, sonolência, euforia, náusea, alterações no apetite e dificuldade de concentração

Fonte: Adaptado a partir de Giorgi et al. (2024) e Filipovic et al. (2025). *Medicamentos aprovados pelo FDA para o tratamento da fibromialgia. IRSN: Inibidor da recaptação de serotonina e noradrenalina; TCAs: Antidepressivo tricíclico; AIRS: Antagonista e inibidor da recaptação de serotonina.

3.5 Efeitos farmacológicos do 4-PSQ: um derivado de quinolina funcionalizado com grupamento organoselênio

O selênio é um micronutriente essencial que exerce diversas funções no organismo, participando de processos antioxidantes, imunológicos e neurológicos. Sua forma orgânica, além de apresentar menor toxicidade, possui alta biodisponibilidade, sendo preferida em aplicações nutricionais e farmacológicas (Luo et al., 2020). Esse potencial bioativo despertou o interesse de pesquisadores na síntese de compostos contendo selênio, dado seu amplo espectro de ações terapêuticas em baixas doses e sua baixa toxicidade (Nogueira et al., 2021). Estudos demonstram que derivados orgânicos de selênio apresentam atividades antioxidante, anti-inflamatória, neuroprotetora, ansiolítica, antidepressiva, anticâncer, antiviral, antimicrobiana, anti-hiperglicêmica, anti-hipertensiva e imunossupressora, evidenciando a relevância desse elemento para o desenvolvimento de novas abordagens farmacológicas (Nogueira et al., 2004; Luchese et al., 2009; Brüning et al., 2012; Bortolatto et al., 2013; Ribeiro et al., 2013; Petronilho et al., 2016; Rosa et al., 2016; Oliveira et al., 2017).

A busca por novos compostos bioativos tem impulsionado o desenvolvimento de moléculas híbridas que integrem diferentes núcleos farmacofóricos. Um exemplo promissor é o 4-PSQ, um derivado da quinolina funcionalizado com um grupamento organoselênio (Savegnago et al., 2013). Desde sua caracterização inicial, esse composto tem sido amplamente investigado quanto ao seu potencial terapêutico em distintos contextos patológicos. Pinz et al. (2016) demonstraram os efeitos

antinociceptivo, anti-inflamatório e antioxidante do 4-PSQ em modelos de nocicepção aguda. Esses achados foram recentemente corroborados por Da Rocha et al. (2024) consolidando o papel multifatorial da molécula. Posteriormente, Silva et al. (2017) elucidaram os mecanismos envolvidos nesses efeitos, destacando a modulação dos sistemas glutamatérgico, serotoninérgico e nitrérgico.

Com base nesses resultados iniciais, a atuação do 4-PSQ no SNC passou a ser mais explorada. Reis et al. (2017) foram os primeiros a demonstrar seu efeito ansiolítico, associado à redução na captação de glutamato no córtex cerebral de camundongos. Expandindo esses achados, Paltian et al. (2020) relataram que esse efeito também envolve a modulação de vias serotoninérgicas e GABAérgicas, além de influenciar os níveis de corticosterona. De forma complementar, Barth et al. (2019) demonstraram que o 4-PSQ pode interferir positivamente na plasticidade sináptica, promovendo o aumento de moléculas de adesão celular e de enzimas como a polisialiltransferase em áreas cerebrais de ratos idosos, além de atuar em vias colinérgicas e nos níveis de colesterol.

A continuidade das investigações revelou ainda novos potenciais do 4-PSQ. Rodrigues et al. (2021) confirmaram sua ação ansiolítica e, pela primeira vez, relataram um efeito de tipo antidepressivo significativo em um modelo experimental de obesidade. Esse efeito tipo antidepressivo foi posteriormente reforçado por De Oliveira et al. (2022), em um modelo de depressão induzido por estresse de restrição, sendo esse efeito associado à inibição do eixo HPA e à modulação do sistema monoaminérgico.

Em continuidade a esses achados, o 4-PSQ também demonstrou eficácia relevante em modelos de neuropatia periférica. Estudos iniciais indicaram sua capacidade de reduzir alterações sensoriais induzidas pela oxaliplatina em modelos agudos e crônicos, por meio da modulação do estresse oxidativo (Reis et al., 2020b, 2022). Em paralelo, foi observada uma ação protetora sobre parâmetros comportamentais e cognitivos relacionados à neurotoxicidade induzida por esse quimioterápico (Reis et al., 2020a). Importante ressaltar que sua ação antinociceptiva também se manteve eficaz em animais senescentes, ampliando sua aplicabilidade (Reis et al., 2022). Em modelos de neuropatia induzida por paclitaxel, o composto demonstrou reduzir a hipersensibilidade térmica e mecânica, além de atenuar o estresse oxidativo, a neuroinflamação e perturbações na homeostase do Ca^{2+} (Paltian et al., 2022). Resultados semelhantes foram observados em modelo de neuropatia diabética induzida

por estreptozotocina, nos quais o 4-PSQ reverteu a hipersensibilidade mecânica e térmica (Voss et al., 2022). Recentemente, Paltian et al. (2025) inovaram ao incorporar o 4-PSQ em nanocápsulas poliméricas de etilcelulose, avaliando sua ação em modelos de nocicepção e inflamação aguda. A forma nanoencapsulada do 4-PSQ apresentou eficácia superior à sua forma livre, promovendo efeitos antinociceptivos e antiedematogênicos mais pronunciados.

Diante do conjunto de evidências disponíveis, o 4-PSQ configura-se como uma molécula promissora para o desenvolvimento de novas abordagens terapêuticas, especialmente por atuar sobre múltiplos alvos sem provocar toxicidade hepática ou renal (Pinz et al., 2016; Reis et al., 2017). Considerando-se a complexidade fisiopatológica de condições como a fibromialgia, que envolvem alterações inflamatórias, oxidativas e disfunções em múltiplos sistemas de neurotransmissão, o 4-PSQ surge como um candidato relevante para estudos futuros (Tabela 2).

Tabela 2. Resumo das principais propriedades farmacológicas apresentadas pelo composto 4-PSQ em estudos anteriores.

Efeitos farmacológicos	Mecanismos envolvidos	Referência
Antinociceptivo e Antiinflamatório	Modulação glutamatérgica, serotoninérgica e nitrérgica	Pinz et al. (2016) ; Da Rocha et al. (2024) ; Silva et al. (2017)
Ansiolítico	Modulação dos sistemas serotoninérgico, GABAérgico e do eixo HPA	Reis et al. (2017); Paltian et al. (2020)
Neuroprotetor/ Modulador da plasticidade sináptica	Aumento de moléculas de adesão celular, enzimas polisialiltransferase, modulação colinérgica e redução dos níveis de colesterol	Barth et al. (2019)
Tipo-antidepressivo	Modulação do eixo HPA e do sistema monoaminérgico	Rodrigues et al. (2021); De Oliveira et al. (2022)
Antinociceptivo (modelo de neuropatia periférica induzida por oxaliplatina)	Modulação do estresse oxidativo e da homeostase iônica	Reis et al. (2020a; 2020b; 2022)
Antinociceptivo (modelo de neuropatia periférica induzida por paclitaxel)	Atenuação do estresse oxidativo, da neuroinflamação e da desregulação do Ca^{2+}	Paltian et al. (2022)
Antinociceptivo (modelo de neuropatia diabética)	Redução dos níveis de glicose e modulação do estresse oxidativo	Voss et al. (2022)

Antinociceptivo e antiinflamatório (nanoencapsulado)	Redução da hiperalgesia mecânica e térmica	Paltian et al. (2025)
Antioxidante	Modulação de marcadores de dano oxidativo e enzimas antioxidantes	Luchese et al. (2020); Vogt et al. (2018)

3.6 Modelos experimentais pré-clínicos de fibromialgia

3.6.1 Reserpina

A reserpina é um alcaloide indólico extraído das raízes da *Rauwolfia serpentina*, uma planta trepadeira nativa do subcontinente indiano (Strawbridge et al., 2023). Durante décadas, foi amplamente empregada no tratamento de distúrbios neuropsiquiátricos e como anti-hipertensivo de primeira linha. No entanto, seu uso clínico foi gradualmente descontinuado devido à alta incidência de efeitos colaterais, principalmente sintomas depressivos relatados por pacientes em tratamento (Healy; Savage, 1998; Strawbridge et al., 2023).

Atualmente, o modelo experimental de fibromialgia induzido por reserpina, descrito por Nagakura et al. (2009), é amplamente reconhecido na literatura pré-clínica por sua robustez e relevância translacional. Este modelo apresenta validade aparente, por reproduzir sintomas dolorosos e comorbidades típicas da fibromialgia; validade de construto, por promover a depleção de aminas biogênicas centrais; e validade preditiva, ao responder a medicamentos utilizados clinicamente no manejo da síndrome (Nagakura et al., 2009).

Dentre os modelos animais disponíveis, o da reserpina é o único que reproduz farmacologicamente, de forma específica, disfunções no controle central da dor mediadas por neurotransmissores monoaminérgicos. Seu mecanismo de ação envolve a inibição dos transportadores vesiculares de monoaminas 1 e 2 (VMAT1 e VMAT2), tanto no SNC quanto SNP. Essa inibição promove o esgotamento de DA, NE e 5-HT, desencadeando uma ampla gama de sintomas sistêmicos, incluindo uma díade dor-depressão pronunciada (Guillot; Miller, 2009). Em estudos pré-clínicos com roedores, são frequentemente observados sinais de nocicepção térmica (ao frio e ao calor) e mecânica (Blasco-Serra et al., 2015; Fusco et al., 2019; Nagakura et al., 2009),

hiperalgesia mecânica muscular, dor espontânea (Nagakura et al., 2019), fadiga (Favero et al., 2019), distúrbios do sono (Blasco-Serra et al., 2020), alterações gastrointestinais (como diarreia ou constipação) (Nagakura et al., 2018; Uchida et al., 2019), além de comportamentos do tipo-ansioso e do tipo-depressivo (Brum et al., 2020; Ferrarini et al., 2022). A capacidade de mimetizar sintomas sensoriais e emocionais torna o modelo de fibromialgia induzido por reserpina especialmente útil no desenvolvimento de fármacos com múltiplos mecanismos de ação (Brum et al., 2022).

3.6.2 EFI

Nishiyori e Ueda, (2008) propuseram o modelo de fibromialgia induzido por EFI, baseado na hipótese de que variações de temperatura podem desencadear síndromes dolorosas como a fibromialgia. Esse modelo baseia-se na exposição repetida dos animais a flutuações bruscas de temperatura, simulando um ambiente estressor imprevisível. Durante o ciclo claro-escuro, os animais alternam entre períodos em câmara fria e temperatura ambiente, o que tem se mostrado eficaz na indução de sintomas persistentes semelhantes aos da fibromialgia. Acredita-se que a exposição intermitente a esse tipo de estressor afete diretamente os mecanismos centrais de modulação da dor, favorecendo o desenvolvimento de uma sensibilização persistente e comportamentos nociceptivos em roedores (Nishiyori; Ueda, 2008). Além disso, o modelo apresenta validade de construto, por envolver processos fisiopatológicos centrais da fibromialgia, como inflamação e sensibilização periférica e central; validade aparente, por reproduzir sintomas típicos da síndrome; e validade preditiva, ao responder a fármacos de uso clínico. Essas características conferem ao modelo EFI alta relevância translacional, consolidando-o como uma ferramenta valiosa na triagem de novos compostos com potencial analgésico e efeitos moduladores sobre os sistemas de estresse (Brum et al., 2022).

Estudos pré-clínicos demonstram que o modelo de EFI promove alterações sensoriais duradouras, como alodinia mecânica e térmica, hiperalgesia muscular e fadiga (Montserrat-de La Paz et al., 2013, 2018). Além disso, foram observados comportamentos do tipo-depressivo e ansioso (Montserrat-de La Paz et al., 2018; Nasu et al., 2019), distúrbios do sono (Clauw, 2014; Miyamoto et al., 2017) e aumento da

concentração plasmática de corticosterona, indicando ativação do eixo HPA (Miyamoto et al., 2017; Nishiyori et al., 2011). Essas alterações apontam para o envolvimento de mecanismos centrais na regulação da dor, especialmente relacionados à resposta ao estresse (Brum et al., 2022).

Apesar de sua relevância, o modelo de EFI ainda não reproduz integralmente todos os aspectos clínicos da fibromialgia. Sintomas menos frequentes, como alterações gastrointestinais, hiperalgesia visceral e temporomandibular, além de déficits cognitivos, não foram evidenciados em roedores submetidos a esse protocolo (Häuser et al., 2017). No entanto, trata-se de um modelo experimental não farmacológico, reprodutível e sensível à variabilidade sexual, o que o torna uma ferramenta valiosa para investigações que buscam avaliar tanto os componentes sensoriais quanto emocionais da dor crônica (Brum et al., 2022).

Os modelos de reserpina e EFI se complementam no estudo pré-clínico da fibromialgia, pois reproduzem diferentes aspectos da síndrome. O modelo de reserpina foca na disfunção monoaminérgica e na díade dor-depressão, enquanto o EFI simula o impacto do estresse ambiental na sensibilização da dor e nos comportamentos emocionais. Juntos, fornecem uma abordagem mais completa para investigar os mecanismos da fibromialgia e testar novos tratamentos com maior relevância translacional.

4. Manuscrito

Os resultados que fazem parte desta dissertação estão apresentados sob a forma de manuscrito. Os itens Materiais e Métodos, Resultados, Discussão e Referências, encontram-se estruturados de acordo com as normas de um periódico**.** Atualmente o manuscrito encontra-se submetido.

7-Chloro-4-(phenylselanyl)quinoline as a novel approach to fibromyalgia treatment: Behavioral and biochemical markers evidence in mice

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Abstract

Fibromyalgia is a musculoskeletal condition characterized by chronic pain and emotional/cognitive comorbidities. Current treatments focus on symptom control but often cause significant long-term adverse effects. The compound 7-chloro-4-(phenylselanyl)quinoline (4-PSQ), a quinoline derivative functionalized with an organoselenium group, was evaluated for its ability to alleviate pain and depressive symptoms in reserpine-induced or intermittent cold stress (ICS) fibromyalgia models in mice. We investigated its effects on key pathophysiological mechanisms, including monoaminergic (serotonin levels, and monoamine oxidase (MAO) activity) and glutamatergic (glutamate levels) dysfunction, hypothalamic-pituitary-adrenal axis hyperactivity (corticosterone levels and spleen, thymus and adrenal gland relative weights), oxidative stress (oxidative damage, antioxidant enzymes), and electrolyte homeostasis (Na^+/K^+-ATPase, Ca^{2+}-ATPase activities). Male and female mice exposed to reserpine or ICS were treated with 4-PSQ (1 mg/kg), vehicle, or duloxetine (30 mg/kg), and thirty minutes later, behavior was assessed. 4-PSQ reversed nociceptive and depressive behaviors in both models, similarly to duloxetine. Its effects were linked to the modulation of serotonin and glutamate levels, normalization of MAO-A and ATPase activities, and regulation of oxidative stress in the central nervous system of mice, in a sex- and model-dependent manner. Additionally, 4-PSQ attenuated HPA axis activation in ICS-exposed females. These findings suggest that 4-PSQ holds promise as a basis for more effective fibromyalgia therapy.

Keywords: Selenium; Reserpine, Intermittent Cold Stress; Glutamate; Serotonin; Corticosterone.

1. Introduction

Fibromyalgia is a chronic musculoskeletal condition characterized by widespread pain, fatigue, sleep disturbances, mood alterations, and cognitive impairments (Jones et al., 2024). It is the third most prevalent musculoskeletal disorder worldwide, affecting approximately 2-3% of the global population, with higher incidence among women aged 30 to 50 (Marques et al., 2017; Sarzi-Puttini et al., 2020). Despite its prevalence, diagnosis and treatment remain challenging due to its multifactorial and partially unknown pathophysiology involving biological, psychological, and social factors (Antonelli et al., 2025).

Recent studies associate fibromyalgia with alterations in the central, peripheral, and autonomic nervous systems that affect pain processing (Antonelli et al., 2025). Dysfunctions in monoaminergic neurotransmission, especially involving serotonin and monoamine oxidase (MAO), play a key role in pain and mood regulation (Azizi, 2022). Patients with fibromyalgia show reduced tryptophan levels, a serotonin precursor, and genetic alterations in MAO expression, contributing to nociceptive hyperexcitability and increased pain perception (Berends et al., 2019; Singh et al., 2021; Vianello et al., 2012). Additionally, glutamatergic dysregulation promotes central sensitization through hyperactivation of dorsal horn neurons in the spinal cord, sustaining chronic pain (Siracusa et al., 2021). Elevated glutamate levels in the insular cortex have been directly associated with pain intensity and depressive symptoms in fibromyalgia (Harris et al., 2008).

Chronic psychological stress significantly contributes to the development and worsening of fibromyalgia (Sarzi-Puttini et al., 2020). Stressful physical or emotional events can trigger symptoms in predisposed individuals (Nishiyori and Ueda, 2008). Patients often present hypothalamic-pituitary-adrenal (HPA) axis dysfunction, which regulates the stress response and is associated with abnormal basal cortisol levels (Menke, 2024). This dysregulation induces neurochemical changes that alter the expression and release of serotonin, dopamine, and norepinephrine, enhancing pain perception and increasing the risk of psychiatric comorbidities (Siracusa et al., 2021).

Dysregulation of monoaminergic and glutamatergic systems, along with HPA axis dysfunction, can disrupt redox balance and electrolyte homeostasis (Singh et al., 2019). Excessive production of reactive species (RS) and impaired antioxidant defenses are key mechanisms in fibromyalgia progression (Sarzi-Puttini et al., 2020). Intracellular oxidative stress compromises sulfhydryl enzymes like ATPases, which are vital for membrane potential, cellular signaling, and ionic balance (Gęgotek and Skrzydlewska, 2019). Altered Na^+/K^+-ATPase and Ca^{2+}-ATPase activity, for example, can lead to neuronal hyperexcitability, contributing to chronic pain and mood disturbances seen in fibromyalgia (Bejček et al., 2021; Mei et al., 2018).

Currently, fibromyalgia treatment relies on a multimodal approach combining patient education, physical exercise, psychotherapy, and pharmacotherapy. As there is no cure, pharmacological interventions focus on symptom management and often require continuous long-term use (Antonelli et al., 2025). Duloxetine (DXT) is a first-line option for relieving pain and depressive symptoms (Dhaliwal et al., 2025). Short-term DXT use reduces pain and depressive symptoms and improves quality of life (Siddiqui et al., 2025). However, long-term treatment often causes adverse effects, particularly psychiatric and neurological disturbances, such as withdrawal syndrome, nausea, drowsiness, sweating, dizziness, constipation, and headache (Siddiqui et al., 2025; Zhu et al., 2025). These side effects, when persistent or severe, compromise tolerability and reduce treatment adherence (Zhu et al., 2025).

Given the complex pathophysiology of fibromyalgia and the limited effectiveness of current treatments, the development of new therapeutic strategies has become increasingly important (Sarzi-Puttini et al., 2020). In this context, 7-chloro-4-(phenylselanyl)quinoline (4-PSQ), a quinoline functionalized with an organoselenium group, has stood out for the promising pharmacological effects demonstrated in previous studies by our group. The compound showed antinociceptive actions and an antidepressant-like profile in several preclinical models, associated with its ability to modulate the glutamatergic, serotonergic, nitrergic, and monoaminergic systems, in addition to regulating the HPA axis and attenuating inflammatory and oxidative processes (Da Costa Rodrigues et al., 2021; Da Rocha et al., 2024; De Oliveira et al., 2022; Paltian et al., 2022; Pinz et al., 2016; Reis et al., 2017; Silva et al., 2017; Vogt et al., 2018). Additionally, 4-PSQ restores electrolyte balance by

normalizing Na^+/K^+-ATPase and Ca^{2+}-ATPase activities in models of peripheral neuropathy (Paltian et al., 2022; Reis et al., 2020b), without causing renal or hepatic toxicity in mice (Pinz et al., 2016; Reis et al., 2017).

Considering the versatility of 4-PSQ and the multifactorial nature of fibromyalgia, this study investigated its effects in experimental fibromyalgia models induced by reserpine or ICS in male and female mice, evaluating parameters related to monoaminergic and glutamatergic neurotransmission, the HPA axis, oxidative stress, and ATPase activities to elucidate its potential mechanisms of action.

2. **Materials and Methods**

2.1. **Chemicals**

4-PSQ (Figure 1) was synthesized and characterized in the Clean Organic Synthesis Laboratory (LASOL) at the Federal University of Pelotas (UFPel) following the methodology described by Duarte et al. (2017). The analysis of the ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra revealed analytical and spectroscopic data consistent with the proposed structure. The chemical purity of 4-PSQ (99.9%) was confirmed through gas chromatography-mass spectrometry. DXT (DXT hydrochloride, EMS®) was diluted in ultrapure water and used as a positive control. The 4-PSQ compound was diluted in canola oil and administered at a constant volume of 10 mL/kg of body weight. Reserpine, which induces fibromyalgia-like symptoms, was obtained from Sigma-Aldrich (St. Louis, MO, USA) and diluted in 0.5% acetic acid solution. All other reagents used were of analytical grade and obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2. **Animals and Ethical Approval**

The experiments were conducted using female and male *Swiss* mice (60 days old, weighing between 25 and 35 g) obtained from the central animal facility at UFPel (Brazil). Three hundred twenty animals were used in this study, comprising one hundred and sixty females and one hundred and sixty males. They were housed in group cages with a maximum of 5 animals per cage to ensure adequate space and comfort.

Environmental conditions were controlled, maintaining a temperature of 22 ± 2 °C, humidity levels between 50–80%, and a 12-hour light/dark cycle (lights on at 07:00 a.m.). Food and water were available *ad libitum*. Before the experiments, the animals underwent a 4-day acclimation period, followed by a 1-hour habituation to the behavioral testing room, prior to the assessments.

All experimental procedures and handling complied with the *National Institutes of Health Guidelines for the Care and Use of Laboratory Animals* (NIH Publication No. 8023, revised in 1978) to minimize the number of animals used and their discomfort. The experimental protocols received approval from the Animal Care and Use Committee of UFPel (CEUA) (protocol no. 28142-2019 and protocol no. 043665/2023-61).

2.3. **Experimental design**

Figure 2 depicts the schematic design of the experimental protocols. The dose of 4-PSQ (1 mg/kg) and the pretreatment time (30 min) were based on previous studies (Pinz et al., 2016; Reis et al., 2022, 2017).

2.3.1. **Reserpine-induced fibromyalgia model**

In this experimental model, fibromyalgia-like symptoms were induced in mice using repeated administration of reserpine, following the experimental model described by Nagakura et al. (2019). This protocol causes the depletion of biogenic amines in both the central and peripheral nervous systems, leading to physiological and behavioral changes that mimic fibromyalgia characteristics, including nociceptive and emotional dysfunctions, such as anxiety and depression-like behaviors (Nagakura, 2022; Nagakura et al., 2019). For this protocol, mice were divided into five experimental groups: I) Control, II) 4-PSQ, III) Reserpine, IV) Reserpine + 4-PSQ, and V) Reserpine + DXT (n = 7–8 animals per group). Mice of the groups III, IV, and V received reserpine at a dose of 0.5 mg/kg subcutaneously (s.c.) injection for three consecutive days, while groups I and II received the reserpine diluent vehicle, a 0.5% acetic acid solution (1 mL/kg, s.c.) for the same period (Figure 2).

Twenty-four hours after the last reserpine injection, animals in groups II and IV received a single oral dose of 4-PSQ (1 mg/kg, i.g.). In contrast, groups I and III were administered canola oil (10 mL/kg, i.g.) as a vehicle control, while group V received DXT (30 mg/kg, i.g.). Thirty minutes after treatment, all animals were subjected to behavioral assessments to evaluate mechanical and thermal nociception and depression-like behaviors (Figure 2).

2.3.2. **Intermittent cold stress (ICS)-induced fibromyalgia model**

Fibromyalgia-like symptoms were induced in this experimental model through repeated exposure to cold, known as ICS, a protocol developed and validated by Nishiyori and Ueda (2008). In this induction model, mice subjected to the protocol develop long-lasting symptoms of allodynia and hyperalgesia, which can be alleviated by treatment with antidepressants and gabapentin but not with opioids (Nishiyori et al., 2011, 2010). Initially, the animals were randomly assigned to five experimental groups: I) Control, II) 4-PSQ, III) ICS, IV) ICS + 4-PSQ, and V) ICS + DXT (n = 7–8 animals per group). Beginning at 4:30 p.m. on the first day of the protocol, animals of the groups III, IV, and V were housed in cages lined with wood shavings, provided with *ad libitum* access to food and water, and exposed to a temperature of 4 ± 2°C overnight.

The following morning, at 10:00 a.m., the animals were transferred from the incubator to an environment maintained at a controlled temperature of 22 ± 2°C. This transfer was repeated at 30-minute intervals until 4:30 p.m. on the second day, after which the animals were returned to the 4 ± 2°C environment and maintained there until the next morning. On the third day of the protocol, the same procedures were repeated. On the fourth day, at 10:00 a.m., the animals were removed from the cold environment and transferred to a room maintained at ambient temperature (22 ± 2°C), where they remained for 1 hour and 30 minutes acclimating before treatment and subsequent behavioral testing.

Animals of the control groups (I and II) were kept at 22 ± 2°C throughout the entire protocol (from 4:30 p.m. on day 1 to 10:00 a.m. on day 4) (Figure 2). After the acclimation period, mice in groups I and II received canola oil (10 mL/kg) via intragastric administration (i.g.). The mice in groups III and IV were treated only once

with 4-PSQ (1 mg/kg, i.g.), while animals in group V received DXT (30 mg/kg, i.g.). Thirty minutes after treatment, the animals underwent behavioral testing to assess mechanical and thermal nociception, as well as depression-like behavior (Figure 2).

2.4. **Behavioral tests**

2.4.1. **Assessment of locomotor and exploratory performance**

The open field test, described by Walsh and Cummins (1976), assessed the mice's general locomotor and exploratory behaviors. The open field apparatus was made of plywood (30 cm high, 45 cm long, 45 cm wide), with the floor divided into nine quadrants (3 rows of 3), marked with masking tape. On day 4 of the protocol, thirty minutes after administering 4-PSQ and DXT, the animals were individually placed in the center of the apparatus and observed for 4 minutes. The number of crossings, indicated by all four paws moving between quadrants (locomotor activity), and the number of rearing, indicated by elevations on the hind limbs (exploratory activity), were recorded. After each animal was evaluated, the apparatus was sanitized with a 30% ethanol solution.

2.4.2. **Assessment of mechanical sensitivity**

Mechanical nociception was assessed using a digital aesthesiometer (#EFF301W, Insight, Ribeirão Preto, SP, Brazil), following the methodology described by Alamri et al. (2018). This device consists of a unit connected to a specific paw pressure transducer. For the test, the animals were individually housed in acrylic chambers placed on an elevated wire mesh platform, allowing access to the plantar surface of their paws. The experiment was conducted in a quiet room, and before paw stimulation, the animals remained still without exploratory movements or paw support.

Using a polypropylene tip, the paw withdrawal threshold was measured by applying progressively increasing pressure to the central area of the animal's hind paw. The force was applied until the paw was withdrawn, accompanied by precise retraction movements, and the pressure value was automatically recorded. The paw withdrawal threshold (g) was evaluated on day 0 of the protocols, before induction with ICS or

reserpine, to establish the animals' baseline mechanical nociceptive threshold. This evaluation was repeated 30 minutes after treatment with 4-PSQ or DXT on day 4 of the protocols.

2.4.3. **Assessment of thermal sensitivity**

Nociception reflexes in response to thermal stimuli during the hot plate test were evaluated according to the method described by Woolfe and MacDonald (1944). The animals were placed individually in a metal plate (Hot Plate #EFF361, Insight, São Paulo, Brazil) preheated to 52 ± 1 ºC and surrounded by an acrylic box to conduct the test. The latency to exhibit a nociceptive response, such as jumping off the surface, shaking, or licking the hind paws, was measured using a stopwatch, starting from the moment the animal was gently placed on the hot plate. These behaviors were considered positive nociceptive reflexes. To prevent paw injury, a cut-off time of 45 seconds was imposed. Paw withdrawal latency (s), an indicator of thermal sensitivity, was assessed on day 0 of the protocol to evaluate the thermal nociceptive threshold of the animals and was again assessed on day 4 of the protocol, 30 minutes after treatment with 4-PSQ or DTX. The apparatus was sanitized with a 30% alcohol solution before each test.

2.4.4. **Assessment of the emotional domain**

On day 4 of the protocol, 30 minutes after treatment of 4-PSQ or DXT, a forced swimming test was performed according to the methodology described by Porsolt et al. (2001). The main objective of this test is to evaluate the development of the depressive phenotype in the animals and to predict the efficacy of new antidepressant drugs (Acikgoz et al., 2022). In this test, mice were individually placed in an open cylindrical apparatus (10 cm in diameter and 25 cm in height) containing 19 cm of water at 25 ± 1 ºC. Over 6 minutes, the latency to the first episode(s) of immobility and the total immobility time(s) were recorded. The animal was immobile when it remained motionless in the water, making only the movements necessary to keep its head above the water.

2.5. *Ex vivo* assays

2.5.1. Blood and sample collection and processing

Immediately after the behavioral tests, the animals were anesthetized and euthanized via isoflurane inhalation. Blood was collected through cardiac puncture, transferred to heparinized tubes, centrifuged at 900 xg for 15 minutes to separate plasma, and subsequently used to measure corticosterone levels. Following blood collection and confirmation of the absence of heartbeats, samples of the cerebral cortex, hippocampus, spinal cord, and cerebellum were rapidly excised, weighed, and homogenized in cold 50 mM Tris-HCl buffer (pH 7.4) at a ratio of 1:10 (weight/volume). The homogenates were centrifuged at 900 xg for 10 minutes, and the resulting supernatants (S_1 fractions) were used to assess Na^+/K^+-ATPase, Ca^{2+}-ATPase, and Catalase (CAT) activities. In addition, the reactive species (RS), and thiobarbituric acid reactive substances (TBARS) levels were measured.

To determine monoamine oxidase (MAO) activity, samples from the cerebral cortex and spinal cord were homogenized in an isolation medium (Na_2PO_4/KH_2PO_4 isotonic with sucrose, pH 7.4) at a ratio of 1:4 (w/v) and centrifuged at 900 xg for 5 minutes at 4°C. The homogenate was then subjected to a second centrifugation at 12,500 xg for 15 minutes. The resulting mitochondrial pellet was washed once with the isolation medium and centrifuged again under the same conditions. Finally, the mitochondrial pellet (MP) was resuspended in a buffer solution (Na_2PO_4/KH_2PO_4 isotonic with KCl, pH 7.4) and subsequently used for the assay.

2.5.2. Adrenal gland, spleen, and thymus relative weight

The adrenal glands, spleen, and thymus were carefully excised and weighed using an analytical balance (AUY220-SHIMADZU). The relative weight of each organ was calculated by dividing its wet weight by the mice's body weight. The results were expressed as a relative weight (mg).

2.5.3. Plasma corticosterone levels

The plasma corticosterone concentration was then measured using the fluorescence method described by Zenker and Bernstein (1958). An aliquot of plasma (200 µL) was initially incubated with chloroform (2 mL). The tubes were shaken for 15 seconds and centrifuged for 5 minutes at 900 xg, then 0.1 M NaOH (1 mL) was added and another round of shaking and centrifugation under the same conditions. Subsequently, the fluorescence reagent (H_2SO_4 and 50% ethanol) was added to the samples, which were then shaken, centrifuged under the same conditions, and incubated at room temperature for 2 hours. Finally, fluorescence intensity was measured at an emission wavelength of 540 nm (excitation at 257 nm) using a Thermo Scientific Varioskan™ LUX multimode microplate reader. Corticosterone levels were expressed as ng corticosterone/mL of plasma.

2.5.4. **MAO activity assay**

MAO activity was assessed following the method described by Krajl (1965), with some modifications. An aliquot of the MP (200 µL) from each sample was pre-incubated at 37°C for 5 min in a medium containing buffer solution and specific inhibitors: clorgiline (MAO-A inhibitor, 250 nM) or pargyline (MAO-B inhibitor, 250 nM). Subsequently, kynuramine dihydrobromide was added as the substrate at concentrations of 90 µM for MAO-A and 60 µM for MAO-B. The reaction mixture was incubated at 37°C for 30 min. The reaction was terminated by adding 10% trichloroacetic acid (TCA), then cooling and centrifuging at 900 ×g for 15 minutes. An aliquot of the supernatant was mixed with 1 M NaOH, and fluorescence intensity was measured using a spectrofluorometer (Shimadzu RF-5301 PC) with an excitation wavelength of 315 nm and an emission wavelength of 380 nm. The concentration of 4-hydroxyquinoline (4-OH quinoline) was determined using a standard fluorescence curve for 4-OH quinoline. MAO activity was expressed as nmol 4-OH quinoline/mg of protein/min.

2.5.5. **Neurotransmitter analysis**

2.5.5.1. **Sample and internal standards preparation**

New experimental induction protocols were performed with ICS or reserpine to analyze neurotransmitters in male and female mice. In these protocols, the animals were induced with reserpine or ICS and then subjected to the open field test to evaluate locomotor and exploratory activities (Figure 3). After that, the animals were euthanized, and brain and spinal cord samples were immediately collected, weighed, and homogenized in chilled acidified butanol at 1:10 (w/v). The aim was to evaluate serotonin and glutamic acid concentrations in these tissues, according to the methodology described by Chang (1964), with modifications by Ciarlone (1978), Maickel et al. (1968), and Singh et al. (2021).

The homogenized mixture was centrifuged at 900 xg for 10 minutes, and the supernatant was collected. For the analysis, an aliquot of the supernatant (1,000 µL for the brain and 400 µL for the spinal cord) was combined with a mixture of heptane (250 µL for the brain and 100 µL for the spinal cord) and 0.1 M hydrochloric acid (300 µL for brain and 120 µL for spinal cord). The tubes were shaken for 2 minutes and then centrifuged at 900 xg for 10 minutes to separate the phases. The aqueous phase was collected to estimate serotonin and glutamic acid concentrations.

Internal standard tubes were prepared to construct standard curves for serotonin and glutamic acid (15 µg - 1.5 mg/mL). Different concentrations of the standards were obtained by diluting a stock solution of serotonin or glutamic acid (1.5 mg/mL) with 0.1 M hydrochloric acid to a final volume of 0.2 mL. Subsequently, 1.5 mL of acidified butanol was added to the tubes, which were shaken and centrifuged at 900 xg for 5 minutes. After centrifugation, 1.25 mL of the supernatant was transferred to tubes containing 800 µL of 0.1 M hydrochloric acid and 2.5 mL of heptane. The tubes were vortexed again for 2 minutes and centrifuged at 900 xg for 5 minutes to separate the phases. The organic supernatant phase was discarded, and an aliquot of the aqueous phase was transferred to tubes for the final stage of the assay.

2.5.5.2 Glutamic acid and serotonin assay

To evaluate the levels of glutamic acid and serotonin in brain and spinal cord samples, as well as in the internal standards, an aliquot (200 µL) was taken from the aqueous phase obtained after the final centrifugation and added to a medium containing

1.2 mL of 4 mg% o-phthalaldehyde, diluted in 10 M hydrochloric acid. This fluorescence reagent was freshly prepared at the time of the assay. Immediately after adding the fluorescence reagent, the tubes were shaken and placed in a boiling water bath (95 ºC) for 10 min. After this period, the tubes were removed from the bath, cooled under running water, and immediately analyzed spectrofluorometrically. The excitation/emission spectra were set at 360–470 nm for serotonin and 515–470 nm for glutamate (Shimadzu RF-5301 PC).

2.5.6. **Involvement of Ca^{2+}-ATPase enzyme activity**

The enzyme Ca^{2+}-ATPase activity was measured as described by Rohn et al. (1993) and adapted by Trevisan et al. (2009). Aliquots of the S_1 fraction (50 µL) were added to an incubation system containing 30 mM Tris-HCl (pH 7.4), 50 mM NaCl, 5 mM KCl, 0.4 mM $CaCl_2$, 3 mM $MgCl_2$, and 0.1 mM EDTA. The reaction was initiated by adding adenosine triphosphate (ATP) at a final concentration of 12 mM. After 30 minutes of incubation at 37°C, the reaction was stopped by adding 50% TCA (w/v). The samples were then centrifuged, and an aliquot of the supernatant was mixed with a color reagent containing 2% ammonium molybdate. The amount of inorganic phosphate (Pi) released was quantified by colorimetric analysis at 650 nm (Thermo Scientific Varioskan™ LUX multimode microplate reader). A parallel incubation system was prepared to confirm enzymatic activity, excluding $CaCl_2$ from the reaction salts. Enzyme activity was expressed as nmol of Pi/mg protein/min.

2.5.7. **Involvement of Na^+/K^+-ATPase enzyme activity**

The enzyme Na^+/K^+-ATPase activity was evaluated using the method described by Fiske and Subbarow (1925). In this assay, 50 µL of the S_1 fraction was added to a medium containing 30 mM Tris-HCl (pH 7.4), 125 mM NaCl, 20 mM KCl, and 3 mM $MgCl_2$. The reaction was initiated by adding ATP at a final concentration of 3 mM. Control samples were prepared under identical conditions, with the addition of 0.1 mM ouabain, a specific Na^+/K^+ pump inhibitor. The samples were incubated at 37°C for 30 minutes, and the reaction was terminated by adding 10% TCA containing 10 mM

$HgCl_2$. Enzymatic activity was calculated as the difference in the amount of Pi released in the absence and presence of ouabain. The reaction was measured spectrophotometrically at 650 nm using the Thermo Scientific Varioskan™ LUX multimode microplate reader. Results were expressed as nmol of Pi/mg protein/minute.

2.5.8. **Oxidative damage markers**

2.5.8.1. **RS levels**

According to Loetchutinat et al. (2005), RS levels were determined from a spectrofluorimetric method. In this assay, S_1 was incubated with 1 mM 2',7'-dichlorofluorescein diacetate (DCFH-DA) and 10 mM Tris HCl (pH 7.4) for 60 minutes. The amount of intracellular RS was evaluated through the oxidation of DCFH-DA in fluorescent dichlorofluorescein (DCF). For DCF detection, the emission of the fluorescence intensity of DCF was captured at 525 nm (with excitation at 488 nm) (Thermo Scientific Varioskan™ LUX multimode microplate reader). The result was expressed as an arbitrary unit.

2.5.8.2. **TBARS levels**

TBARS levels were determined using the spectrophotometric method described by Ohkawa et al. (1979) as a measure of lipid peroxidation. An aliquot of S_1 (200 µL) was added to a reaction medium containing thiobarbituric acid (0.8%), acetic acid (pH 3.4), and sodium dodecyl sulfate (8.1%). The system was incubated for 2 hours at 95 ºC, and the absorbance was measured at 532 nm using the Thermo Scientific Varioskan™ LUX multimode microplate reader. The results were reported as nmol malondialdehyde (MDA)/mg protein.

2.5.9. **Antioxidant marker**

2.5.9.1. **CAT activity**

CAT activity was assessed using a spectrophotometric test described by Aebi (1984). This assay monitors the disappearance rate of the substrate H_2O_2 in the homogenate at 240 nm (Thermo Scientific Varioskan™ LUX multimode microplate reader). The reaction started with adding H_2O_2 and S_1 to a system containing a 50 mM potassium phosphate buffer (pH 7.0). The results were expressed as a unit per milligram of protein (U/mg of protein) (1U decomposes 1 μmol H_2O_2/ min at pH 7 at 25 ºC).

2.5.10. **Protein determination**

The tissue protein concentration was measured using the Bradford (1976) spectrophotometric method. Comassie Blue and 50 mM Tris-HCl (pH 7.4) were added to an aliquot of S_1. After 10 minutes, the reading was performed at 595 nm. The results were expressed in mg/mL, using bovine serum albumin (1 mg/mL) as the standard.

2.6. **Statistical analysis**

The data is presented as the mean ± standard error of the mean (S.E.M.), and $p < 0.05$ was considered statistically significant. The data's normality was assessed using the Shapiro-Wilk test. Statistical analyses were performed with GraphPad Prism® 8 software. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test was applied when appropriate, and unpaired Student's t-tests were used where necessary. One-way ANOVA was used to compare groups considering treatment as the sole independent variable, whereas t-tests were applied to assess differences between male and female animals within control and induced groups (ICS or Reserpine).

3. **Results**

3.1. **Treatment with 4-PSQ attenuates fibromyalgia-like nociceptive responses reserpine- or ICS-induced in male and female mice**

Figures 4 and 5 show the effects of a single dose of 4-PSQ and DXT on mechanical and thermal sensitivities induced by reserpine and ICS. One-way ANOVA followed by Tukey's post hoc test revealed that reserpine induction significantly reduced paw withdrawal thresholds in response to mechanical stimuli in both male and female mice compared to the control groups (Figure 4A) (One-way ANOVA: male: $F_{(4,35)} = 221.3$, $p < 0.0001$; female: $F_{(4,35)} = 200.8$, $p < 0.0001$). These findings confirm that reserpine induction successfully established mechanical hypersensitivity in both sexes. No significant difference in paw withdrawal thresholds was observed between male and female mice in the control group or the reserpine group (Unpaired Student's t-test: *control*: $t = 0.05938$, $d_f = 14$, $p = 0.9535$; *reserpine*: $t = 2.091$, $d_f = 14$, $p = 0.0553$).

Similarly, reserpine induction also affected thermal sensitivity in both male and female mice, as evidenced by a decrease in the latency time in the hot plate test compared to the control group (Figure 4B) (One-way ANOVA: male: $F_{(4,35)} = 70.6$, $p < 0.0001$; female: $F_{(4,35)} = 25.9$, $p < 0.0001$). However, unlike mechanical sensitivity, female mice in the control group exhibited greater thermal sensitivity than males, as shown by latency time reduction in the hot plate test (Unpaired Student's t-test: $t = 2.439$, $d_f = 12$, $p = 0.0312$). This difference persisted following reserpine induction, with female mice displaying significantly higher thermal sensitivity than males (Unpaired Student's t-test: $t = 7.070$, $d_f = 14$, $p < 0.0001$).

A single administration of 4-PSQ effectively reduced reserpine-induced mechanical and thermal hypersensitivities in male and female mice. This effect was demonstrated by increased paw withdrawal thresholds in response to mechanical stimuli and prolonged latencies in the hot plate test compared to the reserpine group. These results were comparable to those produced by DXT, the reference drug.

Building upon these findings, the effects of 4-PSQ or DXT were also evaluated in a mechanical and thermal hypersensitivities model induced by ICS. One-way ANOVA followed by Tukey's post hoc test revealed that exposure to ICS resulted in a decreased paw withdrawal threshold to mechanical stimuli in male and female mice (Figure 5A) (One-way ANOVA: male: $F_{(4,35)} = 157.3$, $p < 0.0001$; female: $F_{(4,35)} = 224.0$, $p < 0.0001$). There was no significant difference in paw withdrawal thresholds between male and female mice in the control or ICS groups (Unpaired Student's t-test: *control:* t

= 1.317, d_f = 14, p = 0.2088; *ICS*: t = 1.634, d_f = 14, p = 0.1246). Regarding thermal sensitivity, ICS exposure reduced latency times in the hot plate test of male and female mice (One-way ANOVA: males: $F_{(4,35)}$ = 15.0, p < 0.0001; females: $F_{(4,35)}$ = 15.3, p < 0.0001) (Figure 5B). The unpaired Student's t-test revealed that control female mice exhibited greater thermal sensitivity than control male mice (unpaired Student's t-test: t = 2.52, d_f = 14, p = 0.0244). However, this difference was not observed between male and female mice exposed to ICS (Unpaired Student's t-test: t = 0.4383, d_f = 14, p = 0.6679). Notably, treatment with a single dose of 4-PSQ or DXT successfully reversed ICS-induced mechanical and thermal hypersensitivities in both male and female mice.

3.2. **Treatment with 4-PSQ attenuates the depressive-like behavior in male and female mice of ICS- or Reserpine-induced fibromyalgia**

The effects of treatment with the compound 4-PSQ or DXT on depression-like behavior induced by reserpine or ICS in male and female mice are presented in Table 1 and Figure 6. One-way ANOVA followed by Tukey's post hoc test revealed that reserpine induction significantly reduced the latency to the first episode of immobility (Table 1) and increased the total immobility time (Figure 6A) in male and female mice compared to the control groups (One-way ANOVA: *latency:* male: $F_{(4,33)}$ = 17.4, p < 0.0001; female: $F_{(4,35)}$ = 10.9, p < 0.0001; *immobility time*: male: $F_{(4,33)}$ = 22.9, p < 0.0001; female: $F_{(4,35)}$ = 13.9, p < 0.0001).

In contrast, 4-PSQ treatment demonstrated an antidepressant-like effect in both sexes induced by reserpine. Interestingly, under this protocol, a single administration of DXT produced an antidepressant-like effect exclusively in females induced with reserpine (Figure 6A). However, neither 4-PSQ nor DXT treatments increased the latency to the first episode of immobility in reserpine-induced male or female mice.

The unpaired Student's t-test revealed no significant differences in the latency or in total immobility time between female and male mice of the control groups (Unpaired Student's t-test: *latency*: t = 1.489; d_f = 13; p = 0.1603*; total immobility time*: t = 1.802; d_f = 12; p = 0.0967). Similarly, no significant differences were observed between the female and male mice reserpine-induced groups for the same parameters (unpaired

Student's t-test: *latency*: t = 1.275; d_f = 14; p = 0.2230; *total immobility time*: t = 0.6133; d_f = 14; p = 0.5495).

In the fibromyalgia-like model induced by ICS, male and female mice exhibited a reduced latency to the first episode of immobility (Table 1) and an increased total immobility time compared to control groups (Figure 6B) (One-way ANOVA: *latency:* male: $F_{(4,31)}$ = 4.4, p < 0.01; female: $F_{(4,33)}$ = 30.4, p < 0.0001; *immobility time*: male: $F_{(4,33)}$ = 9.1, p < 0.0001; female: $F_{(4,34)}$ = 29.9, p < 0.0001). A single dose of 4-PSQ increased the latency to the first immobility episode in females and reduced total immobility time in both sexes exposed to the ICS protocol. The effects observed for 4-PSQ were like those of DXT, which increased the latency to the first episode of immobility and reduced the total immobility time in male and female mice exposed to ICS.

The unpaired Student's t-test revealed no significant differences in latency between male and female mice control groups or total immobility time (Unpaired Student's t-test: *latency*: t =1.104; d_f = 13; p = 0.2897; *total immobility time*: t = 0.9722; d_f = 13; p = 0.3487). Similarly, no significant differences were observed between male and female ICS-induced groups for these parameters (Unpaired Student's t-test: *latency*: t = 0.1117; d_f = 12; p = 0.9129; *total immobility time*: t = 1.265; d_f = 13; p = 0.2282).

3.3. **Locomotor and exploratory domains were not altered by 4-PSQ administration**

The number of crossings and rearings in the open field test is presented in Table 2. Data analysis revealed that male and female mice exposed to the reserpine protocol exhibited reduced locomotor and exploratory activities in the open field test compared to the control group (One-way ANOVA: *crossings*: male: $F_{(4,35)}$ = 256.7, p < 0.0001; female: $F_{(4,35)}$ = 276.6, p < 0.0001; *rearings*: male: $F_{(4,35)}$ = 27.5, p < 0.0001; female: $F_{(4,35)}$ = 120.4, p < 0.0001). Treatment with 4-PSQ or DXT failed to reverse the locomotor and exploratory deficits induced by reserpine in male and female mice. Furthermore, no significant locomotor or exploratory activities differences were observed between male and female mice control groups and their respective reserpine-induced groups (Unpaired Student's t-test: t = 0.5908; d_f = 14; p = 0.5641).

Conversely, data analysis showed that exposure of male and female mice to the ICS protocol and/or treatment with 4-PSQ did not result in significant changes in the number of crossings or rearings (One-way ANOVA: *crossings*: male: $F_{(4,34)} = 1.0$, $p > 0.05$; female: $F_{(4,34)} = 2.4$, $p > 0.05$; *rearings*: male: $F_{(4,31)} = 2.3$, $p > 0.05$; female: $F_{(4,34)} = 1.9$, $p > 0.05$). Additionally, no significant differences in the number of crossings or rearings were observed between male and female mice control groups (Unpaired Student's t-test: *crossings*: $t = 1.290$; $d_f = 14$; $p = 0.2179$; *rearings*: $t = 1.624$; $d_f = 12$; $p = 0.1303$) or between male and female ICS-induced groups (Unpaired Student's t-test: *crossings*: $t = 1.733$; $d_f = 14$; $p = 0.1050$; *rearings*: $t = 1.790$; $d_f = 13$; $p = 0.0968$).

3.4. **Treatment with 4-PSQ modulated changes in glutamic acid levels and monoaminergic system induced by Reserpine or ICS in male and female mice**

Figure 7 illustrates the effects of 4-PSQ and DXT on glutamic acid levels in the brain and spinal cord of male and female mice subjected to reserpine or ICS induction. In the reserpine induction model, females of the control group exhibited higher brain and spinal cord glutamic acid levels compared to males of the control group (Figure 6A and 6C) (Unpaired Student's t-test: *brain:* $t = 6.041$; $d_f = 14$; $p < 0.0001$; *spinal cord:* $t = 2.567$; $d_f = 14$; $p = 0.0224$). However, this difference of glutamic acid levels in the brain and spinal cord disappeared following reserpine induction, with no significant differences observed between males and females exposed to the inductor (Unpaired Student's t-test: *brain:* $t = 1.355$; $d_f = 15$; $p = 0.1955$; *spinal cord*: $t = 1.666$; $d_f = 14$; $p = 0.1180$).

Reserpine-induced male mice showed increased glutamic acid levels in the brain and spinal cord compared to the control group (Figures 7A and 7C) (One-way ANOVA: *brain:* $F_{(4,35)} = 21.4$, $p < 0.0001$; *spinal cord:* $F_{(4,35)} = 5.6$, $p < 0.01$). In contrast, reserpine-induced female mice exhibited a significant increase only in the spinal cord (One-way ANOVA: $F_{(4,35)} = 6.2$, $p < 0.001$). Treatment with 4-PSQ effectively normalized glutamic acid levels in the brain of male mice and in the spinal cord of both sexes exposed to reserpine induction. Conversely, DXT treatment did not reverse the elevated levels of this neurotransmitter in any of the analyzed structures, regardless of

sex. No significant differences were observed in brain glutamic acid levels between experimental groups in reserpine-induced female mice (One-way ANOVA: $F_{(4,35)} = 1.9$, $p > 0.05$).

In the ICS protocol, an unpaired Student's t-test revealed that females of the control group had higher glutamic acid levels in the brain and spinal cord compared to control males (Figures 7B and 7D) (Unpaired Student's t-test: brain: $t = 4.304$; $d_f = 14$; $p = 0.0007$; spinal cord: $t = 2.468$; $d_f = 13$; $p = 0.0282$). This difference was exacerbated by ICS exposure, with a significant increase observed only in the spinal cord of female mice compared to ICS-exposed males (Unpaired Student's t-test: spinal cord: $t = 3.014$; $d_f = 13$; $p = 0.0100$). No significant differences were found in brain glutamic acid levels between male and female mice exposed to ICS (Unpaired Student's t-test: brain: $t = 0.4797$; $d_f = 14$; $p = 0.6388$).

One-way ANOVA followed by Tukey's test showed that ICS-exposed female mice had higher levels of glutamic acid in the brain and spinal cord compared to the control group (One-way ANOVA: *brain:* $F_{(4,35)} = 5.9$, $p < 0.001$; *spinal cord:* $F_{(4,31)} = 5.2$, $p < 0.01$). In contrast, male mice only showed an increase in these neurotransmitter levels in the brain, with no significant differences in the spinal cord compared to controls (one-way ANOVA: *brain:* $F_{(4,35)} = 5.1$, $p < 0.01$; *spinal cord:* $F_{(4,35)} = 0.9$, $p > 0.05$). Treatment with 4-PSQ reduced the increase in glutamic acid levels in the brain and spinal cord of ICS-exposed female mice (Figures 7B and 7D). However, a single dose of 4-PSQ was not enough to reverse the ICS-induced rise in brain glutamic acid levels in male mice. Additionally, DXT treatment did not influence the ICS-induced increase in glutamic acid levels in the brains or spinal cords of male and female mice.

Effects of 4-PSQ and DXT on the serotonin levels in the brain and spinal cord of male and female mice subjected to reserpine or ICS induction are shown in Figure 8. Data analyses revealed that reserpine induction led to a reduction in serotonin levels in the brain and spinal cord of male and female mice compared to the control group (Figures 8A and 8C) (One-way ANOVA: *brain*: male: $F_{(4,35)} = 8.5$, $p < 0.0001$; female: $F_{(4,35)} = 4.4$, $p < 0.01$; *spinal cord*: male: $F_{(4,35)} = 8.0$, $p < 0.0001$; female: $F_{(4,35)} = 8.8$, $p < 0.0001$). A single administration of 4-PSQ increased serotonin levels in the brain of male mice and the spinal cord of female mice subjected to reserpine induction. In contrast, DXT administration effectively reversed the reserpine-induced decrease in

serotonin levels in the spinal cords of male and female mice, as well as in the brains of male mice, when compared to their respective control groups.

The student's t-test revealed no significant differences in serotonin levels in the brain and spinal cord between the control groups of females and males mice (Unpaired Student's t-test: *brain:* $t = 0.1652$; $d_f = 14$; $p = 0.8711$; *spinal cord:* $t = 1.779$; $d_f = 14$; $p = 0.0970$). Furthermore, no differences were observed in serotonin levels between the female and male mice groups induced with reserpine in either of the analyzed structures (Unpaired Student's t-test: *brain:* $t = 0.1132$; $d_f = 14$; $p = 0.1132$; *spinal cord:* $t = 1.584$; $d_f = 14$; $p = 0.1356$).

In the ICS protocol, as shown in Figures 8B and 8D, male mice exposed to ICS exhibited reduced serotonin levels in the brain and spinal cord compared to the control group (One-way ANOVA: *brain:* $F_{(4,35)} = 21.2$, $p < 0.0001$; *spinal cord:* $F_{(4,35)} = 7.0$, $p < 0.0001$). Treatment with 4-PSQ normalized serotonin levels in both structures in ICS-exposed male mice. Similarly, DXT increased serotonin levels in the brains and spinal cords of male mice exposed to ICS. Notably, DXT was more effective than 4-PSQ in normalizing serotonin levels in the brain but showed similar effects to 4-PSQ in the spinal cord.

No significant differences were observed between the experimental groups regarding serotonin levels in the brain and spinal cord of female mice (One-way ANOVA: *brain:* $F_{(4,35)} = 0.8$, $p > 0.05$; *spinal cord:* $F_{(4,35)} = 1.9$, $p > 0.05$). Additionally, the Student's t-test data did not reveal significant differences in serotonin levels in the spinal cord between the male and female control groups, nor between the male and female ICS groups (Unpaired Student's t-test: control: *brain:* $t = 0.9066$; $d_f = 14$; $p = 0.3800$; *spinal cord:* $t = 1.136$; $d_f = 13$; $p = 0.2766$; *ICS:* brain: $t = 1.136$; $d_f = 14$; $p = 0.2750$; spinal cord: $t = 0.4015$; $d_f = 14$; $p = 0.6941$).

Figure 9 and Table 3 illustrates the effects of 4-PSQ and DXT treatments on the activity of the enzymes MAO-A and MAO-B in the cerebral cortex and spinal cord of male and female mice subjected to reserpine or ICS induction. Reserpine induction significantly increased MAO-A activity in the cerebral cortex of male and female mice compared to their respective controls (One-way ANOVA: *male:* $F_{(4,35)} = 9.1$, $p < 0.0001$; *female:* $F_{(4,35)} = 17.5$, $p < 0.0001$) (Figure 9A). Notably, according to the unpaired Student's t-test, this increase was more pronounced in females induced with

reserpine than in males under the same condition (Unpaired Student's t-test: t = 2.961; d_f = 14; p = 0.0103). Treatment with 4-PSQ normalized MAO-A activity in the cerebral cortex of both sexes exposed to reserpine. In contrast, DXT did not affect the activity of this enzyme in the cerebral cortex of mice of both sexes induced by reserpine. No significant differences in MAO-A activity in the cerebral cortex were observed between male and female mice controls in the reserpine induction protocol (Unpaired Student's t-test: t = 0.8529; d_f = 14; p = 0.4081).

Furthermore, no significant alterations in MAO-B activity were observed after induction of reserpine e/or treatment of 4-PSQ or DXT in the cerebral cortex of male and female mice (Table 3) (One-way ANOVA: male: $F_{(4,34)} = 0.7$, $p > 0.05$; female: $F_{(4,35)} = 0.6$, $p > 0.05$). There was no significant difference in MAO-B activity in the cerebral cortex between male and female mice control or male and female exposed to reserpine (Unpaired Student's t-test: control: t = 0.8335; d_f = 14; p = 0.4186; reserpine: t = 1.543; d_f = 14; p = 0.1451).

Regarding the ICS induction protocol, Figures 9B and Table 3 show that ICS induction did not cause significant changes in the activity of the enzymes MAO-A and MAO-B in the cerebral cortex of male and female mice (One-way ANOVA: *MAO-A*: male: $F_{(4,33)} = 1.2$, $p > 0.05$; female: $F_{(4,34)} = 1.6$, $p > 0.05$; *MAO-B*: male: $F_{(4,35)} = 1.8$, $p > 0.05$; female: $F_{(4,35)} = 0.8$, $p > 0.05$). Furthermore, no differences were observed in the unpaired Student's t-test between male and female control groups and ICS male and female groups regarding enzyme activity in the cerebral cortex (Unpaired Student's t-test: *MAO-A*: Control: t = 1.298; d_f = 12; p = 0.2188; ICS: t = 2.022; d_f = 13; p = 0.0642; *MAO-B*: Control: t = 1.531; d_f = 14; p = 0.1480; ICS: t = 1.085; d_f = 14; p = 0.2962).

Like the cerebral cortex, reserpine treatment increased MAO-A enzyme activity in the spinal cord of male and female mice compared to the control groups (One-way ANOVA: male: $F_{(4,34)} = 10.3$, $p < 0.0001$; female: $F_{(4,35)} = 4.3$, $p < 0.01$) (Figure 9C). However, unlike the cerebral cortex, the increase in MAO-A activity in the spinal cord was more pronounced in males induced with reserpine than in females under the same condition (Unpaired Student's t-test: t = 2.881; d_f = 14; p = 0.0121). A single administration of 4-PSQ reversed the reserpine-induced increase in MAO-A activity in the spinal cord of male and female mice exposed to reserpine. Conversely, treatment

with DXT reduced MAO-A activity only in the spinal cord of male mice, with no effect on enzyme activity in female mice exposed to reserpine. No significant differences in MAO-A activity in the spinal cord were observed between male and female mouse controls (Unpaired Student's t-test: $t = 0.1412$; $d_f = 13$; $p = 0.8898$).

Furthermore, no significant changes in MAO-B activity were observed in the spinal cord among the experimental groups in male and female mice subjected to the reserpine protocol (Table 3) (One-way ANOVA: male: $F_{(4,30)} = 1.5$, $p > 0.05$; female: $F_{(4,36)} = 1.1$, $p > 0.05$). However, male mice treated with reserpine exhibited higher MAO-B activity in the spinal cord than female mice under the same treatment (Unpaired Student's t-test: $t = 2.439$, $df = 13$, $p = 0.0298$). No significant differences in MAO-B activity were observed between male and female control groups (Unpaired Student's t-test: control: $t = 0.1719$, $df = 13$, $p = 0.8662$).

The ICS induction protocol and/or treatment of 4-PSQ or DXT did not significantly alter the activities of MAO-A and MAO-B in the spinal cord of male and female mice, as shown in Figures 9D and Table 3 (One-way ANOVA: *MAO-A*: male: $F_{(4,35)} = 1.9$, $p > 0.05$; female: $F_{(4,35)} = 0.8$, $p > 0.05$; *MAO-B*: male: $F_{(4,35)} = 1.7$, $p > 0.05$; female: $F_{(4,35)} = 1.5$, $p > 0.05$). Additionally, no significant differences in enzyme activity were observed between male and female mice control groups or between male and female ICS groups when analyzed using the unpaired Student's t-test (Unpaired Student's t-test: *MAO-A*: Control: $t = 0.1532$, $d_f = 14$, $p = 0.8804$; ICS: $t = 1.778$, $d_f = 14$, $p = 0.0971$; *MAO-B*: Control: $t = 1.829$, $d_f = 14$, $p = 0.0888$; ICS: $t = 2.108$, $d_f = 14$, $p = 0.0535$).

3.5. **Treatment with 4-PSQ attenuates the HPA axis activation caused by Reserpine- or ICS-induction in male and female mice**

Figure 10 shows the effects of 4-PSQ or DXT treatment on the plasma corticosterone levels and the relative weight of the adrenal gland, spleen, and thymus in male and female mice exposed to the reserpine or ICS induction protocols. According to one-way ANOVA followed by Tukey's test, no statistically significant differences were found between the experimental groups in plasma corticosterone levels in male and female mice subjected to the reserpine induction protocol (Figure 10A) (One-way

ANOVA: male: $F_{(4,35)} = 0.6$, $p > 0.05$; female: $F_{(4,35)} = 0.8$, $p > 0.05$). However, the unpaired Student's t-test revealed that reserpine-induced male mice showed increased plasma corticosterone levels compared to females subjected to the same induction protocol (Unpaired Student's t-test: $t = 2.542$; $d_f = 14$; $p = 0.0235$). No significant differences were observed between male and female controls for this parameter (Unpaired Student's t-test: $t = 0.1109$; $d_f = 14$; $p = 0.9133$).

On the other hand, male and female mice exposed to the ICS induction protocol showed increased plasma corticosterone levels compared to the control group (Figure 10B) (One-way ANOVA: male: $F_{(4,35)} = 6.0$, $p < 0.001$; female: $F_{(4,35)} = 12.3$, $p < 0.0001$). This increase was more pronounced in female mice exposed to ICS than in male mice under the same experimental conditions (Unpaired Student's t-test: $t = 4.606$; $d_f = 14$; $p = 0.0004$). A single administration of 4-PSQ and DXT normalized corticosterone levels only in female mice exposed to ICS. No significant differences were observed between male and female controls for this parameter (Unpaired Student's t-test: $t = 1.912$; $d_f = 14$; $p = 0.0765$).

In the relative weight of the adrenal gland, one-way ANOVA followed by Tukey's test showed that male and female mice exposed to reserpine and/or treated with 4-PSQ or DXT exhibited no statistically significant differences between the experimental groups (Figure 10C) (One-way ANOVA: male: $F_{(4,35)} = 1.3$, $p > 0.05$; female: $F_{(4,35)} = 2.1$, $p > 0.05$). However, reserpine-treated females showed an increase in adrenal gland relative weight compared to male mice under the same conditions (Unpaired Student's t-test: $t = 1.912$; $d_f = 14$; $p = 0.0765$). This difference was not observed between male and female mice in the control group (Unpaired Student's t-test: $t = 6.281$; $d_f = 14$; $p < 0.0001$).

On the other hand, male and female mice exposed to ICS showed an increase in the relative weight of the adrenal gland compared to the control group (Figure 10D) (One-way ANOVA: male: $F_{(4,35)} = 1.3$, $p > 0.05$; female: $F_{(4,35)} = 2.1$, $p > 0.05$). Treatment with 4-PSQ and DXT did not reduce the relative weight of the adrenal gland in male and female mice exposed to ICS. The unpaired Student's t-test did not show significant differences in the relative weight of the adrenal gland between male and female control groups or between male and female ICS groups (Unpaired Student's t-test: control: $t = 1.469$; $d_f = 14$; $p = 0.1638$; ICS: $t = 1.740$; $d_f = 15$; $p = 0.1023$).

Regarding the relative weight of the spleen and thymus, the repeated administration of reserpine caused a significant reduction in the relative weight of the spleen (Figure 10E) and thymus (Figure 10G) in male and female mice compared to the control group (One-way ANOVA: *spleen*: male: $F_{(4,35)} = 79.7$, $p < 0.0001$; female: $F_{(4,34)} = 15.1$, $p < 0.0001$; *thymus*: male: $F_{(4,35)} = 10.1$, $p < 0.0001$; female: $F_{(4,33)} = 6.3$, $p < 0.001$). Moreover, the unpaired Student's t-test revealed that female mice controls exhibited higher relative spleen and thymus weights compared to male controls (Unpaired Student's t-test: *spleen*: $t = 3.055$; $d_f = 13$; $p = 0.0092$; *thymus*: $t = 3.406$; $d_f = 13$; $p = 0.0047$). This difference persisted after reserpine induction, with reserpine-exposed females showing higher relative spleen and thymus weights compared to males subjected to the same induction protocol (Unpaired Student's t-test: *Spleen*: $t = 3.232$; $d_f = 14$; $p = 0.0060$; *Thymus*: $t = 3.502$; $d_f = 14$; $p = 0.0035$).

Similar to the reserpine induction protocol, male and female mice exposed to the ICS protocol exhibited a reduction in the relative weights of the spleen (Figure 10F) and thymus (Figure 10H) compared to the control group (One-way ANOVA: *spleen*: male: $F_{(4,35)} = 6.2$, $p < 0.001$; female: $F_{(4,35)} = 10.3$, $p < 0.0001$; *thymus*: male: $F_{(4,35)} = 6.1$, $p < 0.001$; female: $F_{(4,35)} = 10.1$, $p < 0.0001$). Statistical analysis further revealed that control group females had higher relative spleen and thymus weights compared to control group males (Unpaired Student's t-test: *spleen*: $t = 5.971$; $d_f = 14$; $p < 0.0001$; *thymus*: $t = 3.552$; $d_f = 14$; $p = 0.0032$). This difference persisted following ICS induction, with females showing greater relative spleen and thymus weights compared to males exposed to ICS (Unpaired Student's t-test: *spleen*: $t = 5.571$; $d_f = 14$; $p < 0.0001$; *thymus*: $t = 2.762$; $d_f = 14$; $p = 0.0153$). Treatment with a single dose of 4-PSQ and DXT did not affect the relative weights of the spleen or thymus in male or female mice exposed to ICS.

3.6. Treatment with 4-PSQ modulated the activity of Ca^{2+}-ATPase and Na^{+}/K^{+}-ATPase enzymes in the central nervous system after induction with reserpine or ICS in male and female mice

Figure 11 illustrates the effect of Reserpine or ICS induction and treatment with the compound 4-PSQ or DXT on Ca^{2+}-ATPase activity in cerebral cortex, spinal cord,

hippocampus, and cerebellum of male and female mice. According to one-way ANOVA followed by Tukey's test, reserpine administration resulted in a significant increase in Ca^{2+}-ATPase activity in the cerebral cortex of female mice compared to the control group (Figure 11A) (One-way ANOVA: $F_{(4,34)} = 11.2$, $p < 0.0001$). Treatment with 4-PSQ did not normalize Ca^{2+}-ATPase activity in the cerebral cortex of female mice. In contrast, a single administration of DXT reduced the enzyme's activity in the cerebral cortex of female mice. No significant differences were observed between experimental groups in Ca^{2+}-ATPase activity in the cerebral cortex of male mice (Figure 11A) (One-way ANOVA: $F_{(4,32)} = 1.1$, $p > 0.05$). Additionally, analysis using the unpaired Student's t-test revealed no significant differences in Ca^{2+}-ATPase activity between male and female Control groups or between male and female mice's reserpine groups (Unpaired Student's t-test: control: $t = 0.6961$, $d_f = 13$, $p = 0.4986$; reserpine: $t = 0.6936$, $d_f = 14$, $p = 0.4993$).

In the ICS induction protocol, female mice exposed to the induction scheme showed increased Ca^{2+}-ATPase activity in the cerebral cortex compared to the Control group (Figure 11B) (one-way ANOVA: $F_{(4,32)} = 4.6$, $p < 0.01$). Furthermore, unpaired Student's t-test revealed that female mice exposed to ICS exhibited higher enzyme activity in this tissue than male mice subjected to the same induction protocol (Unpaired Student's t-test: $t = 5.301$, $d_f = 13$, $p = 0.0001$). 4-PSQ and DXT treatments had similar effects, reducing enzyme activity in the cerebral cortex of female mice exposed to ICS. In contrast, no significant differences in Ca^{2+}-ATPase activity were detected among the experimental groups in the cerebral cortex of male mice (Figure 11B) (One-way ANOVA: $F_{(4,29)} = 2.4$, $p > 0.05$). Additionally, no significant differences were observed in the cerebral cortex Ca^{2+}-ATPase activity between the male and female mouse control groups (Unpaired Student's t-test: $t = 1.251$, $d_f = 12$, $p = 0.2346$).

In the spinal cord (Figure 11C), male and female mice induced with reserpine exhibited inhibition of Ca^{2+}-ATPase activity compared to the Control group (One-way ANOVA: male: $F_{(4,32)} = 9.6$, $p < 0.0001$; female: $F_{(4,34)} = 5.5$, $p < 0.01$). Interestingly, the inhibition of Ca^{2+}-ATPase activity was more pronounced in reserpine-induced male mice than in females subjected to the same induction protocol (Unpaired Student's t-test: $t = 4.116$, $d_f = 13$, $p = 0.0012$). Treatment with 4-PSQ reversed the enzyme inhibition exclusively in the spinal cord of reserpine-exposed male mice. Conversely,

DXT did not alter Ca^{2+}-ATPase activity in the spinal cord of either male or female mice induced with reserpine. Additionally, no statistical difference was observed in Ca^{2+}-ATPase activity in the spinal cord between control male and female mice (unpaired Student's t-test: $t = 1.626$, $d_f = 13$, $p = 0.1280$).

In contrast, an increase in Ca^{2+}-ATPase activity was observed in the spinal cord of male and female mice subjected to the ICS protocol compared to the Control group (Figure 11D) (One-way ANOVA: male: $F_{(4,32)} = 3.7$, $p < 0.05$; female: $F_{(4,33)} = 3.5$, $p < 0.05$). According to the unpaired Student's t-test, this increase was more pronounced in female mice exposed to ICS than in their male counterparts exposed to the same induction protocol (Unpaired Student's t-test: $t = 3.070$, $d_f = 13$, $p = 0.0089$). A single administration of 4-PSQ normalized Ca^{2+}-ATPase activity exclusively in male mice exposed to ICS. Conversely, a single administration of DXT affected Ca^{2+}-ATPase activity only in female mice subjected to ICS. No significant Ca^{2+}-ATPase activity difference was detected in the spinal cord between male and female control mice (Unpaired Student's t-test: $t = 1.895$, $d_f = 14$, $p = 0.0789$).

In the hippocampus, as shown in Figure 11E, male mice subjected to the reserpine induction protocol exhibited increased Ca^{2+}-ATPase activity compared to the Control group (One-way ANOVA: $F_{(4,31)} = 4.2$, $p < 0.01$). Treatment with 4-PSQ restored Ca^{2+}-ATPase activity in the hippocampus of reserpine-induced male mice to levels like those of the controls. DXT did not affect Ca^{2+}-ATPase activity in the hippocampus of male mice exposed to reserpine induction. No significant differences in Ca^{2+}-ATPase activity were observed among the experimental groups in the hippocampus of female mice (One-way ANOVA: $F_{(4,35)} = 1.2$, $p > 0.05$). Unpaired Student's t-test analysis revealed that the female mice control group displayed higher Ca^{2+}-ATPase activity in the hippocampus than male mice controls (Unpaired Student's t-test: $t = 3.149$, $d_f = 13$, $p = 0.0077$). However, this difference was not observed in hippocampus Ca^{2+}-ATPase activity between male and female mice exposed to reserpine induction (Unpaired Student's t-test: $t = 0.4931$, $d_f = 14$, $p = 0.6296$).

Exposure to the ICS induction protocol caused an inhibition of Ca^{2+}-ATPase activity in the hippocampus of female mice compared to the control group (Figure 11F) (One-way ANOVA: $F_{(4,34)} = 7.3$, $p < 0.01$). Treatment with 4-PSQ and DXT similarly reversed the ICS-induced inhibition of Ca^{2+}-ATPase activity in the hippocampus of

female mice exposed to ICS. No significant differences in Ca^{2+}-ATPase activity were observed among the experimental groups in the hippocampus of male mice (One-way ANOVA: $F_{(4,34)} = 0.4$, $p > 0.05$). As seen in the results from the reserpine induction protocol, female mice in the control group had higher Ca^{2+}-ATPase activity in the hippocampus than male controls (Unpaired Student's t-test: $t = 5.100$, $d_f = 13$, $p = 0.0002$). However, this difference was not detected in Ca^{2+}-ATPase activity between male and female mice exposed to the ICS protocol (Unpaired Student's t-test: $t = 0.07847$, $d_f = 14$, $p = 0.9386$).

In the cerebellum, as shown in Figure 11G, Ca^{2+}-ATPase activity remained unchanged across all experimental groups of male and female mice (One-way ANOVA: male: $F_{(4,35)} = 0.3$, $p > 0.05$; female: $F_{(4,35)} = 1.4$, $p > 0.05$). Additionally, there were no significant differences between male and female mice in the control or reserpine-induced groups (Unpaired Student's t-test: *control:* $t = 0.3821$, $d_f = 14$, $p = 0.7081$; *reserpine:* $t = 1.193$, $d_f = 14$, $p = 0.2527$).

In contrast, male mice exposed to the ICS protocol showed increased Ca^{2+}-ATPase activity in the cerebellum compared to the control group (Figure 11H) (One-way ANOVA: $F_{(4,30)} = 4.9$, $p < 0.01$). Additionally, unpaired Student's t-test indicated that males subjected to the ICS protocol had higher Ca^{2+}-ATPase activity in the cerebellum than females exposed to the same induction protocol (Unpaired Student's t-test: $t = 3.045$, $d_f = 13$, $p = 0.0094$). Treatment with 4-PSQ did not affect Ca^{2+}-ATPase activity in this tissue, while a single dose of DXT normalized enzyme activity in the cerebellum of male mice. No significant differences in Ca^{2+}-ATPase activity were observed among the experimental groups in the cerebellum of female mice (One-way ANOVA: $F_{(4,33)} = 2.4$, $p > 0.05$). Furthermore, no differences in Ca^{2+}-ATPase activity were found between male and female control groups in this tissue (unpaired Student's t-test: $t = 1.019$, $d_f = 12$, $p = 0.3282$).

Figure 12 shows the effect of Reserpine or ICS induction and treatment with the compound 4-PSQ or DXT on Na^+/K^+ ATPase activity in male and female mice's cerebral cortex, spinal cord, hippocampus, and cerebellum. Male mice induced with reserpine exhibited an inhibition of Na^+/K^+ ATPase activity in the cerebral cortex (Figure 12A), spinal cord (Figure 12C), and hippocampus (Figure 12E) compared to the Control group (One-way ANOVA: *cerebral cortex:* $F_{(4,33)} = 5.6$, $p < 0.01$; *spinal cord:*

$F_{(4,33)}$ = 2.9, $p < 0.05$; *hippocampus:* $F_{(4,30)}$ = 5.4, $p < 0.01$). A single administration of 4-PSQ effectively restored the activity of this enzyme in the cerebral cortex and hippocampus of the animals induced by reserpine. In contrast, treatment with DXT failed to reverse reserpine-induced impairments in these regions.

Similar to male mice, exposure to reserpine led to the inhibition of Na^+/K^+ ATPase activity in the cerebral cortex (Figure 12A), spinal cord (Figure 12C), and hippocampus (Figure 12E) of female mice compared to the control group (One-way ANOVA: *cerebral cortex:* $F_{(4,31)}$ = 5.6, $p < 0.01$; *spinal cord:* $F_{(4,34)}$ = 15.3, $p < 0.0001$; *hippocampus*: $F_{(4,35)}$ = 3.1, $p < 0.05$). Treatment with 4-PSQ reversed the Na^+/K^+ ATPase inhibition in female mice's spinal cord and hippocampus. Consistent with the results in male mice, DXT treatment did not alter Na^+/K^+ ATPase activity in female mice's cerebral cortex, spinal cord, or hippocampus. No differences in enzyme activity were observed between the female and male mice control group (Unpaired Student's t-test: *cerebral cortex*: t = 0.8797, d_f = 14, p = 0.3939; *spinal cord*: t = 0.05744, d_f = 13, p = 0.9551; *hippocampus*: t = 0.4202, d_f = 13, p = 0.6812) or between the reserpine-induced female and male mice in the cerebral cortex, spinal cord, and hippocampus (unpaired Student's t-test: *cerebral cortex:* t = 1.438, d_f = 13, p = 0.1741; *spinal cord:* t = 0.3551, d_f = 14, p = 0.7278; *hippocampus:* t = 1.233, d_f = 14, p = 0.2380).

No significant changes were observed among the experimental groups in Na^+/K^+ ATPase activity in the cerebellum of male and female mice exposed to the reserpine protocol (Figure 12G) (One-way ANOVA: male: $F_{(4,32)}$ = 0.3, $p > 0.05$; female: $F_{(4,34)}$ = 0.1, $p > 0.05$). Despite this, female mice exhibited increased enzyme activities in the cerebellum compared to male mouse controls (Unpaired Student's t-test: t = 2.468, d_f = 12, p = 0.0296). No differences were observed in Na^+/K^+ ATPase activity in this tissue between reserpine-induced male and female mice (Unpaired Student's t-test: t = 1.353, d_f = 14, p = 0.1977).

In contrast, female mice exposed to ICS exhibited a significant inhibition of Na^+/K^+ ATPase activity in the cerebral cortex (Figure 12B), spinal cord (Figure 12D), and hippocampus (Figure 12F) compared to the control group (One-way ANOVA: *cerebral cortex*: $F_{(4,30)}$ = 5.0, $p < 0.01$; *spinal cord*: $F_{(4,31)}$ = 5.7, $p < 0.01$; *hippocampus*: $F_{(4,30)}$ = 4.2, $p < 0.01$). Moreover, as demonstrated by the unpaired Student's t-test, this inhibition was more pronounced in the cerebral cortex and spinal cord of female mice

exposed to ICS compared to male mice subjected to the same protocol (Unpaired Student's t-test: *cerebral cortex:* $t = 2.793$, $d_f = 13$, $p = 0.0152$; *spinal cord:* $t = 2.868$, $d_f = 14$, $p = 0.0124$), whereas no significant differences were observed in the hippocampus these animals (Unpaired Student's t-test: $t = 1.115$, $d_f = 13$, $p = 0.2851$). Treatment with 4-PSQ reversed the inhibition of Na^+/K^+ ATPase activity in female mice's cerebral cortex and spinal cord. In contrast, DXT showed no effects on enzyme activity in the tissues evaluated. No significant differences in Na^+/K^+ ATPase activity were detected between control groups of male and female mice in any of the analyzed regions (Unpaired Student's t-test: *cerebral cortex*: $t = 0.06371$, $df = 12$, $p = 0.9502$; *spinal cord*: $t = 1.027$, $df = 13$, $p = 0.3232$; *hippocampus*: $t = 0.6175$, $df = 12$, $p = 0.5485$).

One-way ANOVA followed by Tukey's test revealed that exposure to ICS and/or treatment with 4-PSQ or DXT did not cause significant changes in Na^+/K^+ ATPase activity in the cerebral cortex (Figure 12B), spinal cord (Figure 12D), and hippocampus (Figure 11F) of male mice (One-way ANOVA: *cerebral cortex:* $F_{(4,31)} = 0.4$, $p > 0.05$; *spinal cord:* $F_{(4,33)} = 0.6$, $p > 0.05$; *hippocampus:* $F_{(4,32)} = 1.9$, $p > 0.05$). Additionally, no significant differences were observed among groups in the cerebellum of male and female mice (Figure 12H) (One-way ANOVA: male: $F_{(4,30)} = 0.4$, $p > 0.05$; female: $F_{(4,34)} = 1.0$, $p > 0.05$). However, the unpaired Student's t-test indicated that control females exhibited higher Na^+/K^+ ATPase activity in the cerebellum than control males (Unpaired Student's t-test: $t = 2.476$, $d_f = 13$, $p = 0.0278$). This difference in enzyme activity was not observed in the cerebellum between the ICS male and ICS female groups (Unpaired Student's t-test: $t = 1.986$, $d_f = 13$, $p = 0.0686$).

3.7. **Influence of reserpine or ICS and 4-PSQ treatment on oxidative stress parameters in male and female mice**

The contribution of oxidative stress to the development of fibromyalgia-like symptoms induced by reserpine or ICS was assessed using markers of oxidative damage (RS and TBARS levels) and antioxidant enzyme activity (CAT) in the cerebral cortex, spinal cord, hippocampus, and cerebellum of male and female mice. Elevated RS levels were detected in the cerebral cortex of male mice and the cerebellum of male and female mice exposed to reserpine compared to the control group (One-way ANOVA:

cerebral cortex: male: $F_{(4,35)} = 4.6$, $p < 0.01$; *cerebellum*: male: $F_{(4,34)} = 11.3$, $p < 0.0001$, female: $F_{(4,35)} = 5.8$, $p < 0.01$) (Table 4). Notably, treatment with 4-PSQ reversed the increase in RS levels induced by reserpine in male mice's cerebral cortex and cerebellum. DXT reduced RS levels induced by reserpine only in the cerebral cortex of male mice.

There was no significant difference between groups in RS levels in the cerebral cortex of female mice and the spinal cord and hippocampus of both sexes mice (Table 4) (One-way ANOVA: *spinal cord*: male: $F_{(4,35)} = 0.5$, $p > 0.05$, female: $F_{(4,34)} = 0.6$, $p > 0.05$; *hippocampus*: male: $F_{(4,35)} = 2.4$, $p > 0.05$, female: $F_{(4,32)} = 2.8$, $p > 0.05$; *cerebral cortex*: female: $F_{(4,35)} = 0.7$, $p > 0.05$). However, it is essential to note that using unpaired Student's t-test, female control mice exhibited higher RS levels in the cerebral cortex, spinal cord, hippocampus, and cerebellum compared to male controls (Unpaired Student's t-test: *cerebral cortex*: $t = 3.614$, $d_f = 14$, $p = 0.0028$; *spinal cord*: $t = 2.545$, $d_f = 13$, $p = 0.0244$; *hippocampus*: $t = 6.544$, $d_f = 13$, $p < 0.0001$; *cerebellum*: $t = 2.220$, $d_f = 13$, $p = 0.0448$). This difference in females persisted after reserpine induction only in the spinal cord and hippocampus compared to male mice (Unpaired Student's t-test: *spinal cord*: $t = 7.307$, $d_f = 14$, $p < 0.0001$; *hippocampus*: $t = 6.997$, $d_f = 13$, $p < 0.0001$; *cerebral cortex*: $t = 0.3228$, $d_f = 14$, $p = 0.7516$; *cerebellum*: $t = 0.6161$, $d_f = 14$, $p = 0.5477$).

Unlike reserpine induction, exposure to ICS significantly increased RS levels only in the hippocampus of male and female mice compared to the control group (Table 4) (One-way ANOVA: male: $F_{(4,35)} = 5.1$, $p < 0.01$, female: $F_{(4,35)} = 3.0$, $p < 0.05$). Treatment with 4-PSQ reversed this ICS-induced increase only in the hippocampus of male mice. In contrast, DXT did not reduce the increase in RS levels in the hippocampus of male and female mice exposed to ICS. Data analysis revealed that exposure to ICS and/or treatment with 4-PSQ or DXT did not cause significant changes in RS levels in the cerebral cortex, spinal cord, and cerebellum of male and female mice (One-way ANOVA: *cerebral cortex*: male: $F_{(4,35)} = 2.3$, $p > 0.05$, female: $F_{(4,35)} = 1.6$, $p > 0.05$; *spinal cord*: male: $F_{(4,34)} = 2.6$, $p > 0.05$, female: $F_{(4,32)} = 2.6$, $p > 0.05$; *cerebellum*: male: $F(4,35) = 0.2$, $p > 0.05$, female: $F_{(4,35)} = 2.1$, $p > 0.05$).

However, similar to the reserpine induction protocol, female control mice exhibited elevated RS levels in all evaluated structures compared to male controls

(Table 4) (Unpaired Student's t-tests: *cerebral cortex*: t = 3.270, d_f = 14, p = 0.0056; *spinal cord*: t = 2.291, d_f = 13, p = 0.0393; *hippocampus*: t = 10.30, d_f = 14, p < 0.0001; *cerebellum*: t = 3.271, d_f = 14, p = 0.0056). This increase persisted after ICS exposure only in the cerebral cortex, hippocampus, and cerebellum of female mice compared to males subjected to the same induction scheme (Unpaired Student's t-tests: *cerebral cortex*: t = 3.176, d_f = 14, p = 0.0067; *hippocampus*: t = 8.931, d_f = 14, p < 0.0001; *cerebellum*: t = 4.367, d_f = 14, p = 0.0006; *spinal cord*: t = 0.7645, d_f = 13, p = 0.4582).

Regarding lipid peroxidation levels, one-way ANOVA followed by Tukey's test revealed that reserpine exposure and/or treatment with 4-PSQ or DXT did not induce significant changes in TBARS levels in the cerebral cortex, spinal cord, hippocampus, and cerebellum of male and female mice (Table 5) (One-way ANOVA: *cerebral cortex*: male: $F_{(4,35)}$ = 2.1, p > 0.05; female: $F_{(4,35)}$ = 1.3, p > 0.05; *spinal cord*: male: $F_{(4,35)}$ = 0.9, p > 0.05; female: $F_{(4,35)}$ = 0.6, p > 0.05; *hippocampus*: male: $F_{(4,35)}$ = 0.5, p > 0.05; female: $F_{(4,35)}$ = 1.4, p > 0.05; *cerebellum:* male: $F_{(4,35)}$ = 1.5, p > 0.05; female: $F_{(4,35)}$ = 1.0, p > 0.05). However, female control mice exhibited higher TBARS levels in the cerebral cortex, spinal cord, and cerebellum compared to male controls (Unpaired Student's t-tests: *cerebral cortex*: t = 3.093, d_f = 14, p = 0.0079; *spinal cord*: t = 4.907, d_f = 14, p = 0.0002; *cerebellum*: t = 8.881, d_f = 14, p < 0.0001). This difference persisted following reserpine induction, with females showing higher TBARS levels in the cerebral cortex, spinal cord, and cerebellum than male mice subjected to the same induction protocol (Unpaired Student's t-tests: *cerebral cortex*: t = 3.092, d_f = 14, p = 0.0080; *spinal cord*: t = 5.702, d_f = 14, p < 0.0001; *cerebellum*: t = 11.89, d_f = 14, p < 0.0001). Notably, no significant differences in TBARS levels were observed in the hippocampus between male and female controls or between animals of both sexes exposed to reserpine (unpaired Student's t-tests: *control*: t = 0.3099, d_f = 14, p = 0.7612; *reserpine*: t = 1.155, d_f = 14, p = 0.2674) (Table 5).

Interestingly, male and female mice exposed to ICS and/or treated with 4-PSQ or DXT also showed no significant differences in TBARS levels across the cerebral cortex, spinal cord, hippocampus, and cerebellum (One-way ANOVA: *cerebral cortex*: male: $F_{(4,35)}$ = 1.4, p > 0.05; female: $F_{(4,34)}$ = 2.1, p > 0.05; *spinal cord*: male: $F_{(4,35)}$ = 0.8, p > 0.05; female: $F_{(4,33)}$ = 1.6, p > 0.05; *hippocampus*: male: $F_{(4,35)}$ = 0.8, p > 0.05; female: $F_{(4,35)}$ = 0.7, p > 0.05; *cerebellum*: male: $F_{(4,35)}$ = 0.4, p > 0.05; female: $F_{(4,31)}$ = 1.8, p >

0.05) (Table 5). On the other hand, similar to the findings with reserpine induction, unpaired Student's t-tests revealed significant differences in TBARS levels between male and female mice controls in the cerebral cortex, spinal cord, and cerebellum (Unpaired Student's t-tests: *cerebral cortex*: $t = 9.211$, $d_f = 14$, $p < 0.0001$; *spinal cord*: $t = 5.107$, $d_f = 14$, $p = 0.0002$; *cerebellum*: $t = 7.654$, $d_f = 13$, $p < 0.0001$). This difference persisted following ICS induction, with females exposed to ICS exhibiting higher TBARS levels than male mice in the cerebral cortex, spinal cord, and cerebellum (Unpaired Student's t-tests: *cerebral cortex*: $t = 8.784$, $d_f = 14$, $p < 0.0001$; *spinal cord*: $t = 5.201$, $d_f = 15$, $p = 0.0001$; *cerebellum*: $t = 17.53$, $d_f = 14$, $p < 0.0001$). No significant differences in TBARS levels were detected in the hippocampus between male and female animal controls or between animals of both sexes exposed to ICS (Unpaired Student's t-tests: *control*: $t = 0.3610$, $d_f = 14$, $p = 0.7235$; *ICS*: $t = 1.691$, $d_f = 14$, $p = 0.1130$) (Table 5).

Table 6 shows that female mice subjected to the repeated reserpine induction protocol exhibited an inhibition of CAT activity in the cerebral cortex and spinal cord compared to the control group (One-way ANOVA: *cerebral cortex*: $F_{(4,35)} = 3.8$, $p < 0.05$; *spinal cord*: $F_{(4,35)} = 8.7$, $p < 0.0001$). In contrast, male mice displayed increased CAT activity in the cerebral cortex and spinal cord compared to the control group (one-way ANOVA: *cerebral cortex*: $F_{(4,35)} = 5.6$, $p < 0.01$; *spinal cord*: $F_{(4,34)} = 3.6$, $p < 0.05$). A single administration of 4-PSQ normalized CAT activity only in the cerebral cortex of male mice induced by reserpine. On the other hand, DXT normalized CAT activity in the spinal cord of female mice and the cerebral cortex of mice of both sexes.

Interestingly, female control mice showed increased CAT activity in the cerebral cortex and spinal cord compared to male controls (Unpaired Student's t-tests: *cerebral cortex*: $t = 6.976$, $d_f = 14$, $p < 0.0001$; *spinal cord*: $t = 6.736$, $d_f = 13$, $p < 0.0001$). This difference was not observed between male and female mice exposed to reserpine induction (Unpaired Student's t-tests: *cerebral cortex*: $t = 1.244$, $d_f = 14$, $p = 0.2339$; *spinal cord*: $t = 0.6135$, $d_f = 15$, $p = 0.5488$) (Table 6).

No significant differences were observed between experimental groups induced by reserpine and/or treated with 4-PSQ or DXT in CAT activity in the hippocampus and cerebellum of male and female mice (Table 6) (One-way ANOVA: *hippocampus*: male: $F_{(4,35)} = 1.5$, $p > 0.05$; female: $F_{(4,35)} = 1.2$, $p > 0.05$; *cerebellum*: male: $F_{(4,33)} = 0.5$, $p >$

0.05; female: $F_{(4,35)}$ = 1.1, p > 0.05). Additionally, unpaired Student's t-test did not indicate significant differences between male and female mice control groups (Unpaired Student's t-tests: *hippocampus*: t = 1.577, d_f = 14, p = 0.1371; *cerebellum*: t = 1.088, d_f = 14, p = 0.2949). Nevertheless, female mice exposed to reserpine exhibited increased CAT activity in the hippocampus compared to male mice, but no differences were observed in the cerebellum (Unpaired Student's t-tests: *hippocampus*: t = 2.516, d_f = 14, p = 0.0247; *cerebellum*: t = 1.312, d_f = 14, p = 0.2106).

One-way ANOVA followed by Tukey's test revealed that female mice exposed to ICS showed an inhibition of CAT activity in the cerebral cortex and spinal cord and increased activity in the hippocampus compared to the control group (Table 6) (One-way ANOVA: *cerebral cortex*: $F_{(4,30)}$ = 3.8, p < 0.05; *spinal cord*: $F_{(4,30)}$ = 5.4, p < 0.01; *hippocampus*: $F_{(4,30)}$ = 10.1, p < 0.0001). Treatment with 4-PSQ normalized CAT activity in the spinal cord and hippocampus in female mice exposed to ICS. DXT normalized CAT activity only in the hippocampus of ICS-exposed female mice. No significant differences were observed in the experimental groups in CAT activity in the cerebellum of female mice (One-way ANOVA: $F_{(4,30)}$ = 2.4, p > 0.05).

In male mice, exposure to ICS and/or treatment with 4-PSQ or DXT did not result in significant changes in CAT activity in the cerebral cortex, spinal cord, hippocampus, or cerebellum (Table 6) (One-way ANOVA*: cerebral cortex*: $F_{(4,32)}$ = 2.1, p > 0.05; *spinal cord*: $F_{(4,33)}$ = 2.2, p > 0.05; *hippocampus*: $F_{(4,32)}$ = 0.4, p > 0.05; *cerebellum*: $F_{(4,34)}$ = 0.2, p > 0.05). On the other hand, unpaired Student's t-tests indicated that control male mice exhibited an inhibition of CAT activity in the cerebral cortex and spinal cord compared to female mice control (Unpaired Student's t-tests: *cerebral cortex*: t = 3.025, d_f = 12, p = 0.0106; *spinal cord*: t = 2.824, d_f = 13, p = 0.0144). Still, no significant differences were observed between these groups in the hippocampus and cerebellum of mice (Unpaired Student's t-tests: *hippocampus*: t = 1.329, d_f = 13, p = 0.2067; *cerebellum*: t = 1.485, d_f = 13, p = 0.1613). In contrast, ICS exposure led to the inhibition of CAT activity in the hippocampus and cerebellum. Still, it increased CAT activity in the spinal cord of male mice compared to female mice exposed to the same induction scheme, with no significant differences in the cerebral cortex (Unpaired Student's t-tests: *spinal cord*: t = 3.196, d_f = 13, p = 0.0070;

hippocampus: t = 4.891, d_f = 13, p = 0.0003; *cerebellum*: t = 3.565, d_f= 13, p = 0.0035; *cerebral cortex*: t = 1.441, d_f= 13, p = 0.1733).

4. **Discussion**

The compound 4-PSQ has emerged as a promising therapeutic candidate due to its multitarget pharmacological properties. This study demonstrates that a single dose of 4-PSQ effectively alleviates nociceptive and depressive-like behaviors in both male and female mice subjected to fibromyalgia-like models, showing efficacy comparable to DXT. While DXT primarily targets monoaminergic systems, 4-PSQ modulates both monoaminergic and glutamatergic neurotransmission, restores HPA axis function, normalizes electrolyte balance, and improves oxidative stress markers in the central nervous system (CNS). These findings position 4-PSQ as a potential therapy capable of addressing multiple pathological mechanisms underlying fibromyalgia.

Reserpine or ICS exposure induced mechanical and thermal hypersensitivities in mice of both sexes, recapitulating the polymodal pain sensitivity observed in patients with fibromyalgia (Gracely & Schweinhardt, 2015; Ruschak et al., 2023). Notably, female controls exhibited greater thermal sensitivity than males by day 4, consistent with clinical reports of more severe and persistent pain in women, a phenomenon linked to estrogen-driven symptom fluctuations (Korszun et al., 2000; Pieretti et al., 2016). While reserpine exacerbated sex differences in mechanical sensitivity (suggesting monoaminergic involvement in female-specific responses), ICS-induced allodynia developed similarly in both sexes.

Chronic pain in fibromyalgia is closely linked to depressive symptoms, with a bidirectional relationship between the two conditions (Gracely and Schweinhardt, 2015). In the present study, male and female mice exposed to reserpine or ICS exhibited a depressive phenotype, reinforcing the connection between pain and emotional alterations. Furthermore, animals of both sexes exposed to reserpine showed a reduction in locomotor and exploratory activities. This behavior has been associated in numerous studies with the depressive phenotype observed following reserpine induction (Gao et al., 2021; Khadrawy et al., 2017; Sousa et al., 2018).

Treatment with 4-PSQ effectively reversed mechanical and thermal hypersensitivities and a depressive phenotype in fibromyalgia models, showing

antinociceptive effects comparable to DXT in both sexes. The efficacy of 4-PSQ is consistent with previous studies showing its antinociceptive and antidepressant-like effects across various neuropathic pain models, likely mediated by serotonergic, glutamatergic, and nitric oxide pathways, mechanisms relevant to fibromyalgia (Reis et al., 2020b; Reis et al., 2022; Paltian et al., 2022; Voss et al., 2022; De Oliveira et al., 2022; Silva et al., 2017).

Fibromyalgia is a central sensitivity syndrome characterized by amplified neuronal signaling (Siracusa et al., 2021). A key mechanism underlying this condition is glutamatergic dysregulation, marked by elevated glutamate levels in the CNS (Pyke et al., 2017; Rus et al., 2024). Our findings support this hypothesis: reserpine administration increased glutamate levels in the brain (males) and spinal cord (both sexes), while ICS exposure elevated glutamate concentrations in the brain (both sexes) and spinal cord (females) of mice. This pattern mirrors the pathophysiology of fibromyalgia, in which hyperactivation of A-δ and C fibers triggers excessive glutamate release in pain-processing regions such as the spinal dorsal horn, thalamus, and somatosensory cortex, contributing to allodynia and hyperalgesia (Sarzi-Puttini et al., 2020; Siracusa et al., 2021). Notably, glutamatergic hyperactivity may also contribute to depressive symptoms by impairing synaptic plasticity and neurogenesis through NMDA receptor signaling (Yepez et al., 2022), which aligns with the observed behavioral alterations.

Sex differences modulate glutamatergic activity, with female mice showing higher baseline glutamate concentrations in the brain and spinal cord compared to males, consistent with reports of elevated glutamate in women's basal ganglia and frontal gray matter (Sailasuta et al., 2008). This disparity likely results from estrogen's influence on glutamate transporters, glutamine synthetase, glutamate release, and NMDA receptor expression (Daniel and Dohanich, 2001; Haghighat, 2005; Krause et al., 2006; Woolley et al., 1997). Such regulation may increase female susceptibility to excitotoxicity-related conditions like chronic pain and mood disorders, despite estradiol's complex role in modulating pain and emotion (Athnaiel et al., 2023).

In this context, 4-PSQ demonstrated a modulatory effect on glutamatergic dysregulation in fibromyalgia-like conditions. Treatment with 4-PSQ restored physiological glutamate levels in the brains of male mice and the spinal cords of both

sexes after reserpine induction, as well as in both regions of ICS-exposed females. Consistent with these findings, Reis et al. (2017) reported that 4-PSQ reduced CNS glutamate uptake in male mice by antagonizing glutamate-receptor interactions. This hypothesis is further supported by Silva et al. (2017) and Da Rocha et al. (2024), who highlighted the involvement of the NMDA receptor in its antinociceptive effects. Thus, 4-PSQ appears to modulate glutamatergic transmission in the brain and spinal cord, reducing neuronal hyperexcitability and acting on the ascending pain pathway.

In addition to glutamatergic dysfunction, fibromyalgia involves significant alterations in the monoaminergic system, particularly due to reduced central serotonin levels (Alfaro-Rodríguez et al., 2024). In this study, reserpine administration decreased serotonin levels in the CNS (brain and spinal cord) of both male and female mice. This depletion likely disrupts descending pain inhibition, contributing to neuronal hyperexcitability and pain amplification, hallmark features of fibromyalgia (Siracusa et al., 2021; Alfaro-Rodríguez et al., 2024). Serotonin deficiency is also associated with depressive symptoms (Kayabaşı et al., 2021), which may explain the sensory and affective changes observed in reserpine-treated animals. Interestingly, the ICS model showed reduced serotonin only in the spinal cord of male mice, suggesting stress-related impairment of descending pain control, consistent with Hata et al. (1991).

The dysregulation of MAO activity, the enzyme responsible for monoamine catabolism, can also impair the monoaminergic system (Youdim & Sandler, 1968; Gürsoy et al., 2008; Naoi et al., 2016; Janssen et al., 2021). In our study, selective MAO-A activation (but not MAO-B) in the cerebral cortex and spinal cord of reserpine-induced male and female mice suggests a compensatory mechanism. This mechanism involves MAO-A-mediated degradation of cytosolic serotonin when vesicular storage is blocked (Freitas et al., 2013). This effect was sexually dimorphic, being more pronounced in females, underscoring sex as a determining factor in neurochemical responses. In contrast, ICS exposure did not alter MAO activity, indicating reserpine model specificity in monoamine regulation.

The involvement of the monoaminergic system in 4-PSQ's antinociceptive and antidepressant-like effects is supported by previous findings (Silva et al., 2017; Oliveira et al., 2022) as well as by the present data. In the reserpine model, 4-PSQ's ability to restore serotonin levels in the brain (in males) and spinal cord (in both sexes), alongside

normalizing MAO-A activity in the cortex and spinal cord, reinforces its modulatory effects on this system. Interestingly, in the ICS model, where MAO activity remains unchanged, 4-PSQ still increased serotonin levels in the brains and spinal cords of male mice, indicating the presence of additional MAO-independent mechanisms. Collectively, these findings suggest that the antinociceptive and antidepressant-like effects of 4-PSQ may involve both MAO-A inhibition and direct modulation of serotonergic pathways. Notably, 4-PSQ has shown similar effects to DXT, a drug that acts by increasing synaptic serotonin levels and relieving pain and depressive symptoms (Dhaliwal et al., 2025).

Through catecholamine metabolism, MAO generates harmful byproducts such as hydrogen peroxide (H_2O_2), an RS that induces oxidative damage and can overload antioxidant defenses (Naoi et al., 2016; Assavarittirong et al., 2022). Accordingly, reserpine exposure increased RS levels in the cerebellum (both sexes) and cerebral cortex (males), while ICS elevated RS levels in the hippocampus (both sexes) of mice. Interestingly, these changes did not result in measurable lipid peroxidation in either model. This apparent discrepancy with previous studies (Martins et al., 2025; Sousa et al., 2018) may reflect the activation of compensatory mechanisms that mitigate oxidative damage to cell membranes (Gęgotek and Skrzydlewska, 2019).

Crucially, these compensatory responses appeared to be sex-specific, as evidenced by divergent CAT activity patterns. In the reserpine model, female mice showed reduced CAT activity in the cerebral cortex and spinal cord, while males exhibited increased activity in these regions. Despite higher MAO-A levels in both sexes, the antioxidant response differed: in females, excessive H_2O_2 likely overwhelmed cortical CAT, leading to its inhibition, although other endogenous antioxidants may have prevented oxidative damage (Liguori et al., 2018; Luo et al., 2020). In males, however, higher RS levels correlated with increased CAT activity, suggesting efficient H_2O_2 clearance. Under ICS, only females showed CAT activity inhibition in the cortex and spinal cord, alongside upregulation in the hippocampus, potentially due to glutamate-induced overload of the antioxidant system (Siracusa et al., 2021

Our study observed that healthy female mice exhibited higher baseline levels of RS, TBARS, and CAT activity compared to males in some CNS regions. This suggests that females may experience higher basal oxidative stress but also potentially possess a

more robust antioxidant response due to hormonal differences, such as the neuroprotective effects of estrogen, which modulate the expression and activity of antioxidant enzymes (Massafra et al., 2000; Sheng-Huang et al., 2015).

Our results reinforce the antioxidant potential of 4-PSQ, highlighting its ability to modulate oxidative homeostasis in a sex- and tissue-specific manner, often surpassing DXT. The compound reduced RS levels and normalized CAT activity in brain regions and the spinal cord involved in pain processing. Notably, in the reserpine model, its effects were more pronounced in males, normalizing RS levels and CAT activity in the cerebral cortex and reducing RS in the cerebellum. In females subjected to ICS, 4-PSQ normalized RS levels in the hippocampus and CAT activity in both the hippocampus and spinal cord. These findings corroborate previous reports of its neuroprotective and antioxidant actions in rodent CNS (Luchese et al., 2020; Paltian et al., 2022; Reis et al., 2020b). Thus, 4-PSQ may enhance antioxidant defenses either by directly scavenging reactive species or by modulating endogenous antioxidant systems.

Redox dysfunction in fibromyalgia is associated with mitochondrial impairment, which decreases ATP production, disrupts membrane potential and calcium homeostasis, and affects essential enzymes (Singh et al., 2019; Macchi et al., 2024). In our results, reserpine inhibited Na^+/K^+-ATPase activity in the cerebral cortex, hippocampus, and spinal cord of both male and female mice, whereas ICS exposure reduced its activity only in structures of females. Na^+/K^+-ATPase inhibition may underlie neuronal hyperexcitability in these models (Bejček et al., 2021).

To further explore the relationship between ionic dysregulation and fibromyalgia, we evaluated Ca^{2+}-ATPase activity as a complementary target, observing sex- and model-dependent modulations. This enzyme is essential for maintaining calcium homeostasis (Primeau et al., 2018), but its hyperactivity in the CNS can impair mitochondrial function, neuronal signaling, and excitability (Brini et al., 2013; Carafoli & Krebs, 2016; Sung et al., 2015; Singh et al., 2019). Here, reserpine increased Ca^{2+}-ATPase activity in the cortex of female and the hippocampus of male mice, while ICS elevated its activity in the cortex of females, the cerebellum of males, and the spinal cord of both sexes. Conversely, inhibitory effects were observed in certain tissues: reserpine reduced enzyme activity in the spinal cord of both sexes, and ICS suppressed it in the hippocampus of females. Such inhibition may result from oxidative

modifications of enzyme sulfhydryl groups or prolonged calcium overload, which could saturate the pump or activate negative feedback mechanisms to prevent cytotoxicity (Mei et al., 2018; Gęgotek & Skrzydlewska, 2019).

In this context, 4-PSQ proved to be a promising agent for restoring ionic homeostasis by modulating Na^+/K^+-ATPase and Ca^{2+}-ATPase in a sex- and model-dependent manner. In the reserpine model, it restored Na^+/K^+-ATPase activity in the hippocampus of both sexes, in the cerebral cortex of males, and in the spinal cord of females, while reversing Ca^{2+}-ATPase inhibition in the hippocampus and spinal cord of males. In the ICS protocol, 4-PSQ normalized Na^+/K^+-ATPase activity in the spinal cord and hippocampus of females, and Ca^{2+}-ATPase activity in the cerebral cortex and hippocampus of females and in the spinal cord of males. These findings align with previous studies associating the neuroprotective and antinociceptive effects of 4-PSQ with the preservation of ionic homeostasis (Reis et al., 2020a; Reis et al., 2022; Paltian et al., 2022). Furthermore, by modulating Ca^{2+}-ATPase, 4-PSQ may reduce glutamate release, like pregabalin, which decreases calcium influx and exerts analgesic and antidepressant effects in fibromyalgia (Carafoli and Krebs, 2016). Thus, its ability to maintain ionic homeostasis and attenuate excitotoxicity may underlie the antinociceptive and antidepressant effects observed in the reserpine and ICS models.

In addition to electrolyte disturbances, dysregulation of the HPA axis has also been implicated in the pathophysiology of fibromyalgia. This axis regulates the stress response through the release of glucocorticoids, such as cortisol in humans and corticosterone in rodents (Singh et al., 2019). In our study, both male and female mice exposed to ICS showed elevated corticosterone levels and increased relative adrenal weights, alterations characteristic of prolonged HPA axis activation (Goel et al., 2014). In fibromyalgia, chronic activation of this axis can impair glucocorticoid signaling (Crofford, 1996; Singh et al., 2019), which is associated with adverse neurological effects, including reduced neurogenesis and impaired neuroplasticity, contributing to core symptoms such as chronic pain, fatigue, and mood disturbances (Blackburn-Munro, 2004).

Glucocorticoids also broadly modulate metabolism, inflammation, and immune function (Goel et al., 2014; Kim, 2023). In our study, mice of both sexes exposed to ICS exhibited reduced relative spleen and thymus weights, likely due to

glucocorticoid-mediated immunosuppression, which inhibits immune cell proliferation and pro-inflammatory cytokine production (Menke, 2024; Straub, 2023), consistent with previous reports of stress-induced splenic atrophy (Chen et al., 2023). Moreover, glucocorticoids promote thymocyte apoptosis, impairing T cell generation and adaptive immunity (Straub, 2023). Notably, although reserpine also induced splenic and thymic atrophy, it did not alter adrenal weight or corticosterone levels, suggesting distinct mechanisms between the models. ICS appears to uniquely depend on HPA axis activation and elevated glucocorticoids. Additionally, females showed higher baseline relative spleen and thymus weights compared to males, possibly due to the immunomodulatory effects of estrogen (Goel et al., 2014), underscoring the importance of sex as a biological variable in immune regulation.

In contrast, 4-PSQ attenuated HPA axis activation by reducing plasma corticosterone levels in female mice subjected to ICS. However, it did not reverse the adrenal, spleen, or thymus weight alterations induced by reserpine or ICS. Previous studies have shown its ability to regulate corticosterone levels in males, associated with its anxiolytic and antidepressant effects (De Oliveira et al., 2022; Paltian et al., 2020; Reis et al., 2020). Similarly, benzodiazepines, which act on $GABA_A$ receptors, also attenuate HPA axis activation (Fries et al., 2006; Mikkelsen et al., 2008). Since 4-PSQ seems modular this receptor to exert anxiolytic effects (Paltian et al., 2020), it is plausible that its influence on the HPA axis occurs either through $GABA_A$ receptor modulation or by directly reducing corticosterone release. Moreover, elevated cortisol levels decrease brain tryptophan availability, thereby reducing serotonin synthesis (Maes et al., 1998). Thus, the effects of 4-PSQ on the HPA axis may also involve MAO-A inhibition, increase serotonin levels and generate negative feedback on this axis.

Our findings highlight the multi-target therapeutic potential of 4-PSQ compared to DXT, positioning it as a promising candidate for fibromyalgia treatment. At a dose of 1 mg/kg, 4-PSQ modulated glutamatergic and monoaminergic systems, reduced oxidative stress, restored CNS electrolyte homeostasis, and attenuated HPA axis hyperactivation. It consistently reduced mechanical and thermal hypersensitivities and depression-like behavior in both male and female mice, despite tissue- or model-dependent variations in its effects on the HPA axis, ATPases, and oxidative

stress. Given these promising results, our research group plans to investigate the effects of prolonged daily 4-PSQ administration in future studies. We also intend to explore sex differences further by defining the estrous cycle in female mice and examining the role of sex hormones in treatment response.

5. Conclusion

This study provides the first evidence demonstrating that a single dose of 4-PSQ effectively reduces mechanical and thermal hypersensitivity induced by reserpine or ICS in both male and female mice. Additionally, 4-PSQ attenuated depressive-like behaviors associated with these models in animals of both sexes. These beneficial effects are likely mediated, at least in part, by modulation of glutamatergic and monoaminergic neurotransmission, attenuation of HPA axis activation, mitigation of oxidative stress, and restoration of electrolyte balance through normalization of Na^+/K^+-ATPase and Ca^{2+}-ATPase enzyme activities. Collectively, our findings strongly support the therapeutic potential of 4-PSQ as a multitarget candidate for the treatment of fibromyalgia and its related comorbidities.

Credit authorship contribution statement

Ana Paula Bonato Wille: Writing - original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Vanessa Macedo Esteves da Rocha: Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Ketlyn Pereira da Motta: Methodology, Investigation, Formal analysis. Ingrid Cardoso Oliveira: Methodology, Investigation, Formal analysis. Diego Alves: Supervision, Investigation, Formal analysis, Data curation. Erico Marlon de Moraes Flores: Supervision, Investigation, Formal analysis, Data curation. Marcia Foster Mesko: Supervision, Investigation, Formal analysis, Data curation, Conceptualization. Ethel Antunes Wilhelm: Writing - review & editing, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

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Author contributions

Ana Paula Bonato Wille, Vanessa Macedo Esteves da Rocha, Ketlyn Pereira da Motta, and Ethel Antunes Wilhelm conceived and designed the experimental study, establishing the foundational framework that guided all research stages. Ingrid Cardoso Oliveira and Diego Alves performed the 4-PSQ synthesis. Erico Marlon de Moraes Flores and Marcia Foster Mesko assisted in writing grant proposals to secure funding for the development of the research. Ana Paula Bonato Wille and Ethel Antunes Wilhelm conducted all data analysis and interpretation and drafted the manuscript. Ethel Antunes Wilhelm supervised the study. All authors approved the final version of the manuscript and agree with its submission.

Data availability

The original contributions presented in this study are included in the article material. Further inquiries can be directed to the corresponding author(s).

Declaration of Generative AI and AI-assisted technologies in the writing process

While preparing this work, the authors used GPTchat (OpenAI, 2023) to improve readability and language. After using this tool, the authors reviewed and edited the content as necessary and took full responsibility for the content of the published article.

Ethics approval

All experiments were performed following the guidelines of the Committee on Care and Use of Experimental Animal Resources of the Federal University of Pelotas, Brazil (CEUA 043665/2023-61) and under the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publications no. 80-23, revised in 1996) and International Guiding Principles for Biomedical Research Involving Animals.

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Tables

Table 1. Effect of 4-PSQ (1 mg/kg, i.g.) or DXT (30 mg/kg, i.g.) treatment on the latency for the first episode of immobility in male and female mice exposed to Reserpine-induced or ICS-induced fibromyalgia.

Groups	Latency (s)	
	Male	**Female**
	Reserpine	
Control	86.0 ± 15.3	62.3 ± 6.7
4-PSQ	120.4 ± 17.0	65.8 ± 11.7
Reserpine	9.4 ± 1.5***	13.5 ± 2.9^{aaa}
Reserpine+4-PSQ	36.9 ± 16.8	23.5 ± 8.4^{aa}
Reserpine+DXT	5.2 ± 0.9***	23.1 ± 3.8^{aa}
	ICS	
Control	98.1 ± 9.5	84.0 ± 8.6
4-PSQ	69.2 ± 5.9	87.4 ± 4.9
ICS	51.3 ± 2.2*	51.9 ± 4.6^{a}
ICS+4-PSQ	85.3 ± 15.2	103.3 ± 10.1^{bbbcccc}
ICS+DXT	109.1 ± 16.1$^{##}$	169.1 ± 7.3^{aaaabbbb}

Data are reported as mean ± SEM for 7-8 animals per group. ANOVA one-way followed by Tukey's test: (*) $p<0.05$, and (***) $p<0.001$ denote significance levels when compared with the control male group (ICS or Reserpine protocol); (##) $p<0.01$ denotes significance levels when compared with the ICS male group; (a) $p<0.05$, (aa) $p<0.01$, (aaa) $p<0.001$, and (aaaa) $p<0.0001$ denote significance levels when compared with the Control female group (ICS or Reserpine protocol); (bbb) $p<0.001$, and (bbbb) $p<0.0001$ denote significance levels when compared with the ICS female group; (cccc) $p<0.0001$ denotes significance levels when compared with the ICS + DXT female group.

Table 2. Effect of 4-PSQ (1 mg/kg, i.g.) or DXT (30 mg/kg, i.g.) treatment in the spontaneous locomotor and exploratory activities of male and female mice exposed to Reserpine-induced or ICS-induced fibromyalgia

Groups	Open Field Test			
	Number of crossing		Number of rearing	
	Male	Female	Male	Female
	Reserpine		Reserpine	
Control	80.2 ± 4.6	83.6 ± 4.0	22.5 ± 4.4	19.8 ± 1.5
4-PSQ	80.6 ± 3.8	75.0 ± 4.1	17.9 ± 1.8	16.9 ± 1.4
Reserpine	1.4 ± 0.6****	1.25 ± 0.2^{aaaa}	0.0 ± 0.0****	0.0 ± 0.0^{aaaa}
Reserpine+4-PSQ	0.6 ± 0.2****	2.75 ± 0.5^{aaaa}	0.0 ± 0.0****	0.0 ± 0.0^{aaaa}
Reserpine+DXT	1.9 ± 0.6****	1.0 ± 0.0^{aaaa}	0.0 ± 0.0****	0.0 ± 0.0^{aaaa}
	ICS		ICS	
Control	78.0 ± 6.9	89.9 ± 6.1	21.9 ± 1.9	28.0 ± 3.3
4-PSQ	72.6 ± 5.9	89.4 ± 7.2	20.2 ± 3.4	31.2 ± 2.1
ICS	69.9 ± 5.0	84.2 ± 6.6	20.3 ± 3.6	29.9 ± 1.0
ICS+4-PSQ	62.5 ± 7.0	99.2 ± 5.7	15.0 ± 1.8	35.1 ± 2.7
ICS+DXT	78.3 ± 7.1	110.7± 7.2	12.3 ±1.5	27.4 ± 1.8

Data are reported as mean ± SEM for 7-8 animals per group. One-way ANOVA was used for statistical analyses, followed by the Tukey multiple comparison test when appropriate: (****) $p<0.0001$ denotes a significant difference as compared with the control male group; (aaaa) $p<0.0001$ denotes a significant difference as compared with the control female group.

Table 3. Effect of 4-PSQ (1 mg/kg, i.g.) or DXT (30 mg/kg, i.g.) treatment on the monoamine oxidase B (MAO-B) activity in the cerebral cortex and spinal cord of male and female mice exposed to Reserpine-induced or ICS-induced fibromyalgia.

MAO-B activity (nmol 4-OH quinoline/mg protein/min)				
Groups	**Cerebral cortex**		**Spinal cord**	
	Male	**Female**	**Male**	**Female**
	Reserpine			**Reserpine**
Control	2.3 ± 0.2	2.6 ± 0.4	3.1 ± 0.6	3.0 ± 0.3
4-PSQ	1.9 ± 0.3	2.3 ± 0.3	3.6 ± 0.5	3.9 ± 0.4
Reserpine	2.7 ± 0.4	3.3 ± 0.1	6.7 ± 1.6	3.2 ± 0.2
Reserpine+4-PSQ	2.3 ± 0.6	2.9 ± 0.9	4.0 ± 1.2	2.5 ± 0.7
Reserpine+DXT	3.0 ± 0.9	2.7 ± 0.4	4.5 ± 1.1	3.4 ± 0.6
	ICS			**ICS**
Control	2.5 ± 0.4	3.5 ± 0.4	2.7 ± 0.4	3.7 ± 0.3
4-PSQ	1.6 ± 0.1	3.9 ± 0.4	2.7 ± 0.8	3.6 ± 0.4
ICS	2.4 ± 0.2	2.8 ± 0.3	3.5 ± 0.4	2.7 ± 0.2
ICS+4-PSQ	2.1 ± 0.5	3.3 ± 0.2	2.8 ± 0.5	3.9 ± 0.5
ICS+DXT	2.9 ± 0.4	3.4 ± 0.6	2.8 ± 0.6	3.3 ± 0.5

Data are reported as mean ± SEM for 7-8 animals per group. ANOVA one-way followed by Tukey's test.

Table 4. Effect of 4-PSQ (1 mg/kg, i.g.) or DXT (30 mg/kg, i.g.) treatment on the reactive species (RS) levels in the cerebral cortex, spinal cord, hippocampus, and cerebellum of male and female mice exposed to Reserpine-induced or ICS-induced fibromyalgia.

	RS (Arbitrary units)			
Groups	**Cerebral cortex**		**Spinal cord**	
	Male	**Female**	**Male**	**Female**
	Reserpine		**Reserpine**	
Control	177.8 ± 15.8^{dd}	259.7 ± 16.2	45.0 ± 3.9^{d}	65.9 ± 7.6
4-PSQ	178.2 ± 9.0	268.5 ± 12.7	44.7 ± 3.4	80.4 ± 6.3
Reserpine	257.8 ± 27.1*	245.9 ± 24.6	48.9 ± 1.7^{eeee}	74.9 ± 3.1
Reserpine+4-PSQ	177.8 ± 11.1$^{\#}$	229.6 ± 14.5	46.5 ± 2.3	76.3 ± 10.5
Reserpine+DXT	176.6 ± 14.4$^{\#}$	263.0 ± 19.2	50.3 ± 4.9	77.7 ± 4.8
	ICS		**ICS**	
Control	179.5 ± 5.6^{dd}	267.4 ± 26.3	44.7 ± 4.7^{d}	65.9 ± 8.3
4-PSQ	229.0 ± 22.5	253.6 ± 20.9	41.6 ± 5.8	85.4 ± 6.2
ICS	201.2 ± 15.7^{ee}	274.2 ± 16.7	56.9 ± 4.0	64.6 ± 9.8
ICS+4-PSQ	255.7 ± 30.2	275.3 ± 19.5	39.9 ± 1.4	84.4 ± 3.4
ICS+DXT	184.5 ± 23.8	206.9 ± 27.6	47.4 ± 3.1	84.0 ± 3.2
Groups	**Hippocampus**		**Cerebellum**	
	Male	**Female**	**Male**	**Female**
	Reserpine		**Reserpine**	
Control	61.4 ± 4.1^{dddd}	137.5 ± 11.6	91.8 ± 9.9^{d}	117.9 ± 6.8
4-PSQ	57.2 ± 3.6	157.0 ± 21.6	84.9 ± 6.0	138.7 ± 12.9
Reserpine	67.2 ± 3.9^{eeee}	150.7 ± 12.0	145.7 ± 8.3***	155.1 ± 12.7^{a}
Reserpine+4-PSQ	60.6 ± 5.1	190.1 ± 20.7	90.9 ± 4.5$^{\#\#\#}$	176.5 ± 4.2^{aaa}
Reserpine+DXT	73.0 ± 3.3	197.5 ± 8.1	121.7 ± 9.3	157.7 ± 4.9^{a}
	ICS		**ICS**	
Control	53.6 ± 3.5^{dddd}	140.0 ± 7.6	99.8 ± 3.0^{dd}	120.3 ± 5.5

4-PSQ	54.3 ± 3.2	151.3 ± 7.4	102.0 ± 7.6	117.5 ± 8.0
ICS	74.9 ± 4.2**^{eeee}	171.6 ± 9.9^{a}	95.6 ± 4.4^{eee}	120.3 ± 3.6
ICS+4-PSQ	51.2 ± 4.7$^{\#\#}$	161.5 ± 3.6	100.3 ± 6.2	142.6 ± 11.0
ICS+DXT	60.0 ± 5.3	150.0 ± 4.0	95.7 ± 7.3	121.9 ± 4.6

Data are reported as mean ± SEM for 7-8 animals per group. ANOVA one-way followed by Tukey's test: (*) $p<0.05$, (**) $p<0.01$, and (***) $p<0.001$ denote significance levels when compared with the Control male group (Reserpine or ICS protocol); (#) $p < 0.05$, (##) $p < 0.01$, and (###) $p<0.001$ denote significance levels when compared with the Reserpine or ICS male group; (a) $p<0.05$, and (aaaa) $p<0.0001$ denote significance levels when compared with the Control female group (Reserpine and ICS protocol); Unpaired Student's t-test: (d) $p<0.05$, (dd) $p<0.01$, and (dddd) $p<0.0001$ denote significance levels when compared with the Control female group (ICS or Reserpine protocol); (ee) $p<0.01$, (eee) $p<0.001$, and (eeee) $p<0.0001$ denote significance levels when compared with the ICS or Reserpine female group.

Table 5. Effect of 4-PSQ (1 mg/kg, i.g.) or DXT (30 mg/kg, i.g.) treatment on the thiobarbituric acid reactive species (TBARS) levels in the cerebral cortex, spinal cord, hippocampus, and cerebellum of male and female mice exposed to Reserpine-induced or ICS-induced fibromyalgia.

	TBARS (nmol MDA/mg protein)			
Groups	**Cerebral cortex**		**Spinal cord**	
	Male	**Female**	**Male**	**Female**
	Reserpine		**Reserpine**	
Control	24.1 ± 2.8^{dd}	41.3 ± 5.7	22.7 ± 1.9^{ddd}	48.5 ± 4.9
4-PSQ	20.9 ± 1.4	30.6 ± 1.8	23.2 ± 1.6	40.8 ± 2.0
Reserpine	25.8 ± 1.9^{ee}	40.2 ± 4.2	21.7 ± 1.2^{eeee}	44.5 ± 3.8
Reserpine+4-PSQ	22.2 ± 2.6	34.6 ± 3.4	22.7 ± 0.9	45.0 ± 2.1
Reserpine+DXT	28.8 ± 2.8	42.1 ± 5.4	25.4 ± 1.4	46.8 ± 4.8
	ICS		**ICS**	
Control	27.2 ± 2.2^{dddd}	56.4 ± 2.2	24.6 ± 3.9^{ddd}	48.0 ± 2.4
4-PSQ	23.9 ± 4.5	66.2 ± 3.5	21.6 ± 2.6	60.1 ± 5.5
ICS	20.4 ± 2.9^{eeee}	54.6 ± 2.6	21.4 ± 1.7^{eee}	53.0 ± 6.2
ICS+4-PSQ	20.4 ± 2.7	61.4 ± 4.0	19.5 ± 2.0	58.1 ± 4.5
ICS+DXT	17.9 ± 2.4	61.5 ± 3.6	18.7 ± 1.8	72.0 ± 13.4
Groups	**Hippocampus**		**Cerebellum**	
	Male	**Female**	**Male**	**Female**
	Reserpine		**Reserpine**	
Control	35.8 ± 6.0	33.6 ± 3.8	23.9 ± 3.1^{dddd}	84.8 ± 6.1
4-PSQ	34.0 ± 5.1	39.1 ± 4.1	25.3 ± 2.1	77.0 ± 6.1
Reserpine	36.5 ± 4.8	43.2 ± 3.2	25.7 ± 2.4^{eeee}	89.0 ± 4.8
Reserpine+4-PSQ	27.6 ± 3.8	46.3 ± 4.1	28.1 ± 2.8	80.2 ± 3.3
Reserpine+DXT	31.4 ± 5.5	40.2 ± 4.7	32.8 ± 3.7	81.0 ± 1.7
	ICS		**ICS**	
Control	36.2 ± 5.0	33.2 ± 6.4	24.0 ± 2.3^{dddd}	97.9 ± 10.0

4-PSQ	28.3 ± 1.5	24.6 ± 5.1	28.0 ± 3.8	118.5 ± 7.6
ICS	30.3 ± 1.7	24.7 ± 2.8	25.1 ± 2.2^{eeee}	103.4 ± 3.9
ICS+4-PSQ	34.2 ± 2.8	34.2 ± 8.8	28.1 ± 5.2	134.2 ± 19.5
ICS+DXT	33.3 ± 5.1	23.9 ± 4.5	24.4 ± 1.3	130.7 ± 13.7

Data are reported as mean ± SEM for 7-8 animals per group. Unpaired Student's t-test: (dd) $p<0.01$, (ddd) $p<0.001$, and (dddd) $p<0.0001$ denote significance levels when compared with the Control female group (ICS or Reserpine protocol); (ee) $p<0.01$, (eee) $p<0.001$, and (eeee) $p<0.0001$ denote significance levels when compared with the ICS or Reserpine female group.

Table 6. Effect of 4-PSQ (1 mg/kg, i.g.) or DXT (30 mg/kg, i.g.) treatment on the catalase (CAT) activity in the cerebral cortex, spinal cord, hippocampus, and cerebellum of male and female mice exposed to Reserpine-induced or ICS-induced fibromyalgia.

Groups	CAT (U/mg protein)			
	Cerebral cortex		Spinal cord	
	Male	Female	Male	Female
	Reserpine		Reserpine	
Control	1.1 ± 0.2^{dddd}	5.7 ± 0.6	2.4 ± 0.7^{dddd}	9.2 ± 0.7
4-PSQ	1.3 ± 0.2	4.7 ± 0.8	3.8 ± 0.6	11.1 ± 1.6
Reserpine	3.7 ± 1.0**	2.4 ± 0.3^{a}	6.0 ± 1.2*	5.1 ± 0.3^{a}
Reserpine+4-PSQ	1.3 ± 0.1$^{\#\#}$	4.4 ± 0.8	3.2 ± 0.5	6.3 ± 0.5^{cc}
Reserpine+DXT	1.3 ± 0.2$^{\#\#}$	5.5 ± 0.6^{b}	5.2 ± 0.7	11.6 ± 1.1^{bbb}
	ICS		ICS	
Control	1.4 ± 0.4^{d}	5.1 ± 1.2	2.1 ± 0.3^{d}	6.4 ± 1.6
4-PSQ	1.7 ± 0.3	2.7 ± 0.8	1.8 ± 0.2	5.3 ± 1.1
ICS	2.5 ± 0.5	1.6 ± 0.3^{a}	2.4 ± 0.3^{ee}	1.3 ± 0.2^{aa}
ICS+4-PSQ	2.5 ± 0.4	1.9 ± 0.8^{a}	1.4 ± 0.3	2.17 ± 0.4^{b}
ICS+DXT	1.5 ± 0.2	1.5 ± 0.4^{a}	2.0 ± 0.1	1.3 ± 0.2^{a}
Groups	Hippocampus		Cerebellum	
	Male	Female	Male	Female
	Reserpine		Reserpine	
Control	2.4 ± 0.5	1.6 ± 0.1	2.4 ± 0.3	3.5 ± 1.0
4-PSQ	2.8 ± 0.8	1.9 ± 0.3	3.3 ± 0.6	3.0 ± 0.7
Reserpine	3.6 ± 0.7^{e}	1.9 ± 0.2	2.5 ± 0.3	3.9 ± 1.1
Reserpine+4-PSQ	1.8 ± 0.2	2.3 ± 0.3	3.1 ± 0.9	2.6 ± 0.4
Reserpine+DXT	2.2 ± 0.4	2.2 ± 0.1	2.7 ± 0.4	4.8 ± 0.8
	ICS		ICS	
Control	3.5 ± 0.8	2.2 ± 0.4	3.1 ± 0.8	4.6 ± 0.6
4-PSQ	3.2 ± 0.7	4.9 ± 1.4	3.2 ± 0.7	5.1 ± 0.6
ICS	3.1 ± 0.4^{eee}	7.6 ± 0.9^{aaaa}	2.7 ± 0.8^{ee}	6.1 ± 0.3

ICS+4-PSQ	3.6 ± 1.0	1.8 ± 0.3^{bbb}	3.2 ± 0.9	4.2 ± 0.6
ICS+DXT	4.3 ± 0.9	2.1 ± 0.4^{bbb}	3.8 ± 0.8	4.3 ± 0.4

Data are reported as mean ± SEM for 7-8 animals per group. ANOVA one-way followed by Tukey's test: (*) $p<0.05$, and (**) $p<0.01$ denote significance levels when compared with the Control male group (Reserpine or ICS protocol); (#) $p < 0.05$, and (##) $p < 0.01$ denote significance levels when compared with the Reserpine or ICS male group; (a) $p<0.05$, (aa) $p<0.01$, and (aaaa) $p<0.0001$ denote significance levels when compared with the Control female group (Reserpine and ICS protocol); (b) $p<0.05$, and (bbb) $p<0.001$ denote significance levels when compared with the Reserpine and ICS female group; (cc) $p<0.01$ denotes significance levels when compared with the Reserpine+DXT female group. Unpaired Student's t-test: (d) $p<0.05$, and (dddd) $p<0.0001$ denote significance levels when compared with the Control female group (ICS or Reserpine protocol); (e) $p<0.05$, (ee) $p<0.01$, and (eee) $p<0.001$ denote significance levels when compared with the ICS or Reserpine female group.

Figures

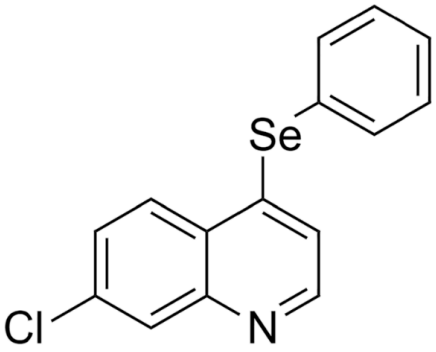

Figure 1. Chemical structure of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ).

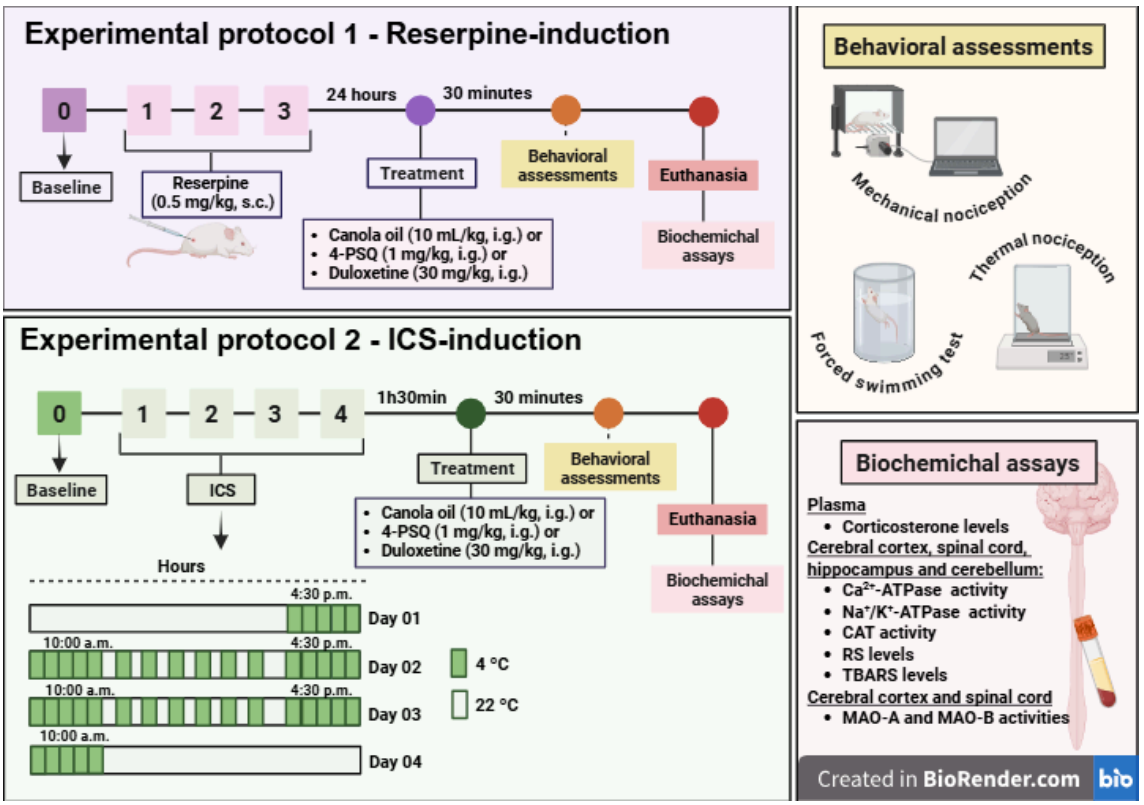

Figure 2. Schematic representation of the experimental protocol to assess the pharmacological effects of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) in two models

of fibromyalgia induction (ICS or Reserpine) in male and female mice. ICS: Intermitent Cold Stress, CAT: Catalase, RS: Reactive Species, TBARS: thiobarbituric acid reactive substances, MAO: Monoamine Oxidase.

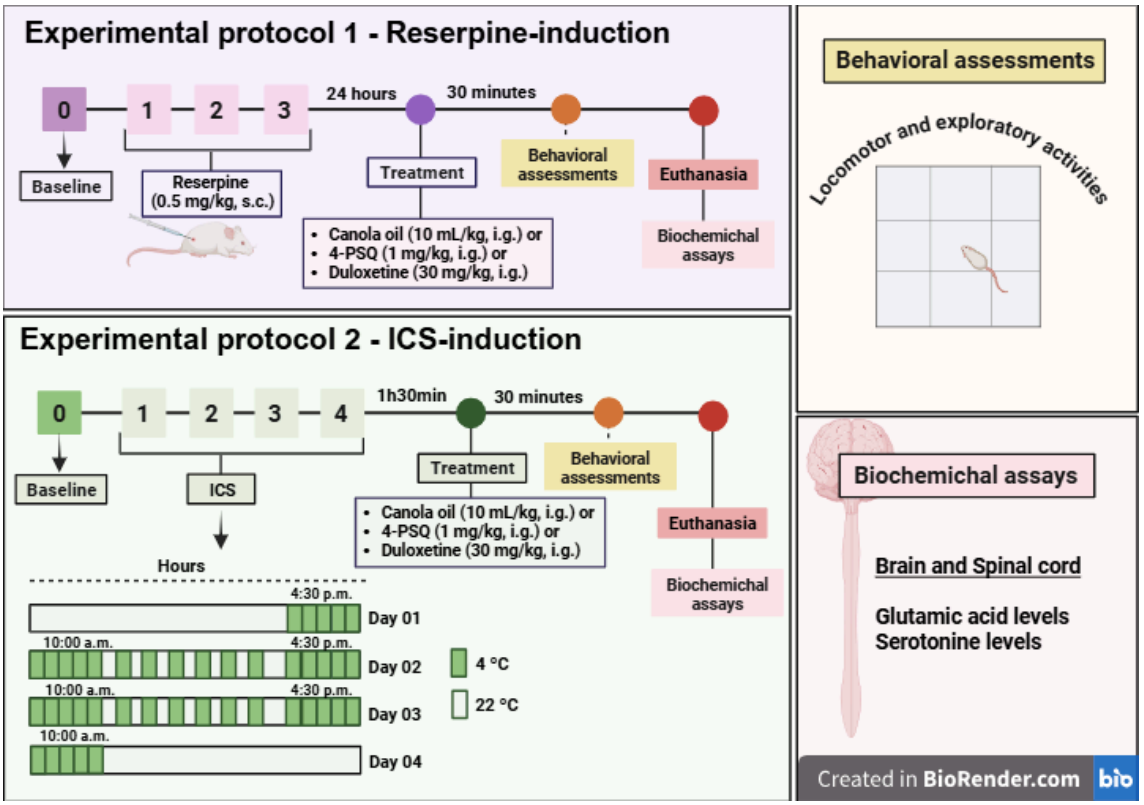

Figure 3. Schematic representation of the experimental protocol to assess the effects of 4-PSQ on the locomotor and exploratory behavior and the glutamic acid and serotonin levels in the brain and spinal cord of male and female mice exposed to reserpine or ICS protocols.

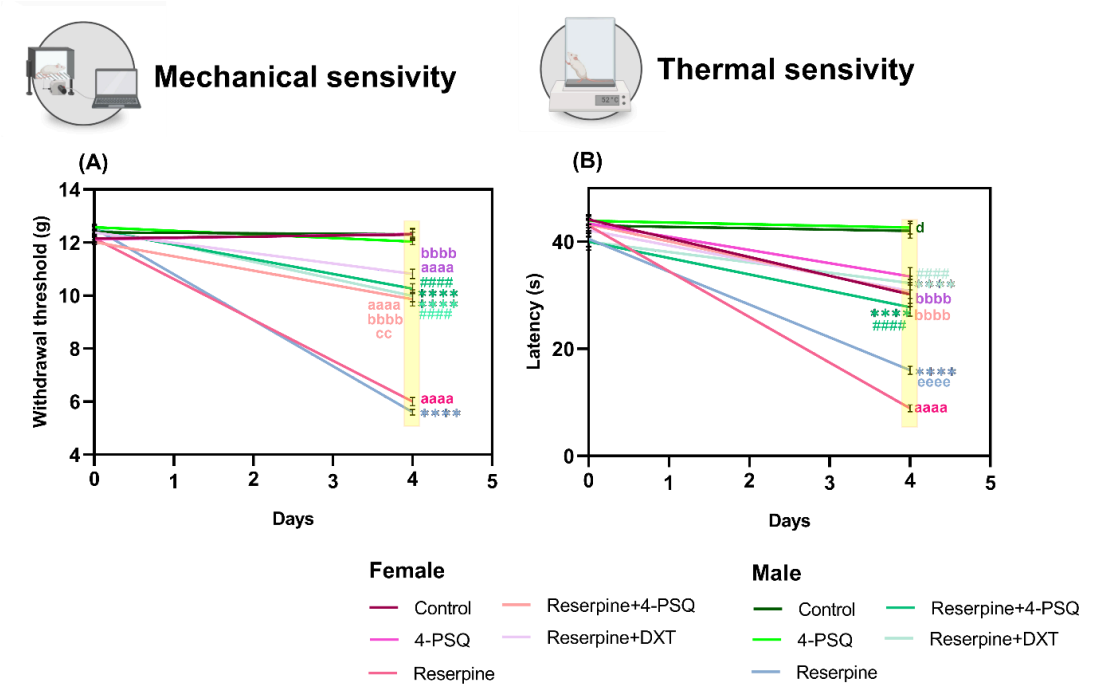

Figure 4. Effect of treatment with 4-PSQ (1 mg/kg, i.g.) or DXT (30 mg/kg, i.g.) on mechanical and thermal nociception in male and female mice exposed to the induction protocol with Reserpine (**A** and **B**, respectively). Each line/ column represents the mean ± S.E.M. of 7-8 animals per group. ANOVA one-way followed by Tukey's test: (****) $p<0.0001$ denotes significance levels when compared with the control male group; (####) $p<0.0001$ denotes significance levels when compared with the Reserpine male group; (aaaa) $p<0.0001$ denotes significance levels when compared with the control female group; (bbbb) $p<0.0001$ denotes significance levels when compared with the Reserpine female group; (cc) $p<0.01$ denotes significance levels when compared with the Reserpine+DXT female group. Unpaired Student's t-test: (d) $p<0.05$ denotes significance levels when compared with the Control female group (ICS or Reserpine protocol); (eeee) $p<0.0001$ denotes significance levels when compared with the Reserpine female group.

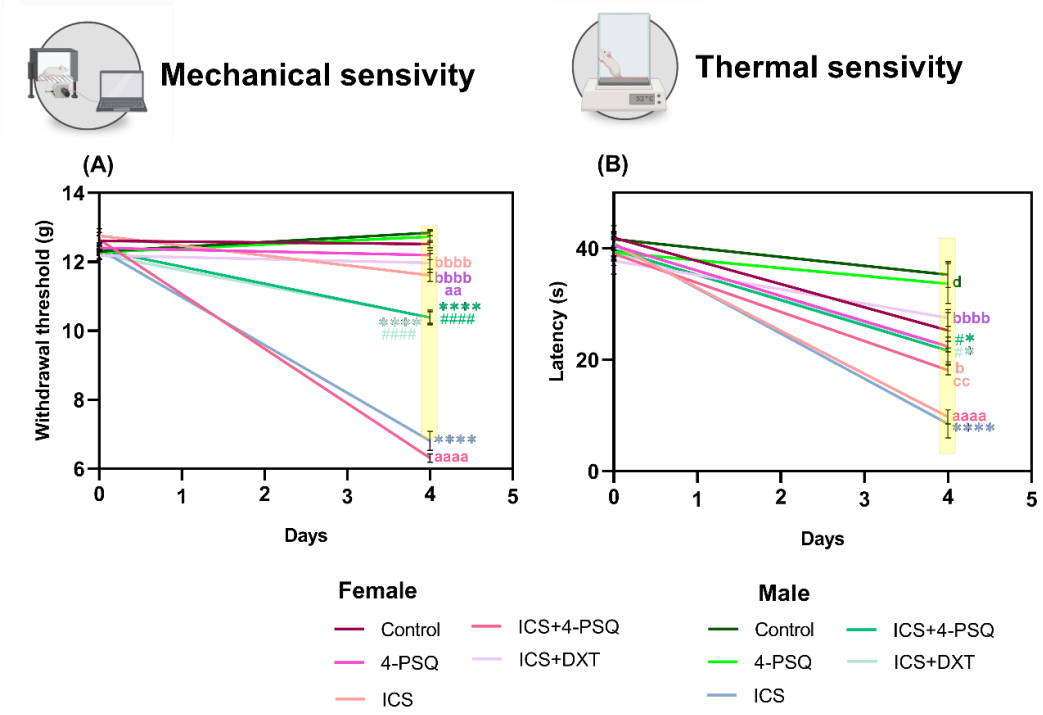

Figure 5. Effect of treatment with 4-PSQ (1 mg/kg, i.g.) or DXT (30 mg/kg, i.g.) on mechanical and thermal nociception in male and female mice exposed to the induction protocol with ICS (**A** and **B**, respectively). Each line/ column represents the mean ± S.E.M. of 7-8 animals per group. ANOVA one-way followed by Tukey's test: (*) $p<0.05$, and (****) $p<0.0001$ denote significance levels when compared with the control male group; (#) $p<0.05$, and (####) $p<0.0001$ denote significance levels when compared with the ICS male group; (aa) $p<0.01$, and (aaaa) $p<0.0001$ denote significance levels when compared with the control female group; (b) $p<0.05$, and (bbbb) $p<0.0001$ denote significance levels when compared with the ICS female group; (cc) $p<0.01$ denotes significance levels when compared with the ICS+DXT female group. Unpaired Student's t-test: (d) $p<0.05$ denotes significance levels when compared with the Control female group.

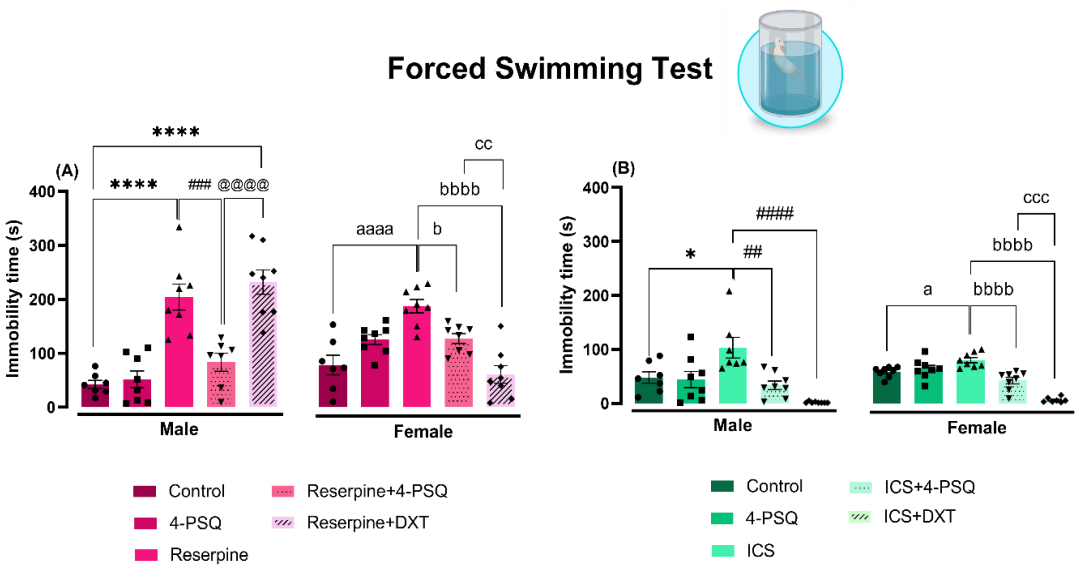

Figure 6. Effect of treatment with 4-PSQ (1 mg/kg, i.g.) or DXT (30 mg/kg, i.g.) in the immobility time on forced swimming test in male and female mice exposed to the induction protocol with Reserpine (**A**) or ICS (**B**). Each line/ column represents the mean ±S.E.M. of 7-8 animals per group. ANOVA one-way followed by Tukey's test: (*) $p<0.05$, and (****) $p<0.0001$ denotes significance levels when compared with the Control male group (ICS or Reserpine protocol); (##) $p<0.01$, (###) $p<0.001$, and (####) $p<0.0001$ denote significance levels when compared with the ICS or Reserpine male group; (@@@@) $p<0.0001$ denotes significance levels when compared with the Reserpine+DXT male group; (a) $p<0.05$, and (aaaa) $p<0.0001$ denote significance levels when compared with the Control female group (ICS or Reserpine protocol); (b) $p<0.05$, and (bbbb) $p<0.0001$ denote significance levels when compared with the Reserpine or ICS female group; (cc) $p<0.01$, and (ccc) $p<0.001$ denote significance levels when compared with the Reserpine+DXT or ICS+DXT female group.

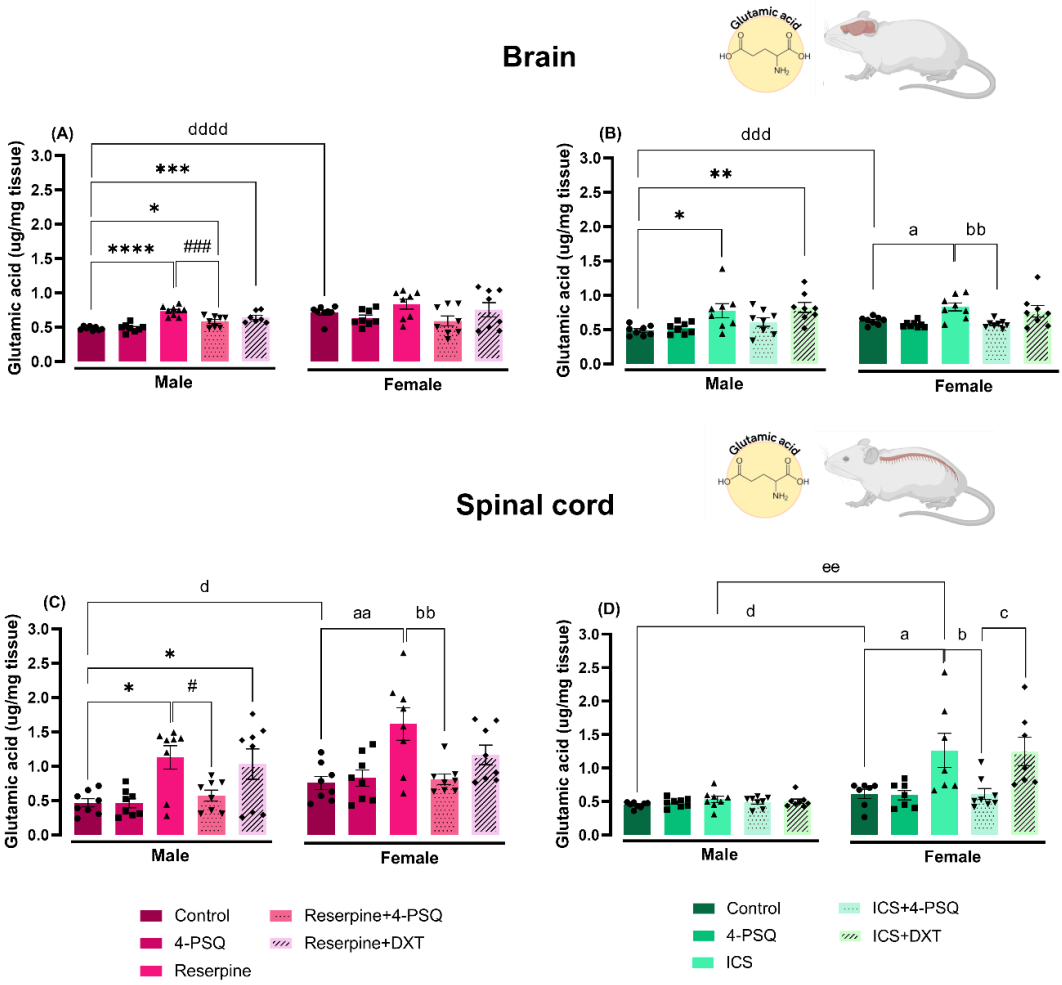

Figure 7. Effect of treatment with 4-PSQ (1 mg/kg, i.g.) or DXT (30 mg/kg, i.g.) on the glutamic acid levels in the brain and the spinal cord of male and female mice exposed to the induction protocol with Reserpine (**A** and **C**, respectively) or ICS (**B** and **D**, respectively). Each line/ column represents the mean ±S.E.M. of 7-8 animals per group. ANOVA one-way followed by Tukey's test: (*) $p<0.05$, (**) $p<0.01$, (***) $p<0.001$, and (****) $p<0.0001$ denote significance levels when compared with the Control male group (ICS or Reserpine protocol); (#) $p<0.05$, and (###) $p<0.001$ denote significance levels when compared with the ICS or Reserpine male group; (a) $p<0.05$, and (aa) $p<0.01$ denote significance levels when compared with the Control female group (ICS

or Reserpine protocol); (b) $p<0.05$, and (bb) $p<0.01$ denote significance levels when compared with the Reserpine or ICS female group; (c) $p<0.05$ denotes significance levels when compared with the ICS+DXT female group. Unpaired Student's t-test: (d) $p<0.05$, (ddd) $p<0.001$, and (dddd) $p<0.0001$ denote significance levels when compared with the Control female group (ICS or Reserpine protocol); (ee) $p<0.01$ denotes significance levels when compared with the ICS female group.

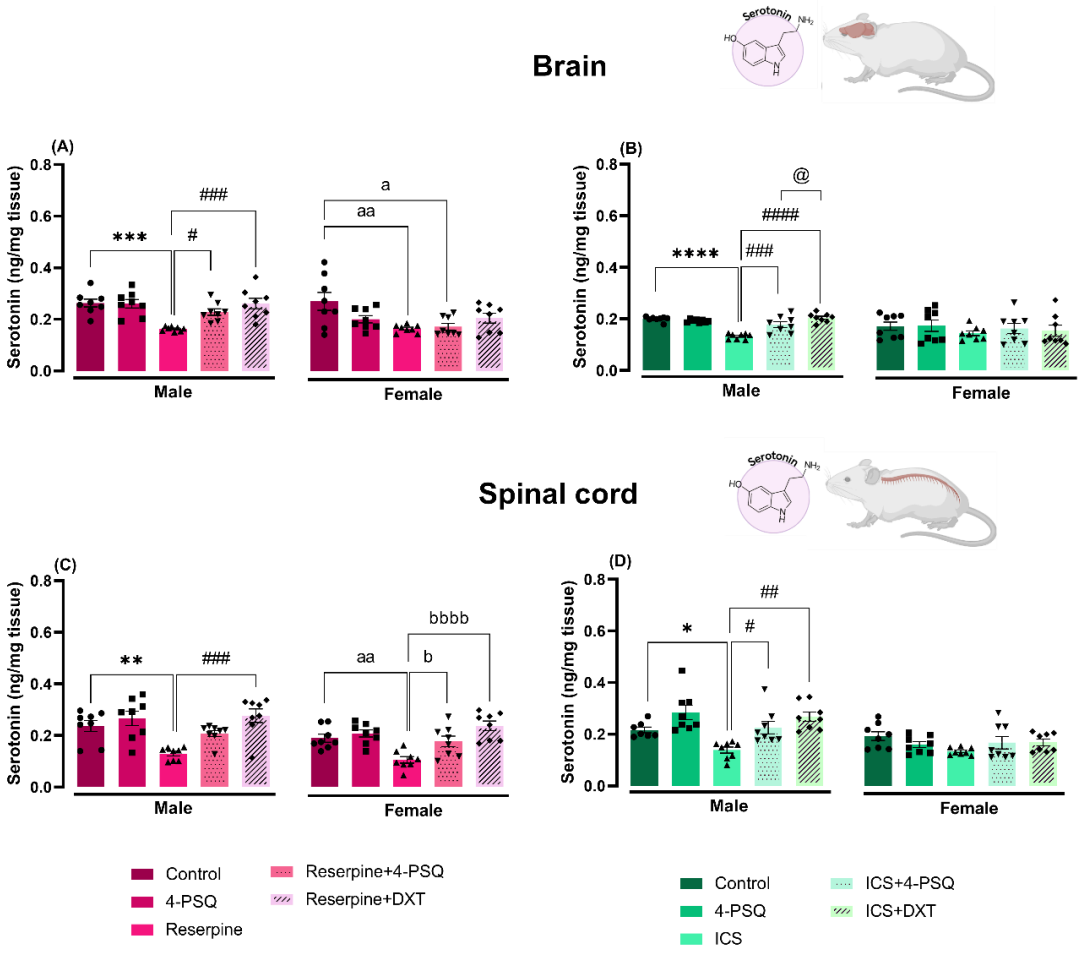

Figure 8. Effect of treatment with 4-PSQ (1 mg/kg, i.g.) or DXT (30 mg/kg, i.g.) in the serotonin levels in the brain and the spinal cord of male and female mice exposed to the

induction protocol with Reserpine (**A** and **C**, respectively) or ICS (**B** and **D**, respectively). Each line/ column represents the mean ±S.E.M. of 7-8 animals per group. ANOVA one-way followed by Tukey's test: (*) $p<0.05$, (**) $p<0.01$, (***) $p<0.001$, and (****) $p<0.0001$ denote significance levels when compared with the Control male group (ICS or Reserpine protocol); (#) $p<0.05$, (##) $p<0.01$, (###) $p<0.001$ and (####) $p<0.0001$ denote significance levels when compared with the ICS or Reserpine male group; (@) $p<0.05$ denotes significance levels when compared with the ICS+DXT male group; (a) $p<0.05$, and (aa) $p<0.01$ denote significance levels when compared with the Control female group (Reserpine protocol); (b) $p<0.05$, and (bbbb) $p<0.0001$ denote significance levels when compared with the Reserpine female groups.

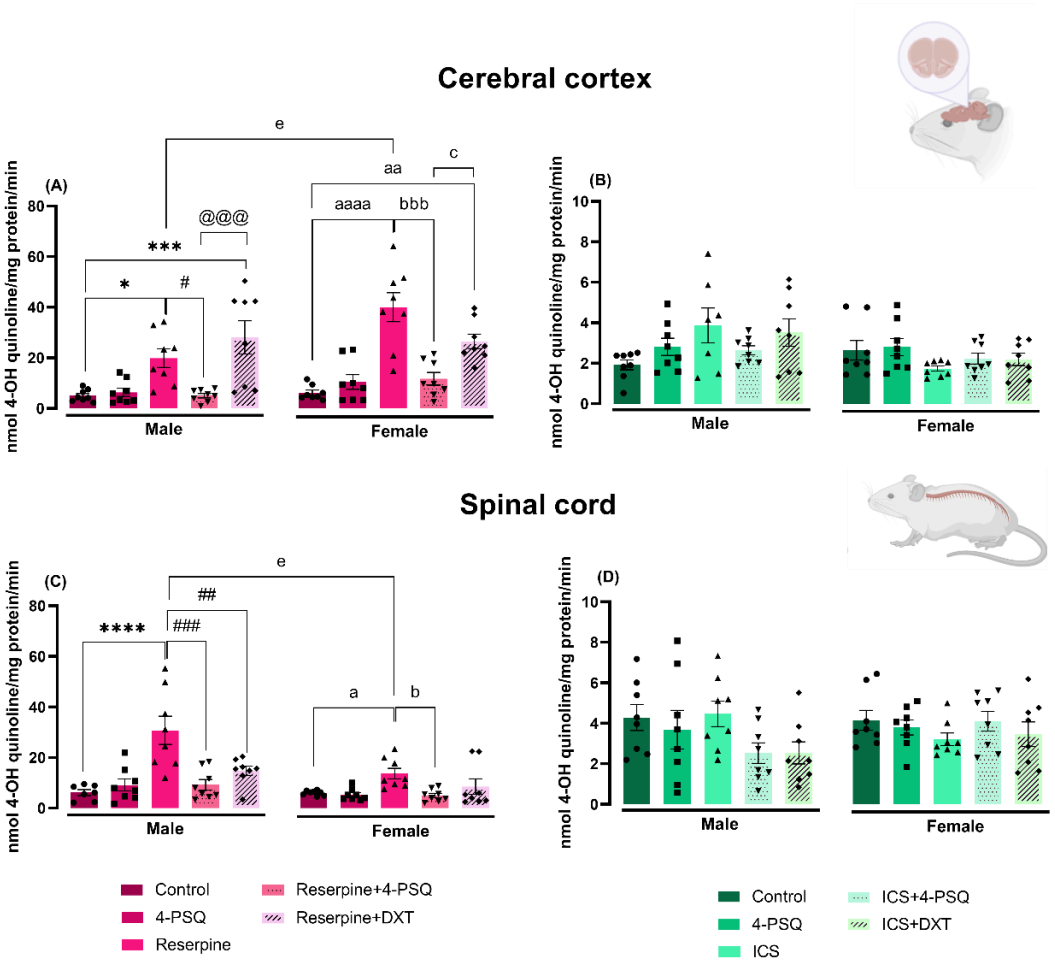

Figure 9. Effect of treatment with 4-PSQ (1 mg/kg, i.g.) or DXT (30 mg/kg, i.g.) on the MAO-A activity in the cerebral cortex and the spinal cord of male and female mice exposed to the induction protocol with Reserpine (**A** and **C**) or ICS (**B** and **D**). Each line/ column represents the mean ±S.E.M. of 7-8 animals per group. ANOVA one-way followed by Tukey's test: (*) $p<0.05$, (***) $p<0.001$, and (****) $p<0.0001$ denote significance levels when compared with the Control male group (Reserpine protocol); (#) $p<0.05$, (##) $p<0.01$, and (###) $p<0.001$ denote significance levels when compared with the Reserpine male group; (@@@) $p<0.001$ denotes significance levels when compared with the Reserpine+DXT male group; (a) $p<0.05$, (aa) $p<0.01$, and (aaaa) $p<0.0001$ denote significance levels when compared with the Control female group

(Reserpine protocol); (b) $p<0.05$, and (bbb) $p<0.001$ denote significance levels when compared with the Reserpine female group; (c) $p<0.05$ denotes significance levels when compared with the Reserpine+DXT female group. Unpaired Student's t-test: (e) $p<0.01$ denotes significance levels when compared with the Reserpine female group.

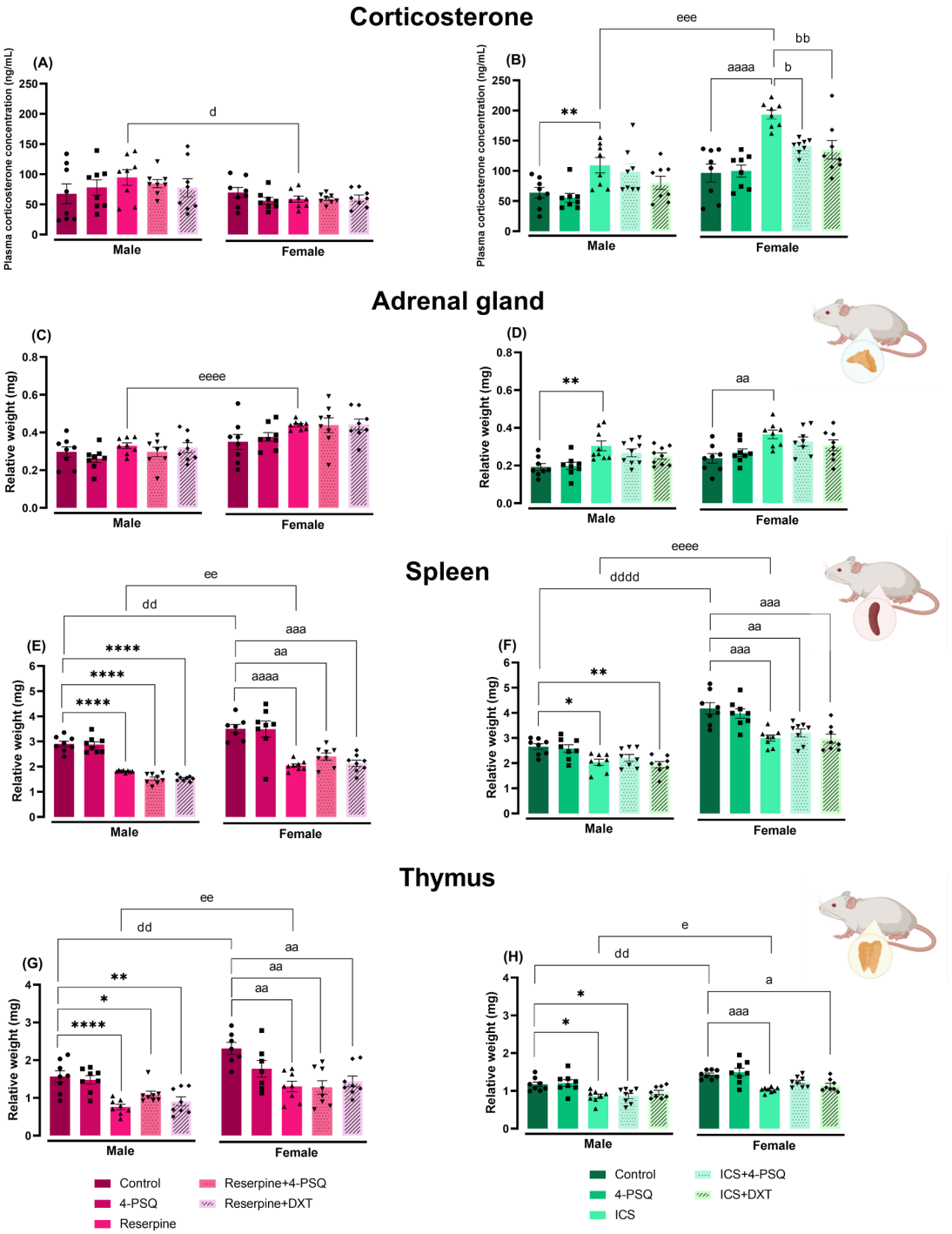

Figure 10. Effect of treatment with 4-PSQ (1 mg/kg, i.g.) or DXT (30 mg/kg, i.g.) in the corticosterone levels (**A** and **B**) and the relative weight of the adrenal gland (**C** and **D**), spleen (**E** and **F**), and thymus (**G** and **H**) of male and female mice exposed to the

induction protocol with Reserpine or ICS. Each line/ column represents the mean ±S.E.M. of 7-8 animals per group. ANOVA one-way followed by Tukey's test: (*) $p<0.05$, (**) $p<0.01$, and (***) $p<0.001$ denote significance levels when compared with the Control male group (Reserpine or ICS protocol); (a) $p<0.05$, (aa) $p<0.01$, (aaa) $p<0.001$, and (aaaa) $p<0.0001$ denote significance levels when compared with the Control female group (Reserpine or ICS protocol); (b) $p<0.05$, and (bb) $p<0.01$ denote significance levels when compared with the ICS female group. Unpaired Student's t-test: (dd) $p<0.01$, and (dddd) $p<0.0001$ denote significance levels when compared with the Control female group (ICS or Reserpine protocol); (e) $p<0.05$, (ee) $p<0.01$, (eee) $p<0.001$. and (eeee) $p<0.0001$ denote significance levels when compared with the Reserpine or ICS female group.

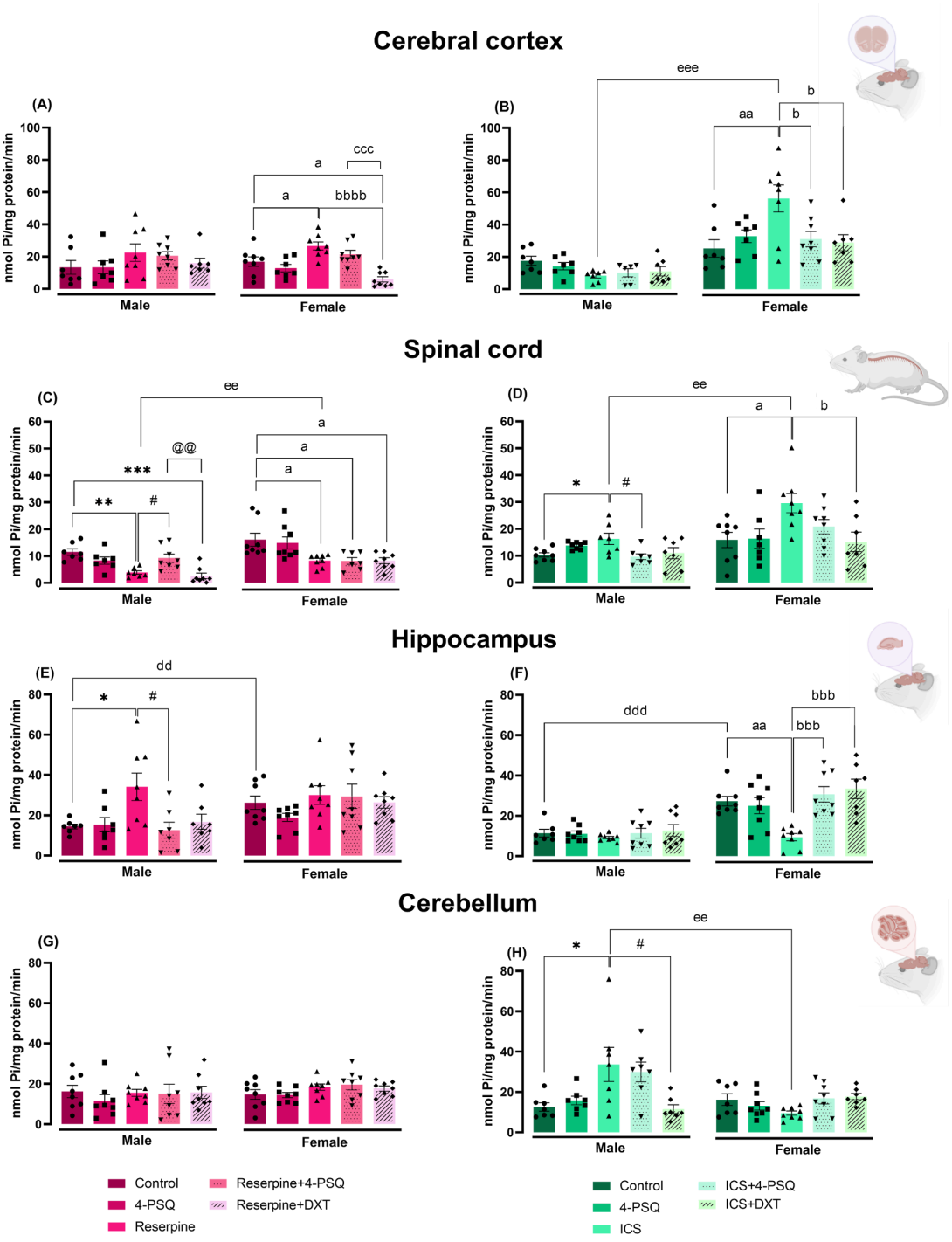

Figure 11. Effect of treatment with 4-PSQ (1 mg/kg, i.g.) or DXT (30 mg/kg, i.g.) in the Ca^{2+} ATPase activity in the cerebral cortex, spinal cord, hippocampus, and cerebellum of male and female mice exposed to the induction protocol with Reserpine (**A**, **C**, **E**,

and **G,** respectively) or ICS (**B**, **D**, **F**, and **H,** respectively). Each line/ column represents the mean ±S.E.M. of 7-8 animals per group. ANOVA one-way followed by Tukey's test: (*) $p<0.05$, (**) $p<0.01$, and (***) $p<0.001$ denote significance levels when compared with the Control male group (Reserpine protocol); (#) $p<0.05$ denotes significance levels when compared with the Reserpine or ICS male group; (@@) $p<0.01$ denotes significance levels when compared with the Reserpine+DXT male group; (a) $p<0.05$, and (aa) $p<0.01$ denote significance levels when compared with the Control female group (Reserpine protocol); (b) $p<0.05$, (bbb) $p<0.001$, and (bbbb) $p<0.0001$ denote significance levels when compared with the Reserpine female group; (ccc) $p<0.001$ denotes significance levels when compared with the Reserpine+DXT female group. Unpaired Student's t-test: (dd) $p<0.01$, and (ddd) $p<0.001$ denote significance levels when compared with the Control female group (ICS or Reserpine protocol); (ee) $p<0.01$, and (eee) $p<0.001$ denote significance levels when compared with the Reserpine female group.

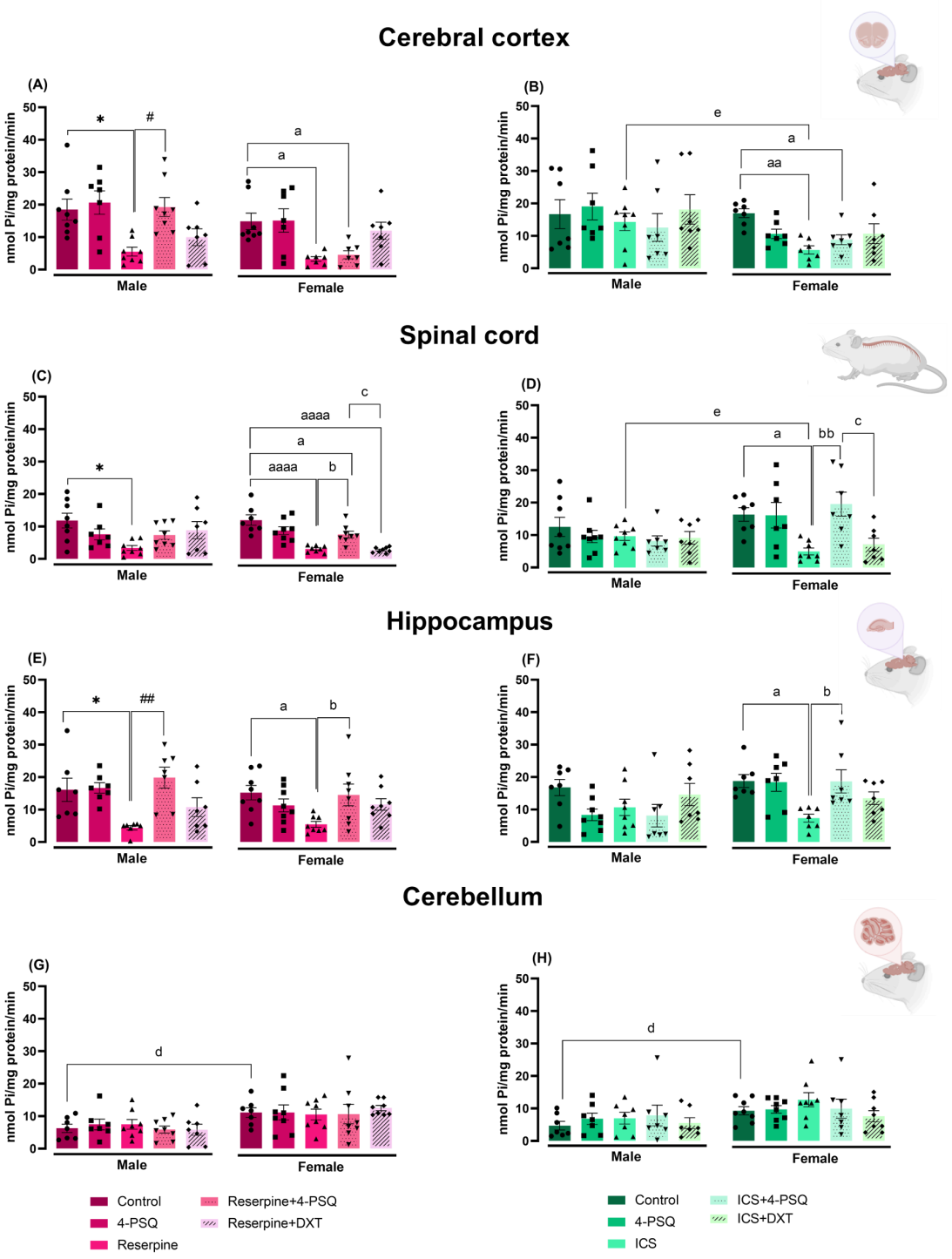

Figure 12. Effect of treatment with 4-PSQ (1 mg/kg, i.g.) or DXT (30 mg/kg, i.g.) on the Na^+/K^+ ATPase activity in the cerebral cortex, spinal cord, hippocampus, and cerebellum of male and female mice exposed to the induction protocol with Reserpine

(**A**, **C**, **E**, and **G**) or ICS (**B**, **D**, **F**, and **H**). Each line/ column represents the mean ±S.E.M. of 7-8 animals per group. ANOVA one-way followed by Tukey's test: (*) $p<0.05$ denotes significance levels when compared with the Control male group (Reserpine protocol); (#) $p < 0.05$, and (##) $p < 0.01$ denote significance levels when compared with the Reserpine male group; (a) $p<0.05$, (aa) $p<0.01$, and (aaaa) $p<0.0001$ denote significance levels when compared with the Control female group (Reserpine and ICS protocol); (b) $p<0.05$, and (bb) $p<0.01$ denote significance levels when compared with the Reserpine and ICS female group; (c) $p<0.05$ denotes significance levels when compared with the Reserpine + DXT or ICS + DXT female group. Unpaired Student's t-test: (d) $p<0.05$ denotes significance levels when compared with the Control female group (ICS or Reserpine protocol); (e) $p<0.05$ denotes significance levels when compared with the ICS female groups.

5. Discussão

Os resultados obtidos nesta dissertação ampliam o conhecimento sobre as propriedades farmacológicas do 4-PSQ, destacando-o como um composto promissor para o tratamento da fibromialgia, uma síndrome dolorosa multifatorial de manejo clínico ainda desafiador. Demonstramos que a administração de 4-PSQ atenuou a hipersensibilidade mecânica e térmica, bem como o fenótipo depressivo em camundongos machos e fêmeas submetidos aos modelos experimentais de fibromialgia. Esses efeitos mostraram-se comparáveis aos observados com a DXT, medicamento com uso clínico consolidado na fibromialgia. Por outro lado, o 4-PSQ revelou um perfil farmacológico mais amplo, modulando além do sistema monoaminérgico, os sistemas glutamatérgico, o eixo HPA, a homeostase eletrolítica e o estresse oxidativo no SNC. Tal atuação multialvo reforça o potencial terapêutico do 4-PSQ, capaz de agir sobre múltiplos mecanismos envolvidos na fisiopatologia da fibromialgia.

A fibromialgia é considerada uma síndrome de difícil manejo exatamente porque envolve mecanismos neurobiológicos, endócrinos, imunológicos e psicossociais que se retroalimentam, perpetuando a dor crônica. Nesse contexto, fármacos de ação única raramente conseguem controlar de forma satisfatória todos os sintomas. O fato de o 4-PSQ apresentar ação multialvo é especialmente relevante, pois se alinha às perspectivas mais recentes em farmacologia, que buscam compostos capazes de modular circuitos interligados em vez de atuar apenas em um receptor ou via específica. Além disso, sua eficácia em doses baixas sugerem um potencial terapêutico promissor, que pode reduzir o risco de efeitos adversos e aumentar a adesão ao tratamento, desafios centrais na prática clínica.

Neste estudo, os modelos de fibromialgia empregados reproduziram a hipersensibilidade polimodal observada em pacientes, incluindo sensibilidades aumentadas a estímulos mecânicos e térmicos, aspectos centrais para o sofrimento dos indivíduos acometidos (Gracely & Schweinhardt, 2015). Fêmeas controle mostraram maior sensibilidade térmica basal que machos, alinhada a dados clínicos que indicam maior dor em mulheres, possivelmente ligada a flutuações hormonais e ao efeito do estrogênio na modulação da dor (Korszun et al., 2000; Pieretti et al., 2016). A exacerbação da hipersensibilidade mecânica exclusivamente no modelo de reserpina

nas fêmeas sugere um papel diferencial do sistema monoaminérgico na modulação da dor entre os sexos. Em contraponto, a alodínia induzida por EFI desenvolveu-se de maneira semelhante em machos e fêmeas, indicando que mecanismos relacionados ao estresse podem atuar independentemente do sexo.

Atrelado a isso, observamos que ambos os modelos de fibromialgia foram capazes de instaurar um fenótipo depressivo nos camundongos machos e fêmeas. De fato, a dor crônica está intimamente ligada aos sintomas depressivos, com uma relação bidirecional entre as duas condições (Gracely e Schweinhardt, 2015).

O tratamento com o 4-PSQ reverteu eficazmente a hipersensibilidade térmica e mecânica e o fenótipo depressivo. Esses resultados estão de acordo com estudos prévios que reportaram seus efeitos antinociceptivo e do tipo-antidepressivo mediados por vias serotoninérgicas, glutamatérgicas e do óxido nítrico (Reis et al., 2020b; Reis et al., 2022; Paltian et al., 2022; Voss et al., 2022; De Oliveira et al., 2022; Silva et al., 2017).

Em vista disso, com o objetivo de elucidar o envolvimento do sistema glutamatérgico no efeito do 4-PSQ sobre os modelos de fibromialgia, avaliamos o nível de glutamato. Observamos que a reserpina aumentou os níveis de glutamato no cérebro de machos e na medula espinhal de ambos os sexos. Já o EFI elevou as concentrações desse neurotransmissor no cérebro de machos e fêmeas e na medula espinhal de fêmeas. Esses resultados refletem o aumento glutamatérgico característico da fibromialgia, associado à hiperativação das fibras A-δ e C (Goebel et al., 2021; Üçeyler et al., 2013; Wakatsuki et al., 2021). Notavelmente, observamos que fêmeas controle apresentaram níveis basais mais altos de glutamato no cérebro e na medula espinhal em relação aos machos, corroborando estudos clínicos que mostram maiores concentrações desse neurotransmissor em mulheres (Sailasuta et al., 2008). Essa diferença pode estar relacionada aos efeitos do estrogênio sobre transportadores de glutamato, glutamina sintetase, liberação de glutamato e expressão de receptores NMDA (Daniel & Dohanich, 2001; Haghighat, 2005; Krause et al., 2006; Woolley et al., 1997). Tal regulação hormonal pode contribuir para maior vulnerabilidade feminina à excitotoxicidade, dor crônica e transtornos de humor, embora o estradiol exerça efeitos complexos sobre a dor e as emoções (Athnaiel et al., 2023).

Nesse contexto, o 4-PSQ demonstrou um efeito modulador na desregulação glutamatérgica em condições semelhantes à fibromialgia. O tratamento com 4-PSQ restaurou os níveis fisiológicos de glutamato no cérebro de camundongos machos e na medula espinhal de ambos os sexos após a indução de reserpina, e em ambas as regiões de fêmeas expostas ao EFI. Consistente com esses achados, Reis et al. (2017) sugeriram que o 4-PSQ modulou a captação de glutamato no SNC em camundongos machos ao antagonizar as interações glutamato-receptor. Essa hipótese é ainda apoiada por Silva et al. (2017) e Da Rocha et al. (2024), que indicaram o envolvimento do receptor NMDA em seus efeitos antinociceptivos. Assim, o 4-PSQ parece modular a transmissão glutamatérgica no cérebro e na medula espinhal, reduzindo a hiperexcitabilidade neuronal e atuando na via ascendente da dor.

A modulação glutamatérgica pelo 4-PSQ é particularmente relevante, uma vez que o excesso de glutamato e a excitotoxicidade são mecanismos centrais não apenas na fibromialgia, mas também em outras doenças neurológicas e psiquiátricas, como epilepsia, depressão e doença de Alzheimer. Assim, os efeitos observados neste estudo podem ter implicações translacionais mais amplas. Além disso, as diferenças sexuais encontradas ressaltam a importância de considerar o sexo biológico como variável experimental, em consonância com as diretrizes internacionais de pesquisa pré-clínica. Tal abordagem abre caminho para uma medicina personalizada, em que fatores hormonais e genéticos possam orientar estratégias terapêuticas mais eficazes para homens e mulheres.

Em complementaridade, avaliamos também o efeito do 4-PSQ sobre a via descendente da dor. Estudos apontam que desregulações no sistema monoaminérgico, especialmente pelo declínio do conteúdo de 5-HT também estão envolvidas na fisiopatologia da fibromialgia (Singh et al., 2019). Neste estudo, a administração de reserpina diminuiu os níveis de 5-HT no SNC (cérebro e medula espinhal) de camundongos machos e fêmeas. Essa depleção provavelmente interrompe a inibição da dor descendente, contribuindo para a hiperexcitabilidade neuronal e a amplificação da dor, características marcantes da fibromialgia (Siracusa et al., 2021; Alfaro-Rodríguez et al., 2024). A deficiência de 5-HT também está associada a sintomas depressivos (Kayabaşı et al., 2021). Curiosamente, o modelo EFI mostrou redução de 5-HT apenas

na medula espinhal de camundongos machos, sugerindo comprometimento do controle da dor descendente relacionado ao estresse, consistente com Hata et al. (1991).

Como componente chave do sistema monoaminérgico, a enzima MAO encontra-se envolvida no catabolismo das monoaminas. Alterações em sua atividade podem comprometer o funcionamento adequado desse sistema (Youdim & Sandler, 1968; Gürsoy et al., 2008; Naoi et al., 2016; Janssen et al., 2021). No presente estudo, a reserpina provocou o aumento seletivo da atividade da MAO-A, mas não da MAO-B, no córtex cerebral e na medula espinhal de camundongos de ambos os sexos. Esse efeito possivelmente reflete um mecanismo compensatório relacionado à degradação da 5-HT livre no citosol quando há inibição do armazenamento vesicular (Freitas et al., 2013). Esse aumento foi mais evidente nas fêmeas, sugerindo um efeito dependente do sexo sobre as adaptações neuroquímicas. Por outro lado, a exposição ao EFI não promoveu alterações na atividade da MAO, reforçando a especificidade do modelo de reserpina na modulação desse sistema.

O tratamento com 4-PSQ modulou os níveis de 5-HT e a atividade da MAO-A no SNC dos camundongos machos e fêmeas, em concordância com Silva et al. (2017) e Oliveira et al. (2022). No modelo de reserpina, o composto restaurou os níveis de 5-HT na medula espinhal de ambos os sexos e no cérebro dos machos, além de normalizar a atividade da MAO-A no córtex cerebral e na medula espinhal. Esses achados indicam que a inibição da MAO-A pelo 4-PSQ pode ter contribuído para a recuperação da 5-HT ao reduzir seu catabolismo. Já no modelo de EFI, embora o 4-PSQ tenha normalizado os níveis de 5-HT no cérebro e na medula espinhal de machos, não houve alteração na atividade da MAO, sugerindo que, nesse contexto, o composto atua modulando a liberação ou recaptação de 5-HT.

Como mecanismo complementar para progressão da fibromialgia, avaliamos o efeito do 4-PSQ sobre marcadores do estresse oxidativo. Um fator contribuinte é o catabolismo das monoaminas, onde a enzima MAO gera subprodutos tóxicos, incluindo o H_2O_2, capaz de induzir dano oxidativo e exceder as defesas antioxidantes (Naoi et al., 2016; Assavarittirong et al., 2022). Neste estudo, a reserpina aumentou os níveis de ER no cerebelo de camundongos de ambos os sexos e no córtex cerebral de machos. O EFI elevou os níveis de ER no hipocampo de machos e fêmeas. Apesar do aumento de ER, não houve aumento de peroxidação lipídica, o que difere de relatos anteriores (Martins et

al., 2025; Sousa et al., 2018) e sugere ativação de mecanismos compensatórios capazes de conter danos às membranas celulares (Gęgotek e Skrzydlewska, 2019).

Essas respostas pareceram moduladas pelo sexo, refletidas em alterações na atividade da CAT. Na exposição à reserpina, fêmeas mostraram redução da CAT em regiões do SNC (córtex cerebral e na medula espinhal), enquanto machos apresentaram aumento nessas regiões. Apesar do aumento de MAO-A em ambos os sexos, em fêmeas o excesso de H_2O_2 pode ter inibido a CAT cortical, embora outros antioxidantes tenham prevenido danos (Liguori et al., 2018; Luo et al., 2020). Em machos, o aumento da CAT sugere depuração mais eficiente de H_2O_2. Sob o EFI, apenas fêmeas exibiram inibição da CAT no córtex cerebral e na medula espinhal, mas aumento no hipocampo.

Notavelmente, fêmeas controle apresentaram alterações basais nos níveis de ER e peroxidação lipídica e na atividade da CAT em algumas regiões do SNC. Esses achados sugerem maior estresse oxidativo basal, mas também indicam potencialmente maior capacidade antioxidante. Esse efeito pode ser mediado pela ação neuroprotetora do estrogênio (Massafra et al., 2000; Sheng-Huang et al., 2015).

Nossos dados confirmam as propriedades antioxidantes do 4-PSQ, evidenciando sua capacidade de regular a homeostase oxidativa de forma sexo e tecido específicos. Observou-se que o composto diminuiu os níveis de ER e restaurou a atividade da CAT em áreas do cérebro e da medula espinhal associadas ao processamento da dor. No modelo induzido por reserpina, seus efeitos foram mais evidentes em machos, com normalização dos níveis de ER da atividade de CAT no córtex cerebral, além da redução de ER no cerebelo. Em fêmeas expostas ao EFI, o 4-PSQ normalizou os níveis de ER no hipocampo e a atividade da CAT tanto no hipocampo quanto na medula espinhal. Esses resultados reforçam estudos anteriores que demonstraram seus efeitos antioxidantes e neuroprotetores no SNC de roedores (Luchese et al., 2020; Paltian et al., 2022; Reis et al., 2020b). Dessa forma, o 4-PSQ parece potencializar as defesas antioxidantes, seja neutralizando diretamente ER ou modulando mecanismos antioxidantes endógenos.

O envolvimento do estresse oxidativo na fibromialgia é cada vez mais reconhecido, e estratégias antioxidantes vêm sendo propostas como adjuvantes terapêuticos. Nesse sentido, os achados do presente estudo reforçam que o 4-PSQ, além de atuar em vias clássicas de neurotransmissão, também exerce efeito regulador

sobre a homeostase redox. Isso é particularmente importante porque sugere que o composto pode atuar não apenas no alívio sintomático, mas também na modulação de processos biológicos que contribuem para a progressão da síndrome. Considerando que muitos pacientes com fibromialgia apresentam comorbidades metabólicas, como obesidade e resistência insulínica, investigar os efeitos do 4-PSQ em contextos de desregulação metabólica poderia trazer insights relevantes para seu potencial clínico.

Como consequência do desequilíbrio redox podem ocorrer alterações no funcionamento mitocondrial, resultando em alterações energéticas bruscas e prejuízo na função de enzimas vitais (Singh et al., 2019; Macchi et al., 2024). Neste estudo, verificamos que a reserpina suprimiu a atividade da Na^+/K^+-ATPase no córtex cerebral, hipocampo e medula espinhal em ambos os sexos, enquanto o EFI promoveu essa redução apenas nessas regiões de fêmeas. A redução da atividade dessa enzima pode estar relacionada ao aumento da excitabilidade neuronal característico desses modelos (Bejček et al., 2021).

Como uma avaliação complementar, investigamos a atividade da Ca^{2+}-ATPase, que desempenha um papel crucial no controle dos níveis de Ca^{2+} intracelular (Primeau et al., 2018). Nossos resultados mostraram que a reserpina elevou a atividade dessa enzima no córtex de fêmeas e no hipocampo de machos, enquanto o EFI provocou aumento no córtex de fêmeas, no cerebelo de machos e na medula espinhal de ambos os sexos. É bem descrito que a ativação excessiva desta enzima no SNC pode comprometer o funcionamento mitocondrial, alterar a comunicação neuronal e aumentar a excitabilidade (Brini et al., 2013; Carafoli & Krebs, 2016; Sung et al., 2015). Em contrapartida, a inibição da enzima foi observada em algumas regiões: a reserpina diminuiu a atividade na medula espinhal de ambos os sexos, e o EFI reduziu no hipocampo de fêmeas. Essa redução na atividade pode estar associada a modificações oxidativas nos grupos sulfidrila da enzima ou ao acúmulo excessivo de Ca^{2+}, que poderia saturar a bomba ou induzir mecanismos de retroalimentação negativa como forma de evitar danos celulares (Mei et al., 2018; Gęgotek & Skrzydlewska, 2019).

Nesse cenário, o 4-PSQ mostrou potencial para reequilibrar a homeostase iônica por meio da modulação das enzimas Na^+/K^+-ATPase e Ca^{2+}-ATPase, com efeitos que variaram conforme o modelo experimental e o sexo dos animais. No modelo de reserpina, o composto restaurou a atividade da Na^+/K^+-ATPase no hipocampo de machos

e fêmeas, no córtex cerebral de machos e na medula espinhal de fêmeas, além de reverter a inibição da Ca^{2+}-ATPase no hipocampo e na medula espinhal de machos. Já no protocolo do EFI, o 4-PSQ normalizou a Na^{+}/K^{+}-ATPase no hipocampo e na medula espinhal de fêmeas, e a Ca^{2+}-ATPase no córtex cerebral e hipocampo de fêmeas, bem como na medula espinhal de machos. Esses resultados corroboram estudos anteriores que associam os efeitos neuroprotetores e antinociceptivos do 4-PSQ à manutenção da homeostase iônica (Reis et al., 2020a; Reis et al., 2022; Paltian et al., 2022). Além disso, ao regular a Ca^{2+}-ATPase, o 4-PSQ pode diminuir a liberação de glutamato de maneira semelhante à pregabalina, que reduz o influxo de Ca^{2+} e apresenta efeitos analgésicos e antidepressivos em casos de fibromialgia (Carafoli & Krebs, 2016). Dessa forma, sua capacidade de preservar o equilíbrio iônico e mitigar a excitotoxicidade pode contribuir para os efeitos antinociceptivos e do tipo antidepressivos observados nos modelos de reserpina e EFI.

A modulação das ATPases pelo 4-PSQ sugere uma atuação mais profunda na regulação da excitabilidade neuronal, indo além dos mecanismos já descritos para fármacos utilizados clinicamente na fibromialgia. Essa característica abre a possibilidade de que o composto possa atuar de forma sinérgica com medicamentos disponíveis, ou mesmo como alternativa em pacientes resistentes às terapias atuais. Estudos futuros que avaliem potenciais interações farmacológicas, assim como os efeitos do 4-PSQ em modelos crônicos de dor associados a disfunções mitocondriais, poderiam expandir o entendimento sobre a relevância desse mecanismo.

Além das alterações eletrolíticas, nossos resultados indicam que os efeitos farmacológicos do 4-PSQ também envolvem a modulação do eixo HPA. Aqui, observamos que a exposição ao EFI aumentou os níveis de corticosterona e o peso relativo das adrenais em machos e fêmeas, sugerindo ativação prolongada desse sistema. Essa ativação crônica pode prejudicar a sinalização dos glicocorticoides, afetando processos como neurogênese e plasticidade neuronal, o que contribui para sintomas centrais da fibromialgia, como dor crônica, fadiga e alterações de humor (Crofford, 1996; Blackburn-Munro, 2004; Singh et al., 2019).

Os glicocorticoides são hormônios fundamentais para o controle do metabolismo, da inflamação e das defesas imunes (Goel et al., 2014; Kim, 2023). Neste estudo, verificou-se que o EFI reduziu o peso relativo do baço e do timo em machos e fêmeas,

efeito provavelmente relacionado à ação imunossupressora dos glicocorticoides, que diminuem a proliferação de células imunes e a liberação de citocinas pró-inflamatórias (Menke, 2024; Straub, 2023). Esses resultados corroboram estudos anteriores que relatam atrofia esplênica induzida por estresse (Chen et al., 2023). Além disso, os glicocorticoides favorecem a apoptose de timócitos, comprometendo a produção de células T e a resposta imune adaptativa (Straub, 2023). Interessantemente, embora a reserpina também tenha causado redução nos pesos do baço e do timo, não houve alteração nas adrenais ou nos níveis de corticosterona, indicando que os mecanismos envolvidos diferem entre os modelos. Enquanto o EFI parece ativar diretamente o eixo HPA, elevando os glicocorticoides, a reserpina atua por outras vias. Por fim, observou-se que as fêmeas apresentaram, em condições basais, baço e timo mais pesados que os machos, possivelmente devido ao efeito imunomodulador do estrogênio (Goel et al., 2014), reforçando a relevância do sexo na regulação do sistema imune.

Neste estudo, o 4-PSQ diminui a hiperatividade do eixo HPA ao reduzir os níveis plasmáticos de corticosterona em fêmeas submetidas ao EFI, embora não tenha revertido as alterações nos pesos das adrenais, baço e timo causadas pela reserpina ou pelo EFI. Estudos anteriores indicam que esse composto é capaz de modular a corticosterona em machos, efeito relacionado às suas propriedades ansiolíticas e antidepressivas (De Oliveira et al., 2022; Paltian et al., 2020; Reis et al., 2020). De modo similar, benzodiazepínicos reduzem a ativação do eixo HPA por sua ação nos receptores GABAA (Fries et al., 2006; Mikkelsen et al., 2008), os quais também parecem mediar parte dos efeitos do 4-PSQ (Paltian et al., 2020). Assim, é possível que esse composto atue sobre o eixo HPA por meio da modulação do $GABA_A$ ou por inibir diretamente a liberação de corticosterona. Além disso, como a elevação do cortisol reduz a disponibilidade de triptofano no cérebro e, consequentemente, a síntese de 5-HT (Maes et al., 1998), os efeitos do 4-PSQ podem ainda envolver a inibição da MAO-A, aumentando os níveis de 5-HT e exercendo retroalimentação negativa sobre o eixo HPA.

A hiperatividade do eixo HPA é reconhecida como um marcador fisiopatológico de diversas condições relacionadas ao estresse crônico, incluindo a fibromialgia, a depressão maior e a síndrome da fadiga crônica. Os possíveis efeitos do 4-PSQ sobre esse eixo sugerem que seu uso poderia impactar sintomas que extrapolam a dor, como fadiga, distúrbios do sono e alterações cognitivas, frequentemente relatados pelos

pacientes. Além disso, as alterações no baço e no timo observadas nos modelos experimentais apontam para a necessidade de investigar com mais profundidade os efeitos imunomodulatórios do 4-PSQ, uma vez que a fibromialgia também envolve desregulações no sistema imune. Tal investigação poderia esclarecer se o composto contribui para restaurar a comunicação neuroimune alterada nessa síndrome.

Em síntese, os resultados desta dissertação evidenciam que o 4-PSQ apresenta um perfil farmacológico promissor para o tratamento da fibromialgia, atuando de forma abrangente sobre mecanismos centrais dessa síndrome. Na dose de 1 mg/kg o 4-PSQ reduziu de maneira consistente a hipersensibilidade mecânica e térmica, além do comportamento depressivo, em camundongos machos e fêmeas. Esses efeitos foram acompanhados pela modulação dos sistemas monoaminérgico e glutamatérgico, pela restauração da homeostase iônica, pela redução do estresse oxidativo e pela atenuação da hiperatividade do eixo HPA. Todo esse conjunto de vias moduladas pelo 4-PSQ reafirmam sua ação multialvo que vai além do simples alívio sintomático, interferindo também em processos que perpetuam e amplificam a dor e as alterações neuropsiquiátricas associadas à fibromialgia (Figura 4). Apesar das variações tecido-, sexo- e modelo-dependentes observadas, este trabalho amplia o conhecimento sobre as propriedades farmacológicas do 4-PSQ e reforça sua relevância como candidato terapêutico para essa condição de difícil manejo. Diante desses achados promissores, destaca-se a importância de estudos futuros que avaliem os efeitos da administração prolongada do 4-PSQ, bem como investiguem de forma mais aprofundada as diferenças sexuais, incluindo a definição do ciclo estral das fêmeas e o papel dos hormônios sexuais na resposta ao tratamento, visando ao desenvolvimento de estratégias terapêuticas mais seguras e eficazes para pacientes com fibromialgia.

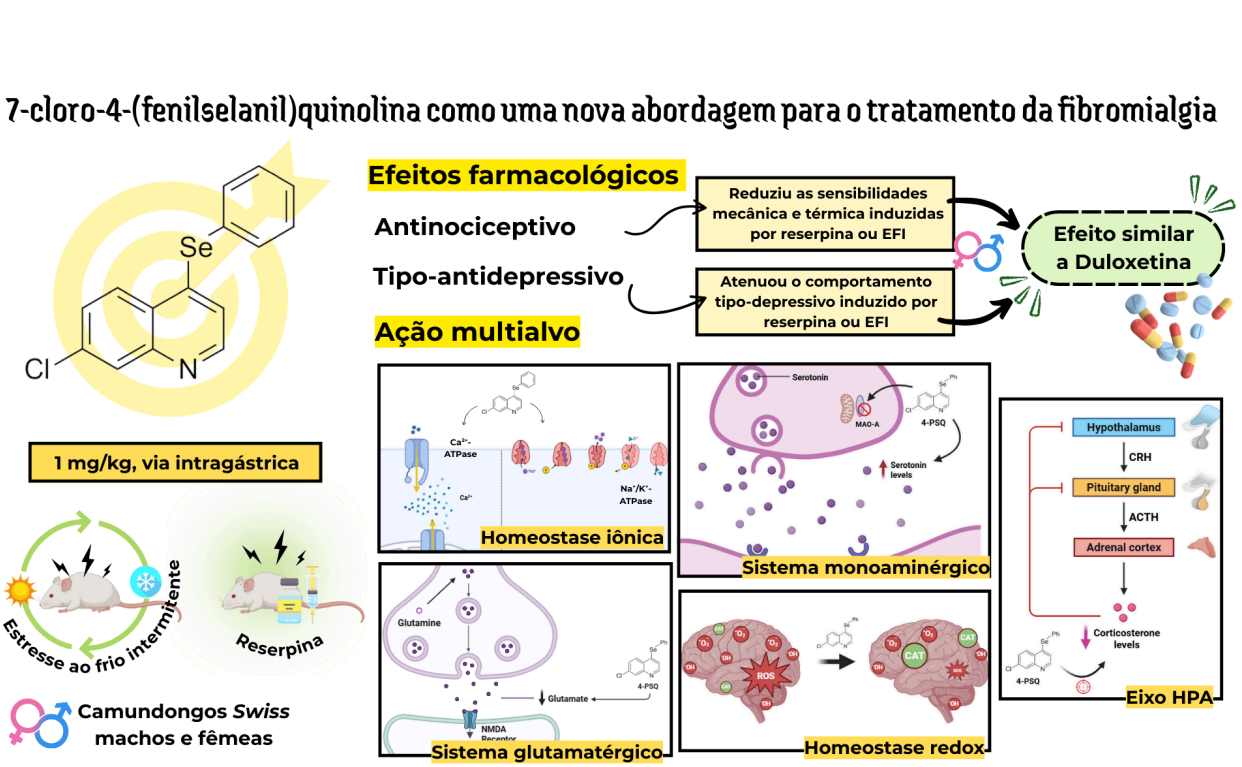

Figura 4. Representação esquemática dos efeitos farmacológicos apresentados pelo 4-PSQ neste estudo.

6. Conclusões

Com base nos resultados apresentados nesta dissertação podemos sugerir que:

- O tratamento com uma dose do 4-PSQ apresentou efeito antinociceptivo, reduzindo tanto a hipersensibilidade mecânica quanto térmica induzida pela reserpina ou pelo EFI em camundongos de ambos os sexos;
- O tratamento com o 4-PSQ demonstrou efeito do tipo-antidepressivo, revertendo o fenótipo depressivo induzido por ambos os modelos experimentais em machos e fêmeas;
- O tratamento com o 4-PSQ modulou a neurotransmissão glutamatérgica, normalizando os níveis elevados de glutamato no cérebro e na medula espinhal induzidos pelos modelos de fibromialgia, o que pode ter contribuído para seus efeitos antinociceptivo e do tipo-antidepressivo;
- A administração de 4-PSQ restaurou os níveis de 5-HT e modulou a atividade da MAO-A no SNC dos animais, principalmente no modelo de reserpina, indicando que esses mecanismos participam dos seus efeitos terapêuticos;

- O tratamento com 4-PSQ atenuou a hiperatividade do eixo HPA ao reduzir os níveis de corticosterona em fêmeas expostas ao EFI, sugerindo sua capacidade de modular vias neuroendócrinas relacionadas à fibromialgia;
- O 4-PSQ apresentou efeitos antioxidantes ao reduzir níveis de ER e restaurar a atividade da CAT em regiões do SNC envolvidas na dor, de forma modelo-, sexo- e tecido-dependente, contribuindo para seus efeitos benéficos e neuroprotetores;
- O 4-PSQ modulou a atividade das enzimas Na^{+}/K^{+}-ATPase e Ca^{2+}-ATPase de forma modelo-, sexo- e tecido-dependente, restaurando a homeostase iônica prejudicada nos modelos de fibromialgia e contribuindo para seus efeitos terapêuticos;
- O 4-PSQ exerceu seus efeitos farmacológicos de forma semelhante em ambos os sexos, não havendo dimorfismo sexual marcantes na resposta ao tratamento;

Dessa forma, podemos concluir que o 4-PSQ é um composto multi alvo. Assim, acreditamos que investigações futuras devem ser realizadas para avaliar o potencial farmacológico do 4-PSQ em um regime de tratamento prolongado. Além disso, deve-se considerar mais especificamente a influência dos hormônios sexuais nas vias moduladas pelo 4-PSQ.

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ANEXOS

Anexo A – Comprovação do Comitê de Ética em Experimentação Animal

PARECER Nº **40/2024/CEUA/REITORIA**
PROCESSO Nº 23110.043665/2023-61

Certificado

Certificamos que a proposta intitulada **"Avaliação das propriedades farmacológicas do composto 7-cloro-4-(fenilselanil) quinolina no tratamento da fibromialgia"**, registrada com o n° 23110.043665/2023-61, sob a responsabilidade de **Ethel Antunes Wilhelm** - 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 recebeu parecer **FAVORÁVEL** a sua execução pela Comissão de Ética no Uso de Animais da Universidade Federal de Pelotas.

Finalidade	(x) Pesquisa ( ) Ensino
Vigência da autorização	Início: 03/04/2024 Término: 01/10/2027
Espécie/linhagem/raça	*Mus musculus* / Swiss
N° de animais	**780 (390 machos e 390 fêmeas)**
Idade	60 dias
Sexo	Fêmeas e machos
Origem	Biotério Central da Universidade Federal de Pelotas, Campus Capão do Leão.

Código para cadastro nº **CEUA 043665/2023-61**