MARIANA BARBOSA DETONI

TRANS-CHALCONA E CALCIPOTRIOL NA ESQUISTOSSOMOSE MANSÔNICA:

AVALIAÇÃO DOS EFEITOS ANTIPARASITÁRIO E ANTIFIBRÓTICO

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Orientador: Profa. Dra. Ivete Conchon Costa.
Co-orientador: Prof. Dr. Wander Rogério Pavanelli.

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Ao meu avô,
minha maior inspiração,
cuja presença me acompanha todos os dias.

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“*Para que a luz brilhe tão intensamente,*
a escuridão tem de estar presente”.
— Francis Bacon

DETONI, Mariana Barbosa. ***Trans*-chalcona e calcipotriol na esquistossomose mansônica:** avaliação dos efeitos antiparasitário e antifibrótico. 2025. 124 f. Tese (Doutorado em Patologia Experimental) – Universidade Estadual de Londrina, Londrina, 2025.

RESUMO

A esquistossomose é uma doença parasitária causada por trematódeos do gênero *Schistosoma*, cujas formas adultas habitam os vasos portais e mesentéricos do ser humano e as formas intermediárias se desenvolvem em moluscos gastrópodes. A fase aguda pode ser assintomática ou apresentar manifestações como febre, dor de cabeça e calafrios, podendo evoluir para a fase crônica, caracterizada por formação de inúmeros granulomas hepáticos ao redor dos ovos depositados nos tecidos, levando a fibrose, hipertensão portal e consequente morbidade. O tratamento baseia-se no uso do praziquantel (PZQ, pirazino-isoquinolina), eficiente apenas contra os vermes adultos. Assim, apesar de sua eficácia, apresenta limitação contra formas juvenis e na resposta granulomatosa, além de risco iminente de surgimento de cepas resistentes. Tendo em vista a magnitude de sua prevalência, a severidade das formas clínicas e ineficiência do tratamento contra formas imaturas e em casos crônicos, torna-se necessário a busca por novas alternativas terapêuticas. Neste contexto, o presente trabalho avaliou os efeitos da *trans*-Chalcona (TC), um flavonoide natural com amplo espectro de atividade biológica, e do calcipotriol (CP), um análogo sintético da vitamina D com propriedades antifibróticas, no contexto da esquistossomose experimental. *In vitro* TC demonstrou atividade esquistossomicida, principalmente contra formas juvenis de *Schistosoma mansoni*, induzindo alterações nos níveis de óxido nítrico, despolarização da membrana mitocondrial, danos ao tegumento e perda da integridade da membrana celular, comprometendo a oviposição. Em modelo murino de esquistossomose, o tratamento com TC reduziu a carga parasitária e o tamanho dos granulomas, promovendo regulação do perfil inflamatório. Além disso, TC apresentou baixa toxicidade em células saudáveis, reforçando seu potencial como agente terapêutico seguro e eficaz. CP, por sua vez, foi avaliado quanto à capacidade hepatoprotetora no modelo experimental. Embora não tenha apresentado ação direta sobre a carga parasitária, o tratamento com CP foi capaz de reduzir o estresse oxidativo e o infiltrado inflamatório hepático, resultando em diminuição dos granulomas e da deposição de colágeno. CP também demonstrou eficácia na modulação do perfil oxidativo em células estreladas hepáticas, com efeitos superiores ao PZQ. Os resultados obtidos indicam que TC possui potencial aplicação como fármaco esquistossomicida, enquanto o CP pode representar uma importante estratégia adjuvante para a proteção hepática em casos graves de esquistossomose. Em conjunto, os dados reforçam a importância do desenvolvimento de terapias combinadas ou complementares, capazes de atuar simultaneamente sobre *S. mansoni* e sobre os danos induzidos no hospedeiro, contribuindo para um tratamento mais eficaz e abrangente da esquistossomose.

Palavras-chave: *Schistosoma mansoni*; chalconas; vitamina D; antifibrótico; células estreladas hepáticas.

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ABSTRACT

Schistosomiasis is a parasitic disease caused by trematodes of the genus *Schistosoma*. The adult forms of the parasite live in the portal and mesenteric blood vessels of humans, while intermediate forms develop in gastropod mollusks. The acute phase is often asymptomatic, sometimes presenting, headache, and chills, progressing to the chronic phase which is highly morbid. The chronic phase is marked by the formation of multiple hepatic granulomas around *Schistosoma mansoni* eggs, resulting in fibrosis and portal hypertension. Treatment is based on praziquantel (PZQ), which only affects adult worms. Therefore, despite its effectiveness, PZQ has limitations in relation to juvenile forms and granulomatous responses, as well as presenting an imminent risk of resistant strains emerging. Given the magnitude of its prevalence, severity of clinical manifestations, and inefficiency of treatment against immature forms and chronic cases, it is necessary to search for new alternatives. In this context, the effects of *trans*-Chalcone (TC), a natural flavonoid with a broad spectrum of biological activity, and calcipotriol (CP), a synthetic analogue of vitamin D with antifibrotic properties, were evaluated in the experimental model of schistosomiasis. *In vitro* studies indicate that TC exhibits schistosomicidal activity, mainly against the juvenile stages of *S. mansoni*. Specifically, TC induces alterations in nitric oxide levels, mitochondrial membrane depolarization, integument damage, and cell membrane integrity loss, thereby impairing oviposition. In a murine model of schistosomiasis, TC treatment significantly reduced the parasite load and improved liver parameters, notably the morphology of the granulomas, which exhibited reduced volume and a modulated inflammatory profile. Furthermore, the compound exhibited low toxicity in healthy cells, reinforcing its potential as a safe and effective therapeutic option. CP, in turn, was evaluated for its antifibrotic effect in the experimental model. Although it did not directly affect the parasite load, CP reduced oxidative stress and the inflammatory infiltrate in the liver, resulting in a reduction in granulomas and collagen deposition. CP also demonstrated efficacy in modulating the oxidative profile of hepatic stellate cells (HSCs), exhibiting superior effects to those of PZQ and highlighting its potential for controlling complications associated with granuloma formation. The results indicate that TC has the potential to be used as a schistosomicidal drug, while CP could be an important strategy for protecting the liver in severe cases of schistosomiasis. Taken together, the data reinforces the importance of developing combined or complementary therapies capable of simultaneously targeting S. mansoni and the damage it induces in the host, leading to a more effective and comprehensive treatment of schistosomiasis.

Key-words: *Schistosoma mansoni*; chalcones; vitamin D; antifibrotic; hepatic stellate cells.

LISTA DE FIGURAS

LISTA DE ABREVIATURAS E SIGLAS

ABC	*ATP-binding cassette*
aHSC	*activated Hepatic Stellate Cells*
APCs	*antigen-presenting cells*
ARE	Elementos de resposta antioxidante
Arg-1	Arginase-1
ATPase	ATP difosfohidrolase
CaM	Calmodulina
Car	Cardamonina
Cav-1	Caveolina-1
CCl4	Tetracloreto de carbono
CP	Calcipotriol
CTGF	Fator de crescimento do tecido conjuntivo hepático
DALYs	*Disability-Adjusted Life-Years*
DBP	V*itamin D Binding Protein*
DTNs	Doenças Tropicais Negligenciadas
ECM	*Extracellular matrix*
ERN	Espécies reativas de nitrogênio
ERO	Espécies reativas de oxigênio
ES	Excretados/secretados
FcεRI	Receptor de alta afinidade para IgE
GAPDH	Gliceraldeído-3-P-desidrogenase
GPCR	*G-Protein-Coupled Receptors*
H_2O_2	Peróxido de hidrogênio
HSC	*Hepatic Stellate Cells*
ICAM-1	Molécula de adesão intercelular 1
IC_{50}	Concentração inibitória para 50% dos parasitas
IFN-γ	Interferon-gama
IgE	Imunoglobulina E
IL	Interleucina
IL-13Rα1	Cadeia alfa-1 do receptor de IL-13
IL-4Rα	Cadeia alfa do receptor de IL-4
ILC2s	*Innate Lymphoid Cells 2*

iNOS	Óxido nítrico sintase induzível
IPSE/alfa-1	*IL-4-inducing principle of S. mansoni eggs*/*alpha-1*
iTregs	Células T regulatórias induzíveis
KO	*knockout*
LicoA	Licochalcona A
LicoB	Licoflavona B
M2	Macrófagos alternativamente ativados
MDA	*Mass Drug Administration*
MRP	*Multidrug resistance-associated proteins*
mVDR	*membrane Vitamin D Receptor*
NF-κB	Fator nuclear-κB
NO	Óxido nítrico
Nrf2	Fator nuclear eritroide 2
nTregs	Células T regulatórias naturais
nVDR	*nuclear Vitamin D Receptor*
OH-1	Heme-oxigenase-1
OMS	Organização Mundial da Saúde
OXA	Oxamniquina
PG	Prostaglandinas
Pgp	Glicoproteína P
PZQ	Praziquantel
qHSC	*quiescente Hepatic Stellate Cells*
RXR	*Retinoid X Receptor*
SAC	*School-Aged Children*
SEA	*Soluble Egg Antigens*
SERCA	Retículo sarcoplasmático/endoplasmático
SmTALs	*Schistosoma mansoni–tegumental allergen-like*)
SOD	Superóxido dismutase
STAT6	*Signal transducer and activator of transcription 6*
TC	*Trans*-chalcona
Tfh	Células T auxiliares foliculares
TGF-β	*Transforming growth factor beta*
TGR	*Thioredoxin glutathione reductase*
Th1	*T-helper 1*

Th2	*T-helper 2*
TIMP-1	*Tissue inhibitor of metalloproteinase-1*
TLR4	*Toll-Like Receptors 4*
TNF-α	*Tumor necrosis factor alpha*
Tregs	T regulatórias
TRP	*Transient receptor potential*
UVB	Ultravioleta B
VCAM-1	Molécula de adesão celular vascular-1
VDR	*Vitamin D Receptor*
VDRE	*vitamin D response element*
VOOC	*Voltage-Operated Ca^{2+} Channels*
α-SMA	*α-smooth muscle actin*
ω -1	Ômega 1

SUMÁRIO

1 INTRODUÇÃO

1.1 ESQUISTOSSOMOSE: ASPECTOS GERAIS

A esquistossomose, também conhecida como bilharzíase, doença do caramujo ou barriga d'água, foi primeiramente descrita por Theodor Bilharz em 1851, sendo denominado o termo *Schistosoma* (*Schisto*: fenda; *soma*: corpo) em 1858 (Jordan, 2000; Steinmann *et al.*, 2006; Carvalho; Coelho; Lenzi, 2008). A doença é causada por trematódeos do filo Platyhelminthes, classe Trematoda, subclasse Digenea, ordem Strigeiformes, família Schistosomatidae e gênero *Schistosoma*. Destacam-se três principais espécies: *Schistosoma mansoni*, *Schistosoma japonicum* e *Schistosoma haematobium*, e três outras espécies de menor distribuição geográfica: *Schistosoma intercalatum*, *Schistosoma mekongi* e *Schistosoma guineensis* (McManus *et al.*, 2018).

De maneira geral, pode resultar em formas intestinais (*S. mansoni*, *S. japonicum*) ou urogenitais (*S. haematobium*); bem como em formas aguda, causando manifestações inespecíficas como febre, dor de cabeça, calafrios e mialgia; e crônica, resultando em dor abdominal, diarreia, fibrose periportal, hipertensão portal e ascite (Colley *et al.*, 2014; McManus *et al.*, 2018). Em vista disso, a natureza crônica e debilitante da doença gera elevados custos para os sistemas de saúde pública e contribui para perdas significativas de produtividade, especialmente em países em desenvolvimento, afetando o crescimento e desenvolvimento cognitivo de crianças em idade escolar, e aumentando a vulnerabilidade social (Ezeamama *et al.*, 2012, 2018)

Como resultado, a esquistossomose ocupa a segunda posição entre as doenças parasitárias em termos de morbidade e mortalidade, depois da malária, sendo considerada uma Doença Tropical Negligenciada (DTN) (Gryseels, 2012; Ogongo *et al.*, 2022; Steinmann *et al.*, 2006). As DTNs compreendem um grupo de agravos de etiologia infecciosa ou decorrentes de acidentes por animais peçonhentos, das quais prevalecem em regiões tropicais e subtropicais, e são caracterizadas pelo subfinanciamento e baixo reconhecimento pelas indústrias farmacêuticas. Afetam predominantemente populações de baixa renda, frequentemente expostas a condições precárias de moradia, saneamento inadequado e acesso limitado aos serviços de saúde. Assim, a escassez de recursos destinados ao tratamento, controle e eliminação, aliada a fatores socioeconômicos e ambientais, têm contribuído

significativamente para a manutenção e disseminação dessas doenças (Hotez *et al.*, 2014; Magalhães *et al.*, 2023; WHO, 2025a).

1.2 Aspectos epidemiológicos

A transmissão da esquistossomose está associada ao contato com corpos d'água, sendo favorecida por atividades como pesca, irrigação, lavagem de roupas e contato direto da água com pés descalços (Reitzug; Ledien; Chami, 2023). Assim, fatores como alta umidade, temperaturas elevadas e a presença de hospedeiros intermediários são determinantes para sua propagação (Stensgaard *et al.*, 2013). Inicialmente restrita a áreas rurais, a esquistossomose tem se expandido para zonas urbanas em decorrência da intensa migração e urbanização, sendo agravada pela precariedade do saneamento básico (Barbosa *et al.*, 2023; Gomes *et al.*, 2012, 2022; Oliveira *et al.*, 2013).

Segundo a Organização Mundial da Saúde (OMS), a transmissão da doença já foi relatada em 78 países de regiões tropicais e subtropicais, com necessidade de quimioterapia preventiva em 51 países endêmicos. Em 2021, cerca de 250 milhões de pessoas precisaram de tratamento preventivo (WHO, 2023). Em estudo avaliando dados da *Global Burden of Disease* no período de 1990 a 2021 em 204 países, a doença foi responsável por aproximadamente 1,75 milhão de anos de vida ajustados por incapacidade (DALYs, do inglês: *Disability-Adjusted Life Years*), resultando em cerca de 12 mil mortes. Adicionalmente, os indivíduos mais afetados pertenciam à faixa etária de 15 a 24 anos, sendo semelhante entre homens e mulheres, com maior incidência na África, Ásia e Américas (Li *et al.*, 2025, no entanto, essa taxa pode ser subestimada devido a doenças associadas como insuficiência hepática e renal, e câncer de bexiga (Duarte *et al.*, 2014; Santos *et al.*, 2021).

No Brasil, os casos autóctones de esquistossomose são causados por *S. mansoni*, ocorrendo principalmente na faixa litorânea do Nordeste. O país abriga três espécies de moluscos do gênero *Biomphalaria*: *Biomphalaria glabrata*, *Biomphalaria tenagophila* e *Biomphalaria straminea* (Brasil, 2024). Desta forma, a distribuição desses vetores está diretamente relacionada aos locais de incidência da doença no país (Scholte *et al.*, 2012).

Segundo dados obtidos pelo Inquérito Nacional de Prevalência da Esquistossomose mansoni e Geo-helmintoses do Ministério da Saúde, estima-se que

1,5 milhão de pessoas foram infectadas no Brasil entre 2010 e 2015 (Katz, 2018). Em complementar, Martins-Melo et al. (2014, 2018) e Brandão et al. (2017) avaliaram que dentre as infecções e mortes registradas no país por DTNs, a esquistossomose é considerada a segunda principal causa, atrás apenas da doença de Chagas (Brandão *et al.*, 2017; Martins-Melo *et al.*, 2018, 2014). Entre 2003 e 2018, a esquistossomose foi relacionada em 11.487 óbitos (0,06%), com taxa média de mortalidade de 0,38 óbitos/100.000 habitantes, afetando principalmente idosos e moradores da região Nordeste do país (Pinheiro *et al.*, 2020). No entanto, Andrade e colaboradores (2024) relataram que a pandemia da COVID-19 impactou diretamente o Programa de Controle da Esquistossomose no país nos anos de 2020 e 2021, com redução da população examinada e do número de testes coproparasitológicos realizados, bem como dos casos positivos detectados e dos indivíduos tratados (Andrade *et al.*, 2024).

Apesar da redução na mortalidade e morbidade nos últimos anos, a doença ainda representa um importante causa de morte no Brasil, com diferenças regionais consideráveis (Martins-Melo *et al.*, 2014; Verjee, 2019). Além disso, cabe destacar que, mesmo em regiões com baixa carga parasitária, formas clínicas graves persistem, comprometendo o estado geral de saúde, e afetando o crescimento e desenvolvimento físico dos indivíduos afetados, com impactos no desenvolvimento humano e social (Ferreira *et al.*, 2017; McManus *et al.*, 2018). Assim, o controle da esquistossomose ainda exige esforços contínuos em prevenção, diagnóstico precoce e tratamento eficaz, a fim de minimizar os impactos da doença.

1.3 Agente etiológico e Ciclo biológico

O ciclo de vida é heteroxênico, envolvendo um hospedeiro definitivo (humanos, roedores e mamíferos alternativos), onde formas adultas se desenvolvem e reproduzem de maneira sexuada; e um hospedeiro intermediário (caramujos aquáticos), no qual formas larvais se reproduzem assexuadamente (Douchet *et al.*, 2023). Os hospedeiros intermediários pertencem ao filo Mollusca, classe Gastropoda, família Planorbidae e gêneros *Biomphalaria* (*S. mansoni*), *Bulinus* (*S. haematobium*) ou *Oncomelania* (*S. japonicum*) (Carvalho; Coelho; Lenzi, 2008), dos quais estão presentes em rios, lagoas, cursos d'água e outros ambientes de água doce, demonstrando elevado impacto epidemiológico (Douchet *et al.*, 2023).

Quanto ao ciclo de *S. mansoni*, hospedeiros definitivos previamente

infectados liberam ovos em estágios de processo de maturação junto ao material fecal (Jurberg *et al.*, 2009). Os ovos (150 x 65 µm) possuem um espículo lateral e quando atingem fontes de água doce, fatores como água, oxigênio, pH e temperatura ambiente elevada (± 28 ºC), estimulam sua eclosão, liberando os miracídios. Os miracídios (160 x 60 µm), por sua vez, consistem em larvas ciliadas e apresentam uma papila apical na região anterior (*terebratorium*), responsável pela adesão e penetração no hospedeiro intermediário (Carvalho; Coelho; Lenzi, 2008). Assim, através de mecanismos quimiotáticos, os miracídios detectam moléculas dos caramujos e os infectam. Após 72 horas de penetração, dão origem a esporocistos primários e secundários através de reprodução assexuada, resultando na formação de formas cercarianas. As cercárias emergem dos caramujos após 30 dias de infecção. Estima-se que cada miracídio resulta na liberação de 300 mil cercárias (Carvalho; Coelho; Lenzi, 2008; Schwartz; Fallon, 2018).

As cercárias (500 µm) apresentam cauda bifurcada e duas ventosas: oral (anterior) e ventral (acetábulo), especializadas na fixação e invasão no hospedeiro definitivo (Dorsey *et al.*, 2002). Uma vez liberadas no ambiente aquático, as cercárias apresentam fototropismo positivo, direcionando-se à superfície de águas rasas, bem como seguem um gradiente térmico, maximizando seu contato humano. Assim, ao entrarem em contato com a pele humana (geralmente regiões interdigitais dos pés, pernas, ou mesmo mucosa oral), respondem a sinais químicos, particularmente ácidos graxos livres de cadeia média (ácido linoleico), como um sinal para invasão na pele (Gryseels *et al.*, 2006; Martins Silva, 2016; McKerrow; Salter, 2002).

Inicialmente, as cercárias secretam uma substância espessa semelhante a muco proveniente do complexo da glândula acetabular, bem como aderem-se via ventosa, realizando o processo de fixação à superfície da pele (McKerrow; Salter, 2002). Após a fixação, as cercárias perdem a cauda e iniciam o processo de penetração com auxílio de movimentos vibratórios e proteases secretadas das glândulas acetabulares, como a elastase cercariana, que degrada componentes proteicos epidérmicos e dérmicos, como elastina, laminina, fibronectina, colágeno tipo IV e queratina, rompendo o contato célula-célula (Hambrook; Hanington, 2023; Ingram *et al.*, 2012; Mowafy; Abdel-Hafeez, 2015). Após a penetração, que ocorre em minutos, algumas cercárias podem permanecer na derme por horas ou dias, onde sofrem alterações morfológicas e bioquímicas e diferenciam-se em esquistossômulos (400 µm), que atingem os vasos sanguíneos e linfáticos (He; Chen;

Ramaswamy, 2002; McKerrow; Salter, 2002).

Como resultado, a mudança do ambiente aquático para intravascular envolve perda da cauda e do glicocálice de superfície, bem como desenvolvimento de uma membrana hepta laminada e mudança para respiração anaeróbica. Assim, os esquistossômulos exibem foto-orientação negativa e respondem a gradientes químicos, como L-arginina e peptídeos do soro e células endoteliais, e após 4-5 dias atingem os pulmões via vasos sanguíneos, onde permanecem por 2-3 dias, e então migram para os vasos portais do fígado, onde continuam o processo de crescimento e diferenciação, alcançando por volta de 21 dias o estágio juvenil. Nessa fase, o tegumento já apresenta a organização típica em camada sincicial, com espinhos tegumentares proeminentes e intensa atividade metabólica, incluindo secreção de glicocálix e expressão de moléculas associadas à evasão da resposta imune do hospedeiro. A completa maturação ocorre ao redor de 35–42 dias, quando os parasitos atingem a fase adulta e iniciam a oviposição (Carvalho; Coelho; Lenzi, 2008; Grabe; Haas, 2004; Skelly; Alan Wilson, 2006).

Os vermes adultos (1-2 cm x 0,3-0,6 mm) ingerem grandes quantidades de eritrócitos e macromoléculas como albumina, dextrana, imunoglobulinas e fosfolipídios (Barrett, 2009; McManus *et al.*, 2018; Skelly *et al.*, 2014). Apresentam duas ventosas: oral, utilizada para alimentação e excreção, e ventral (acetábulo) usada para fixação nos vasos sanguíneos. Apresentam dimorfismo sexual, com vermes adultos machos apresentando um canal ginecóforo para acomodar a fêmea durante a cópula, além de lobos testiculares e tubérculos (protuberâncias no tegumento), enquanto vermes fêmeas são mais escuras devido à síntese de hemozoína, delgadas, apresentam ventosas reduzidas e possuem a superfície do corpo lisa com poucos espinhos (Carvalho; Coelho; Lenzi, 2008). O pareamento dos vermes adultos é essencial para a maturidade sexual, uma vez que, evidências moleculares indicam que a atividade gênica nas glândulas vitelínicas das fêmeas depende de sinais provenientes dos machos (Biolchini *et al.*, 2006).

O tegumento dos vermes adultos consiste em uma estrutura única, essencial na interação com o hospedeiro, importante para adaptação na corrente sanguínea. Esta estrutura é composta por duas bicamadas lipídicas: uma membrana plasmática interna e um membranocélice externo, resultante da fusão de vesículas multilaminadas, no qual permite uma renovação constante do tegumento (Van Hellemond *et al.*, 2006), e contribui para a evasão imunológica, inibindo o sistema

complemento, além de participar da nutrição, excreção, sinalização e osmorregulação (Jones *et al.*, 2004; Mulvenna *et al.*, 2010). Em vista disso, a dinâmica do tegumento, bem como as estratégias de evasão complexas, permite a longa permanência dos vermes adultos no hospedeiro humano (Kusel; Al-Adhami; Doenhoff, 2007; Pearce; MacDonald, 2002). Assim, estudos topográficos do tegumento são essenciais para entender danos induzidos por drogas (Amin; Mikhail, 1989; Dantas *et al.*, 2024).

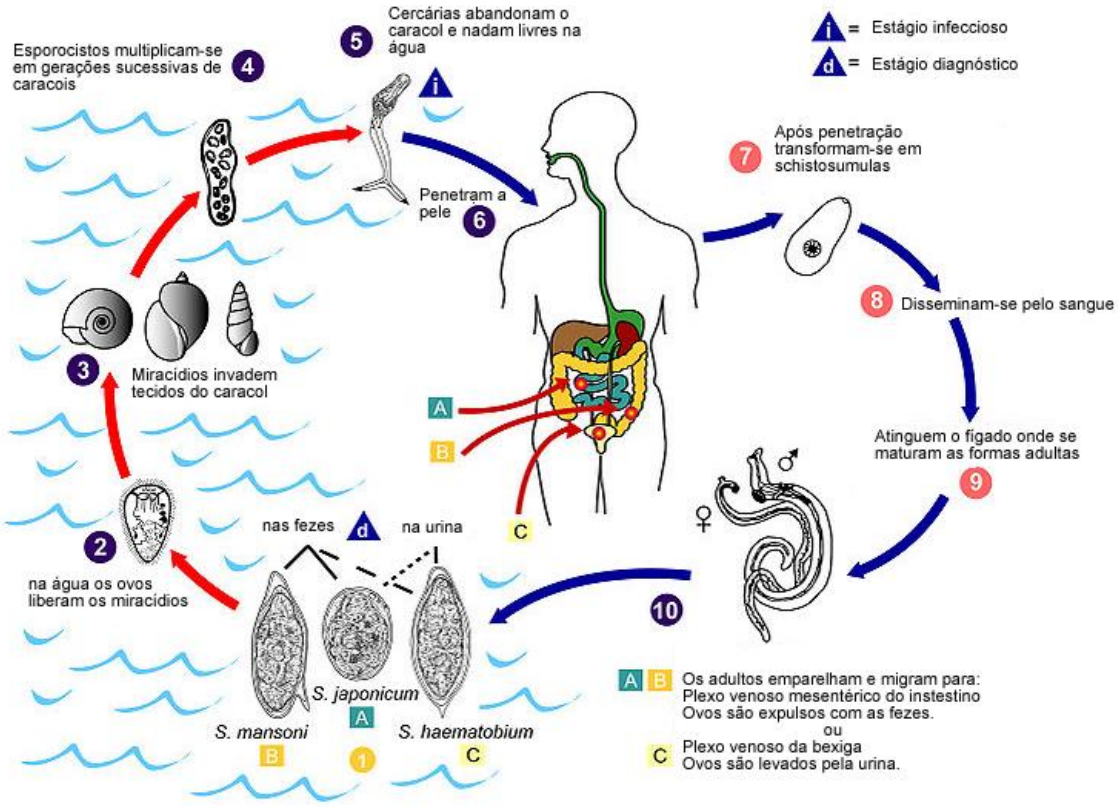
Assim, após maturação, vermes adultos machos de *S. mansoni* albergam vermes fêmeas em seu canal ginecóforo, e acasalados migram contra a corrente circulatória, alojando-se em vasos mesentéricos e hemorroidários, próximos ao intestino, onde permanecem em média de 3 a 10 anos (Carvalho; Coelho; Lenzi, 2008). A migração dos vermes adultos pode ser controlada por sinais provenientes do hospedeiro, como por exemplo, hormônios ou produtos absorvidos através da digestão. Além disso, tendem a se localizar em regiões com baixo estresse mecânico e alta vascularização, favorecendo a deposição dos ovos e evitando que sejam levados pela corrente sanguínea (Costain; MacDonald; Smits, 2018).

Uma vez nos vasos mesentéricos, as fêmeas são capazes de produzir cerca de 300 ovos por dia, dos quais depositam-se no endotélio dos vasos mesentéricos e podem ser disseminados através do fluxo sanguíneo para outros órgãos ou translocados para o lúmen do intestino. Um dos fatores que favorecem o contato ovo-endotélio, facilitando sua passagem, são as fortes contrações musculares exercidas no poro genital de vermes fêmeas (dorsiflexão) e a pressão exercida pelos ovos depositados próximos ao endotélio (bombeamento) (Neves, 2011; Yeh; del Álamo; Caffrey, 2024). Além disso, os ovos secretam uma série de proteínas, como enzimas metabólicas enolase e gliceraldeído-3-P-desidrogenase (GAPDH), das quais atuam como receptores de superfície, induzindo a ativação de plasminogênio e geração de plasmina, aumentando a atividade fibrinolítica e promovendo a degradação da matriz extracelular e recrutamento de monócitos (Schwartz; Fallon, 2018). Assim, uma vez em contato com o endotélio, os ovos se prendem às moléculas de adesão da superfície endotelial, incluindo molécula de adesão intercelular 1 (ICAM-1), molécula de adesão celular vascular-1 (VCAM-1) e E-selectina, induzindo uma resposta inflamatória granulomatosa, no entanto, com deposição mínima de colágeno, promovendo sua passagem através do endotélio e deposição na luz do intestino (Costain; MacDonald; Smits, 2018; Schwartz; Fallon, 2018).

Cerca de 50 a 60% dos ovos permanecem na mucosa intestinal,

enquanto os demais são direcionados a tecidos como intestino, fígado e baço, a favor do fluxo sanguíneo, onde atuam como estímulo antigênico contínuo, induzindo inflamação e formação de granulomas (Chuah *et al.*, 2014; Pearce; MacDonald, 2002; Schwartz; Fallon, 2018) (**Figura 1**).

Figura 1. Ciclo biológico de *Schistosoma* spp.



Fonte: Adaptado de (CDC, 2019). Ovos de *Schistosoma* spp. são primeiramente eliminados juntamente com fezes ou urina (1). Em condições apropriadas os ovos sofrem eclosão e liberam os miracídios (2). Os miracídios penetram em hospedeiros intermediários específicos (3), onde sofrem sucessivas divisões, produzindo milhares de esporocistos (4) e liberando as cercárias (5). As cercárias infectantes penetram na pele do hospedeiro humano (6), perdendo a cauda bifurcada e diferenciando-se em esquistossômulos (6). Os esquistossômulos migram através da circulação venosa para os pulmões (8) e então atingem o sistema porta-hepático, onde sofrem maturação (8). Vermes adultos machos e fêmeas copulam e migram para vênulas mesentéricas (*S. mansoni e S. japonicum*) ou plexo venoso vesicular e pélvico da bexiga (*S. haematobium*) (10), onde iniciam a deposição de ovos que são eliminados com as fezes ou urina, respectivamente.

1.4 IMUNOPATOGÊNESE

Como resultado da coevolução com o hospedeiro humano, *S. mansoni* apresenta notável adaptação, com tempo de vida estimado em 5 a 10 anos nestes hospedeiros (Crellen *et al.*, 2016; Webster; Gower; Blair, 2004). Essa

adaptação, combinada com a forma evolutiva do parasito que o hospedeiro abriga, as taxas de infecção e exposição, bem como a resposta imune induzida, influenciam diretamente nas manifestações clínicas e evolução da doença (Pearce; MacDonald, 2002). A progressão clínica da infecção e o perfil de resposta imune ocorrem ao longo das fases aguda e crônica. No entanto, alguns autores estabelecem a presença de quatro estágios imunológicos: (i) fase inicial da resposta imune pré-patente (pré-postural); (ii) fase tardia da resposta imune pré-patente; (iii) fase ativa estabelecida da infecção aguda; e (iv) fase crônica (Gobbi *et al.*, 2020; McManus *et al.*, 2018).

Na fase inicial, a penetração ativa das cercárias através da epiderme desencadeia resposta imune local (Rogers *et al.*, 2024). Assim, os queratinócitos, como primeiras células de contato, secretam citocinas pró-inflamatórias, como interleucina (IL) -6, IL-12, IL-1β, fator de necrose tumoral alfa (TNF-α, do inglês: *tumor necrosis factor alpha*) e interferon-gama (IFN-γ), promovendo uma resposta inflamatória local, com infiltrado dérmico e recrutamento de células apresentadoras de antígenos (APCs, do inglês: *antigen-presenting cells*), como macrófagos e células dendríticas, incluindo as células de Langerhans (Hogg; Kumkate; Mountford, 2003; Mountford; Trottein, 2004). À medida que as cercárias atravessam a epiderme, membrana basal e derme, alcançando a circulação sanguínea e os vasos linfáticos, parte das larvas não sobrevive ao processo e permanece nos tecidos superficiais, desencadeando uma reação de hipersensibilidade imediata, mediada por imunoglobulina E (IgE). Essa reação resulta na liberação de mediadores inflamatórios, como histamina, culminando no desenvolvimento de lesões cutâneas maculopapulares e prurido intenso nas áreas expostas, manifestações conhecidas como dermatite cercariana ou "coceira do nadador" (Martins Silva, 2016; Pleass; Kusel; Woof, 2000).

Durante a penetração e diferenciação das cercárias em esquistossômulos ocorre a liberação de produtos excretados/secretados (ES), como a elastase cercariana, que facilita a remodelação da matriz extracelular e penetração na pele, bem como regula a resposta inflamatória local (Leontovyč *et al.*, 2020; Liu *et al.*, 2015; Sanin; Mountford, 2015). Esses produtos ativam queratinócitos, que por sua vez, produzem prostaglandinas (PG) E_2 e D_2 e induzem a liberação de IL-10, que inibe a expressão de citocinas pró-inflamatórias e moléculas coestimulatórias, além de atuar como potente vasodilatador, facilitando o acesso do parasito à circulação (Angeles; Mercado; Rivera, 2020; He; Chen; Ramaswamy, 2002; Mountford; Trottein,

2004).

Durante a migração dos esquistossômulos e sua maturação em vermes adultos, a infecção pode ser assintomática ou evoluir para um quadro agudo sintomático conhecido como síndrome de Katayama, caracterizado por febre debilitante, cefaleia, fadiga, dor abdominal, diarreia e perda de peso (Hams; Aviello; Fallon, 2013; Ross *et al.*, 2007). Achados também indicam manifestações pulmonares, com nódulos e áreas de vidro fosco observadas em exames radiológicos, associadas à presença de ovos nesse tecido (Gryseels *et al.*, 2006). Nesta fase, ocorre uma reação de hipersensibilidade sistêmica no hospedeiro, associada a altas concentrações de eosinófilos que expressam receptores de alta afinidade para IgE (FcεRI), induzindo efeitos citotóxicos sobre os esquistossômulos. Essa resposta afeta principalmente indivíduos não previamente expostos ao parasito, como viajantes não imunes ou aqueles que não desenvolveram uma resposta imunológica eficaz na primeira exposição (Gobbi *et al.*, 2020; McManus *et al.*, 2020). Nesse período, a resposta imune é predominantemente do tipo T-helper 1 (Th1), com alta produção de IL-1, IL-2, IL-6, IL-12, IFN-γ e TNF-α, atingindo seu pico entre a 3ª e 5ª semanas após infecção (Egesa *et al.*, 2018; Pearce; MacDonald, 2002; Zheng *et al.*, 2020).

Com o desenvolvimento dos vermes adultos, estes frequentemente não estimulam inflamação local, uma vez que possuem células-tronco somáticas que permitem uma regeneração imediata do tegumento e consequente ligação aos antígenos do hospedeiro, ocultando suas moléculas de superfície e inibindo uma resposta imunológica efetiva, não havendo sintomas de forma direta (Colley; Secor, 2014). Vermes adultos também podem proteger o hospedeiro de novas infecções, através da imunidade concomitante tardia ou imunidade contra o estágio larval (Buck; De Leo; Sokolow, 2020). Dessa forma, uma vez que vermes adultos produzem um conjunto de proteínas e moléculas imunomoduladoras, estas podem direcionar a resposta imune contra formas larvais, favorecendo a longa permanência no hospedeiro, reduzindo a competição intraespecífica e limitando a carga de vermes (Brown; Grenfell, 2001; Buck; De Leo; Sokolow, 2020).

Proteínas do tegumento, como as SmTALs (do inglês, *Schistosoma mansoni–tegumental allergen-like*) são capazes de induzir aumento nos níveis de IgE (SmTAL-IgE), no qual está negativamente associada à intensidade de reinfecção, sugerindo um papel na imunidade concomitante (Oettle; Wilson, 2017). Portanto, em áreas endêmicas, a imunidade concomitante contribui para a estabilidade da carga

infectante ao longo da vida do hospedeiro, ao mesmo tempo em que limita reinfecções significativas e possibilita a manutenção crônica do parasita no hospedeiro (Buck; De Leo; Sokolow, 2020).

Assim, a partir da 5ª a 6ª semana pós-infecção, inicia-se a fase postural e os ovos depositados tornam-se maduros, apresentando desenvolvimento miracidial completo e formação do envelope de von Lichtenberg, camada altamente ativa, rica em retículo endoplasmático rugoso, considerada a principal fonte de secreções imunogênicas (Ashton *et al.*, 2001; Meevissen; Yazdanbakhsh; Hokke, 2012; Takaki *et al.*, 2021). Assim, os antígenos solúveis excretados/secretados pelos ovos (SEA, do inglês: *soluble egg antigens*) durante essa fase são cruciais na transição da resposta Th1 para o tipo T-helper 2 (Th2) (Ashton *et al.*, 2001; Meevissen *et al.*, 2010; Zheng *et al.*, 2020).

Entre os principais componentes do SEA destacam-se as glicoproteínas: ômega 1 (ω -1), com atividade ribonuclease T2, que se liga ao receptor de manose, uma lectina do tipo C expressa por macrófagos e células dendríticas, levando à polarização dessas células para um fenótipo indutor de células Th2 (Everts *et al.*, 2009; Steinfelder *et al.*, 2009); e IPSE/alfa-1 (do inglês: *IL-4-inducing principle of S. mansoni eggs*/*alpha-1*), capaz de induzir a produção de IL-4 em basófilos dependente de IgE (Schramm *et al.*, 2006). Como resultado, esses antígenos direcionam a resposta imune para o perfil Th2, estimulando a produção de IL-4, IL-5, IL-10, IL-13 e fator de crescimento transformador beta (TGF-β, do inglês: *transforming growth factor beta*) e redução de IFN-γ (Everts *et al.*, 2009; Fahel *et al.*, 2010; Zheng *et al.*, 2020).

As citocinas IL-4 e IL-13 desempenham um papel central na resposta Th2, compartilhando os mesmos complexos de ligação: cadeia alfa-1 do receptor de IL-13 (IL-13Rα1) e cadeia alfa do receptor de IL-4 (IL-4Rα). A ativação desses receptores leva à sinalização via transdutor de sinal e ativador de transcrição 6 (STAT6, do inglês: *signal transducer and activator of transcription 6*), induzindo deposição de colágeno e formação de granuloma (Wilson *et al.*, 2007). Sabe-se que em camundongos *knockout* (KO) para IL-4Rα, há formação de granulomas menores (Jankovic *et al.*, 1999), no entanto, sua deficiência durante o estágio agudo da infecção, diminui respostas Th2 e leva a doença grave e prematura (Nono *et al.*, 2017). Além disso, a ausência de IL-4 e IL-10 leva a respostas Th1 exacerbadas, resultando em 100% de mortalidade na doença aguda (Hoffmann; Cheever; Wynn, 2000). Assim,

a sinalização via IL-4/IL-4Rα é crucial para prolongar a sobrevivência do hospedeiro durante a esquistossomose aguda e reduzir a inflamação do tecido (Ndlovu; Brombacher, 2014; Schwartz *et al.*, 2014).

A IL-13 também desempenha um papel crucial na formação do granuloma e desenvolvimento da fibrose hepática. De maneira geral, a produção de IL-13 depende da ligação de IL-33 ao supressor do receptor de tumorigenicidade (ST2), expresso células residentes e infiltrantes do fígado. Assim, a ligação de IL-33/ST2 em células linfoides inatas tipo 2 (ILC2s, do inglês: *innate lymphoid cells* 2) e eosinófilos ativa a produção de IL-13 (Maggi *et al.*, 2023). Consequentemente, a ligação de IL-13 ao seu receptor IL-13Rα induz a expressão de colágeno tipo I e III, metaloproteinases de matriz, IL-6 e TGF-β (Liu *et al.*, 2011). A via de ativação IL-33/ST2 também estimula a expressão de moléculas de adesão em células endoteliais, promovendo a infiltração e ativação de células T $CD4^+$ e eosinófilos, além de ativar células residentes, como fibroblastos (Maggi *et al.*, 2023). IL-4 e IL-13 também induzem a expressão de arginase-1 em macrófagos alternativamente ativados (M2). A arginase-1, por sua vez, usa L-arginina como substrato para produzir L-ornitina, que é convertida em prolina pela ornitina-aminotransferase. A prolina é um aminoácido essencial envolvido na produção de colágeno e, portanto, no desenvolvimento de fibrose (Licá *et al.*, 2023; Pearce; MacDonald, 2002).

Assim, a resposta Th2 durante a esquistossomose atua como um efeito duplo, uma vez que a resposta granulomatosa pode ser considerada prejudicial ao fígado devido à progressão da fibrose hepática, no entanto, a formação do granuloma permite controlar a secreção contínua de antígenos secretados pelos ovos, que podem levar a respostas inflamatórias descontroladas e danos permanentes aos tecidos (Zheng *et al.*, 2020).

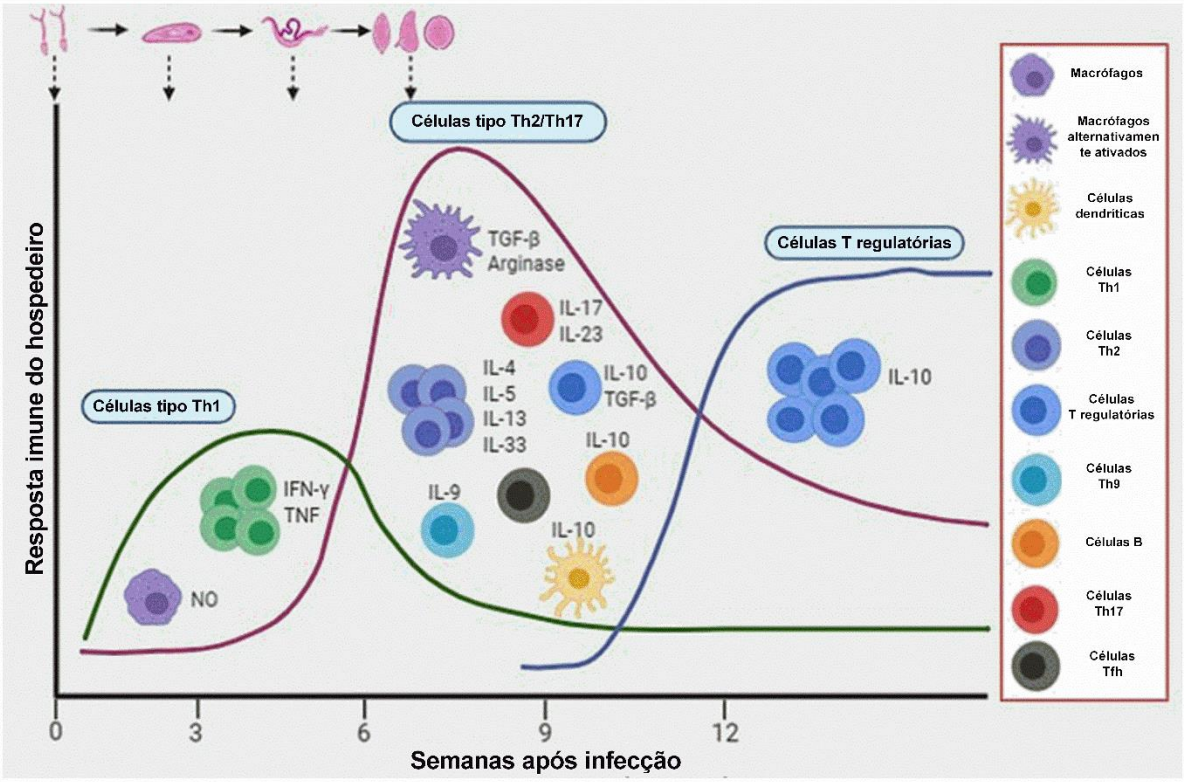
A resposta mediada por Th17 também tem se mostrado relevante, uma vez que, IL-23 derivada de células dendríticas estimuladas por SEA é capaz de induzir diferenciação de células Th17 e a produção de IL-17 em camundongos (Shainheit *et al.*, 2008), e sua neutralização (anti-IL-17) mostrou ser capaz de inibir inflamação granulomatosa (Rutitzky; Lopes da Rosa; Stadecker, 2005; Zhang *et al.*, 2012). As células T auxiliares foliculares (Tfh) também estão envolvidas na resposta imune, promovendo a produção de IL-21 em centros germinativos e contribuindo para a progressão da fibrose hepática (Wang *et al.*, 2017). Zhan et al. (2017) também

avaliaram a participação de respostas pertencentes ao perfil Th9, identificando elevados níveis de IL-9 em camundongos infectados, consistente com a inflamação granulomatosa hepática (Zhan *et al.*, 2017).

Por fim, células T regulatórias (Tregs) desempenham papel modulador importante na fase crônica da esquistossomose. Uma vez que, entre a 8ª e 16ª semana pós-infecção, SEA e moléculas provenientes de esquistossomos induzem potente geração de Tregs naturais (nTregs) no granuloma, mediando respostas Th2 e inibindo o desenvolvimento do granuloma e a progressão da fibrose, promovendo um estado de imunossupressão (Baumgart *et al.*, 2006; Turner *et al.*, 2011). As Tregs induzíveis (iTregs) também possuem um papel importante através da secreção de IL-10, que suprime a produção de IL-12 e consequentemente, o desenvolvimento de respostas Th1, além de levar à inibição da proliferação de células T $CD4^+$, demostrando que ambas as respostas comandadas por nTregs e iTregs são capazes de regular respostas Th1 e Th2 (Hesse *et al.*, 2004; Masamba; Kappo, 2021; Zheng *et al.*, 2020).

Em vista disso, na fase crônica tardia, com predomínio de células Tregs, há uma redução no número de vermes e consequente redução no número de ovos excretados nas fezes ou depositados no tecido. Ao mesmo tempo, os granulomas reduzem em tamanho, demonstrando menor infiltrado inflamatório e substituição por tecido fibroso (cicatrização) (McManus *et al.*, 2018). Em indivíduos que desenvolvem a esquistossomose hepatoesplênica grave, a fibrose do trato portal resulta em lesões vasculares obstrutivas, hipertensão portal, ascite, desenvolvimento de varizes esofagogástricas, hemorragia e hepatomegalia (Hams; Aviello; Fallon, 2013).

Figura 2. Perfil de resposta imune durante a infecção por *Schistosoma mansoni*.



Fonte: Adaptado de Molehin (2020). Nas primeiras semanas de infecção por cercárias de *Schistosoma mansoni*, predomina-se uma resposta do tipo Th1, caracterizada pela produção de IFN-γ, TNF e óxido nítrico, atuando no controle inicial das formas imaturas do parasita. Com o início da postura de ovos, há transição para uma resposta predominantemente Th2 e Th17, marcada pela produção de IL-4, IL-5, IL-13, IL-33, IL-17, IL-23, IL-9 e IL-10, bem como pela ativação de macrófagos alternativamente ativados (M2) produtores de arginase e TGF-β. Essa fase promove a formação de granulomas e deposição de colágeno, contribuindo tanto para o isolamento dos ovos quanto para o desenvolvimento de fibrose hepática. A partir de oito semanas, intensifica-se a ação das células T regulatórias, com produção de IL-10, modulando e suprimindo respostas inflamatórias exacerbadas, o que reduz o tamanho dos granulomas e limita o dano tecidual, favorecendo o estabelecimento da fase crônica da doença. Abreviações: NO, óxido nítrico; IFN-γ, interferon-γ; TNF, fator de necrose tumoral; TGF-β, fator de crescimento transformador-β; Tfh, células T auxiliares foliculares.

1.4.1 Granuloma hepático

Na esquistossomose, com a eliminação de ovos por casais de vermes adultos resulta em sua deposição na mucosa intestinal ou em órgãos como fígado, baço e intestino (*S. mansoni* e *S. japonicum*) ou na bexiga e sistema urogenital (*S. haematobium*). Esses ovos desencadeiam intensa estimulação antigênica e resposta inflamatória, culminando na formação do granuloma esquistossomótico (McManus *et*

al., 2018). No geral, o granuloma é uma estrutura organizada composta principalmente por fagócitos mononucleares maduros, podendo ou não apresentar demais características como necrose, infiltrado de linfócitos, macrófagos, células plasmáticas e componentes de matriz extracelular. Sua formação ocorre como mecanismo de defesa frente a agentes infecciosos ou corpos estranhos que não podem ser fragmentados, degradados ou eliminados pelo organismo (Pagán; Ramakrishnan, 2018).

Os granulomas induzidos por *S. mansoni* são estruturas formadas, principalmente, por linfócitos T e B, eosinófilos, macrófagos, células estreladas hepáticas (HSC, do inglês: *hepatic stellate cells*) e fibroblastos (Malta *et al.*, 2021, 2022). Embora atuem como um mecanismo de proteção ao minimizar os danos causados pelos produtos liberados pelos ovos, a inflamação e deposição de colágeno resultam em lesões teciduais e perda de função (Hams; Aviello; Fallon, 2013). No fígado, o principal sítio de formação do granuloma é circundando ovos alojados nos capilares pré-sinusoidais do tecido. Como resultado, esses granulomas dão origem a fibrose periportal, levando a interrupção do fluxo sanguíneo, hipertensão portal, hepatoesplenomegalia, ascite, formação de varizes esofágicas e sangramento gastrointestinal, contribuindo para a morbidade (Burke *et al.*, 2009). No intestino, a deposição de ovos causa uma resposta granulomatosa da mucosa, levando a pseudopolipose, microulceração e sangramento superficial (Gryseels *et al.*, 2006; Ross *et al.*, 2002).

Os granulomas esquistossomóticos apresentam variações morfológicas, com diferentes tamanhos e composições celulares, dependendo de seus estágios de desenvolvimento. Estudos histopatológicos documentam dois estágios principais: pré-granulomatoso, com início da organização celular ao redor do ovo, e granulomatoso, altamente organizado e caracterizado por fases progressivas (Amaral *et al.*, 2017). De maneira geral, são identificadas 3 regiões principais: a região central com predomínio de macrófagos, a camada média composta principalmente por fibras arranjadas concentricamente, e a camada periférica, onde observa-se uma riqueza celular e fibras reticulares frouxamente dispostas (Amaral *et al.*, 2017; Lenzi *et al.*, 1998; Schwartz; Fallon, 2018).

As células estreladas hepáticas (HSC, do inglês: *hepatic stellate cells*), também conhecidas como célula de Ito, são consideradas o principal tipo celular responsável pela deposição de colágeno e formação do granuloma (Carson *et al.*,

2018). As HSC consistem em células mesenquimais, localizadas no espaço de Disse no sinusoide hepático, constituindo 5-8% de todas as células deste tecido (Geerts, 2001). No fígado normal, as HSC estão em estado quiescentes (qHSC), das quais armazenam cerca de 80% da vitamina A total do corpo e são responsáveis pela manutenção da matriz extracelular (ECM, do inglês: *extracellular matrix*). Após estimulação, são transfiderenciadas em miofibroblastos proliferativos, fibrogênicos e contráteis, tornando-se células ativadas (aHSC), que perdem a capacidade de armazenar vitamina A e aumentam a expressão de α-actina de músculo liso (α-SMA, do inglês: *α-smooth muscle actin*) e genes pró-fibróticos. As aHSC são capazes de produzir colágeno, atuam como células apresentadoras de antígeno (APCs), e induzem expressão de citocinas, quimiocinas e espécies reativas de oxigênio (ERO) (Bataller; Brenner, 2005; Carson *et al.*, 2018).

De maneira geral, as HSC podem ser ativadas em resposta à lesão celular e estímulos derivados de hepatócitos danificados, células de Kupffer, células endoteliais ou células inflamatórias. Dentre os fatores de ativação estão ERO, espécies reativas de nitrogênio, além de produtos de peroxidação lipídica (Bataller; Brenner, 2005; Friedman, 2008; Guimarães *et al.*, 2006). Sabe-se que o peróxido de hidrogênio (H_2O_2) pode atuar como um mediador sinalizador, potencializando a ativação de TGF-β e aumentando a transcrição de colágeno (*Col1α1*). Além disso, o aumento de ERO juntamente com a depleção de antioxidantes contribui para a proliferação de HSC e síntese de colágeno (Urtasun; Nieto, 2007). Em vista disso, a maior expressão TGF-β1, α-SMA, colágeno I e III, bem como a síntese excessiva de ECM e sua menor degradação são os principais componentes que promovem a formação de fibrose (Weiskirchen; Tacke, 2014).

Na esquistossomose, fatores como TGF-β1 e IL-33 estão entre os principais mediadores envolvidos na ativação de HSCs. O TGF-β1 pode ser produzido pelas próprias HSCs, atuando de forma autócrina nesse processo. Essas células expressam em sua superfície os receptores TβRI e TβRII. Assim, após a ligação do TGF-β1 ao receptor TβRII, ocorre o recrutamento de TβRI, que promove a fosforilação das proteínas Smad2 e Smad3. As formas fosforiladas dessas proteínas associam-se à Smad4, formando um complexo oligomérico (p-Smad2/p-Smad3/Smad4) que é translocado para o núcleo. No núcleo, esse complexo interage com promotores transcricionais e cofatores, regulando a expressão de genes-alvo associados à proliferação e à ativação das HSCs (Carson *et al.*, 2018). O fator de crescimento do

tecido conjuntivo hepático (CTGF) também é capaz de ativar HSC através de vias de sinalização MAPK e fator nuclear-κB (NF-κB), induzindo proliferação, migração e expressão de α-SMA e colágenos tipo I e IV. A regulação positiva de CTGF, por sua vez, é controlada por TGF-β1 e IL-33 (Carson *et al.*, 2018).

Quanto a IL-33, seu papel está envolvido na ativação de ILC2s. Assim, durante a infecção, hepatócitos expressam IL-33, que ativam ILC2s e produzem IL-13 (Mchedlidze *et al.*, 2013; Price *et al.*, 2010). A atividade pró-fibrótica de IL-13 pode ser dependente de TGF-β1, induzindo sua expressão em vários tipos celulares, como HSC e macrófagos, sendo regulada positivamente durante a infecção (Lee *et al.*, 2001). No entanto essa atividade pode também ser independente de TGF-β1, levando a regulação positiva de CTGF, regulando a expressão de colágeno tipos I e III, metaloproteinase de matriz 2 e 9 (MMP-2 e -9) e inibidor tecidual de metaloproteinase 1 (TIMP-1, do inglês: *tissue inhibitor of metalloproteinase* 1), mesmo em camundongos deficientes para TGF-β1 (Liu *et al.*, 2011). Assim, o mecanismo de fibrose hepática na esquistossomose pode ser impulsionado principalmente por um equilíbrio de IL-33 e IL-13 em vez de TGF-β1 (Borthwick; Wynn, 2015).

As aHSC estão presentes em maior número em fígados murinos e humanos infectados por *S. mansoni*, quando comparados a fígados saudáveis (Chang *et al.*, 2006). Evidências indicam que reverter aHSC ao estado quiescente ou induzir sua apoptose pode contribuir para a regressão da fibrose hepática (Lemoinne *et al.*, 2013; Zhou *et al.*, 2022). Em vista disso, o cultivo de HSC tem sido fundamental para o avanço na compreensão de seu papel após lesão hepática. Linhagens de HSC imortalizadas foram desenvolvidas, das quais recapitulam algumas características do fenótipo ativado *in vitro*. Entre essas, destaca-se a linhagem celular GRX, derivada de granulomas fibróticos de camundongos C3H/HeN infectado por *S. mansoni* (Borojevic *et al.*, 1985). As células GRX correspondem à primeira linhagem celular que representa aHSC, das quais apresentam fenótipo proliferativo, morfologia de miofibroblastos, e expressam proteínas da matriz extracelular, como colágeno tipo I, III e IV, vimentina e fibronectina. Além disso, expressam α-SMA, indicando que são derivadas de aHSC, Caveolina-1 (Cav-1), α-tubulina, E-caderina, IL-6 e TGF-β1 (Bitencourt *et al.*, 2012; Bragança De Moraes *et al.*, 2012; Schröder *et al.*, 2022). Assim, modelos como a linhagem GRX mostram-se fundamentais na investigação dos mecanismos que regulam a fibrogênese hepática e na identificação de novas terapias para a fibrose associada à esquistossomose.

1.5 TRATAMENTO

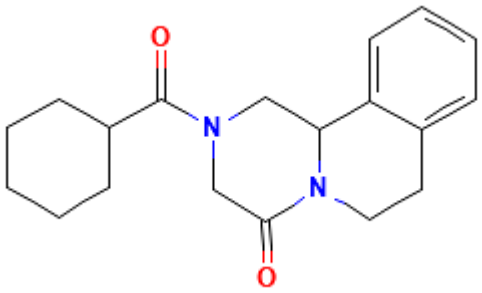
O controle da esquistossomose tem se baseado na administração em massa de medicamentos (MDA, do inglês: *mass drug administration*) em regiões endêmicas, com o objetivo de reduzir a morbidade e transmissão da doença (Toor *et al.*, 2018). Dentre os medicamentos utilizados, o praziquantel (PZQ) é o principal agente terapêutico. No entanto, sua cobertura através de MDA ainda está entre as mais baixas entre as infecções helmínticas (Anderson *et al.*, 2015).

Os programas de MDA priorizam o tratamento comunitário, especialmente de crianças em idade escolar (do inglês: SAC, *school-aged children*, 5-14 anos), embora adultos jovens (25-30 anos) também representem uma parcela significativa dos infectados. Dessa forma, restringir o tratamento apenas às SAC permite que uma fração considerável da carga de infecção por *S. mansoni* permaneça sem tratamento (Anderson *et al.*, 2016). A efetividade da MDA também depende de fatores como: cobertura populacional, frequência das intervenções e variabilidade genética dos parasitos (McDonald; Linde, 2002; Spielman *et al.*, 2004). Assim, embora a MDA tenha reduzido a morbidade e prevalência da esquistossomose em diversas áreas, a transmissão persiste em algumas regiões. Isso ocorre porque a eliminação do parasito não induz imunidade adquirida, tornando os indivíduos tratados suscetíveis a reinfecções (Kittur *et al.*, 2017; Pennance *et al.*, 2016).

1.5.1 Praziquantel

O praziquantel (PZQ) (2-(ciclohexilcarbonil)-1,2,3,6,7,11-b-hexahidro-4H-pirazino(2,1-a)isoquinolina-4-ona) consiste um derivado da pirazino-isoquinolina (**Figura 3**), utilizado no tratamento da esquistossomose desde 1970 (Gönnert; Andrews, 1977). Desde então, tem sido o único medicamento de escolha para a doença, devido à sua baixa toxicidade, fácil administração, baixo custo e alta eficácia contra estágios adultos de todas as espécies de *Schistosoma* (Fukushige *et al.*, 2021; Spangenberg, 2021). O PZQ é comercializado como uma mistura racêmica dos isômeros: (*R*)-PZQ e (*S*)-PZQ (Andrews, 1985). O enantiômero *R*(−) exibe atividade anti-*Schistosoma in vitro* e *in vitro*, enquanto o isômero *S*(+) é associado a efeitos colaterais e ao sabor amargo do medicamento, com pouca ou nenhuma atividade anti-helmíntica (Nogueira *et al.*, 2022).

Figura 3. Estrutura química do Praziquantel.



Fonte: PubChem (2004).

Quanto ao mecanismo de ação, sabe-se que o PZQ é capaz de alterar a homeostase do cálcio em vermes adultos ao interagir e modular canais de Ca^{2+} ativados por voltagem (VOOC, do inglês: *voltage-operated* Ca^{2+} *channels*). Especificamente, PZQ interage com subunidades β presente em VOOC, localizado na membrana do parasito, promovendo influxo de cálcio e contração muscular, tornando-o seletivo para os parasitos, sem afetar significativamente células de mamíferos (Cioli *et al.*, 2014; Kohn *et al.*, 2001; Xiao; Mei; Jiao, 2009). No entanto, quando os níveis intracelulares de Ca^{2+} aumentam, este liga-se à calmodulina (CaM). A ativação de CaM leva a ativação de CaMKII, que, por sua vez, ativa a via de sinalização do NF-κB. Essa cascata resulta na ativação da cálcio-ATPase do retículo sarcoplasmático/endoplasmático (SERCA), promovendo o sequestro de Ca^{2+} no interior do retículo e reduzindo a concentração citosólica de cálcio. Além disso, essa via também induz a expressão da glicoproteína P (Pgp), facilitando o efluxo do PZQ e promovendo mecanismos de resistência (Abou-El-Naga, 2025; Hirst; Lawton; Walker, 2022). Adicionalmente, CaMKII também atua como um efetor a jusante, sendo utilizada pelo TGF-β para promover a proliferação de HSC e a ativação da via AKT (An *et al.*, 2012). Assim, após exposição ao PZQ, CaMKII pode apresentar maior ativação e favorecer a proliferação de HSC (You *et al.*, 2013).

Outros alvos também podem estar envolvidos no mecanismo de ação do PZQ, uma vez que, pode interagir com a cadeia leve reguladora da miosina (Gnanasekar *et al.*, 2008), glutationa S-transferase (McTigue; Williams; Tainer, 1995),

receptor de adenosina (Angelucci *et al.*, 2007), canais de potencial do receptor transitório (TRP, do inglês: *transient receptor potential*) (Bais; Greenberg, 2018) e receptor acoplado à proteína G 5-HT_{2B} (do inglês: *5-Hydroxytryptamine receptor 2B*) humano, que medeia funções fisiológicas da serotonina, promovendo aumento da atividade motora do esquistossomo (Chan *et al.*, 2017). Assim, após tratamento com PZQ, o tegumento de vermes adultos sofre vacuolização e formação de bolhas, mas a mesma alteração não ocorre em formas mais juvenis (Xiao; Mei; Jiao, 2009).

Apesar de sua eficácia, o uso prolongado tem resultado no surgimento de cepas menos sensíveis, representando um desafio no controle da doença. Os transportadores de cassete de ligação ao ATP (ABC, do inglês: *ATP-binding cassette*) são proteínas que usam a energia gerada pela hidrólise de ATP para translocar compostos, como toxinas e xenobióticos, através da membrana. Assim, uma série de homólogos de proteínas de transporte ABC são reconhecidos em *S. mansoni*, semelhantes à Pgp e às proteínas associadas à resistência a múltiplos fármacos (MRP, do inglês: *multidrug resistance-associated proteins*), facilitando o efluxo de PZQ (Greenberg, 2013; Kasinathan; Greenberg, 2012). Cepas menos sensíveis podem apresentar alterações na composição do tegumento e nível aumentado de Pgp, comprometendo a eficácia do PZQ (Messerli *et al.*, 2009; Pinto-Almeida *et al.*, 2016). Em modelo animal, tratamentos repetidos com PZQ apresentaram um amadurecimento dos vermes adultos, que por sua vez, reduziram a sensibilidade ao medicamento, efeito que parece ser amplificado durante as gerações das formas adultas (Pica-Mattoccia *et al.*, 2009).

Em relação aos vermes juvenis (3-4 semanas após infecção) de *S. mansoni*, foi avaliado que estes são menos sensíveis ao PZQ, demonstrando uma expressão significativamente maior de SMDR2 (Pgp) e SmMRP1 quando comparado a vermes adultos (7-8 semanas após infecção), contribuindo para a sensibilidade reduzida desse estágio evolutivo ao PZQ (Kasinathan; Morgan; Greenberg, 2010). A ineficácia contra os estágios juvenis permite a sobrevivência dos parasitos mesmo após o tratamento, comprometendo a cura e contribuindo para o aumento da morbidade nos indivíduos afetados (Ross; Olveda; Li, 2015).

Além disso, o PZQ possui baixa solubilidade, alta permeabilidade e rápida metabolização (via hidroxilação), resultando em baixa concentração em contato com o verme (Caffrey, 2015). A mistura racêmica de dois estereoisômeros do PZQ inclui um que é esquistossomicida e outro que contribui para os efeitos adversos,

como o sabor desagradável. Assim, dos 600 mg do comprimido, apenas 40 mg são eficazes contra os esquistossomos, o que dificulta a administração, especialmente para crianças (Meyer *et al.*, 2009; Wu *et al.*, 1991). Os efeitos colaterais mais comuns incluem dor de cabeça, náusea, anorexia, vômito, dor abdominal, diarreia, fadiga, mialgia e tontura, frequentemente associados à intensidade da infecção, sendo resultado da morte dos vermes e liberação de antígenos e metabólitos (Cioli *et al.*, 2014).

Apesar da eficácia do PZQ contra todas as espécies de esquistossomos, poucas intervenções eficazes estão disponíveis para tratar a fibrose hepática e hipertensão portal associada à esquistossomose. Assim, essa condição é frequentemente negligenciada, e muitos pacientes são submetidos a cirurgia para remoção de baços ou fígado aumentados, decorrente do processo fibrótico, evitando o risco de morte (Andersson; Chung, 2007; Parsons; Takashima; Rippe, 2007). Dessa forma, o desenvolvimento de terapias eficazes para a fibrose hepática continua sendo uma necessidade urgente, e novos medicamentos devem ser prontamente aplicados na prática clínica.

1.6 AVALIAÇÃO DE NOVOS COMPOSTOS ESQUISTOSSOMICIDAS

Como supracitado, o PZQ apresenta limitações farmacêuticas e farmacológicas, com baixa estabilidade metabólica, eficácia reduzida em dose única e sabor desagradável, dificultando o tratamento de crianças, consideradas população-alvo (Caffrey; Secor, 2011; Mduluza; Mutapi, 2017). O PZQ também não apresenta efeito contra formas juvenis e durante a inflamação granulomatosa. Além disso, estudos clínicos no Egito, África Subsaariana e Brasil, relataram taxas de curas inferiores às esperadas, reforçando a necessidade de novas alternativas terapêuticas (Caffrey *et al.*, 2019; Ismail *et al.*, 1999; Stelma *et al.*, 1995).

Diante disso é estabelecido um perfil ideal para novos candidatos terapêuticos, como: eficácia contra formas juvenis e múltiplas espécies, maior potência em doses menores que o PZQ, ação sobre alvos moleculares distintos e baixo risco de resistência cruzada (Caffrey, 2007, 2015). Outras propriedades desejáveis, envolvem ampla margem de segurança, incluindo em crianças pequenas e mulheres grávidas e lactantes, baixo risco de interações medicamentosas, estabilidade e custo acessível para populações vulneráveis (Caffrey *et al.*, 2019).

Em vista disso, uma das estratégias na pesquisa de novos medicamentos consiste na avaliação de produtos naturais. As substâncias ativas dos produtos naturais e seus efeitos farmacológicos independentes têm atraído muita atenção, uma vez que, plantas medicinais tradicionais são altamente diversificadas e muitos de seus metabólitos demonstram ter efeitos terapêuticos contra vermes adultos e na fibrose hepática (Azevedo *et al.*, 2023; Liu *et al.*, 2024). Tais derivados de plantas são geralmente obtidos de folhas, cascas, caules ou raízes e podem incluir alcaloides, compostos fenólicos, flavonoides e terpenoides (Eze; Ogugofor; Ossai, 2022). Sabe-se que cerca de 60% das moléculas antiparasitárias usadas entre 1981 e 2014 originaram-se de produtos naturais (Adegboye *et al.*, 2021). Além disso, em países endêmicos para esquistossomose, os efeitos anti-*Schistosoma* de produtos naturais ou extratos de plantas têm sido extensivamente estudados na tentativa de descobrir medicamentos alternativos de baixo custo (Mølgaard *et al.*, 2001).

Outra estratégia é o reposicionamento de fármacos, no qual identifica novos usos terapêuticos para medicamentos já existentes eliminando riscos, custos e tempo associados ao desenvolvimento de novos produtos, também amplamente empregado para o tratamento de doenças negligenciadas (Ramamoorthi; Graef; Dent, 2015). Entre 2000 e 2011, apenas quatro dos 29 fármacos aprovados para DTN eram novos, os demais consistem em formulações já existentes para o tratamento de outras doenças (Pedrique *et al.*, 2013). Em vista disso, dado o longo tempo necessário para o desenvolvimento de um novo fármaco, a alta taxa de falha de medicamentos em estágio clínico, a escassez de fármacos em desenvolvimento para esquistossomose, bem como o potencial surgimento de resistência ao PZQ e o impacto contínuo da esquistossomose na saúde pública, o reposicionamento se apresenta como uma abordagem promissora (Dickson; Gagnon, 2004). Idealmente, a busca por alternativas capazes de reduzir a carga de ovos nos tecidos e/ou modular a resposta imune é necessária, para favorecer um prognóstico benigno ao hospedeiro e minimizar as manifestações clínicas mais severas da esquistossomose, ampliando a eficácia terapêutica (Gouveia *et al.*, 2018).

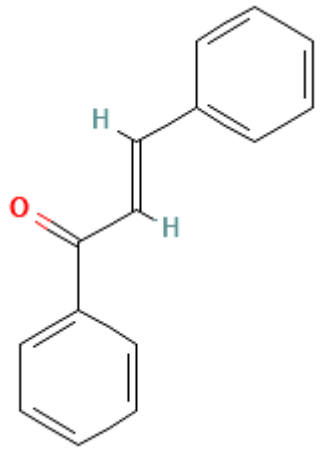
1.6.1 Trans-*chalcona*

As chalconas consistem em cetonas aromáticas, consideradas precursores biogenéticos de flavonoides e isoflavonoides, naturalmente encontradas

em diversas partes das plantas (Rudrapal *et al.*, 2021). A biossíntese ocorre a partir do aminoácido L-fenilalanina, que é convertido em p-cumaril-CoA. Esse intermediário reage com três moléculas de malonil-CoA, em uma reação catalisada pela enzima chalcona sintase, culminando na formação do esqueleto estrutural das chalconas (Dao; Linthorst; Verpoorte, 2011). Assim, as chalconas podem ser convertidas em auronas, originando pigmentos amarelos que colorem as flores, ou em naringenina, um precursor chave na biossíntese dos flavonoides, além de desempenharem papel em vias de sinalização celular processos de simbiose (Falcone Ferreyra; Rius; Casati, 2012).

O núcleo da chalcona é composto por dois anéis aromáticos (A e B), ligados por um sistema α, β-insaturado carbonílico com três carbonos, derivado de 1,3-difenil-2-propen-1-ona (**Figura 4**). Estereoquimicamente, a chalcona pode existir tanto em isômeros *E* (*trans*) ou *Z* (*cis*), sendo o isômero *E* (*trans*-chalcona, TC) o mais estável termodinamicamente devido à menor repulsão estérica entre os substituintes da ligação dupla (Aksöz; Ertan, 2011). Além disso, a chalcona pode adotar diferentes conformações em torno da ligação simples adjacente à carbonila, conhecidas como *s-cis* e *s-trans*, que influenciam suas propriedades espectroscópicas e reatividade. A estrutura das chalconas contendo os anéis aromáticos e a sistema carbonílico α, β-insaturado em conjugação contínua, contribui para seu baixo potencial redox, estabilidade química, e capacidade de realizar reações de transferência de elétrons, características fundamentais para suas notáveis atividades biológicas (Aksöz; Ertan, 2011; Rudrapal *et al.*, 2021).

Figura 4. Representação estrutural da *trans*-Chalcona.



Fonte: PubChem (2004).

As chalconas e seus derivados são amplamente reconhecidos por suas propriedades antioxidantes atuando na modulação da atividade de enzimas como superóxido dismutase (SOD), catalase e glutationa peroxidase. Esse efeito pode estar associado à ativação do fator nuclear eritroide 2 (Nrf2) e sua ligação aos elementos de resposta antioxidante (ARE), via Nrf2-ARE, no qual regula positivamente a expressão dessas enzimas (Villa; Heckman; Bandyopadhyay, 2024). Em estudo realizado por Martinez e colaboradores (2017), em modelo de inflamação cutânea por irradiação ultravioleta em camundongos *hairless*, tanto a administração sistêmica e formulação tópica de *trans*-Chalcona (TC) aumentaram a expressão de Nrf2 e heme-oxigenase-1 (OH-1), evidenciando seus efeitos anti-inflamatórios e antioxidantes (Martinez *et al.*, 2017). Além disso, as chalconas podem inibir a produção de ERO interferindo em enzimas como NADPH oxidases e xantina oxidases, e possuem propriedades quelantes de metais, prevenindo a geração de ERO catalisada por metais (Villa; Heckman; Bandyopadhyay, 2024). Essas atividades estão relacionadas às suas propriedades físico-químicas, presença do sistema carbonil α, β-insaturado, além do tipo e natureza dos substituintes nos anéis aromáticos (Mittal; Vashistha; Das, 2022).

No modelo de esquistossomose, chalconas naturais e sintéticas demonstraram efeitos promissores. A cardamonina (Car), chalcona isolada de *Piper aduncum* L., induziu 100% de mortalidade de vermes adultos de *S. mansoni in vitro* (100 µM; 24 h), promoveu alterações tegumentares e interferiu na oviposição a *in vitro*. Car também reduziu a atividade da ATP difosfohidrolase (ATPase), resultando em

concentração inibitória para 50% dos parasitas (IC_{50}) de 23,54 µM, interagindo com SmATPDase 1 (De Castro *et al.*, 2015), enzima presente no tegumento, envolvida na evasão imunológica e regulação de respostas inflamatórias (Fonseca *et al.*, 2012). De forma semelhante, a Licoflavona B (LicoB), chalcona isolada de *Glycyrrhiza inflata,* apresentou efeitos semelhantes a 50 µM, sem ser tóxica para células Vero, além de inibir as atividades ATPase e ADPase (IC_{50} de 23,78 e 31,51, respectivamente) (Carvalho *et al.*, 2015).

Licochalcona A (LicoA), também isolada de *G. inflata*, mostrou IC_{50} de 9,12 e 9,52 µM (fêmeas e machos), promovendo separação dos casais e redução da oviposição, sem causar toxicidade significativa em células CHO-K1 (CC_{50} = 508,3 µM) (Souza *et al.*, 2017). LicoA também induziu a formação de vacúolos no tegumento, lise focal do tecido intersticial e da camada muscular, tumefação e degeneração das mitocôndrias, acúmulo de gotículas lipídicas, bem como condensação da cromatina e degeneração de células vitelínicas. Adicionalmente, levou ao aumento nos níveis de ânion superóxido e diminuição da atividade da enzima SOD (Felicissimo *et al.*, 2021; Souza *et al.*, 2017). Nanopartículas lipídicas sólidas contendo LicoA causaram 100% de mortalidade com intenso dano no tegumento e ruptura dos tubérculos nos de vermes. *In vitro*, o tratamento oral (2,5 mg/kg) e intraperitoneal (5 mg/kg) reduziu a carga parasitária (Silva *et al.*, 2021).

Chalconas sintéticas também se destacaram. Pereira et al. (2018) avaliaram que 7 de 15 compostos causaram 100% de mortalidade *in vitro* em vermes adultos a 100 µM, e 2 permaneceram ativas a 25 µM, causando redução dos tubérculos. Em macrófagos peritoneais foi avaliada toxicidade somente em altas concentrações (> 150 µM). Em modelo murino de esquistossomose, as duas chalconas mais ativas também reduziram a carga parasitária em dose única (400 mg/kg) administrada oralmente no dia 49 pós infecção. *In silico,* os autores demonstraram que as chalconas foram capazes de inibir a atividade ATPase, com interação para SmATPDase 1. Morais e colaboradores (2021), por sua vez, avaliaram pirazolinas derivadas de chalconas sintéticas, das quais um foi mais ativo contra vermes adultos (IC_{50} = 6,2 µM), com elevado índice de seletividade (IS) (21,6 e 32,2 para Vero e SH-SY5Y, respectivamente) (Morais *et al.*, 2021).

Marcovicz et al. (2022) investigaram que chalconas bromo e nitro reduziram a motilidade e oviposição em vermes adultos a 25 µg/mL, bem como causaram alteração dos tubérculos e espinhos, rigidez da ventosa acetabular,

formação de bolhas no tegumento, e alterações no esôfago, intestino e zona do ovário (Marcovicz *et al.*, 2022). Adicionalmente, auronas derivadas de chalconas também causaram 100% de mortalidade em vermes adultos, além de danos tegumentares, com ruptura de tubérculos. Usando uma dose oral única (400 mg/kg), as auronas foram capazes de diminuir a carga parasitária e a eliminação de ovos nas fezes (Pereira *et al.*, 2021).

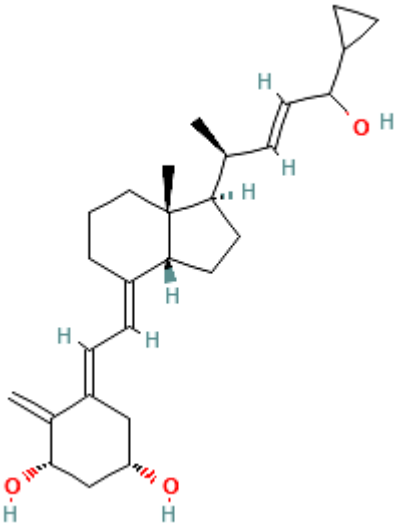
Uma série de estudos também têm avaliado o efeito das chalconas, especialmente a *trans*-Chalcona, em modelos de fibrose e inflamação hepática. Singh et al. (2016) observaram que a administração de TC (5, 10 e 20 mg/kg) por 14 dias no modelo de inflamação e fibrose hepática induzida por tetracloreto de carbono (CCl_4) e paracetamol reduziu a variação do peso corporal e o peso relativo do fígado. TC também atenuou os níveis séricos das enzimas aspartato aminotransferase (AST), alanina aminotransferase (ALT), fosfatase alcalina (ALP) e bilirrubina, o conteúdo de colágeno e de TGF-β1, semelhante ao tratamento com silimarina. O tratamento reduziu os níveis de substâncias reativas ao ácido tiobarbitúrico (TBARS), nitritos/nitratos e TNF-α, e aumentou glutationa reduzida (GSH). Por fim, quando avaliada as alterações histopatológicas, TC foi capaz de reverter a necrose hepática (Singh *et al.*, 2016). Em complementar, Karkhaneh et al. (2016) relataram que o tratamento TC (12 e 24 mg/kg) por 6 semanas em ratos alimentados com dieta rica em colesterol reduziu enzimas hepáticas (AST, ALP e ALT) e os níveis de malondialdeído, e aumentou SOD e GSH, não sendo observada fibrose ou esteatose hepática nos animais (Karkhaneh *et al.*, 2016).

Diversos estudos também têm destacado a ampla gama de atividades farmacológicas da TC, incluindo propriedades antimicrobianas (Bitencourt *et al.*, 2019), anti-inflamatórias (Javadi *et al.*, 2023; Karimi-Sales; Mohaddes; Alipour, 2018; Martinez *et al.*, 2017; Staurengo-Ferrari *et al.*, 2018), neuroprotetoras (Dhakal *et al.*, 2021), anticancerígenas (Komoto *et al.*, 2021; Senrung *et al.*, 2023; Siqueira *et al.*, 2021), bem como antiparasitárias contra espécies dos gêneros *Babesia*, *Theileria* (Batiha *et al.*, 2019) e *Leishmania* (Miranda-Sapla *et al.*, 2019; Piñero *et al.*, 2006). Diante disso, buscamos avaliar o efeito da TC no modelo experimental de esquistossomose mansônica.

1.6.2 Calcipotriol

O calcipotriol (CP), também conhecido como calcipotrieno, consiste um análogo sintético da vitamina D3, derivado de 1,25 dihidroxyvitamina D3 ($1,25(OH)_2D3$), diferenciado pela presença de uma ligação dupla e um anel de ciclopropano na cadeia lateral, características que o tornam um dos derivados mais ativos da vitamina D, com ação biológica similar, porém com menor efeito sobre o metabolismo do cálcio (Guilhou, 2001) (**Figura 5**). Descrito pela primeira vez 1988, o CP demonstrou capacidade de inibir a proliferação de queratinócitos *in vitro* e efeito hipercalcêmico reduzido *in vitro*, tornando-o clinicamente aprovado para o tratamento tópico de psoríase crônica (Guilhou, 1998; Kragballe, 1995; Scott; Dunn; Goa, 2001).

Figura 5. Representação estrutural do calcipotriol.



Fonte: PubChem (2004).

A vitamina D (VD) é um hormônio esteroide lipossolúvel, que desempenha um papel essencial na homeostase do cálcio e do fósforo, além de exercer múltiplas funções em diversos tecidos do organismo (Bikle, 2014). Sua produção ocorre a partir de um processo fotobiológico na pele, onde a radiação ultravioleta B (UVB) induz a conversão de um precursor, o 7-desidroxicolesterol, em pré-vitamina D_3 que, por isomerização térmica, é transformada em vitamina D_3 (colecalciferol). A vitamina D_3 liga-se à proteína ligadora de vitamina D (DBP, do inglês: *vitamin D binding protein*) e é transportada para o fígado, onde sofre

hidroxilação e é convertida em 25-hidroxivitamina D_3 ($25(OH)D_3$, calcidiol). Nos rins, $25(OH)D_3$ passa por uma segunda hidroxilação, formando 1,25-diidroxivitamina D_3 ($1,25(OH)_2D_3$, calcitriol), considerada a forma biologicamente ativa da vitamina D (Bikle, 2014; Christakos *et al.*, 2016).

Os efeitos biológicos da VD são mediados através do receptor de vitamina D (VDR, do inglês: *vitamin D receptor*), que pode ser classificado em: 1) receptor de membrana (mVDR) e 2) receptor nuclear (nVDR) (Bouillon *et al.*, 2008). O mVDR regula a homeostase mineral óssea por meio da sinalização da reabsorção de cálcio e fosfato. Em contraste, o nVDR consiste em um fator de transcrição nuclear que influencia na expressão de genes associados à proliferação e diferenciação celular, além de regular respostas imuno-inflamatórias e fibrose. Após a ligação da VD ou de seus análogos ao nVDR, ocorre o recrutamento do receptor retinoide X (RXR, do inglês: *retinoid X receptor*), resultando na formação do complexo VDR-RXR, que se liga ao elemento de resposta à vitamina D (VDRE, do inglês: *vitamin D response element*) na região promotora de genes-alvo, modulando sua transcrição (Bakke; Sun, 2018).

O VDR é expresso em células não parenquimatosas do fígado, incluindo células estreladas hepáticas (Gascon-Barré *et al.*, 2003). Nesse contexto, estudos demonstraram que análogos de vitamina D3, bem como CP, são capazes de inibir a ativação e proliferação de células HSC (LX-2), levando a apoptose (Gong *et al.*, 2022). De fato, Ding e colaboradores (2013) avaliaram que em HSC primárias e células LX-2, o tratamento com CP atenuou a ativação induzida por TGF-β1, reprimindo genes associados à fibrogênese. Em complementar, os autores também avaliaram a interação entre os fatores de transcrição de VDR e Smad3. Assim, uma vez que ocorre lesão hepática, TGF-β1 ativa as HSC resultando na translocação de Smad3 para o núcleo, levando a uma remodelação da cromatina e aumento da acessibilidade a VDREs. Assim, uma vez que os VDREs tornam-se mais acessíveis ao VDR, este se liga a essas regiões genômicas que antes estavam "ocultas" ou inativas, atuando como um antagonista da atividade do Smad3, reduzindo sua atividade nessas regiões do DNA e suprimindo a expressão de genes pró-fibróticos (Ding *et al.*, 2013). De fato, a sinalização do TGF-β produz uma redistribuição nos sítios de ligação do VDR em todo o genoma (cistroma do VDR) em HSC, facilitando a ligação do VDR em genes-alvo dependentes de Smad3 envolvidos na fibrogênese. Devido a esse mecanismo, os ligantes do VDR inibem a ativação de HSC induzida

por TGF-β e anulam a fibrogênese hepática (Dewidar *et al.*, 2019).

Adicionalmente, em modelo de fibrose hepática induzida por CCl_4 em camundongos C57BL/6J, o tratamento com CP (20 µg/kg, gavagem oral) durante 4 semanas (cinco vezes/semana) reduziu significativamente a expressão de genes fibróticos (*Col1α1* e *Tgfb1*) e não alterou a concentração sérica de cálcio. O pré-tratamento com CP também anulou a resposta fibrogênica, indicando seu potencial preventivo. No entanto, em camundongos deficientes para VDR, houve perda do controle da resposta inflamatória local, além da desregulação da fibrogênese, sugerindo um papel direto do VDR (Ding *et al.*, 2013). Em complementar, em modelo de fibrose hepática induzida com tioacetamida, camundongos que receberam CP intraperitoneal nas doses de 20, 60 ou 80 µg/kg diariamente por 4 semanas demonstraram redução nos níveis de ALT, AST, ALP e malondialdeído, bem como no infiltrado inflamatório, necrose e na porcentagem de fibrose no tecido hepático, principalmente na maior dose 80 µg/kg. O tratamento com CP também reduziu a necroinflamação, a expressão de TIMP-1, bem como os níveis de fator de crescimento transformador TGF-β1 e de Col1α1. Através de imuno-histoquímica foi avaliada redução na expressão Smad2/3 e Smad1/5/9 (Wahsh *et al.*, 2016).

Sabe-se que em doenças hepáticas crônicas há uma insuficiência de vitamina D, independente da etiologia da doença (Arteh; Narra; Nair, 2010; Malham *et al.*, 2011). Dessa forma, em infecções agudas e crônicas por *S. mansoni e S. haematobium* foi avaliado que também há diminuição dos níveis séricos de 25-hidroxivitamina D, podendo ser considerado um alvo para marcador de esquistossomose (Noha; Aly; Mohamed, 2019). Corroborando com esses dados, Zhou et al. (2019, 2021) determinaram uma associação entre vitamina D e esquistossomose, sendo avaliado um desequilíbrio imunológico e progressão de danos hepáticos em pacientes com esquistossomose avançada (Zhou; Wu; Zhang, 2019; Zhou *et al.*, 2021). Chienwichai et al. (2024) também avaliaram que dois derivados de vitamina D (25-hidroxivitamina D2 e 1α-hidroxi-2β-(3-hidroxipropoxi) vitamina D3) foram biomarcadores potenciais para esquistossomose, demonstrando níveis reduzidos após 4 semanas de infecção (Chienwichai *et al.*, 2024).

Em modelo de esquistossomose, a administração de uma combinação de praziquantel e calcitriol (1 µg por dia oralmente por 5 dias consecutivos) em pacientes com infecção por *S. haematobium* levou a aumento nas respostas específicas de IgE e na porcentagem de vacuolização de eosinófilos

favorecendo uma reação do tipo Th2, no entanto, os níveis de proteína catiônica eosinofílica não foram alterados, indicando, paradoxalmente, uma resposta Th1. Assim, os autores concluíram que o calcitriol causa algum da resposta imunológica quando combinado com a terapia com PZQ em pacientes com esquistossomose (Snyman *et al.*, 1997). Adicionalmente, He e colaboradores (2018) demonstraram que camundongos infectados com *S. japonicum* e tratados com CP (20 µg/kg) via oral cinco vezes por semana, apresentaram redução significativa na fibrose comparado ao grupo controle, com diminuição do tamanho do granuloma e dos níveis de expressão de genes marcadores de fibrose (*Col1α1, Co31α1* e *α-SMA*) (He *et al.*, 2018). Assim, apesar desses achados promissores, até o momento não há estudos que avaliem os efeitos do CP em infecções por *S. mansoni*, tampouco sua atuação sobre células derivadas de granulomas esquistossomóticos.

2 JUSTIFICATIVA

A atual quimioterapia para a esquistossomose, baseada exclusivamente no PZQ, apresenta limitações significativas, como baixa atividade sobre formas juvenis e ausência de efeito antifibrótico. Além disso, muitos pacientes continuam a desenvolver complicações hepáticas crônicas, como inflamação persistente e fibrose hepática, mesmo após o tratamento antiparasitário. Considerando a relevância clínica da esquistossomose e o impacto das suas manifestações crônicas sobre a saúde pública, torna-se essencial a busca por estratégias terapêuticas que atuem na modulação da resposta inflamatória e fibrogênese.

Nesse contexto, o reposicionamento de moléculas com propriedades imunomoduladoras e antifibróticas tem se mostrado uma alternativa promissora. O calcipotriol, um análogo da vitamina D, tem se destacado por sua capacidade de regular negativamente a expressão de citocinas pró-inflamatórias, reduzir espécies reativas de oxigênio e atuar na modulação da ativação de células estreladas hepáticas. Seu potencial efeito benéfico em modelos de doenças hepáticas, incluindo fibrose, sugere que essa molécula possa ser útil como adjuvante no tratamento da esquistossomose, embora seus efeitos nessa doença ainda sejam pouco explorados.

Além disso, compostos naturais como a *trans*-Chalcona têm ganhado atenção por seus múltiplos efeitos farmacológicos, incluindo ação antiparasitária e antioxidante. Estudos recentes conduzidos em nosso laboratório demonstraram que a *trans*-Chalcona possui atividade contra parasitos do gênero *Leishmania*. Desta forma, prosseguimos com novos estudos, avaliando em formas juvenis e adultos de *Schistosoma mansoni*, além dos efeitos imunomoduladores que podem ser relevantes para o controle da inflamação e prevenção da fibrose associada à infecção crônica.

Esses achados nos motivaram a investigar os efeitos do calcipotriol e da *trans*-Chalcona em modelos experimentais de esquistossomose, com foco na modulação da resposta inflamatória, do estresse oxidativo, da fibrose hepática e na função hepática. O objetivo é contribuir para o desenvolvimento de estratégias terapêuticas mais eficazes, que associam o tratamento antiparasitário à proteção tecidual e prevenção das complicações crônicas da doença.

3 OBJETIVOS

3.1 OBJETIVOS GERAIS

Investigar a atividade antiparasitária da *trans*-Chalcona e seus mecanismos de ação sobre vermes adultos de *Schistosoma mansoni*, bem como avaliar os efeitos do calcipotriol na ativação de células estreladas hepáticas, *tanto in vitro* quanto em modelo murino de esquistossomose.

3.2 OBJETIVOS ESPECÍFICOS

- Avaliar *in vitro* o efeito da *trans*-Chalcona sobre formas juvenis, vermes adultos machos e fêmeas de *S. mansoni*;
- Determinar a seletividade da *trans*-Chalcona frente ao parasita *S. mansoni* e investigar seus mecanismos de ação, incluindo a diferença de sensibilidade entre os sexos dos vermes e alterações morfológicas;
- Avaliar, em modelo murino de esquistossomose, a carga parasitária, as alterações granulomatosas hepáticas e o perfil de citocinas em camundongos tratados com diferentes doses de *trans*-Chalcona;
- Investigar os efeitos do calcipotriol em modelo murino de infecção por *S. mansoni*, com ênfase em parâmetros histopatológicos, inflamatórios, oxidativos e função hepática;
- Determinar *in vitro*, a viabilidade celular e o índice de seletividade do praziquantel e calcipotriol em células estreladas hepáticas (GRX) e fibroblastos (L929);
- Avaliar parâmetros relacionados ao estresse oxidativo e ao metabolismo de células GRX tratadas com calcipotriol e praziquantel.

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4 PRODUÇÕES CIENTÍFICAS

4.1 ARTIGO 1. *TRANS*-CHALCONE AFFECTS SCHISTOSOMULA AND ADULT WORMS BY IMPAIRING MEMBRANE INTEGRITY AND ATTENUATES THE EFFECTS OF *SCHISTOSOMA MANSONI*-INDUCED LIVER FIBROSIS

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4.2 ARTIGO 2. CALCIPOTRIOL ATTENUATES FIBROGENESIS, INFLAMMATION, AND OXIDATIVE STRESS IN HEPATIC STELLATE CELLS AND IN MURINE MODEL OF SCHISTOSOMIASIS

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Research paper

Trans-chalcone affects schistosomula and adult worms by impairing membrane integrity and attenuates the effects of *Schistosoma mansoni*-induced liver fibrosis

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ABSTRACT

Schistosomiasis is an acute and chronic parasitic disease caused by helminths of the genus *Schistosoma*. The current treatment, praziquantel, is ineffective against immature forms and during granuloma formation in chronic phase. Chalcones belong to the flavonoid family and are known for their wide biological activities. *Trans*-Chalcone (TC), the thermodynamically stable isomeric form, exhibits anti-fibrotic and hepatoprotective effects in liver injury models. This study evaluates the *in vitro* and *in vivo* effects of TC on *S. mansoni*. *In vitro*, TC cytotoxicity was tested on LLC-MK2 cells, while schistosomula and adult worms were assessed for viability, oxidative

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; AW/LC_{50}, lethal concentration for inhibiting 50 % of adult worms; BW, body weight; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; CC_{50}, cytotoxic concentration for 50 % of cells; DMEM, Dulbecco's Modified Eagle Medium; DMSO, dimethyl sulfoxide; ELISA, enzyme-linked immunosorbent assay; EP, exudative-productive phase; FBS, fetal bovine serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; H&E, hematoxylin and eosin; H_2O_2, hydrogen peroxide; H_3PO_4, N(1-Naphthyl) ethylenediamine in orthophosphoric acid; HSCs, hepatic stellate cells; HTAB, hexadecyltrimethylammonium bromide; IL-12, interleukin-12; IL-14, interleukin-4; IL-13, interleukin-13; K_2HPO_4, potassium hydrogen phosphate; KOH, potassium hydroxide; LC_{50}, lethal concentration for 50 % of parasites; MPO, myeloperoxidase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NAG, N-acetylglucosaminidase; $NaNO_2$, sodium nitrite; NE, necrotic-exudative; NO, nitric oxide; NTS, newly transformed schistosomula; NTS/LC_{50}, lethal concentration for inhibiting 50 % of NTS; P, productive; p.i., post-infection; PBS, phosphate-buffered saline; PE, pre-granulomatous exudative phase; PI, propidium iodide; PZQ, praziquantel; RPMI, Roswell Park Memorial Institute 1640 medium; SEM, standard error of the mean; SI, selectivity index; SmATPDase, *Schistosoma mansoni* ATP diphosphohydrolase; SmGAPDH, *Schistosoma mansoni* GAPDH; TC, *trans*-Chalcone; TGF-β, transforming growth factor-beta; TMRE, tetramethylrhodamine-ethyl ester; ΔΨm, mitochondrial membrane potential.

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stress, mitochondrial membrane potential, tegument integrity, morphological changes, and egg production. *In vivo*, *S. mansoni*-infected BALB/c mice received oral doses of TC (5, 10, and 20 mg/kg) for 15 days. Parasite burden, liver function (measuring NO, NAG, MPO, IL-12, IL-4, IL-13, and TGF-β), and hepatic histology (granuloma size, stage, and collagen deposition) were analyzed. TC reduced viability in normal cells only at high concentrations (175.60 and 264.90 μM). It was selective for juvenile (17.74 μM) and adult worms (50.00 μM), especially males. TC increased nitric oxide levels, altered mitochondrial and membrane integrity, and reduced egg laying in paired worms. In mice, TC improved liver function, reduced worm and egg counts, and altered granuloma area and profile, with lower levels of pro-inflammatory and pro-fibrotic cytokines, suggesting a modulatory effect on granuloma formation.

1. Introduction

Schistosomiasis is a parasitic disease caused by blood flukes of the genus *Schistosoma*, with acute and chronic manifestations. In 2021, it was estimated that over than 250 million people needed preventive treatment, as transmission has been reported from 78 countries. Preventive chemotherapy is required in 51 endemic countries with moderate to high transmission, where people and communities are targeted for large-scale treatment [1].

Since the 1970s, chemotherapy against schistosomiasis has been limited to praziquantel (PZQ), a pyrazino-isoquinolone compound, wich the action mechanism involves the disruption of calcium homeostasis, promoting muscle contraction and parasite death. However, PZQ is known to target only adult worms, being ineffective against larval stages, presenting therapeutic resistance, as well as inactivity during granuloma formation [2]. Consequently, the search for alternative compounds that have schistosomicidal activity and act in the formation of hepatic granuloma represents great interest, aiming at a possible reduction in the morbidity and mortality associated with schistosomiasis.

Chalcones are a class of natural compounds considered to be one of the major secondary metabolites of plants belonging to the flavonoid family, a diverse group of phytochemicals widely known for their broad spectrum of biological activity and low toxicity [3]. Chalcones (1, 3-diphenyl-2-propen-1-one) consist of an open-chain in which the two aromatic rings are joined by a three-carbon α, β-unsaturated carbonyl system [4]. It can exist in two isomeric forms, *cis* and *trans*, the latter being considered more thermodynamically stable [5]. Recently, chalcones have been widely studied for exhibiting biological activities such as antioxidant and anti-inflammatory [6].

Previous studies using chalcone derivatives [7,8] and plant-isolated chalcones as cardamonin (Car) (*Piper aduncum* L.) [9,10], licochalcone A (LicoA) and licoflavone B (LicoB) (*Glycyrrhiza inflata*) [11–13], have demonstrated the potential of such compounds against *Schistosoma* species. In adult worms, chalcones targeted the integument, affected egg deposition, and promoted mitochondrial and morphological changes. In a mouse model, reduced the burden of worms and mature eggs. Chalcones, including *trans*-Chalcone (TC), have also been reported to have anti-fibrotic and hepatoprotective properties in different models of liver injury by reducing collagen content in liver tissue [14,15]. No study, however, has investigated the effects of TC on the schistosomiasis mansoni model. For this reason, we aimed to investigate its mechanism of action in juvenile and adult schistosomes *in vitro*, and *in vivo* during the patent phase of the infection.

2. Materials and methods

2.1. Trans-chalcone (TC), praziquantel (PZQ), and reagents

Commercial *trans*-Chalcone (purity ≥97 %) was obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA- Catalog Number sc-204681 - Dallas, USA). A *trans*-Chalcone stock solution was prepared in dimethyl sulfoxide (DMSO, GIBCO, Invitrogen, New York, USA) and stored at −20 °C in the dark. DMSO was used as vehicle control, not exceeding 0.02 % *in vitro* and 2 % *in vivo* experiments. Praziquantel (purity ≥98 %) and all other cell culture grade reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Animals and ethics statement

Animal care and handling procedures were performed according to the approval of the Ethical Committee for the Use of Animals at the State University of Londrina, Paraná, Brazil (process number 020.2021). Female BALB/c mice (4–6 weeks old, 20–22 g) were housed in cages in a standard environment (24 °C room temperature, 12-h light/dark cycle) with *ad libitum* access to water and food. The animals were used to maintaining the *S. mansoni* (BH strain) life cycle and for both *in vitro* and *in vivo* experimental procedures.

2.3. Cell lines and cytotoxicity assay

The cytotoxicity of TC was determined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. Cell lines used included rhesus monkey kidney epithelial cells (LLC-MK2) (ATCC CCL-7) and mouse (*Mus musculus*) subcutaneous connective tissue fibroblasts (L929) (ATCC CCL-1). The cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Life Technologies, CA, USA) and Dulbecco's modified Eagle's medium (DMEM) (Life Technologies, CA, USA), respectively, supplemented with 10 % fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin (GIBCO, Invitrogen, New York, USA), and maintained at 37 °C in 5 % CO_2. The cells (1×10^4) were treated with TC (25, 50, 75, 100, 125, 150, 175, and 200 μM) and incubated for 32 h. MTT solution (Sigma-Aldrich, St. Louis, MO, USA) (0.33 mg/mL) was added and kept for 3 h in a dark room at 37 °C. The solution was removed, and DMSO (100 μl) was added. Optical density was measured in a spectrophotometer (Thermo Scientific, Multiskan GO, USA) at 550 nm. Culture medium (negative control), 0.02 % DMSO (vehicle control), hydrogen peroxide (H_2O_2), and doxorubicin (Dox, 20 μM) were used as experimental controls. H_2O_2 and Dox served as positive controls. The cytotoxic concentration for 50 % of the cells (CC_{50}) was calculated by logarithmic regression.

2.4. Immature and adult Schistosoma mansoni viability

2.4.1. Viability of newly transformed schistosomula

S. mansoni cercariae were mechanically transformed into newly transformed schistosomula (NTS) using a vortex mixer [16]. NTS were cultured in 199 medium, supplemented with 1 % L-glutamine, 10 % sodium bicarbonate, 10 % FBS, 1 % human urine, 10 U/mL penicillin, and 10 μg/mL streptomycin (GIBCO, Invitrogen, New York, USA) and incubated overnight (37 °C, 5 % CO_2). Subsequently, NTS were transferred to a 96-well culture plate (200 NTS/well) and incubated with TC (3.12, 6.25, 12.5, 25, 50, 100, and 200 μM) or 199 medium for 24 h. Viability was assessed by trypan blue exclusion assay [17,18]. The lethal concentration for 50 % of NTS (NTS/LC_{50}) was calculated by logarithmic non-linear regression on the dose-response curve.

2.4.2. Motility, viability, and oviposition of adult worms

S. mansoni adult worms recovered by perfusion with sterile saline (0.9 % NaCl) of the hepatic portal system of infected BALB/c mice were washed and transferred to Petri dishes containing RPMI 1640 medium (20 mM HEPES, 100 U/mL penicillin, 100 μg/mL streptomycin, and 10 % FBS supplemented) (GIBCO, Invitrogen, New York, USA). Intact worms (couples, males, and females) were distributed in 24-well plates (3 worms/well) and incubated overnight (37 °C, 5 % CO_2). Motility was assessed by visual inspection under an inverted microscope. TC was added (50, 75, 50, 100, 125, 150, 175, and 200 μM) and the worms were monitored after 2, 4, 6, 12, 24, and 32 h of incubation. The following adapted scores were used: 1) active worms with normal body movements; 2) slow worms with delayed body movements; 3) minimal activity, occasional head and tail movements; and 4) complete absence of movements [19]. Oviposition was also examined, and eggs were counted under an inverted microscope.

For the next experiments, male and female adult worms were treated with TC at the lethal concentration for 50 % of the adult worms (AW/LC_{50}) 50 μM and (AW/2xLC_{50}) 100 μM for 32 h (37 °C, 5 % CO_2). The degree of TC selectivity was expressed as selective index (SI)=CC_{50} of TC on cell lines/LC_{50} of TC on *S. mansoni* (NTS and adult worms) [11].

2.4.3. Nitric oxide levels in adult worms

The determination of nitrite was used to measure the concentration of nitric oxide (NO) by the Griess method. First, 60 μL of the supernatant from TC-treated male and female adult worms was collected and added to 96-well plates. Then, 60 μL of Griess reagent (1 % of sulfanilamide and 0.1 % of N(1-naphthyl) ethylenediamine in 5 % orthophosphoric acid (H_3PO_4)) was added and kept at room temperature for 10 min. A calibration curve was made using sodium nitrite ($NaNO_2$) dilutions and the absorbance was measured at 550 nm on a microplate reader (Thermo Scientific, Multiskan GO).

2.4.4. Mitochondrial membrane potential of adult worms

The mitochondrial membrane potential (ΔΨm) of male and female adults TC-treated was assessed using the fluorescent marker tetramethylrhodamine ethyl ester (TMRE) (10 μg/mL) (Sigma-Aldrich, St. Louis, MO, USA) [20–22]. TMRE is a cationic, cell-permeable fluorescent dye that selectively accumulates in active mitochondria due to their negative membrane potential. In contrast, mitochondria with reduced or lost membrane potential, characteristic of inactive or damaged states, do not retain TMRE, limiting its accumulation. Therefore, worms were incubated with TMRE for 30 min, and washed with phosphate-buffered saline (PBS). Untreated worms were used as a negative control, and carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) (100 μM) was used as a positive control. Analyses were performed on a fluorescence microplate reader (Victor X3, PerkinElmer) using emission/excitation wavelengths of 480/580.

2.4.5. Membrane integrity of adult worms

Cell membrane integrity was analyzed through staining with propidium iodide (PI) (2 μg/mL) [23,24]. PI is a fluorescent dye that intercalates with DNA, which makes it effective for staining nucleic acids in cells with impaired membranes. Thus, its incorporation into adult worms is used to assess membrane permeability. Male and female adult worms were treated with TC, incubated with PI for 30 min, and washed with phosphate-buffered saline (PBS). Untreated worms were used as negative control, while digitonin (40 μM) was used as positive control. Analyses were performed on a fluorescence microplate reader (Victor X3, PerkinElmer) using emission/excitation wavelengths of 480/580.

2.4.6. Scanning electron microscopy of adult worms

Morphological analysis of male and female *S. mansoni* adult worms was performed by scanning electron microscopy. After incubation with TC, the worms were washed in 0.1 M sodium cacodylate buffer (pH 7.2) for further fixation in 0.1 M sodium cacodylate buffer and 2.5 % glutaraldehyde. The samples were washed three times with 0.1 M cacodylate buffer, postfixed with 1 % osmium tetroxide in the dark for 60 min, and washed again with 0.1 M cacodylate buffer. The samples were then dried by replacing ethanol with carbon dioxide, metallized with gold, and analyzed in a scanning electron microscope (FEI QUANTA 200) at magnifications of 100 μm and 20 μm.

2.5. Animal experiment

2.5.1. Experimental design

Thirty-six 4- to 6-week-old female BALB/c mice (20–22 g) were infected by tail exposure to a suspension containing 80 ± 5 *S. mansoni* cercariae. Confirmation of infection was performed on day 45 post-infection (patent infection - adult infection model) by parasitological examination of stool samples using the spontaneous sedimentation technique. The animals were randomly divided into six groups (n = 6/group), and the treatment was started via oral gavage (200 μl) for 15 days. Groups: I) infected untreated control (PBS); II): infected vehicle control (2 % DMSO); III) PZQ (40 mg/kg body weight - bw); IV) TC (5 mg/kg bw = 24 μM/kg bw); V) TC (10 mg/kg bw = 48 μM/kg bw); VI) TC (20 mg/kg bw = 96 μM/kg bw). All animals were weighed on days 30, 45, 50, 55, and 60 post infection (p.i.). The bw variations were taken relative to day 30. At 24 h after the end of treatment, the animals were anesthetized and euthanized according to the ethics committee.

2.5.2. Assessment of parasitological criteria

Different parasitological parameters were evaluated and performed by different investigators. I) Measurement of the relative organ weight (liver and spleen). II) Fecal egg count on days 45, 52, and 60 p.i. by the quantitative Kato-Katz method. III) Count of recovery worms, estimated by the animal perfusion method. IV) Count of the eggs in the liver after digestion in 5 % potassium hydroxide (KOH) solution.

2.5.3. Nitric oxide levels in vivo

NO levels were estimated as described previously [25]. Serum samples collected from the animals were deproteinized, and the supernatants (60 μL) were collected and diluted in glycine buffer solution (45 g/L). Activated cadmium granules were added, stirred for 10 min, and Griess reagent was added. A calibration curve was prepared by serial dilutions of $NaNO_2$ to determine the nitrite concentration of the samples. Absorbance was read at 550 nm using a standard microplate reader (Multiskan GO, Thermo Scientific). Final results were expressed as μM of nitrite.

2.5.4. Myeloperoxidase (MPO) and N-acetylglucosaminidase (NAG) activity

MPO and NAG activity can indirectly assess local neutrophil and macrophage infiltration, respectively. Liver tissue collected from animals was collected in potassium hydrogen phosphate (K_2HPO_4) buffer (50 mM) containing hexadecyltrimethylammonium bromide (HTAB) 0.5 % and stored at −80 °C until use. Frozen samples were homogenized using a tissue turrax (Tissue-tearor, BioSpec). MPO and NAG activity was measured in the supernatants as previously described [25].

2.5.5. Cytokine measurement

Liver tissues collected from animals were rinsed in PBS and stored at −80 °C until analysis. Frozen samples were homogenized using a Tissue-Tearor (BioSpec) homonenizer. Cytokine levels in the supernatant of liver homogenates were measures using eBiosciences® commercial kits capture enzyme-linked immune sorbent assay (ELISA) (San Diego, CA, USA). According to the manufacturer's instructions, absorbance was read at 450 nm using a spectrophotometer and the results were expressed in pg/ml based on a standard curves. The cytokines quantified included interleukin-12 (IL-12), IL-4, IL-13 and transforming growth factor beta (TGF-β).

2.5.6. Histopathological investigations and collagen quantification

Liver fragments were collected from the medial lobe, fixed in 10 % buffered formalin solution (pH 7.2), dehydrated in graded alcoholic solutions, embedded in paraffin, and sectioned on a microtome (5 μm) (SLEE CUT 4062). Sections were stained with hematoxylin and eosin (H&E) and examined by light microscopy (O400S Opticam) at a final magnification of 200x for general histopathologic analysis. For liver granulomas, the total area was determined using by Image-Pro Plus

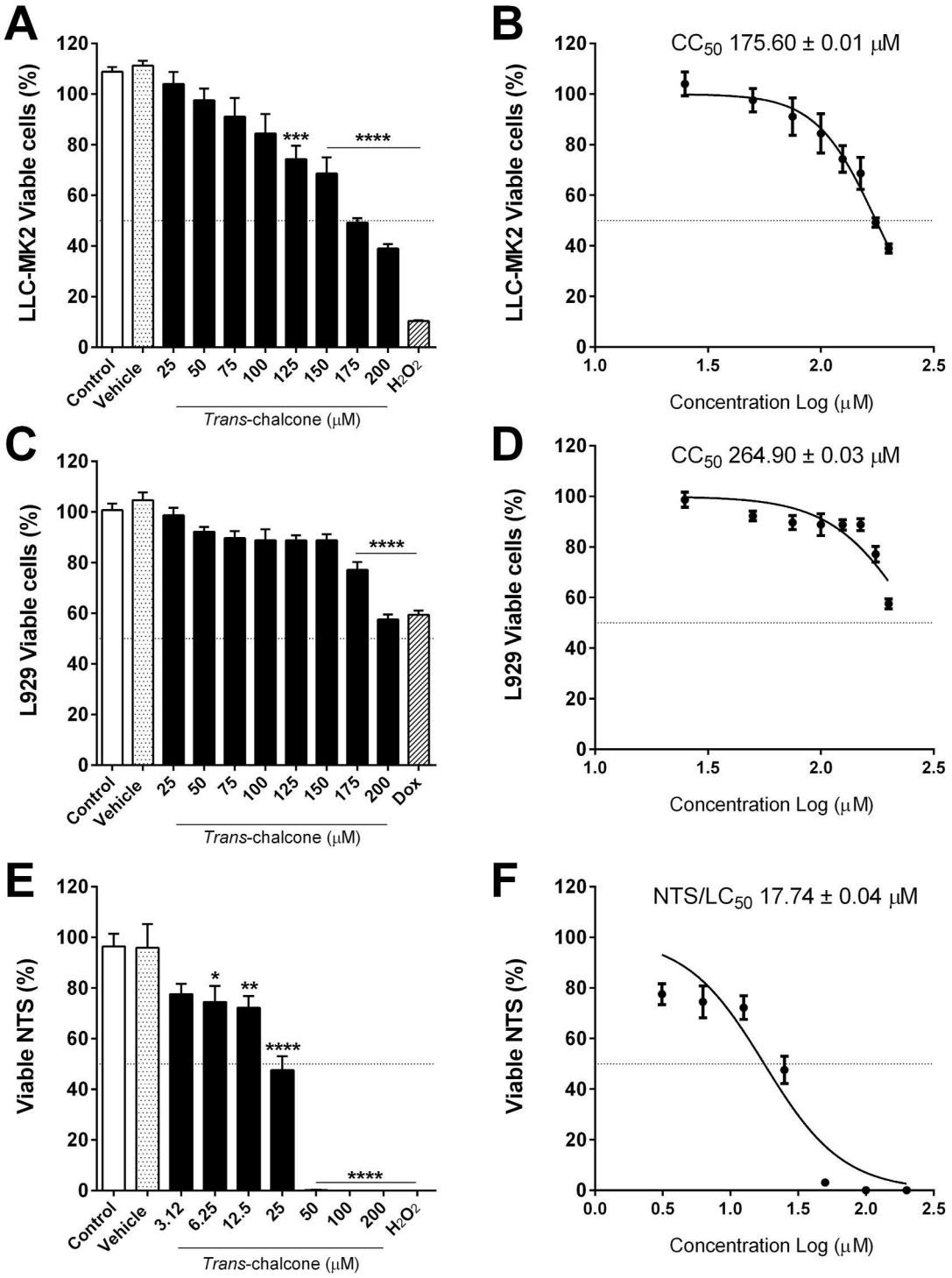

Fig. 1. Cytotoxicity of *trans*-Chalcone against rhesus monkey kidney epithelial (LLC-MK2) and mouse fibroblasts (L929) cell lines, and newly transformed schistosomula (NTS) of *Schistosoma mansoni*. LLC-MK2 and L929 cell lines were treated with *trans*-Chalcone (TC) at concentrations of 25, 50, 100, 125, 150, 175, and 200 μM for 32 h, and cell viability was determined by MTT assay (A and C). NTS were also incubated with different concentrations of TC (3.12, 6.25, 12.5, 25, 50, 100, and 200 μM) for 24 h and the count was performed using Trypan blue exclusion staining (E). The cytotoxic concentration for 50 % of cells (CC_{50}) (B and D) and lethal concentration for 50 % of NTS (NTS/LC_{50}) (F) were calculated from a non-linear regression dose-response inhibition graph (B and D). Culture medium (negative control), DMSO 0.02 % (vehicle), and H_2O_2 (positive control) were used as control. Values represent the mean ± SEM of three independent experiments performed in trip-licate. ** Significant difference from negative control ($p \leq 0.01$), *** ($p \leq 0.001$), and ****($p \leq 0.0001$). CC_{50}: cytotoxic concentration for 50 % of cells; LC_{50}: lethal concentration for 50 % of parasites; NTS: newly transformed schistosomula.

(version 4.5), and characterized as pre-granulomatous exudative phase (PE), necrotic-exudative (NE), exudative-productive phase (EP), and productive (P) [26]. To evaluate the type I and III collagen fibers, liver fragments were stained with Sirius red and examined under polarized light using a photomicroscope (Nikon Eclipse 80i) with a coupled camera (Nikon DSFi1C). Images of ten random microscopic fields of red-stained collagen fibers in the liver section of each mouse mice were captured and analyzed using Image-Pro Plus (version 4.5). The results were expressed as a percentage of the area with the presence of collagen compared to the total measured area.

2.6. Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM). Three independent experiments were performed, each with triplicate datasets. Significant differences between the groups were determined through One-way analysis of variance (ANOVA) followed by Tukey's post-test for multiple comparisons. All analyses were performed using Prism 8 (GraphPad Software, San Diego, CA) software. Differences were considered statistically significant when $p < 0.05$. The values were also categorized by: * ($p < 0.05$); ** ($p \leq 0.01$); *** ($p \leq 0.001$); **** ($p \leq 0.0001$).

3. Results

3.1. Trans-chalcone exerts direct schistosomicidal activity on newly transformed schistosomula

Our first step was to determine whether TC treatment induced a toxic effect on LLC-MK2 and L929 cells. Treatment significantly affected LLC-MK2 cell viability only at the highest concentrations (from 125 μM) ($p \leq 0.001$; $p \leq 0.0001$) (Fig. 1A). The calculated cytotoxic concentration for 50 % (CC_{50}) of the cells was 175.60 ± 0.01 μM (Fig. 1B). For L929 cells, TC exhibited cytotoxic effects only at higher concentrations. A CC_{50} value of 264.90 ± 0.03 μM was observed, with significant effects only above 175 μM ($p \leq 0.0001$) (Fig. 1C and D).

To assess the direct effect of TC on *S. mansoni* schistosomula, NTS were incubated with TC and evaluated by trypan blue staining. Treatment with 6.25, 12.5, 25 and 50 μM significantly reduced NTS viability to 74.48 %, 72.24 %, 47.59 % and 3.08 %, respectively ($p \leq 0.0001$). At the highest concentrations (100 and 200 μM), TC exhibited 100 % lethality against the parasites ($p < 0.05$; $p \leq 0.01$; $p \leq 0.0001$) (Fig. 1E). Therefore, we found that TC at a concentration of 17.74 ± 0.04 μM caused mortality in 50 % of NTS (NTS/LC_{50}) (Fig. 1F).

3.2. TC alters motility and vability of adult worms

The effect of TC was also tested on paired and unpaired *S. mansoni* adult worms. Worms were treated and monitored at 2, 4, 6, 12, 24 and 32 h post-exposure. Table 1 summarizes the effect of TC on motility and viability of *S. mansoni* adult worms, with a scoring criteria indicating the behavior. Paired worms showed slow movements up to 24 h after treatment, however, after 32 h there was a complete absence of motor activity from 50 μM. Male worms exhibited minimal motor activity at the highest concentration (200 μM) during the initial observation periods (2 and 4 h), and the same was observed at 6 and 12 h after treatment from 100 μM. At 24 h, male worms showed minimal activity at 75 μM, while at 32 h they showed no movement at 50 μM. Finally, in females, after 6 h of exposure to TC, minimal activity was detected at 175 μM, while at 12 and 24 h we detected the same effect from 75 μM. At 32 h, no movement was detected at 75 μM (Table 1). When incubated as a pair, separation was observed after 12 h of incubation at a concentration of 50 μM (data not shown). Therefore, male worms were more sensitive than female and paired worms, as the effect was time-dependent. AW/LC_{50} of 50 μM and AW/2xLC_{50} of 100 μM were determined after 32 h of treatment and used for subsequent *in vitro* experiments.

3.3. TC acts selectively on schistosomes

To evaluate the selectivity of TC, the selectivity index was calculated using the CC_{50} of the cell lines (LLC-MK2 and L929, respectively) and the LC_{50} of NTS and adult worms. Regarding NTS, TC presents an SI of 9.89 and 14.93, while for adult worms we found an SI of 3.51 and 5.29. Thus the compound was found to have selective activity against parasites.

3.4. TC affects egg-laying, nitric oxide production, and membrane integrity in adult worms

NO is produced by the enzyme nitric oxide synthase (NOS) using oxygen (O_2) and L-arginine as substrates. The isoforms neuronal NOS (nNOS), inducible NOS (iNOS) and NADPH-diaphorase (NADPH-d) have been identified in schistosomes. In summary, NO plays a critical role in schistosomes, participating in several physiological pathways essential for parasite survival and adaptation, as well as mediating parasite-host interactions, highlighting the importance of NO in the parasite life cycle and its potential as a therapeutic target. In males, the presence of NOS is prominent in the tegument, while in females, its involvement is more directed to reproduction and egg development, present in the vitelline gland [27]. With this in mind, females have been shown to have higher levels of oxidants and to be more resistant to pro-oxidant challenge [28]. In this context, it was found that negative control females exhibited higher basal NO levels compared to males ($p \leq 0.0001$). However, when exposed to TC, a significant decrease was observed in females, but an increase in males ($p \leq 0.01$) (Fig. 2A).

The maintenance of mitochondrial membrane potential is essential for the survival of worms, as it plays a critical role in energy production and maintaining the parasite's metabolism [29]. The mitochondrial

Table 1
Effect of *trans*-Chalcone on motility and viability of *Schistosoma mansoni* adult worms.

TC (μM)	Couple worms						Male worms						Female worms					
	Hours						Hours						Hours					
	2	4	6	12	24	32	2	4	6	12	24	32	2	4	6	12	24	32
Control	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
50	1	1	1	1	2	4	1	1	2	2	2	4	1	1	2	2	2	3
75	1	1	1	1	2	4	2	2	2	2	3	4	1	2	2	3	3	4
100	1	1	2	2	2	4	2	2	3	3	3	4	1	2	2	3	3	4
125	2	2	2	2	2	4	2	2	3	3	3	4	1	2	2	3	3	4
150	2	2	2	2	2	4	2	2	3	3	3	4	2	2	2	3	3	4
175	2	2	2	2	2	4	2	2	3	3	3	4	2	2	3	3	3	4
200	2	2	2	2	2	4	3	3	3	3	3	4	2	2	3	3	3	4

Score criteria: 1) Active worms with normal body movements; 2) slow worms with delayed body movements; 3) minimal activity, occasional movement of head and tail, and 4) dead worms with a total absence of movements. TC: *trans*-Chalcone.

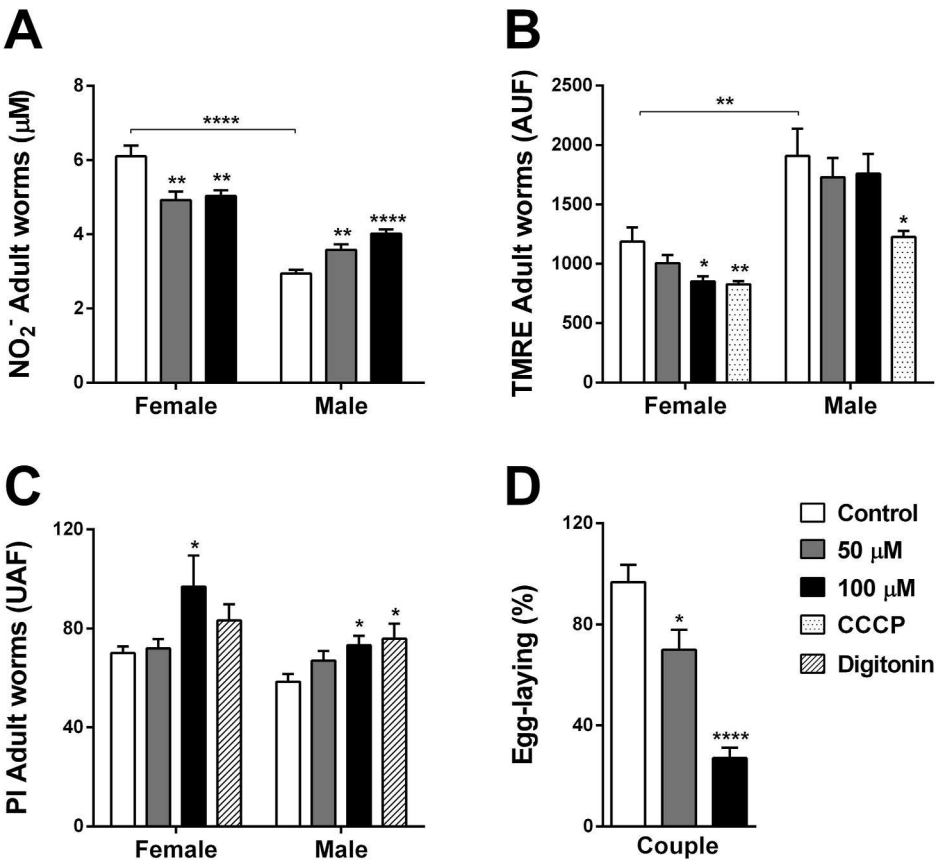

Fig. 2. Implications for male and female *Schistosoma mansoni* adult worms treated with *trans*-Chalcone. Male and female adult worms were incubated separately with 50 % parasite lethal concentration (AW/LC_{50} - 50 μM) and 2x the 50 % parasite lethal concentration (AW/2xLC_{50} - 100 μM) of TC for 32 h and assessed for nitric oxide production by the Griess method (A), and mitochondrial membrane potential (B) and plasma membrane integrity (C) by fluorescence. Worms in couple were also incubated with AW/LC_{50} and AW/2x LC_{50} of TC for 32 h and assessed for egg release (D). Data represent the mean ± SEM of three independent experiments performed in triplicate. Significant difference from negative control, * ($p < 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$), and **** ($p \leq 0.0001$). NO: nitric oxide; TMRE: tetramethylrhodamine-ethyl; PI: propidium iodide; AUF: arbitrary units of fluorescence; CCCP: carbonyl cyanide-*m*-chlorophenylhydrazone.

membrane is responsible for creating an electrochemical gradient by pumping protons into the intermembrane space, which drives ATP synthesis. In addition, the polarization of the mitochondrial membrane also regulates various cellular processes such as apoptosis and the maintenance of redox balance, which is crucial for protection against oxidative stress [30]. In female schistosomes, the mitochondrial respiratory chain is essential for energy production, which favors egg production, while in males it plays a fundamental role in maintaining and rebuilding the surface membrane complex, which is essential for protecting the parasite from the host's immune defenses [31]. In this sense, we evaluated a higher basal level of mitochondrial activity in negative control males ($p \leq 0.01$), which can also be explained by a greater motor activity due to the abundance of muscle fibers and the need for more energy to maintain muscle contraction compared to females [28]. Furthermore, when the worms were exposed to TC, only the females showed a depolarization of the mitochondrial membrane, demonstrating a low accumulation of TMRE at a concentration of 100 μM ($p < 0.05$) (Fig. 2B).

In addition, we assessed schistosome membrane damage by using proidium iodide, since its labeling of nucleic acids is an indicator of increased membrane permeability [32]. In this regard, both males and females showed a significant increase in PI labeling at a concentration of 100 μM, indicating membrane permeability ($p < 0.05$) (Fig. 2C). Knowing that TC is capable of affecting pathways involved in reproduction, viability and pairing of couples, it was found that the treatment reduced egg-laying to 69.93 % (±7.87) and 27.00 % (±4.18) at concentrations of 50 μM and 100 μM, respectively ($p < 0.05$ and $p \leq 0.0001$) (Fig. 2D).

3.5. TC promotes morphological changes in adult worms

After determining the reduction in viability and alterations in metabolic and membrane structures, we assessed morphological changes. Adult worms in the negative control group exhibited a healthy tegument with intact oral and ventral suckers (Figs. 3 and 4 A-C). When females were exposed to 50 μM TC, it was possible to assess changes in the tegument conformation (Fig. 3D and F), which was more evident with 100 μM, being assessed blebs formation around the ventral sucker (Fig. 3G–I). In negative control male worms, the posterior region showed tubercles densely covered with randomly distributed spines (Fig. 4A–C). However, the exposure to 50 μM of TC induced retraction of the ventral sucker, loss of sensory papillae in the tubercles, and blebs formation in the tegument (Fig. 4D–F). At 100 μM, the effects were more highlighted, with desquamation on the surface of the ventral sucker, reduction in the distribution of spines, and changes in the conformation of the tegument (Fig. 4G–I).

3.6. High-dose TC treatment restores body weight and improves liver function in an experimental Schistosoma mansoni mouse model

Hepatosplenomegaly can lead to impaired protein synthesis, lipid and carbohydrate metabolism, leading to loss of function, reduced absorption of nutrients and consequent loss of muscle mass, resulting in weight loss. With this in mind, we investigated the effect of TC on body

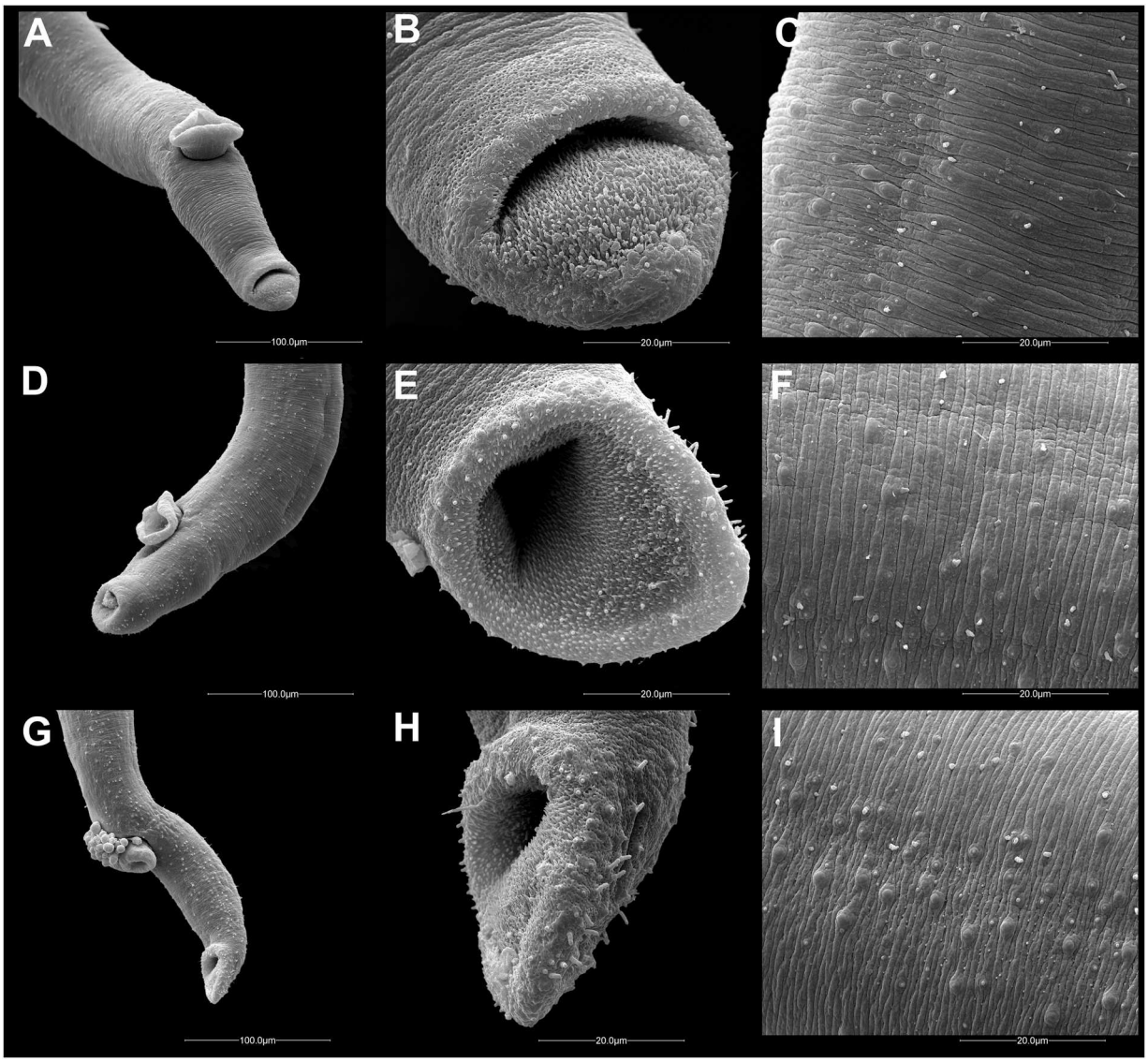

Fig. 3. Effect of *trans*-Chalcone treatment on the morphology of female adult worms of *Schistosoma mansoni*. Morphological changes were evaluated by scanning electron microscopy. Female worms were treated with culture medium (A–C), 50 μM (D–F), and 100 μM (G–I) of *trans*-Chalcone for 32 h. Scale bars: A, D, and G: 100 μm; B, C, E, F, H, and I: 20 μm.

weight and organs (liver and spleen). Treatment with TC started on day 45 of *S. mansoni* infection (patent stage) and continued for 15 days. Thus, when evaluating the change in body weight, the non-infected ($p \leq 0.0001$), infected PZQ-treated ($p \leq 0.001$) and infected TC-treated (20 mg/kg) ($p \leq 0.01$) groups had a significant weight gain compared to the untreated infected group after 55 days of infection (Fig. 5A).

At the end of treatment, all groups showed a loss of body weight. This has been reported in other studies, especially after 56 days of infection, when there is a high deposition of eggs in the liver and spleen and an influx of cells [33]. However, uninfected and infected animals treated with PZQ and TC showed a significant improvement compared to the untreated infected group. Uninfected animals had a mean weight gain of 0.5 g ($p \leq 0.0001$), PZQ-treated animals had a reduction of 0.7 g ($p < 0.05$), while animals treated with TC at a dose of 20 mg/kg had a weight reduction of only 0.2 g ($p < 0.01$) (Fig. 5A). When assessing relative liver and spleen weights, there was a significant reduction after PZQ treatment ($p < 0.05$) (Fig. 5B and C).

NO is involved in pro-inflammatory responses and has either a protective effect, acting as an effector molecule for macrophages, or a deleterious effect, inducing cell damage through the formation of toxic radicals [34]. In this context, it was evaluated that treatments with PZQ and TC did not promote a significant increase or decrease in systemic NO (Fig. 5D) and neutrophil infiltrates in macrophages (Fig. 5E) and neutrophils (Fig. 5F).

3.7. TC treatment reduced parasite load and induced the development of newly formed and smaller granulomas

When parasite load was assessed, after 7 days of treatment, animals receiving TC at doses of 10 and 20 mg/kg showed a reduction in eggs per gram of feces compared to the untreated group ($p < 0.05$ and $p \leq 0.01$, respectively). After 15 days, TC treatment (10 mg/kg) maintained the reduction in egg number ($p < 0.05$) (Fig. 6A). The TC effect on adult worms in the hepatic portal system and eggs trapped in the liver tissue were also determined. The treatment with 10 mg/kg reduced only the number of eggs, averaging 77.63 (±17.12) per animal ($p < 0.05$). While 20 mg/kg treated animals reduced 40.60 % of the adult worms ($p < 0.05$) and had a mean of 72.56 (±11.72) eggs retained in the liver ($p < 0.05$) (Fig. 6B and C).

Following TC treatment at doses of 10 and 20 mg/kg, the liver

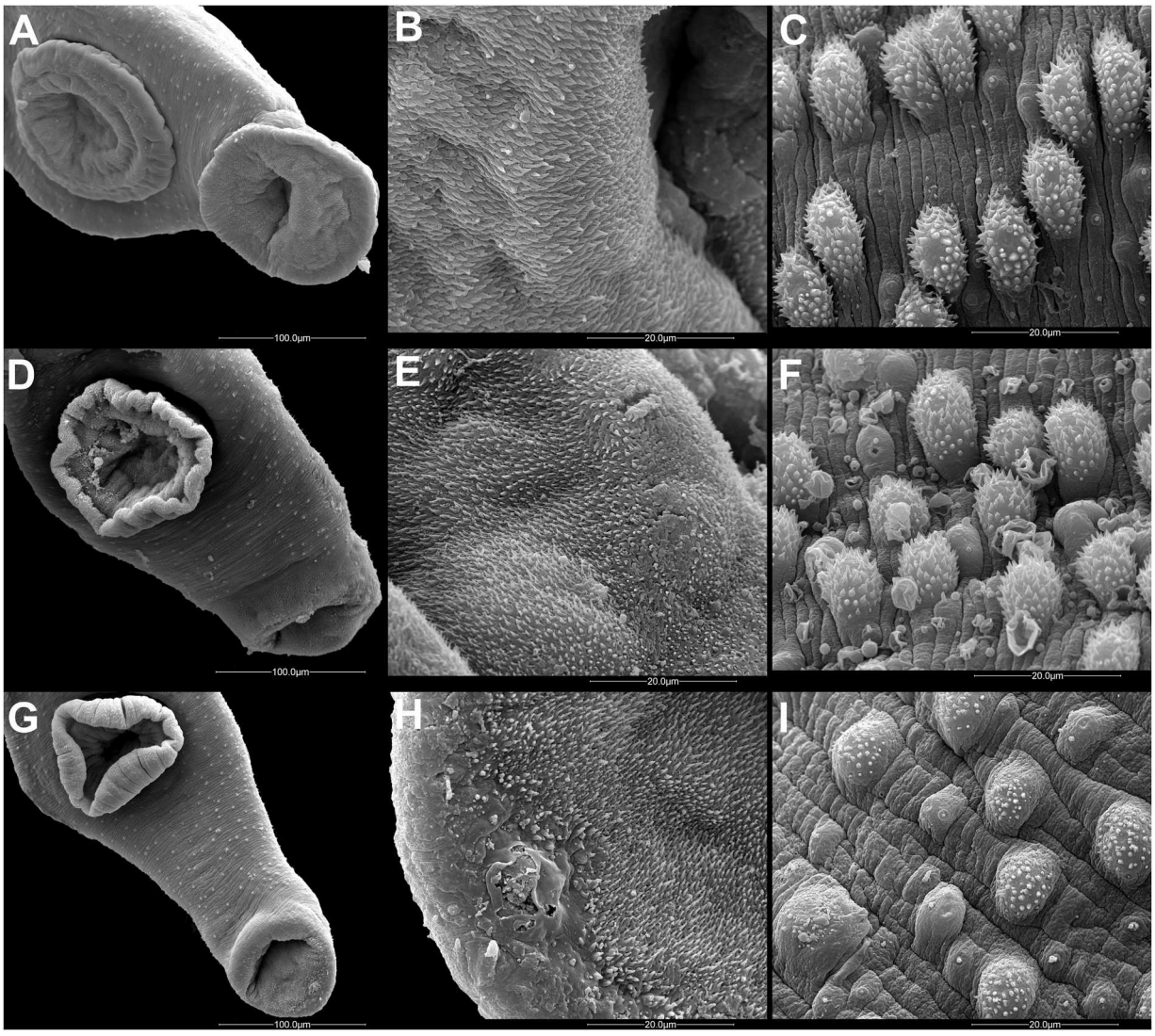

Fig. 4. Morphological changes in male adult worms of *Schistosoma mansoni* treated with *trans*-Chalcone. Scanning electron microscopy images show the morphological changes in male worms after 32 h of treatment with culture medium (A–C), 50 μM (D–F), and 100 μM (G–I) of *trans*-Chalcone. Scale bars: A, D, and G: 100 μm; B, C, E, F, H, and I: 20 μm.

granulomas exhibited a smaller area ($p \leq 0.01$) (Fig. 6D). Additionally, 28.71 % of the granulomas were in the early phase (pre-granulomatous exudative - PE) after TC treatment, compared to 13.95 % when treated with PZQ (Fig. 6E). When collagen deposition in liver granulomas was assessed, no significant difference in the proportion of collagen type I and III was found between the groups. However, TC-treated animals at doses of 5, 10 and 20 mg/kg showed proportions of 40 %, 32 % and 34 % immature collagen (type III) collagen, respectively (Fig. 6F). In addition, histological images showed a reduction in granuloma size after treatment with PZQ and TC, confirming previous findings (Fig. S1).

Treatment with TC also altered cytokine levels. It was found that TC induced a significant reduction in IL-12 and IL-4 at all doses tested ($p < 0.05$ and and $p \leq 0.01$) in comparison to the untreated infected group (Fig. 7A and B), while PZQ only reduced IL-4 ($p < 0.05$). TC also showed a tendency to reduce IL-13 and TGF-β levels, particularly at the 20 mg/kg dose (Fig. 7C and D).

4. Discussion

Chalcones are one of the major classes of plant secondary metabolites, essential for defense against reactive oxygen species by preventing molecular damage [35]. Such compounds exhibit a benzylideneacetophenone scaffold, with two aromatic nuclei linked by a three-carbon α, β-unsaturated carbonyl bridge, responsible for the broad biological activity [4]. *Trans*-Chalcone (TC), especially, is the core structure of chalcone derivatives, identified as having low toxicity for healthy cells [36–38]. TC was found to present a high CC_{50} in Madin-Darby bovine kidney (MDBK), mouse embryonic fibroblast (NIH/3T3), and human foreskin fibroblast (HFF) cell lines, with values of 293.9 μM, 434.4 μM, and 498 μM, respectively [37]. Additionally, in sheep erythrocytes, TC induced hemolysis only from the concentration of 10,000 μM, while in murine peritoneal macrophages, concentrations above 1000 μM compromised viability (CC_{50} 555 μM) [36,38]. These findings corroborate our data showing that TC induces cytotoxicity only at high concentrations (CC_{50} 175.60 and 264.9 μM) in addition to being able to selectively target schistosomes and reduce the effects induced by granulomas during experimental infection.

In silico, Miranda-Sapla et al. (2019) assessed the Lipinski and Veber criteria for TC, reporting molecular weight, logP, and number of hydrogen donors/acceptors within recommended limit, with no violations. These findings indicate that TC has structural features consistent with orally administered drugs, good drug-likeness, and potential for good oral bioavailability. Moreover, based on predicted ADMET properties, TC exhibits no genotoxicity, good aqueous solubility, and high

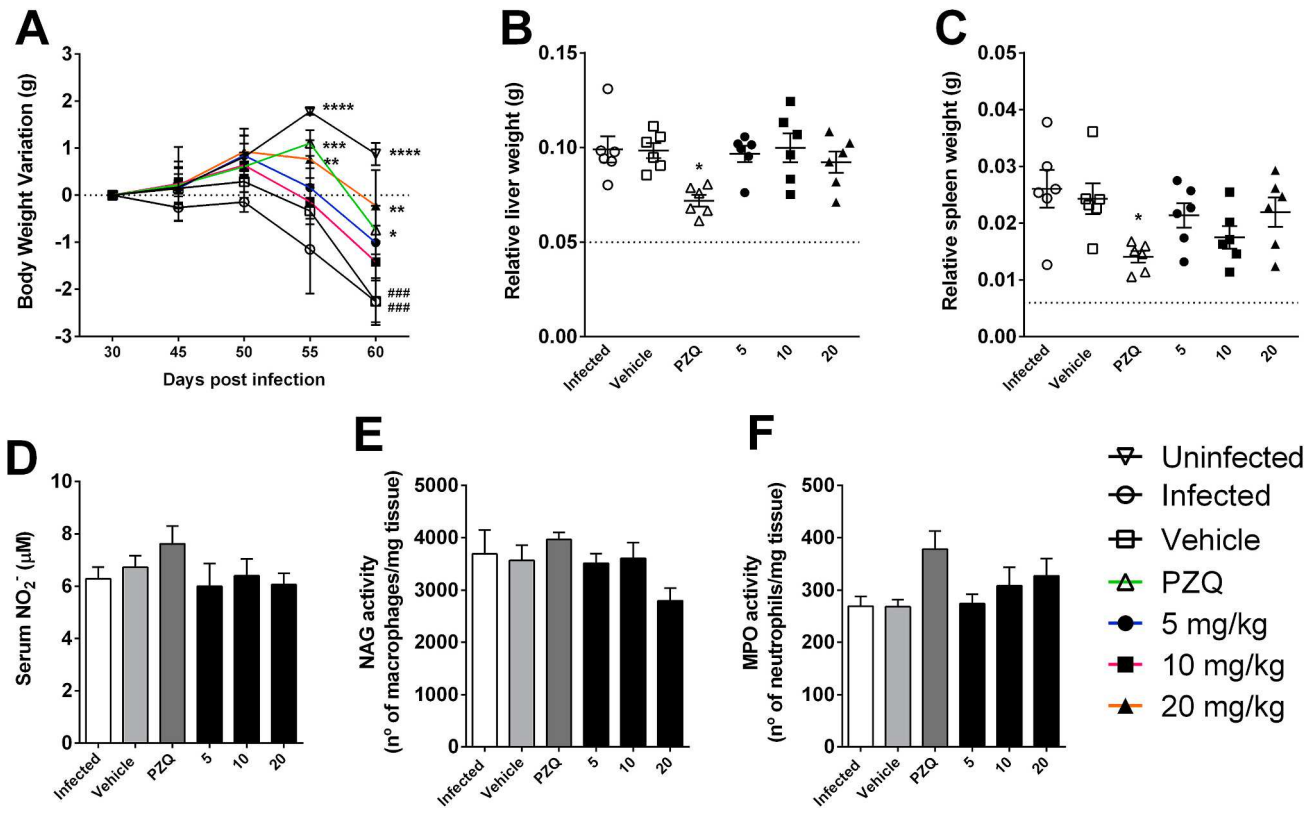

Fig. 5. Assessment of body and organ weight, and liver damage in *Schistosoma mansoni*-infected BALB/c mice treated with *trans*-Chalcone. BALB/c mice were infected with cercariae (80 ± 5) of *S. mansoni*, after 45 days post-infection the mice were treated with *trans*-Chalcone (5, 10, and 20 mg/kg), DMSO (Vehicle – 2 %), Praziquantel (PZQ - 40 mg/kg), and phosphate buffered saline (PBS - infected/untreated) for 15 days. After treatment, body weight (A), and liver (B) and spleen (C) weights were evaluated. Serum levels of NO (D), and liver NAG (E) and MPO (F) activity were also evaluated. Data represent the mean ± SEM. Significant difference from infected control, * ($p < 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$), and **** ($p \leq 0.0001$). Significant difference from day 30 post-infection, ### ($p \leq 0.001$). bw: body weight measurement; PZQ: praziquantel; NO: nitric oxide; NAG: N-acetylglucosaminidase; and MPO: myeloperoxidase.

intestinal absorption and permeability in Caco-2 cells, further supporting potential for oral administration. However, the predicted inhibition of CYP2C19 and CYP1A2, as well as the promiscuous of cytochrome P450 inhibition, suggests the need for further investigations into potential drug–drug interactions, especially in multi-drug regimens [36].

Human and experimental studies have shown that alterations in the hepatic metabolism of PZQ and its interactions with other druges can affect its systemic levels, resulting in variable cure rates [39]. Additionaly, PZQ is effective against adult worms, however, has minimal effect on immature parasites during the 2–4 weeks following infection of the mammalian host [2]. One of the mechanisms associated with PZQ resistance involves ATP-binding cassette (ABC) transporters, which are responsible for the efflux of toxins and xenobiotics. These include P-glycoproteins (Pgp) and multidrug resistance-associated proteins (MRPs) (MDR) [40]. *S. mansoni* has homologues of both MRPs (e.g., SmMRP1) and Pgp (e.g., SmMDR2) [41]. Juvenile schistosomes express basal levels of SMDR2 and SmMRP1 2.5 times higher than those in adults [42]. Additionally, Hines-Kay et al. demonstrate that exposure to PZQ *in vitro* significantly increased the transcript levels of ABC transporters SMDR1, SmMRP1, SmMRP2, and SMDR3 in juvenile schistosomes [43]. In this context, TC exhibits activity against *S. mansoni* schistosomula, with a selectivity index of 9.89 and 14.93 relative to normal cells, demonstrating greater efficacy against juvenile forms compared to adult worm, in contrast to PZQ. A compound's selectivity index is a widely accepted criterion for expressing a drug's *in vitro* efficacy, with values above 10 suggesting safe use in mammalian hosts [44,45].

When the effect of TC on adult worms was evaluated, all schistosomes exhibited slow or minimal activity after 24 h of treatment at all concentrations tested. However, at the lowest concentration (50 µM, SI = 3.51), couples and males died, while females still exhibited minimal movement at the final time point. In a previous study by Pereira et al. (2018), among 15 synthetic chalcone derivatives tested, only compounds 1 and 3 showed significant activity at 25 µM after 24 h, causing 50 % mortality, tegumental alterations, and reduced motility in adult worms. Chalcone 1 showed low cytotoxicity ($CC_{50} > 250$ µM; SI ≥ 10), while chalcone 3 had a CC_{50} of 163.5 µM (SI = 6.54) [7]. Chalcones isolated from *G. inflata* also demonstrated efficacy: LicoA caused 100 % mortality and complete loss of motility at 12.5 µM after 24 h, with LC_{50} values of 9.12 and 9.52 µM for female and male worms, respectively, and a CC_{50} of 508.3 µM in CHO–K1 fibroblasts (SI = 55.7 and 53.3) [11]. Similarly, LicoB induced 100 % mortality at 25 µM after 24 h of exposure. Praziquantel (1.56 µM) also led to complete motility loss at all time points [13].

When assessing the effect of TC on adult worms a sex difference in sensitivity was observed, as male worms showed a reduction in viability at lower concentrations of TC compared to female worms. Adult male and female worms exhibit different genetic profiles that provide specific biological characteristics during the development. Female worms require a higher energy supply to support egg production. In contrast, male worms are exposed to host cells and are able to high levels of genes associated with integument repair [46]. In this context, differences in susceptibility are also considered, as male worms have been found to be more sensitive to exposure to different treatments [47].

In the human host, schistosomes are continuously exposed to conditions that induce cellular repair and control of excessive reactive species production. As a result, the parasite has developed several resistance mechanisms to detoxify compounds derived from respiratory

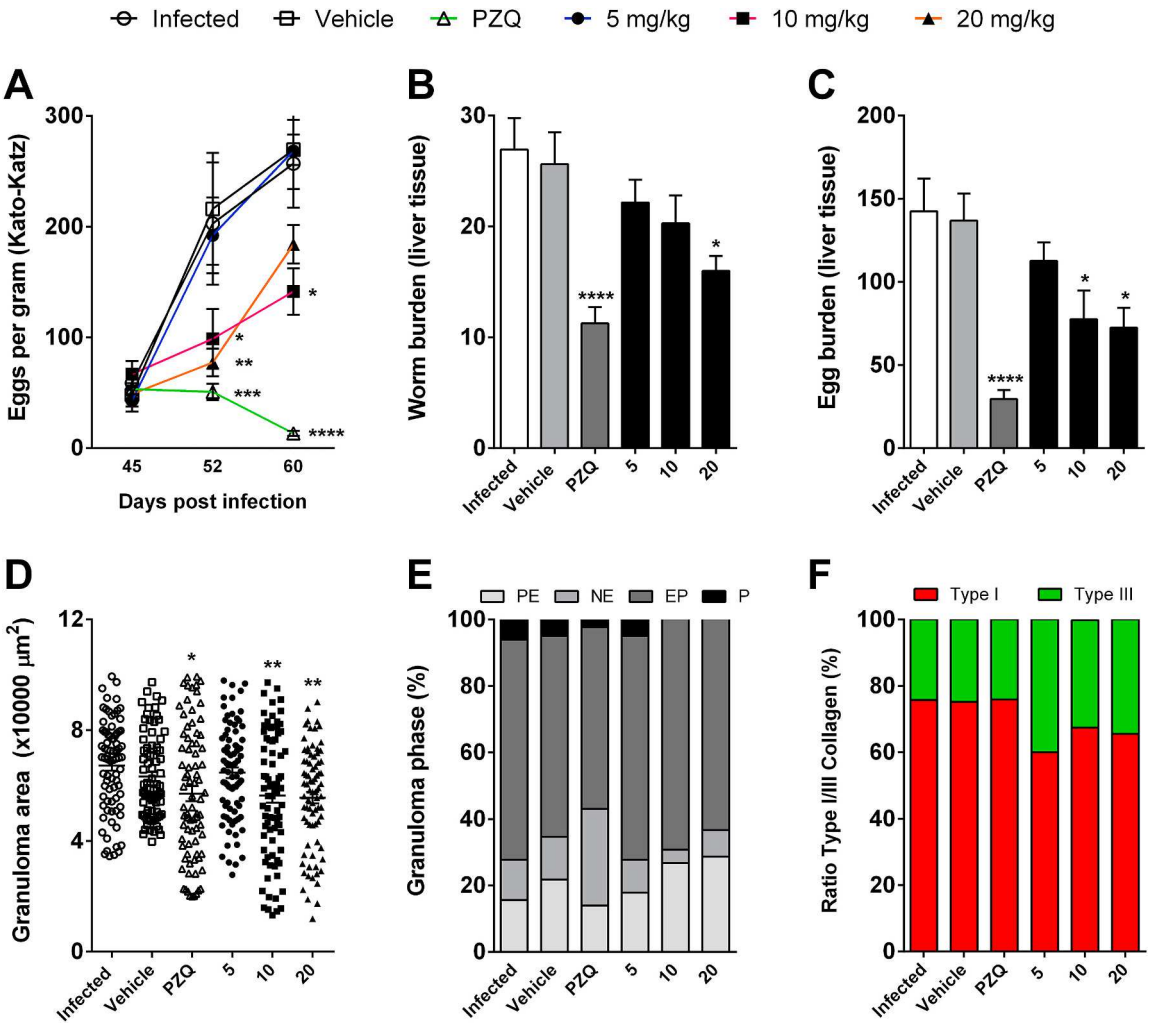

Fig. 6. Parasite load and hepatic granuloma profile in BALB/c mice infected with *Schistosoma mansoni* and treated with *trans*-Chalcone. BALB/c mice were infected with *S. mansoni* cercariae (80 ± 5) and treated with *trans*-Chalcone (5, 10 and 20 mg/kg), DMSO (vehicle – 2 %), praziquantel (PZQ - 40 mg/kg) and phosphate buffered saline (PBS - infected/untreated). The number of eggs per gram of feces (A) was measured on days 45, 52 and 60 post-infection. Treatment was performed from day 45 to day 60. After treatment, the animals were euthanized and the number of adult worms in the hepatic portal system (B) and eggs retained in the liver (C) were determined. In liver tissue, the area (D), phase (E), and collagen deposition (F) of granulomas were evaluated. Data are mean ± SEM. Significant difference from infected control, * ($p < 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$), and **** ($p \leq 0.0001$). PE: pre-granulomatous exudative phase; NE: necrotic-exudative; EP: exudative-productive; P: productive; PZQ: praziquantel.

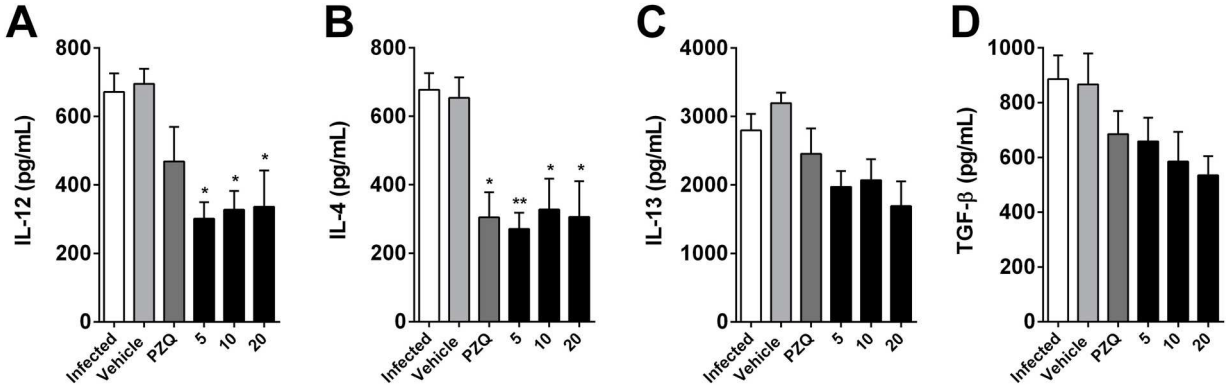

Fig. 7. Cytokine production in BALB/c mice infected with *Schistosoma mansoni* and treated with *trans*-Chalcone. BALB/c mice were infected with *S. mansoni* cercariae (80 ± 5) and treated with *trans*-Chalcone (5, 10 and 20 mg/kg), DMSO (vehicle – 2 %), praziquantel (PZQ - 40 mg/kg) and phosphate buffered saline (PBS - infected/untreated). Treatment was performed from day 45 to day 60 post infection. After treatment, the animals were euthanized and liver homogenates were assessed for IL-12 (A), IL-4 (B), IL-13 (C) and TGF-β (D) by ELISA. Data are mean ± SEM. Significant difference from infected infected control, * ($p < 0.05$) and ** ($p \leq 0.01$). PZQ: praziquantel; IL: interleukin; and TGF-β: transforming growth factor-beta.

processes and the host immune response [48]. Female worms are known to express higher levels of multidrug resistance transporters (SmMRD2) and antioxidant enzymes compared to males, making them less susceptible to PZQ, as well as to oxidative and nitrosative stress [28,42]. In this study, male worms were not only more sensitive than females, but also more susceptible to reactive species, exhibiting increased nitric oxide levels following exposure to TC.

Once exposed to high levels of nitric oxide, adult worms exhibit a range of effects, including developmental inhibition, damage to the tegument and reproductive organs, impaired in nutrient absorption and egg production, leading to schistosome death by damaging mitochondria and inhibition of the respiratory chain [31]. Increased oxidative stress in adult *S. mansoni* worms was also evaluated after exposure to LicoA, as demonstrated by Souza et al. (2017). The authors evaluated increased levels of superoxide anion and reduced superoxide dismutase (SOD) enzyme, contributing to mitochondrial damage [11]. In this context, treatment with TC also induced mitochondrial membrane depolarization, supporting previous findings that chalcones can dissipate the proton motive force across membranes, resulting in depolarization and increased membrane permeability [49,50].

S. mansoni eggs are responsible for the transmission and maintenance of the parasite's life cycle and play a central role in inducing inflammation and fibrosis in target organs [51]. Given this, one strategy for evaluating schistosomicidal compounds is to alter the reproductive capacity of adult worms by inhibiting egg-laying. Thus, in addition to causing mitochondrial damage and altering nitric oxide levels, exposure to TC also significantly impaired egg release, consistent with other studies (Car, LicoA, LicoB).

The tegument plays a vital role in the survival of the schistosome by enabling it to obtain nutrients and establish direct contact with the host. This structure consists of a heptalminate membrane, essential for nutrient uptake, osmoregulation, immunoprotection and structural support [52]. It also presents receptors that favour delivery systems and is continuously repaired and replaced, providing protection against the host's immune system [53]. Therefore, molecules required for the development, maintenance and proper functioning of the tegument are potential targets for anthelmintic drugs.

Previous studies indicated that synthetic chalcones induce tegumental blebbing, swelling and tubercle loss [7]. Regarding natural chalcones, Car caused massive tubercle disintegration and LicoB induced dose-dependent tegumental damage with ruptured tubercles that were more evident in males than in females [9,13]. LicoA was found to induce ultrastructural changes, including degeneration of vitelline cells, chromatin condensation, lysis of internal tissues and muscle layers, mitochondrial swelling, lipid droplets and tegumental vacuolization [11,54]. Additionally, exposure to PZQ (10 μM) caused rupture of tubercles, indicating a loss of integument integrity [54]. *Schistosoma* worms depend on spinous tubercles and suckers to attach to blood capillaries; severe damage to these structures may result in displacement by blood flow [55]. Therefore, the reduction of spines and tubercles, as well as the retraction of the suckers mediated by TC, may impair the worm's ability to anchor to blood vessels, facilitating its elimination. In addition, consistent with previous studies, TC also induced tegumental damage such as rupture and blistering, which may further compromise parasite viability.

Another well-known anti-parasitic mechanism of chalcones is the inhibition of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), an enzyme involved in the glycolytic breakdown of glucose. Dehydrogenase inhibition is capable of altering energy metabolism, affecting mitochondrial function, leading to parasite death [56]. In *S. mansoni* worms, GAPDH (SmGAPDH) has been identified in the tegument, as its suppression is able to reduce the activity of the parasite [57]. Another target of chalcones in *S. mansoni* schistosomes is the ATP diphosphohydrolase (SmATPDase), an ectoenzyme located on the outer surface of the tegument that hydrolyzes tri- and diphosphate nucleosides to monophosphate nucleosides and is involved in evasion mechanisms [9,13]. In view of this, TC also appears to selectively target the schistosome tegument.

Chalcones have been extensively studied for their hepatoprotective properties in models of acute and chronic liver disease. In a drug-induced hepatotoxicity model, TC was found to reduce serum AST, ALT, ROS and nitrite levels, as well as attenuate the inflammatory response and control TGF-β1 and collagen levels [58,59]. Given the *in vitro* activity of TC, we explored the treatment in an experimental mouse model of schistosomiasis and assessed parasite burden and liver granuloma.

The different phases of schistosomiasis are determined by the developmental stage of the schistosomes and their excreted products. First, there is a response to migrating schistosomula and maturation of adult worms, characterizing the acute phase of infection with an initial Th1-type response (IFN-γ, IL-12 and TNF-α). Subsequently, there is a change in the response to antigens shed by the eggs, with a switch to a Th2 response (IL-4, IL-13 and TGF-β), representing the chronic phase, in which hepatic stellate cells (HSCs) are activated and initiate collagen deposition [60]. Here, we found that TC administration was able to reduce parasite burden, resulting in decreased egg deposition in liver tissue and a consequent reduction in the levels of both pro-inflammatory and pro-fibrotic cytokines in the liver.

The antigenic stimulation by eggs retained in the trapped leads to granuloma formation, with immune cell accumulation and consequent chronic fibrosis, portal hypertension, and ascites [60]. Initially, granulomas show a disorganized inflammatory cell infiltrate around the eggs, characterizing the PE phase, followed by a central necrotic halo, the NE phase. The organization of the granuloma begins in the EP phase. This is characterized by a greater deposition of collagen fibers, still with inflammatory cells in the periphery. Finally, the granuloma takes on a characteristic appearance, with a thick band of collagen and a small number of inflammatory cells, known as the P phase. In this context, oral treatment with TC attenuated liver damage and promoted the formation of newly formed granulomas in the initial reactive and exudative phases (PE) and of smaller size. Therefore, the data presented here support further studies with chalcones in the treatment of schistosomiasis.

5. Conclusion

In summary, our results suggest that TC is a compound capable of eliminating both immature forms and adult worms of *S. mansoni* without inducing significant cytotoxicity. This effect is attributed to its ability to interact with specific molecules of the parasite, as determined in previous studies, leading to loss of membrane integrity and alterations in the integument, with consequent reduction in oviposition. In addition, TC has been found to modulate the immunopathogenesis associated with schistosomiasis, by reducing the levels of pro-fibrotic cytokines and altering the size and profile of granulomas induced during the patent phase of infection.

CRediT authorship contribution statement

Mariana Barbosa Detoni: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Luryan Silvério Fidélis Ortiz:** Methodology, Investigation. **Amanda Caroliny Gomilde:** Methodology, Investigation. **Bruna Taciane da Silva Bortoleti:** Writing – review & editing, Validation, Methodology, Investigation, Data curation. **Fernanda Tomiotto-Pellissier:** Writing – review & editing, Validation, Methodology, Investigation, Data curation. **Virgínia Marcia Concato-Lopes:** Writing – review & editing, Validation, Methodology, Investigation. **Manoela Daiele Gonçalves-Lens:** Validation, Methodology, Investigation, Data curation. **Taylon Felipe Silva:** Validation, Methodology, Investigation. **Ana Carolina Jacob Rodrigues:** Writing – review & editing, Validation, Methodology, Investigation. **Amanda Cristina Machado Carloto:** Validation, Methodology, Investigation. **Ellen Mayara Souza Cruz:** Validation,

Methodology, Investigation. **Danielle Lazarin-Bidóia:** Validation, Methodology, Investigation. **Waldiceu Aparecido Verri:** Resources. **Francisco José de Abreu Oliveira:** Supervision, Methodology, Conceptualization. **Milena Menegazzo Miranda-Sapla:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Ivete Conchon-Costa:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization. **Wander Rogério Pavanelli:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbi.2025.111770.

Data availability

Data will be made available on request.

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Calcipotriol attenuates fibrogenesis, inflammation, and oxidative stress in hepatic stellate cells and in murine model of schistosomiasis

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Abstract

Schistosomiasis-associated liver fibrosis remains a significant public health concern, with limited therapeutic options available besides antiparasitic

treatment. This study aimed to evaluate the antifibrotic and immunomodulatory effects of calcipotriol (CP), a vitamin D receptor agonist, compared to praziquantel (PZQ), in an experimental model of *Schistosoma mansoni* infection. BALB/c mice were infected with 80 ± 5 cercariae and treated orally for 15 consecutive days with either PZQ (40 mg/kg) or CP (20 µg/kg). Parasitological, biochemical, histopathological and immunological parameters were assessed. In parallel, *in vitro* experiments using GRX hepatic stellate cells were conducted to investigate oxidative stress, mitochondrial dysfunction, lipid metabolism, cytokine production and morphological changes. CP treatment significantly reduced nitric oxide and superoxide anion levels, induced mitochondrial membrane depolarization, and decreased lipid droplet accumulation and TGF-β levels in HSCs. This contrasts with PZQ, which exacerbated superoxide anion and induced mitochondrial hyperpolarization. *In vivo*, CP effectively reduced macrophage infiltration, granuloma size, arginase-1, and type I and III collagen deposition, resulting in decreased liver and spleen mass. Furthermore, CP modulated the immune response by reducing IL-12, IL-4, and TGF-β levels. Remarkably, CP promoted controlled cellular death or inactivation without inducing significant oxidative damage. Taken together, these results suggest that CP exhibits potent antifibrotic, antioxidant, and immunomodulatory properties, making it a promising adjunctive therapy for hepatic fibrosis induced by schistosomiasis.

Key-words

Schistosoma mansoni; Vitamin D; Granuloma; Collagen; Cell death; Immunomodulation.

Abbreviations

ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; BH, Belo Horizonte; CC_{50}, half-maximal cytotoxic concentration; CCCP, Carbonyl cyanide m-chlorophenyl hydrazone; CP, Claicpotriol; DCF, 2′,7′-dichlorofluorescein; DH, Ductal hyperplasia; DMEM, Dulbecco's Modified Eagle Medium; DMSO, Dimethyl sulfoxide; EI, Eosinophilic infiltrate; ELISA, Enzyme-linked immunosorbent assay; EP, Exudative-productive; FBS, Fetal bovine sérum; H_2DCF, 2′,7′-dichlorodihydrofluorescein; H_2DCFA, 2′,7′-

dichlorofluoresceindiacetate; KCH, Kupffer cell hyperplasia; KOH, potassium hydroxide; LD, Lipid droplets; LDH, Lactate dehydrogenase; LI, Lymphocyte infiltrate; MPO, Myeloperoxidase; N, Necrosis; NAG, N-acetylglucosaminidase; NBT, nitroblue tetrazolium; NE, Necrotic-exudative; NOx, Nitric oxide metabolites; NR, Nile red; $O_2^{\bullet-}$, Superoxide anion; P, Productive; PE, Pre-granulomatous exudative; PMA, Phorbol myristate acetate; PZQ, Praziquantel; ROS, Reactive oxygen species; SSLF, Schistosomiasis-associated liver fibrosis; TMRE, tetramethylrhodamine ethyl ester; TS, Total score; VD, Vacuolar degeneration; Δψm, Mitochondrial membrane potential.

1. INTRODUCTION

Schistosomiasis is an acute and chronic parasitic disease caused by blood flukes of the genus *Schistosoma.* The main species involved in human infections are *Schistosoma mansoni*, *Schistosoma haematobium*, and *Schistosoma japonicum*. The disease affects about 250 million people worldwide, and more than 700 million live in endemic areas, being prevalent in tropical and subtropical areas, in poor communities without proper sanitation and drinking water [1].

Schistosomiasis-associated liver fibrosis (SSLF) results from the deposition of *Schistosoma* eggs in hepatic sinusoids, which triggers granuloma formation and fibrogenesis. Initially, infection elicits a Th1-type immune response (IFN-γ, TNF-α, IL-1, IL-2), which partially targets the adult worms. However, as the eggs are deposited, the response shifts to a Th2 profile (IL-4, IL-5, IL-13), promoting granulomatous inflammation and fibrosis [2,3].

Praziquantel (PZQ) is the treatment of choice for schistosomiasis. It is effective against *Schistosoma* adult worms, exhibits low toxicity, ease administration and affordability, making it the standard therapy worldwide [4]. However, despite its efficacy in eliminating the parasites, PZQ is unable to reverse established liver damage, such as fibrosis [5]. Therefore, individuals with chronic complications and advanced hepatic fibrosis require additional therapies alongside PZQ. In such cases, β-blockers are used to reduce portal circulation flow and elastic ligation of varices can prevent bleeding, consequently reducing mortality in patients with periportal fibrosis [6]. These findings highlight the urgent

need for new therapeutic strategies that prevent or reduce the inflammation triggered by egg deposition in host tissues.

The proliferation and activation of hepatic stellate cells (HSCs) are critical factors in the progression of SSLF. Under physiological conditions, HSCs remain in a quiescent state, acting as storage sites for lipid droplets and vitamin A. However, following liver injury, these cells become activated and acquire a myofibroblast-like phenotype (MFB). These MFBs are highly proliferative and secrete large amounts of extracellular matrix (ECM) components, mainly type I and III collagen. Consequently, this process contributes to the development of portal hypertension, hepatomegaly, and severe hepatic dysfunction [7]. Given their central role in fibrogenesis, HSCs represent essential therapeutic targets for antifibrotic strategies [8].

Calcipotriol (CP) is a synthetic analogue of vitamin D3 (1α,25-dihydroxyvitamin D3) that acts as a potent agonist of the vitamin D receptor (VDR). The nuclear vitamin D receptor (nVDR) is a transcription factor that regulates the expression of genes involved in cell proliferation and differentiation, as well as modulating inflammatory and fibrotic responses, which is highly expressed in HSCs [9]. Following liver injury, HSCs become activated, expressing *Acta2* (α-smooth muscle actin, *α-SMA*), leading to the translocation of Smad3 into the nucleus. This process promotes chromatin remodeling and increases accessibility to vitamin D response elements (VDREs). Upon ligand activation, VDR forms a heterodimer with the retinoid X receptor (VDR-RXR), which binds competitively to Smad3 binding sites on chromatin. This inhibits the pro-fibrotic TGF-β/Smad3 signaling pathway, reducing the expression of fibrogenic genes [10]. CP, as a vitamin D analogue, also plays a pivotal role in regulating inflammatory responses as an immunomodulatory, since it negatively modulates the expression of pro-inflammatory cytokines, including TNF-α, IL-1β, IL-6 and IL-8 [11].

In this context, the present study aimed to evaluate the potential of CP in mitigating SSLF. For this purpose, hepatic granuloma dimensions and collagen accumulation were analyzed, and its effects on HSCs were further investigated, including its anti-proliferative capacity, antioxidant properties, and modulation of profibrotic cytokine. Taken together, our findings indicate that CP has therapeutic

potential as an anti-inflammatory and antifibrotic agent in the treatment of liver fibrosis induced by *S. mansoni* infection.

2. MATERIALS AND METHODS

2.1. Calcipotriol and Praziquantel

Calcipotriol (CP, CAS 147657-22-5) and Praziquantel (PZQ, CAS 55268-74-1), both with 98% purity (Cayman Chemical, USA), were used in this study. Stock solutions were prepared in dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO, USA) and stored at -20 °C. DMSO was used as vehicle control, not exceeding 0.02% *in vitro* and 2% *in vivo* experiments.

2.2. Animals and ethics statement

Animal care and handling procedures were performed according to the approval of the Ethical Committee for the Use of Animals at the State University of Londrina, Paraná, Brazil (process number 007.2024). Female BALB/c mice (4 to 6 weeks old, 20-22 g) were housed in cages in a standard environment (24°C room temperature, 12-h light/dark cycle) with *ad libitum* access to water and food. The animals were used to maintain the *S. mansoni* (BH strain) life cycle and for *in vivo* experimental procedures.

2.3. Maintenance of the *Schistosoma mansoni* life cycle (BH strain)

The BH strain (Belo Horizonte strain) of *S. mansoni* was maintained through passage in BALB/c mice and *Biomphalaria glabrata* snails. Initially, BALB/c mice were infected with 80 ± 5 cercariae. Forty-five days' post-infection, eggs excreted in the feces were collected using the Hoffman technique and used to infect the snails. For snail infection, 24-well plates were used. In each well, 10 eggs were placed, followed by the introduction of the snails. The snails were exposed to the eggs for 2 hours under artificial light and elevated temperature and then transferred to aquaria separate from uninfected animals. Thirty days' post-infection, the snails were exposed to artificial light at approximately 28 °C for

2 hours to induce cercarial shedding. BALB/c mice were then infected with 80 ± 5 *S. mansoni* cercariae via percutaneous exposure for 1 hour under artificial light and heat.

2.4. *In vivo* experiments

2.4.1. Animal procedure and in vivo experiments

Four-to six-week-old female BALB/c mice were randomly divided into four groups (n = 6 per group). Animals were infected by exposing the tail to a suspension containing 80 ± 5 *S. mansoni* cercariae. Infection was confirmed on day 45th post-infection (patent infection) using the spontaneous sedimentation technique. The treatment started on day 45th and finished on day 60th post infection. It was administered by oral gavage (200 µL) for 15 days. Group: I) infected-untreated control (phosphate buffered saline; PBS); II) vehicle control (2% DMSO - PZQ vehicle); III) PZQ (40 mg/kg bw); and IV) CP (20 µg/kg bw). 24 hours after the end of treatment, the animals were anesthetized with ketamine (180 mg/kg) and xylazine (26 mg/kg) and euthanized by cervical dislocation according to the ethics committee's guidelines.

Blood was collected via cardiac puncture, and the liver, spleen, and intestine were removed for relative organ mass measurements. The blood was then centrifuged at 1000 g for 10 minutes and the plasma was used to determine the levels of aspartate aminotransferase, alanine aminotransferase and nitrite. Liver samples were used to measure superoxide anion, cytokine and myeloperoxidase and N-acetylglucosaminidase activity. Additionally, liver fragments were fixed in 10% buffered formalin for histological analysis.

2.4.2. Parasitological criteria assay

Parasitological parameters were evaluated by counting the number of eggs in the faeces on days 45, 52 and 60 after infection using the quantitative Kato–Katz method, and by determining the number of worms recovered through the perfusion method and the number of eggs in the liver after digestion in a 5% potassium hydroxide (KOH) solution.

2.4.3. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) determination

AST and ALT levels were measured in the plasma of the animals with a biochemical auto-analyzer (Dimension Dade AR Dade Behring, Deerfield, IL, USA), using Dade Behring® kits.

2.4.4. *Nitric oxide metabolites (NOx) assessment in vivo*

Plasma NOx levels were estimated by measuring nitrite as previously described [12]. Plasma supernatant (60 µL) was deproteinized in a $ZnSO_4$ solution (75 mM), followed by a NaOH solution (55 mM). The final supernatants were collected and diluted in a glycine buffer (45 g/L). Cadmium granules stored in H_2SO_4 solution (100 mM) were added to $CuSO_4$ solution (5 mM) in glycine-NaOH buffer (15 g/L). Activated granules were added to glycine buffer-diluted supernatants, and gently vortexed for 10 min. Griess reagent (60 µL) was added, incubated for 10 min at room temperature, protected from light, and read on a plate reader (Thermo Scientific, Multiskan GO, USA) at 550 nm. Serial dilutions of $NaNO_2$ were used for quantification. Results were expressed as µM nitrite.

2.4.5. Superoxide anion measurement in vivo

The superoxide anion ($O_2^{\bullet-}$) production in the liver homogenates was evaluated by testing with nitroblue tetrazolium (NBT, Sigma-Aldrich, USA). For the assay, liver fragments were mechanically homogenized (Tissue-treator, BioSpec) in KCl 1.15% (100 mg/mL). Then, 10 µL of supernatants were plated, and 100 µL of the solution containing NBT (1 mg/mL) was added. After 15 min of reaction at room temperature, the formazan, product of the reaction, was solubilized by the addition of 60 µL of 2.0 M KOH and 60 µL of DMSO. The readings were performed on a spectrophotometer (GloMax Explorer instrument, Promega) at 600 nm.

2.4.6. Myeloperoxidase (MPO) and N-acetylglucosaminidase (NAG) activity

MPO and NAG enzyme activities can indirectly assess local neutrophil and macrophage infiltration, respectively. Liver samples were collected in a 50 mM K_2PO_4 buffer (50 mM, pH 6.0) containing 0.5% hexadecyltrimethylammonium and homogenized (Tissue-tearor, BioSpec). For MPO activity, the supernatants (15 µL) were mixed with phosphate buffer (50 mM), containing O-dianisidine dihydrochloride (0.167 mg/mL) and hydrogen peroxide (0.015%). For NAG activity, supernatants (10 µL) were mixed with K2HPO4 buffer (50 mM) containing 4-nitrophenyl-N-acetyl-b-d-glucosaminide and glycine buffer (0.2 M). Absorbance was measured using a spectrophotometer (Thermo Scientific, Multiskan GO, USA) to determine MPO (450 nm) and NAG (400 nm) activity. The MPO and NAG activities were compared to a standard curve of neutrophils and macrophages, respectively. Results were expressed as MPO (number of neutrophils/mg tissue) and NAG (number of macrophages/mg tissue) activity.

2.4.7. Cytokine measurement in vivo

Cytokine levels were assessed in the supernatant of the liver homogenates by Cytometric Beads Array (CBA) assay. A commercial kit (BD Bioscience, USA) (mouse Th1/Th2/Th17) was used to detect IFN-γ (0.5 pg/mL). Data were collected in a BD Accuri C6 flow cytometer and analyzed using FCAP Array v3.0.1 software. IL-12p70, IL-10, IL-13, IL-4 and TGF-β levels were determined by enzyme-linked immunosorbent assay (ELISA) according to the instructions of the ELISA kit manufacturer (eBiosciences®, USA).

2.4.8. Histopathology, hepatic granuloma and collagen quantification

Liver fragments collected from the medial lobe were fixed in a 10% buffered formalin solution (pH 7.2), dehydrated, included in paraffin, sectioned into a microtome (SLEE CUT 4062), and stained with hematoxylin and eosin (H&E). The sections were examined by light microscopy (O400S Opticam) at a final magnification of 200×. It was determined: 1x = vacuolar degeneration; 1x = Kupffer cells hyperplasia; 1x = ductal hyperplasia; 1x = eosinophilic infiltrate; 1x

= lymphocyte infiltrate; and 3x = necrosis. For intensity: 0 = absent; 1 = mild, 2 = mild to moderate; 3 = moderate; 4 = moderate to severe, and 5 = severe.

The number and total area of granulomas were evaluated. The mean diameter of each granuloma was calculated by measuring two diameters perpendicular to each other. The liver granulomas were also characterized as PE (pre-granulomatous exudative phase); NE (necrotic-exudative); EP (exudative-productive phase); and P (productive) [13,14]. For quantification of type I and III collagen fibers, liver sections were stained with Sirius red using the picrosirius technique and examined under polarized light using a photomicroscope (Nikon Eclipse 80i) with a coupled camera (200 × final magnification). Images were analyzed using Image-Pro Plus (version 4.5). Red fibers correspond to type I collagen and yellow/green to type III collagen.

2.4.9. Immunohistochemistry for Arginase-1

Liver sections were processed as previously described and subjected to antigen retrieval in citrate-acetate buffer (10 mM, pH 6) for 45 min in a pressure cooker at 90°C. Endogenous peroxidase activity was blocked using a solution of 3% H_2O_2, 10% methanol (Merck), and 0.1% Tween-80 (Sigma-Aldrich) for 30 min followed by blocking of nonspecific binding sites with PBS (0.1% Tween-80) containing 3% BSA (GIBCO Invitrogen) for 30 min at room temperature. Sections were then incubated with the primary antibody against Arginase-1 (Arg-1, 1:600, Santa Cruz, SC18351). Subsequently, a universal biotinylated secondary antibody solution (antigoat IgG, System-HRP, 1:200, DAKO, Japan) was added for 1 h at room temperature. After three washes with PBS, sections were incubated with 3,3'-diaminobenzidine diaminobenzidine (DAB, DAKO, K3468), and counterstained with hematoxylin (Merck).

For quantitative analysis, images were evaluated using the color deconvolution tool in FIJI software (NIH, USA). For the negative control, sections were incubated without the primary antibody to ensure that any observed signal resulted from specific binding. Data are expressed as the percentage of labeled pixels relative to the image area. Five images per animal were analyzed, with five animals evaluated per group.

2.5. *In vitro* experiments

2.5.1. Cell culture

The GRX cell line (hepatic stellate cells, HSCs), derived from the spontaneous migration of cells from granulomas in the livers of *Schistosoma mansoni*-infected C3H/HeN mice, was obtained from the Cell Bank of Rio de Janeiro and kindly provided by Lizandra Guidi Magalhães Caldas (Natural Products Research Group, Research Center for Science and Technology, University of Franca). Under standard culture conditions, GRX cells exhibit a myofibroblastic phenotype, with proliferative activity and extracellular matrix production [15]. Additionally, L929 cells (fibroblasts from C3H/Na mice) (ATCC CCL1) were also used. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Life Technologies, Carlsbad, CA, USA), supplemented with 10% fetal bovine serum (FBS) (GIBCO, Invitrogen, New York, USA), 100 U/mL penicillin, and 100 µg/mL streptomycin (Santa Cruz Biotechnologies, Dallas, TX, USA), and incubated at 37 °C under 5% CO_2.

2.5.2. Cell viability assay

GRX and L929 cells (10^4 cells/well) were treated with CP and PZQ (range 12.5–200 µM), and cytotoxicity was assessed at 24 h. Then, resazurin (60 µM) was added and left for 2 h, and the absorbance was quantified in a Glomax® spectrophotometer (Promega, USA) at 600 nm. This method detects the metabolic activity of cells by reducing resazurin (7-hydroxy-3 H-phenoxazine-3-one 10-oxide) to resorufin (reduced form) using mitochondrial enzymes. The half-maximal cytotoxic concentration (CC_{50}) curve was calculated by logarithmic regression using GraphPad Prism 8 software for the above times. The CC_{50} at 24 h was used for the remaining experiments.

2.5.3. Analysis of reactive oxygen species (ROS)

GRX cells were plated (10^4 cells/well) in black 96-well plates, treated with CC_{50}-CP and CC_{50}-PZQ, and incubated for 24 h. Then, cells were washed with

PBS and a solution of 10 µM 2′,7’-dichlorofluoresceindiacetate (H_2DCFDA; Sigma-Aldrich) was added and left for 30 min in the dark at 37°C. H_2DCFDA, a cell-permeant probe, after diffusing into the cell, is deacetylated by cellular esterases to form 2′,7′-dichlorodihydrofluorescein (H_2DCF). In the presence of ROS, predominantly H_2O_2, H_2DCF is oxidized to 2′,7′-dichlorofluorescein (DCF), which is highly fluorescent. Fluorescence intensity was quantified using a Glomax® spectrophotometer (Promega, USA) (excitation: 488 nm; and emission: 530 nm). Untreated cells were used as a negative control. The data obtained was expressed in arbitrary units.

2.5.4. Superoxide anion measurement in vitro

The production of superoxide anion radical ($O_2^{\bullet-}$) was determined by the reduction of NBT (1 mg/mL; Sigma-Aldrich). GRX cells (10^4 cells/well) were treated with CC_{50}-CP and CC_{50}-PZQ, incubated for 24 h, washed with PBS, and subsequently, 100 µL of NBT solution was added and incubated at 37 °C for 1 h. The supernatant was removed, and the formazan precipitate was then solubilized by adding 60 µL of 2.0 M KOH and 60 µL of DMSO. The optical density was measured spectrophotometrically at 600 nm in a Glomax® spectrophotometer (Promega, USA). Untreated cells were used as a negative control, and phorbol myristate acetate (PMA; 1 µM) was used as a positive control.

2.5.5. Determination of nitric oxide metabolites (NOx) in vitro

NOx levels were determined based on the Griess method. GRX cells (10^4 cells/well) were treated with CC_{50} of CP and PZQ and incubated for 24 h. The supernatant of the samples was collected and analyzed as previously described [12]. Briefly, 60 µL of supernatant was added to 60 µL of Griess reagent (1% sulfanilamide and 0.1% N-(1-Naphthyl) ethylenediamine in 5% orthophosphoric acid (H_3PO_4)), incubated for 10 min, protected from light, at room temperature, and read on a plate reader (Glomax® spectrophotometer; Promega, USA) at 550 nm. For quantification, serial dilutions of $NaNO_2$ were used. Untreated cells were used as a negative control.

2.5.6. Determination of mitochondrial membrane potential (ΔΨm)

ΔΨm was determined by tetramethylrhodamine ethyl ester (TMRE; Sigma-Aldrich, St. Louis, MO, USA) staining. TMRE is a cell-permeable, cationic, red-orange, fluorescent dye that readily accumulates in active mitochondria due to its relative negative charge. In contrast, inactive mitochondria have limited ΔΨm and do not sequester TMRE. GRX cells were plated (10^4 cells/well) in black 96-well plates, treated with CC_{50} CP and PZQ, and incubated for 24 h. After, TMRE (25 nM) was added and incubated for 30 min at 37 °C. The wells were then washed with PBS and immediately analyzed fluorometrically at 480 nm excitation and 580 nm emission in a GloMax® Microplate Reader (Promega, USA). Data was expressed in arbitrary units. Carbonyl cyanide m-chlorophenyl hydrazone (CCCP; 100 μM) was used as the positive control.

2.5.7. Detection of lipid droplets (LD)

GRX cells were plated (10^4 cells/well) in black 96-well plate and treated as described above. Lipid droplets were evaluated by staining with Nile red (NR; Sigma-Aldrich, St. Louis, MO, USA); a lipophilic stain used to localize and quantitate lipids, particularly neutral lipid droplets within cells. In the presence of lipids, the fluorescence colors range from golden yellow to deep red. Firstly, cells were washed with PBS, stained with 10 μg/mL NR (Sigma-Aldrich) for 30 min, and washed again. The cells were analyzed using a GloMax® Microplate Reader (Promega, USA) at 530 and 635 nm for excitation and emission, respectively. Data was expressed in arbitrary units.

2.5.8. Lactate dehydrogenase (LDH) Activity

To evaluate the LDH activity, GRX cells (10^6 cells/well) were seeded in 24-well microplates and treated with CC_{50}-CP and CC_{50}-PZQ for 24 h. After, the cell supernatant was collected to measure extracellular LDH. To determine intracellular LDH activity, the adherent cells were washed twice with PBS and then subjected to three cycles of freezing at −80 °C and thawing in a heated water bath to lyse the cells. Intra- and extracellular LDH assays were performed

according to the kit instructions (Gold Analisa Diagnóstico, Minas Gerais, Brazil). Untreated cells were used as a negative control.

2.5.9. Cytokine measurement in vitro

The level of cytokine TGF-β in the cell supernatant was measured using a commercial ELISA kit (eBiosciences®, USA), following the manufacturer's instructions. The optical density was measured using a microplate reader with a 450 nm filter and the results were expressed in pg/mL based on a cytokine standard curve (GloMax® Microplate Reader, Promega, USA).

2.5.10. Morphological and ultrastructural analysis by scanning electron microscopy

Scanning electron microscopy of the GRX cell line was performed to analyze morphological changes in the cell surface topography. Briefly, cells (10^5 cells/well) were seeded into 24-well plates, incubated (37 °C, 5% CO_2) and treated with CC_{50}-CP and PZQ for 24 h at 37 °C and fixed with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4). The cells were then dehydrated through increasing concentrations of ethanol (30–100%), subjected to critical point drying (Baltec SCD-030, Balzers, Liechtenstein), and metallized with gold for visualization using a high-resolution FEI SCIOS (Oregon, USA) double beam electron microscope (Thermo Fisher Scientific, Massachusetts, United States).

2.6. Statistical analysis

Data are expressed as the mean ± standard error of the mean (SEM). Three independent experiments were performed, each with duplicate data sets, and analyzed with GraphPad Prism 8 statistical software (GraphPad Software, Inc., USA, 500.288). Significant differences between groups were determined through t-tests or one-way ANOVA followed by Tukey's test for multiple comparisons. A result was considered statistically significant if $p < 0.05$. Values

were also categorized fallows: * ($p < 0.05$); ** ($p \leq 0.01$); *** ($p \leq 0.001$); **** ($p \leq 0.0001$).

3. RESULTS

3.1. Calcipotriol reduces liver and spleen mass without affecting parasite burden

The antiparasitic effect of calcipotriol was initially evaluated by assessing organ mass, and the number of eggs and adult worms in feces and liver tissue. For the uninfected control group, the relative liver and spleen masses were 0.053 g (± 0.001) and 0.006 g (± 0.0005), respectively, whereas in the infected control group they were 0.099 g (± 0.006) and 0.026 g (± 0.003), resulting in a significantly different ($p \leq 0.0001$) difference between the group. In animals treated with PZQ, the relative liver and spleen masses were 0.070 g (± 0.003) and 0.014 g (± 0.001), respectively, while in those treated with CP were 0.077 g (± 0.005) and 0.016 g (± 0.002), respectively. Both PZQ ($p \leq 0.01$) and CP ($p < 0.05$) treatments significantly reduced liver and spleen masses compared with the infected control group (**Fig. 1 A and B**). A significant difference was also observed in the relative intestinal mass between the uninfected (0.007 ± 0.0005 g) and infected (0.010 ± 0.0006 g) control groups ($p < 0.05$) (**Fig. 1 C**).

When assessing the number of eggs per gram of feces, on day 60 post-infection, only the PZQ group exhibited a significant reduction in fecal egg count compared to the other groups ($p \leq 0.0001$) (**Fig. 1 D**). Regarding hepatic egg load, PZQ also significantly reduced the number of eggs in the liver to 29.59 (±5.44) ($p \leq 0.0001$) (**Fig. 1 E**). For total worm burden, the infected control group showed a mean of 26.94 (±2.84) worms, compared to 11.25 (±1.48) in the PZQ group and 18.30 (±2.38) in the CP group. Similarly, only PZQ significantly reduced the number of worms in the liver ($p \leq 0.001$) (**Fig. 1 F**).

Taken together, these findings indicate that CP was not effective in reducing the overall parasite burden. However, its ability to decrease organ masses suggests a potential role in alleviating parasite-induced tissue alterations.

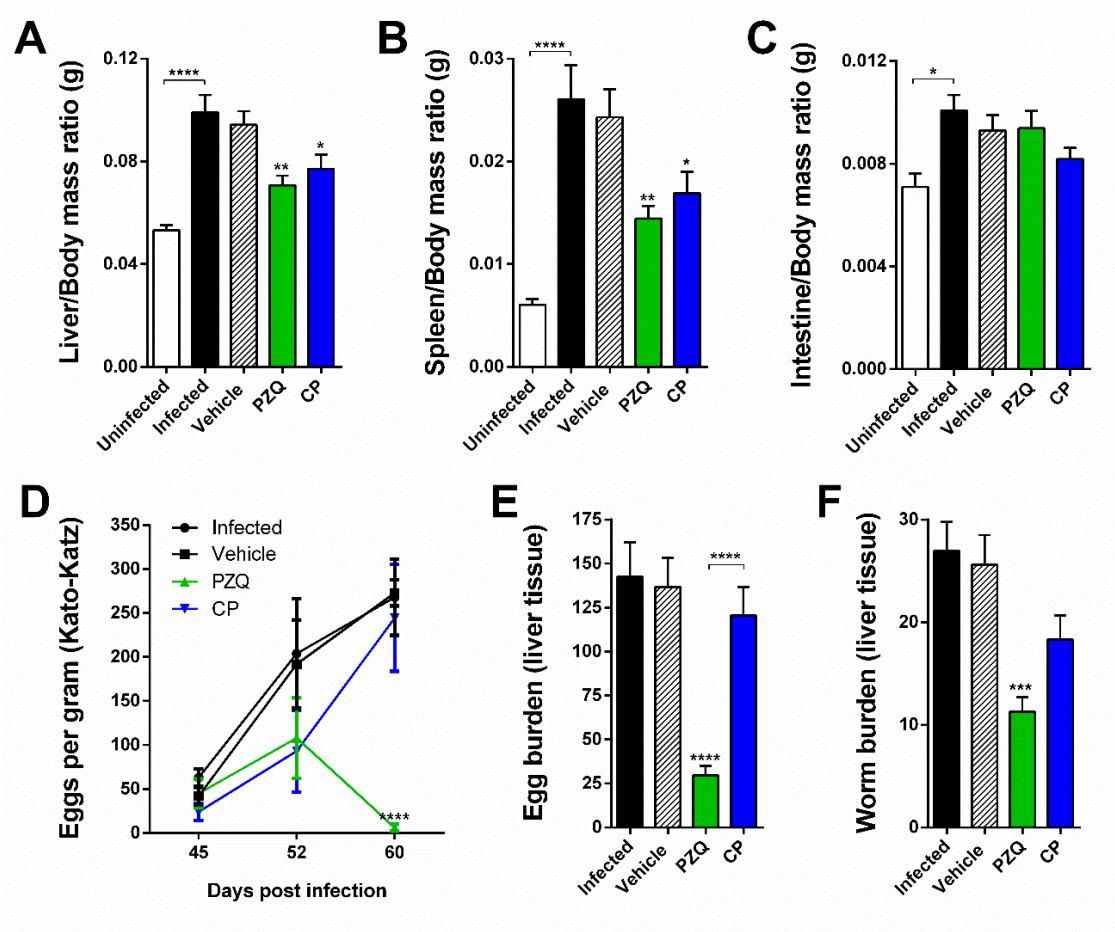

Figure 1. Effects of Praziquantel (PZQ) and Calcipotriol (CP) treatment on organ weight and parasitic burden in *Schistosoma mansoni*-infected BALB/c mice. Female BALB/c mice were infected with 80 ± 5 *Schistosoma mansoni* cercariae. After 45 days of infection, the animals were treated orally for 15 consecutive days with phosphate buffered saline (infected control group), 2% DMSO (vehicle group), PZQ (40 mg/kg) and CP (20 µg/kg) (n=6 animals/group). Twenty-four hours after the end of treatment, the animals were euthanized, and the relative masses of the liver (A), spleen (B), and intestine (C) were measured. Parasitic load was assessed by counting the number of eggs per gram of feces using the Kato-Katz method on days 45, 52, and 60 post-infection (D). Egg burden was quantified by liver tissue digestion (E), and the worm burden was assessed by perfusion of the hepatic portal system (F). Non-infected animals were included in the uninfected control group. Data represent the mean ± SEM. Significant difference: * ($p < 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$), and **** ($p \leq 0.0001$).

3.2. Treatment with calcipotriol modulates cellular infiltration in liver tissue and regulates nitric oxide production levels

To assess systemic and hepatic inflammatory alterations following treatment with PZQ and CP, serum levels of AST, ALT, and NOx were measured,

along with the hepatic production of superoxide anion, and the levels of MPO (a neutrophil marker) and NAG (a macrophage marker). No significant reductions were observed in AST, ALT, or superoxide anion levels (**Fig. 2 A - C**); however, CP treatment showed a trend toward decreased ALT levels ($p = 0.094$) (**Fig. 2 B**). Regarding nitrite, MPO, and NAG levels, CP significantly reduced all three compared to PZQ ($p < 0.05$) (**Fig. 2 D-F**) and also decreased NAG levels compared to the infected control group ($p < 0.05$) (**Fig. 2 E**). These findings suggest that CP was able to modulate the inflammatory response in liver tissue, demonstrating a more effective outcome than PZQ.

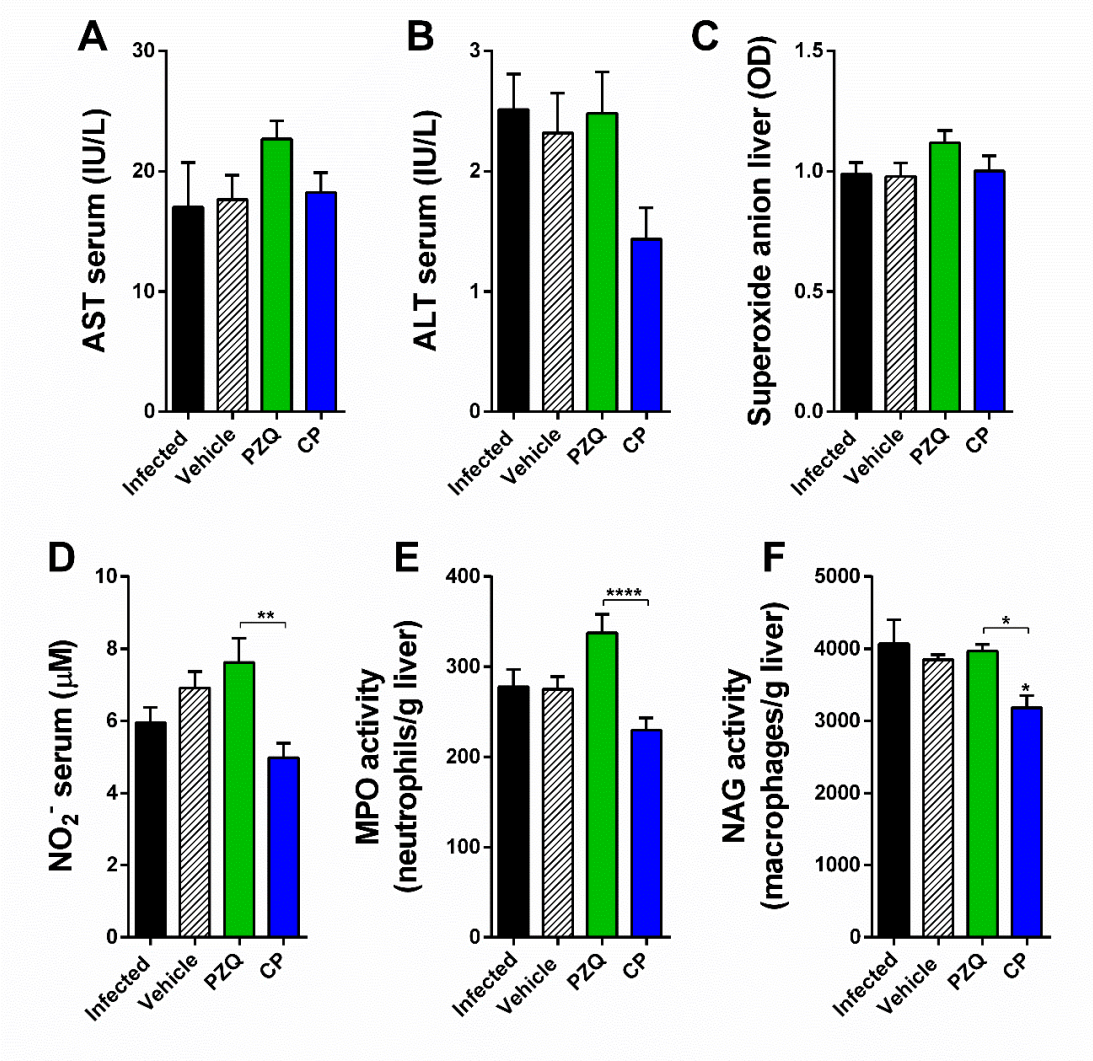

Figure 2. Hepatic and systemic inflammatory alterations in *Schistosoma mansoni*-infected BALB/c mice treated with Praziquantel (PZQ) and Calcipotriol (CP). BALB/c mice were infected with 80 ± 5 *Schistosoma mansoni* cercariae. After 45 days of infection, the animals were treated orally for 15 consecutive days with phosphate buffered saline (infected control group), 2%

DMSO (vehicle group), PZQ (40 mg/kg) and CP (20 µg/kg) (n=6 animals/group). Twenty-four hours after the end of treatment, the animals were euthanized, and plasma and liver tissue samples were collected. Serum levels of AST (A), ALT (B), and nitrite (NO_2^-) (D) were quantified. In liver homogenates, superoxide anion production (C), N-acetyl-β-D-glucosaminidase (NAG) activity (E), and myeloperoxidase (MPO) activity (F) were evaluated. Data represents the mean ± SEM. Significant difference: * ($p < 0.05$), ** ($p \leq 0.01$), and **** ($p \leq 0.0001$).

3.3. Calcipotriol attenuates histopathological alterations in the liver by reducing collagen deposition and modulating hepatic granulomas

Based on the previously anti-inflammatory effects of CP treatment in *S. mansoni*-infected mice, we assessed histological changes and deposition of type I and III collagen post-treatment. Both PZQ- and CP-treated groups exhibited a significant reduction in the total area (**Fig. 3 A**) and diameter (**Fig. 3 B**) of hepatic granulomas ($p < 0.05$). Regarding collagen deposition, the CP-treated group exhibited a significant reduction in type I and III collagen compared to both the infected control and PZQ groups ($p < 0.05$) (**Fig. 3 C and D**).

During the acute phase, granulomas with necrotic-exudative (NE) characteristics predominate, indicating an exacerbated inflammatory response at this stage of infection. This granuloma type is typically large, rich in inflammatory cells, and commonly observed in acute human schistosomiasis mansoni [13,16]. For the infected control group, 77.10% of the granulomas were classified as NE, compared to 64.12% in the CP-treated group and 70.45% in the PZQ-treated group. In contrast, relative to early phase (PE), the CP group exhibited 19.08%, while the PZQ group presented only 9.09% (**Fig. 3 E**). Representative images illustrating the area, stage, and collagen deposition in hepatic granulomas from each group are presented in **Figure 3 F**.

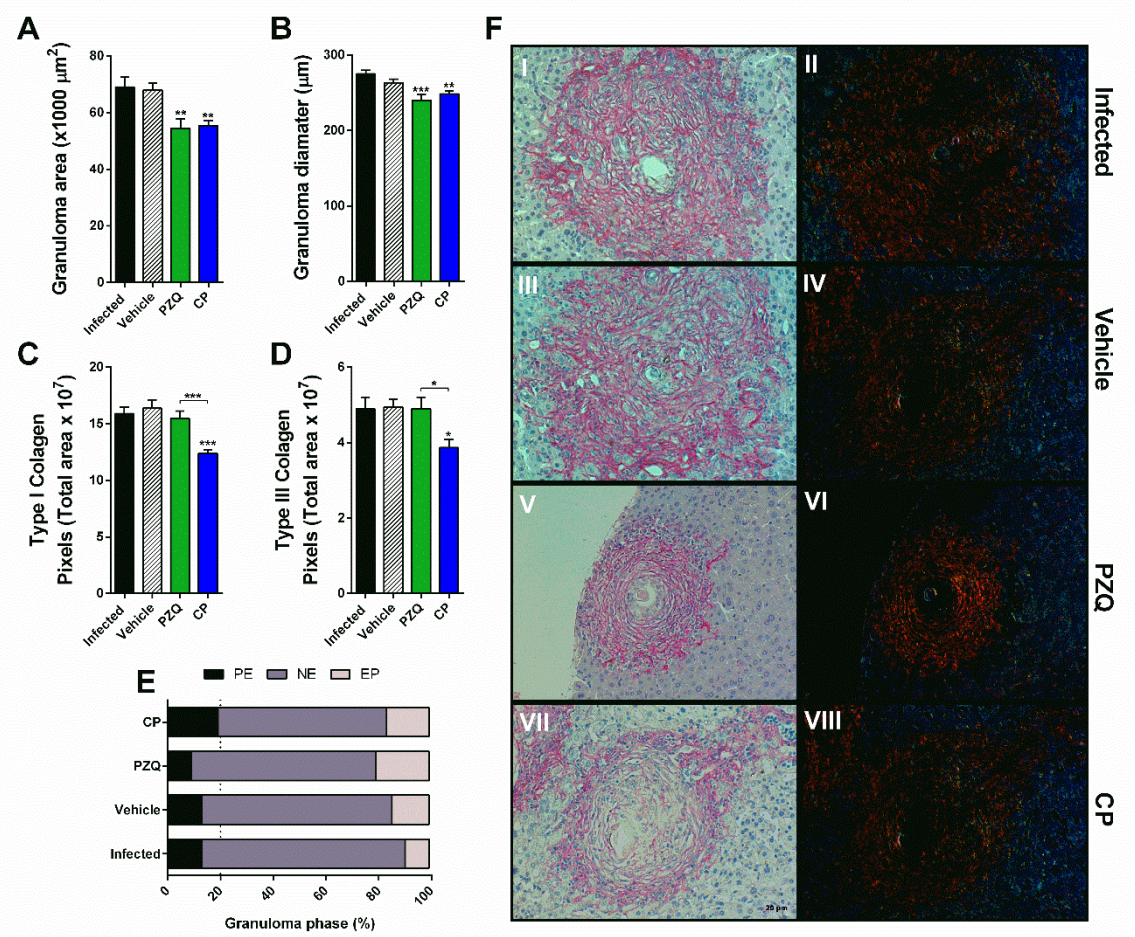

Figure 3. Assessment of granuloma area, diameter, phase, and collagen deposition in *Schistosoma mansoni*-infected BALB/c mice treated with Praziquantel (PZQ) and Calcipotriol (CP). BALB/c mice were infected with 80 ± 5 *Schistosoma mansoni* cercariae. After 45 days of infection, the animals were treated orally for 15 consecutive days with phosphate buffered saline (infected control group), 2% DMSO (vehicle group), PZQ (40 mg/kg) and CP (20 µg/kg) (n=6 animals/group). Twenty-four hours after the end of treatment, the animals were euthanized, and liver samples were then collected for histological analysis. Tissue sections were stained with Hematoxylin and Eosin (H&E) to evaluate granuloma area (A), diameter (B), and developmental phase (E). Sirius Red staining was used to quantify type I (C) and type III (D) collagen deposition using Image-Pro Plus software. Representative photomicrographs of liver granulomas from the respective groups: untreated infected (I and II); vehicle (III and IV); PZQ (V and VI); and CP (VII and VIII) (F). Granuloma phases were classified as PE: pre-granulomatous exudative; NE: necrotic-exudative; EP: exudative-productive; and P: productive. Data represent the mean ± SEM. Significant difference: * ($p < 0.05$), ** ($p \leq 0.01$), and *** ($p \leq 0.001$).

In the histological analysis of liver tissue, PZQ treatment showed an egg:granuloma ratio of 0.98 (±0.22), indicating that each granuloma contained one egg, and a granuloma:egg ratio of 1.01 (±0.29), reinforcing this near one-to-

one correspondence. These findings suggest that granuloma formation is closely linked to the presence of eggs, with limited modulation of the inflammatory process beyond the initial antigenic response. In contrast, treatment with CP resulted in an egg:granuloma ratio of 1.14 (±0.13), indicating more eggs than granulomas, and a granuloma:egg ratio of 0.86 (±0.12), meaning fewer granulomas were formed per egg (**Table 1**). These results suggest that CP was able to reduce granuloma formation even in the presence of eggs, reflecting an anti-inflammatory effect that limits the host's immune response to the parasite antigen.

Table 1. Quantitative histological assessment of hepatic eggs, granulomas, and their ratios in *schistosoma mansoni*-infected mice treated with praziquantel and calcipotriol.

Groups	Egg	Granuloma	Egg / Granuloma	Granuloma / Egg
Infected	80.20±16.07	73.75±10.36	1.08±0.22	0.91±0.41
Vehicle	76.00±18.40	70.86±14.50	1.07±0.09	0.93±0.08
PZQ	33.88±11.90	34.50±7.2	0.98±0.22	1.01±0.29
CP	62.09±12.95	54.00±9.5	1.14±0.13	0.86±0.12

Notes: Values are mean ± SEM. Abbreviations: PZQ, Praziquantel; CP, Calcipotriol.

When evaluating hepatic histopathological alterations in *S. mansoni*-infected mice post-treated with PZQ or CP, it was initially observed that neither treatment significantly reduced vacuolar degeneration compared to the infected group, indicating persistent morphological changes in hepatocytes. Regarding Kupffer cell hyperplasia (KCH), both PZQ and CP significantly reduced this alteration ($p < 0.05$), suggesting decreased activation of the liver's resident inflammatory response. As for ductal hyperplasia, the PZQ-treated group showed a higher score (2.25) than the infected group (1.71), indicating a possible adverse effect on the biliary epithelium. In contrast, CP reduced this alteration (1.25), suggesting a protective effect on ductal structures.

Both treatments decreased eosinophilic infiltrates in liver tissue, particularly CP (2.09 vs. 2.71 in the infected group), potentially reflecting lower inflammatory activity associated with parasitic antigens. A similar trend was

observed for lymphocytic infiltrates, with CP promoting a more pronounced reduction (1.16), indicating lower chronic inflammation compared to the other groups. Both treatments also reduced necrosis levels (PZQ: 1.87; CP: 1.80) compared to the infected group (3.00), reflecting improved tissue integrity. Consequently, the total hepatic lesion score decreased in both treatment groups compared to the infected group (Infected: 30.14 vs. PZQ: 23.50 vs. CP: 24.27), indicating overall improvement in liver pathology (**Table 2**).

Table 2. Histopathological scoring of liver tissue in *schistosoma mansoni*-infected mice treated with praziquantel or calcipotriol.

Groups	VD	KCH	DH	EI	LI	N	TS
Infected	1.66±0.33	3.57±0.20	1.71±0.42	2.71±0.47	1.57±0.36	3.00±0.37	30.14±2.90
Vehicle	1.71±0.42	3.00±0.00	1.62±0.37	2.87±0.35	1.40±0.24	2.25±0.75	27.14±2.69
PZQ	1.62±0.41	2.75±0.16*	2.25±0.31	2.37±0.32	1.37±0.26	1.87±0.44	23.50±2.71
CP	1.54±0.28	2.80±0.24*	1.25±0.27	2.09±0.34	1.16±0.16	1.80±0.35	24.27±2.51

Notes: Values are mean ± SEM. Significant difference from infected * ($p < 0.05$). Abbreviations: VD, vacuolar degeneration; KCH, Kupffer cell hyperplasia; DH, ductal hyperplasia; EI, eosinophilic infiltrate; LI, lymphocyte infiltrate; N, necrosis; TS, total score; PZQ, Praziquantel; CP, Calcipotriol.

In summary, CP treatment demonstrated relevant protective effects on liver tissue, particularly by reducing ductal hyperplasia, inflammatory infiltrates, and necrosis, with comparable performance to PZQ in most parameters. Thus, although PZQ was more effective in reducing parasite burden, CP showed a superior profile in preserving hepatic architecture.

3.4. Calcipotriol modulates inflammatory and pro-fibrotic cytokines and reduces arginase-1 production

Following 15 days of treatment with PZQ and CP, the production of pro- and anti-inflammatory cytokines was assessed in liver fragments from *S. mansoni*-infected BALB/c mice at 60 days' post-infection. Regarding pro-inflammatory cytokines, PZQ treatment significantly reduced IFN-γ production

only ($p \leq 0.001$) (**Fig. 4 A**). By contrast, CP treatment significantly reduced IL-12 levels compared with the infected control group ($p < 0.05$) and the PZQ group ($p \leq 0.01$) (**Fig. 4 B**). Regarding pro-fibrotic cytokines, CP also induced a marked decrease in TGF-β and IL-4 production ($p < 0.05$) (**Fig. 4 D and E**). No significant alteration was observed in IL-10 and IL-13 levels (**Fig. 4 C and F**).

Additionally, immunohistochemistry revealed high Arg-1 labeling in the infected control group (22.46 ± 1.97 %). However, PZQ treatment reduced the labeling area to 15.71% (± 3.05) and CP treatment induced a further significant reduction to 13.54% (± 2.24) ($p < 0.05$) (**Fig 4 G and H**). These findings suggest that CP exerts immunomodulatory effects on cytokines involved in hepatic fibrogenesis (TGF-β and IL-4) and collagen production, while also reducing Arg-1 levels.

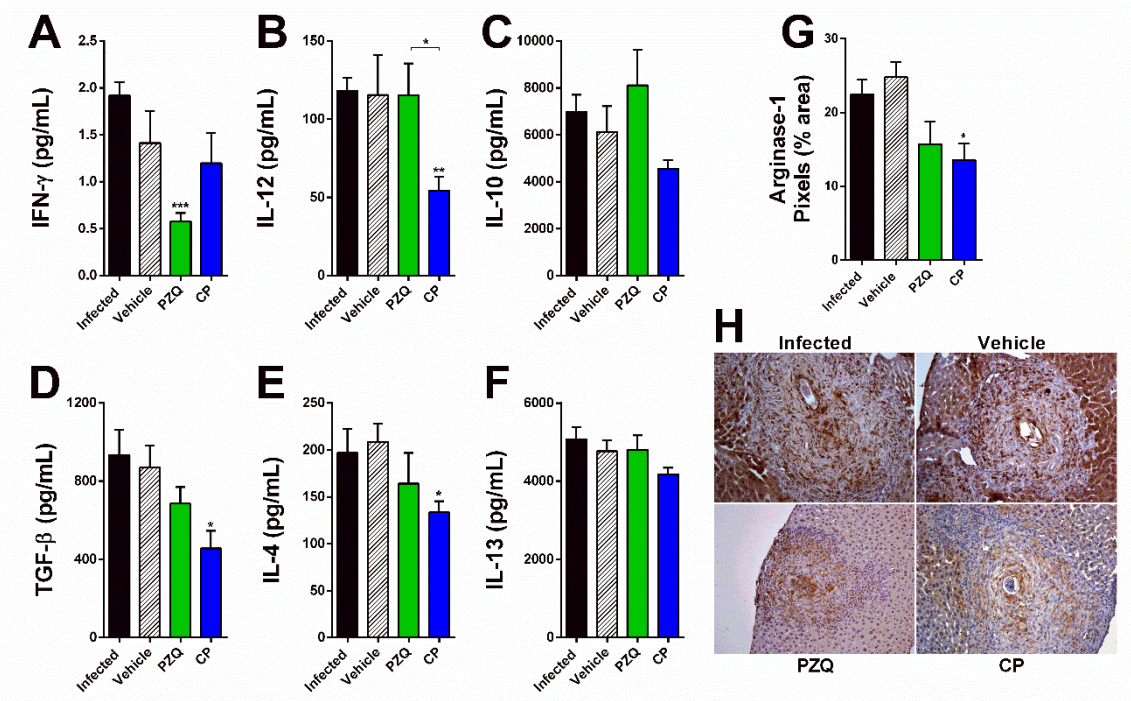

Figure 4. Evaluation of pro- and anti-inflammatory cytokine levels and arginase-1 production in liver samples from *Schistosoma mansoni*-infected BALB/c mice treated with Praziquantel (PZQ) and Calcipotriol (CP). BALB/c mice were infected with 80 ± 5 *Schistosoma mansoni* cercariae. After 45 days of infection, the animals were treated orally for 15 consecutive days with phosphate buffered saline (infected control group), 2% DMSO (vehicle group), PZQ (40 mg/kg) and CP (20 µg/kg) (n=6 animals/group). Twenty-four hours after the end of treatment, the animals were euthanized, and liver tissues were collected. Cytokine levels of IFN-γ (A), IL-12 (B), IL-10 (C), TGF-β (D), IL-4 (E) and IL-13 (F) were measured in liver homogenates. Liver samples were also collected for histological analysis and stained with Hematoxylin and Arginase-1.

Immunohistochemical quantification (G) and representative figures (H) were evaluated. Data represent the mean ± SEM. Significant difference: * ($p < 0.05$), ** ($p \leq 0.01$), and *** ($p \leq 0.001$).

3.5. Calcipotriol reduces the viability of hepatic stellate cells derived from schistosomal granulomas (GRX)

Hepatic stellate cells are the main producers of collagen and other extracellular matrix components, playing a central role in the initiation of fibrogenesis. Among them, the GRX cell line, derived from hepatic granulomas of *S. mansoni*-infected mice, is a representative model of hepatic stellate cell activation in schistosomal fibrosis [15]. Given that CP has been shown to modulate the inflammatory response and influence hepatic granuloma formation, we investigated its effects on GRX cells, as well as on fibroblasts derived from mouse subcutaneous connective tissue (L929).

Firstly, we evaluated the cytotoxicity of PZQ and CP after 24 h of treatment on L929 cells. As a result, PZQ significantly reduced cell viability only at 200 µM ($p \leq 0.001$) (**Fig. 5 A**), while CP showed cytotoxic effects from 100 µM ($p < 0.05$) (**Fig. 5 C**). In GRX cells, PZQ exhibited a similar profile, with significant viability reduction only at 200 µM ($p \leq 0.001$) (**Fig. 5 B**), whereas CP induced cytotoxicity beginning at 25 µM ($p < 0.05$) (**Fig. 5 D**).

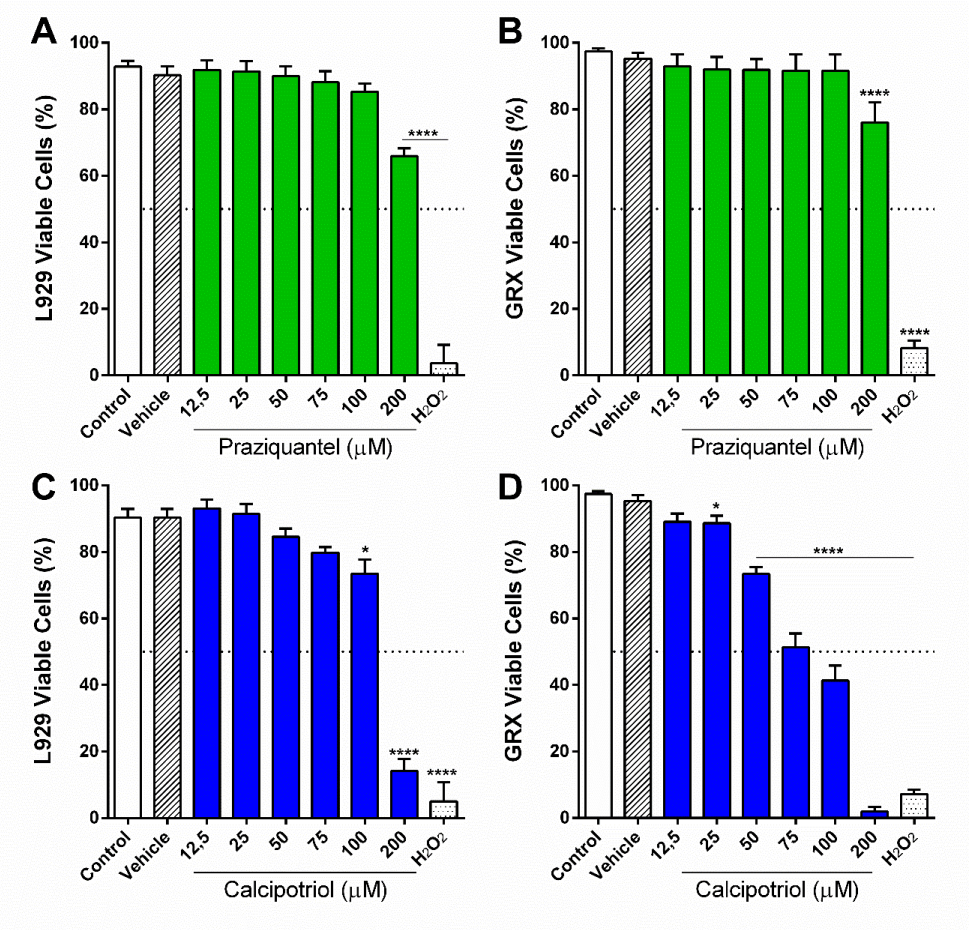

Figure 5. Evaluation of L929 and GRX cell viability following treatment with Praziquantel (PZQ) and Calcipotriol (CP). L929 and GRX cells were cultured and treated with concentrations of 12.5 to 200 μM of PZQ and CP. Cell viability was assessed after 24 hours of treatment. Viability was evaluated for PZQ in L929 cells (A) and GRX cells (B), and for CP in L929 cells (C) and GRX cells (D). Data represent the mean ± SEM. Significant difference: * ($p < 0.05$) and **** ($p \leq 0.0001$).

In addition, the half-maximal cytotoxic concentration (CC_{50}) of PZQ was 465.9 μM (±0.01) for L929 cells and 469.3 μM (±0.01) for GRX cells. In contrast, CP exhibited a CC_{50} of 123.1 μM (±0.02) for L929 cells and 76.6 μM (±0.01) for GRX cells. Based on these values, the selectivity index (SI) of PZQ for GRX cells was 0.99, indicating low selectivity for this cell type. In contrast, CP exhibited a higher SI of 1.60, demonstrating greater selectivity toward GRX cells (**Table 3**). Together, these findings indicate that CP may directly affect hepatic stellate cells, which play a central role in fibrosis during *S. mansoni* infection.

Table 3. Half-maximal cytotoxic concentration (CC_{50}) and selectivity index of Praziquantel and Calcipotriol in fibroblast (L292) and hepatic stellate (GRX) cells

	L929	GRX	SI
Praziquantel CC_{50} µM (±SEM)	465.9 (±0.01)	469.3 (±0.01)	0.99
Calcipotriol CC_{50} µM (±SEM)	123.1 (±0.02)	76.6 (±0.01)	1.60

Notes: Values are mean ± SEM. Abbreviations: SI, selective index; PZQ, Praziquantel; CP, Calcipotriol; CC_{50}, cytotoxicity concentration for 50% of the cells; SEM, standard error of the mean.

3.6. Calcipotriol modulates oxidative stress and decreases TGF-β levels in GRX cells.

Knowing that CP exhibits greater effect against GRX cells compared to PZQ, we proceeded to investigate the cellular effects induced by both compounds. First, to validate the previously determined CC_{50} values, we performed a new resazurin assay, which confirmed the calculated concentrations (**Fig. 6 A**). Next, we assessed nitrite levels in the cell supernatants. Both treatments significantly reduced nitrite production, with CP being more effective in this reduction ($p \leq 0.001$) (**Fig. 6 B**). Similarly, both compounds significantly decreased total ROS levels ($p \leq 0.0001$), and CP demonstrated a stronger reduction compared to PZQ ($p \leq 0.01$) (**Fig. 6 C**). Regarding superoxide anion levels, PZQ induced a significant increase, similar to the positive control ($p \leq 0.01$), whereas CP significantly reduced superoxide levels compared to PZQ ($p \leq 0.01$) (**Fig 6. D**).

In subsequent analyses, CP induced a marked reduction in mitochondrial membrane potential relative to both the control ($p \leq 0.001$) and PZQ ($p \leq 0.0001$), indicating mitochondrial depolarization (**Fig. 6 E**). CP also led to increased extracellular LDH and decreased intracellular LDH levels ($p \leq 0.0001$), suggesting membrane disruption (**Fig. 6 F and G**). Finally, CP treatment resulted in a significant reduction of cytoplasmic lipid droplets ($p \leq 0.001$) (**Fig. 6 H**), and in TGF-β levels ($p \leq 0.001$) even compared with PZQ ($p \leq 0.01$) (**Fig. 6 I**) in GRX cells.

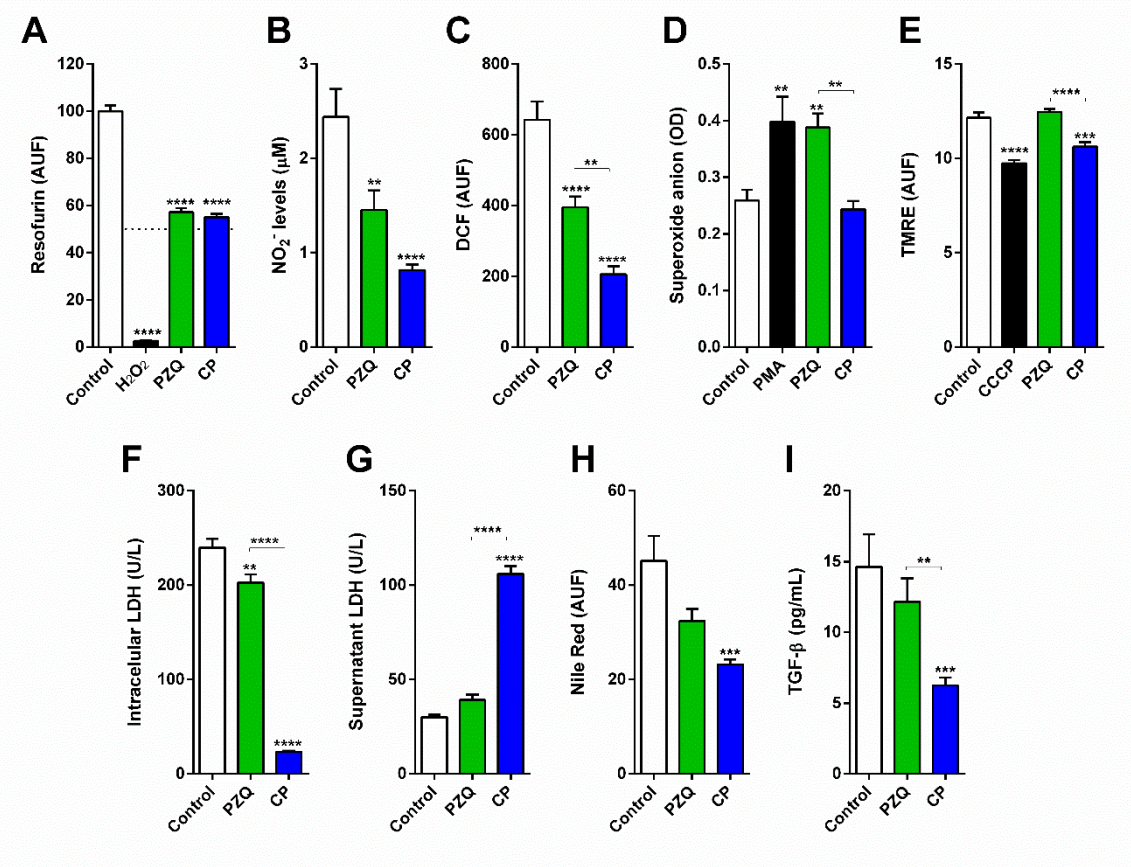

Figure 6. Evaluation of the effects induced by Praziquantel and Calcipotriol on GRX cells. GRX cells were seeded and treated with the half-maximal cytotoxic concentrations (CC_{50}) of PZQ (469.3 µM) and CP (76.6 µM). The following parameters were assessed: metabolic activity by resazurin assay (A), nitrite levels in the supernatant (B), total reactive oxygen species (C), superoxide anion levels (D), mitochondrial membrane potential (E), extracellular (F) and intracellular (G) levels of LDH, accumulation of lipid droplets (H), and TGF-β levels in the supernatant. Data represent the mean ± SEM. Significant difference: ** ($p \leq 0.01$), *** ($p \leq 0.001$), and **** ($p \leq 0.0001$).

3.7. Calcipotriol induces morphological changes in the GRX cell line

Since CP is capable of modulating oxidative parameters and GRX cell activation, we evaluated whether it also induces morphological changes. Untreated cells (**Fig. 7 A and B**) displayed adherence to the substrate, intact plasma membranes, and presence of lamellipodia, filopodia, and microvilli across the cell surface. When the cells were treated with PZQ, exhibited mild alterations, such as partial loss of cytoplasmic filaments (**Fig 7 C-F**), however CP led to pronounced changes, including plasma membrane alterations, cell rounding, reduction or loss of lamellipodia and filopodia, irregular cell surfaces, and

absence or marked reduction of microvilli (**Fig 7 G-J**) These findings indicate that CP treatment significantly altered cell morphology, resulting in the loss of fibroblast-like features.

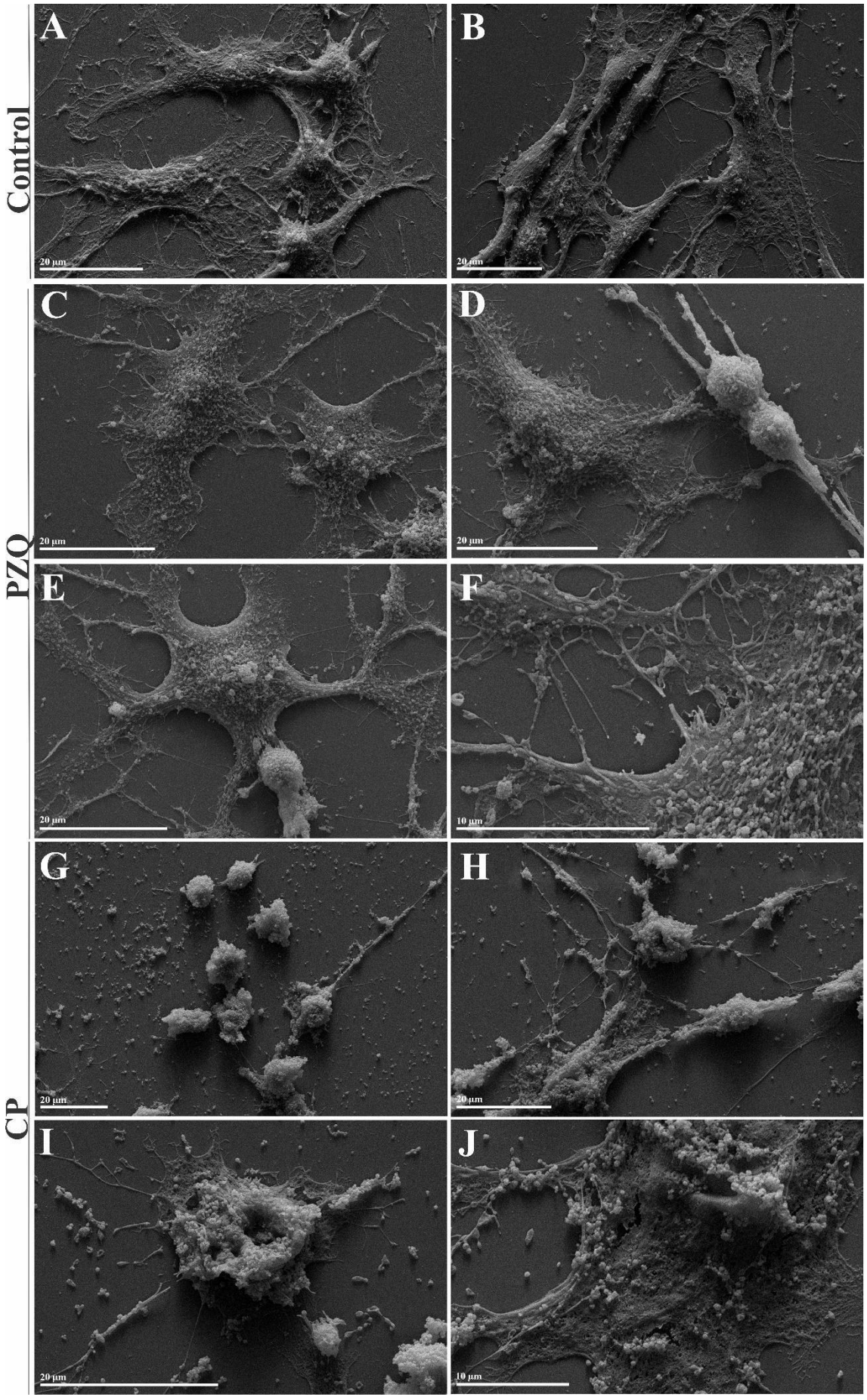
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Figure 7. Effect of Praziquantel and Calcipotrol treatment on the morphology of GRX cells. GRX cells were seeded and treated with the half-maximal cytotoxic concentrations (CC_{50}) of PZQ (469.3 µM) and CP (76.6 µM). Morphological changes were assessed by scanning electron microscopy after treatment for 24 h. (A-B) Control, untreated cells; (C-F) cells treated with CC50-PZQ; (G-J) cells treated with CC_{50}-CP.

4. DISCUSSION

Chronic schistosomiasis affects mostly individuals with long-standing infections, particularly in endemic areas where repeated exposure leads to progression to the chronic phase [17]. In these stage, immune responses to schistosome eggs trapped in tissues result in inflammation and obstruction of the gastrointestinal tract, hepatosplenic involvement and, potentially, periportal fibrosis [18]. Although diagnostic tools and effective treatments such as PZQ are widely available against active infection, chronic disease often remains asymptomatic, allowing progressive tissue damage to remain undetected. Thus, despite being the standard of care, PZQ has limited efficacy against egg-induced inflammation and established fibrosis [19]. Additionally, there are currently no FDA-approved antifibrotic therapies, and individuals with severe disease may require surgery to address fibrosis and prevent life-threatening complications. These limitations highlight the urgent need for additional therapeutic strategies to improve long-term outcomes [6,20]

Previous studies have demonstrated that CP (20 µg/kg) effectively modulates drug-induced liver fibrosis, leading to significant reductions in collagen deposition and hydroxyproline levels, as well as in the expression of fibrotic marker genes (*Col1a1* and *Tgfb1*), without affecting serum calcium levels [10]. Furthermore, treatment also decreased the levels of ALT, AST and TGF-β [21]. Notably, in a murine model of *Schistosoma japonicum* infection, the oral administration of CP (20 µg/kg, five times per week/eight weeks) resulted in a significant decrease in fibrosis, granuloma size, hydroxyproline content and in the expression of *Col1a1*, *Col3a1* and *α-SMA* (*Acta2*) [22]. Thus, while these results support the antifibrotic potential of CP, our study further contributes to this evidence by demonstrating its efficacy in a schistosomiasis model using *S. mansoni*, as well as by investigating its mechanisms of action *in vitro* in HSCs,

highlighting its potential as a therapeutic strategy even under shorter treatment durations.

In this study, we demonstrated that CP, despite lacking direct antiparasitic activity, was able to reduce inflammation, liver injury, and decreased immune cell infiltration and fibrosis in a murine model of *S. mansoni* infection. Notably, CP decreased hepatic granuloma size, type I and III collagen deposition, and levels of pro-fibrotic cytokines such as IL-4 and TGF-β. These reductions were associated with the formation of smaller and newly formed (PE phase) granulomas, and decreased hepato- and splenomegaly. In HSCs cells, CP induced mitochondrial alterations and reduced oxidative stress and TGF-β levels, consistent with senescence or cell death and HSCs inactivation. These findings support the potential of CP as an antifibrotic adjuvant, particularly in the chronic phase of the disease, where controlling fibrosis is crucial to preventing hepatic complications.

One of the host's responses to SEA (soluble egg antigens) released by *S. mansoni* eggs is the generation of reactive oxygen species (ROS) [23]. These molecules may contribute to the death of both schistosomes and miracidia within the eggs. However, excessive ROS production can be detrimental, as it enhances the fibrogenic cascade in the liver [24]. For instance, eosinophils and macrophages that constitute the granulomas are known to produce elevated levels of ROS, including superoxide anion and hydroxyl radicals [25]. This large amount of reactive oxygen intermediates, in turn, is critical in the differentiation of alternatively activated macrophages (M2) [26]. Furthermore, ROS can impair mitochondrial function, increase lipid peroxidation, and induce microvesicular steatosis [27].

Nitric oxide (NO) is another key molecule produced during inflammatory and oxidative stress conditions, particularly in response to parasitic infections such as *S. mansoni* [28]. Its production is primarily mediated by inducible nitric oxide synthase (iNOS), which is upregulated by pro-inflammatory cytokines in macrophages and endothelial cells. NO can react with superoxide anion to generate peroxinitrite ($ONOO^-$), which induces oxidative stress by oxidizing lipids, proteins, and DNA. This leads to lipid peroxidation, mitochondrial dysfunction, and inhibition of critical enzymes [29]. In addition, excessive hepatic NO production contributes not only to direct tissue damage but also to the

amplification of tissue fibrosis, stimulating the release of fibrogenic cytokines and enhancing collagen synthesis [30].

In addition, the evaluation of liver enzyme function, reflected by serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), is commonly used to evaluate hepatic damage caused by schistosomiasis. These enzymes are considered important indicators of hepatocellular injury. The elevated enzymatic activity is attributed to hepatocyte irritation induced by toxins or metabolic products released by developing larvae, adult worms, and deposited eggs, and is further exacerbated by the inflammation triggered by these parasite stages. This irritation leads to the leakage of AST and ALT from damaged hepatocytes into the bloodstream and is often accompanied by the replacement of healthy liver tissue with granulomatous lesions formed around the trapped *Schistosoma* eggs [31].

Egg deposition also triggers factors that suppress this pro-inflammatory state, leading to the production of other mediators such as interleukin IL-4 and IL-13 [32]. This shift is also associated with the alternative activation of macrophages (M2). Schistosomal granulomas are rich in M2 macrophages [26], which promote fibrosis by suppressing the production of IFN-γ and generating pro-fibrotic factors. One such factor is the enzyme arginase-1, which converts L-arginine into L-ornithine and provides proline for collagen synthesis. Moreover, these mediators also drive angiogenesis, a hallmark of schistosomiasis-associated fibrosis, characterized by severe remodeling of the hepatic vasculature, with distortion of the portal venous system and compensatory hyperplasia and hypertrophy of the arterial circulation [33].

M2 macrophages express high levels of pro-fibrotic cytokines, such as TGF-β, CCL2, and IL-1β, which activate HSCs, in addition to exhibiting elevated VDR expression [34]. In this context, a study by Liu et al. (2023) showed that THP-1 macrophages polarized with IL-4 (M2) and treated with CP (50 nmol/L) displayed downregulation of the cluster of differentiation 206 (CD206). By contrast, IFN-γ-stimulated macrophages (M1) treated with CP exhibited a slight increase in CD86 expression. Furthermore, the authors found that LX-2 cells (HSCs) cultured with conditioned medium (CO) from M2 macrophages, exhibited increased cell proliferation and *α-SMA* upregulation. Additionally, CO from CP-treated M2 macrophages suppressed LX-2 proliferation, suggesting VDR

activation and inhibition of M2 pro-fibrotic activity. In an *in vivo* model, oral treatment with CP (20 µg/kg) following CCl_4-induced liver fibrosis resulted in reduced *Col1a1* and *α-SMA* (*Acta2*) expression, as well as decreased collagen deposition in liver tissue [35]. Given this, an increase in macrophage infiltration and a shift to an M2 profile may stimulate HSCs activation and collagen deposition.

Together, these findings suggest that in *S. mansoni* infections, CP exerts antifibrotic effects by reducing inflammation and IL-12 production, and by suppressing the pro-fibrotic activity of M2 macrophages. This reduces local levels of mediators such as arginase-1, TGF-β and IL-4. In addition, it limits hepatocellular injury and collagen deposition by attenuating NO-driven inflammation and fibrogenesis, demonstrating efficacy in the chronic phase of schistosomiasis, where fibrosis is often established and persists despite antiparasitic therapy.

HSCs play a central role in hepatic schistosomiasis since they are involved in the formation of granulomas. This is demonstrated by an increase in the number of these cells in the livers of mice and humans infected with *S. mansoni*, compared to healthy individuals [36]. Upon liver damage, inflammatory factors are released, leading to the transdifferentiation of HSCs into MFB, which initiate large-scale ECM production [37,38]. Factors involved in the activation of HSCs include extracellular signals from resident and inflammatory cells, such as macrophages, hepatocytes, liver endothelial cells and sinusoidal cells, as well as T and NK cells. Other factors include mediators, such as oxidative stress, and receptor-mediated signals [38]. In view of this, when evaluated *in vitro*, CP was shown to exert a direct and selective effect on HSCs cells. It demonstrated a higher selectivity index compared to PZQ. Given that GRX cells are considered an *in vitro* model of HSCs derived from schistosomal fibrosis that expresses high levels of *α-SMA* (*Acta2*) and produces collagen, this is particularly relevant.

High levels of ROS, such as superoxide anion, are known to induce proliferation, activation and fibrogenic activity in HSCs, thereby increasing necrotic injury [39]. In turn, the generation of mitochondrial ROS can activate the nucleotide-binding domain inflammasome and the leucine-rich repeat pyrin domain-containing protein 3 (NLRP3), which regulates the cleavage and activation of caspase-1. This leads to the maturation and extracellular secretion

of interleukin IL-1β and IL-18, which act as pro-inflammatory cytokines and promote HSCs activation [40,41]. However, it has recently been shown that CP inhibits the activation of the NLRP3 inflammasome in a hepatic cholestasis model *in vivo* and in LX-2 cells *in vitro*, improving fibrosis and liver damage [42]. Emerging evidence also demonstrates that CP may exert its anti-inflammatory and antifibrotic effects by suppressing NF-κB transcriptional activity, either directly or indirectly through the regulation of intracellular ROS, which subsequently influences NF-κB signaling [43]. In view of this and given that oxidative stress is involved in the activation of HSCs and hepatic fibrosis in schistosomiasis by stimulating the production of TGF-β, extracellular matrix synthesis and the proliferation of activated stellate cells, it was found that CP not only reduced GRX cell viability, but also markedly attenuated oxidative and profibrotic responses. This was evidenced by a reduction in superoxide anion and total ROS levels, mitochondrial membrane depolarization and a reduction in TGF-β levels.

In addition, ROS can act as an intracellular signalling mediator of the fibrogenic action of TGF-β, promoting liver fibrosis by stimulating HSCs activation [41]. One of the main effects of CP is to act as an nVDR agonist, affecting the transcription of genes involved in cell proliferation and differentiation, generating immunoinflammatory responses, and regulating fibrosis. Thus, VDR plays a broad role: since it binds to the active form of vitamin D, it can combine with RXR and the VDR-interacting repressor (VDIR) to activate or inhibit specific genes. VDR and Smad3 are known to co-occupy around 10,000 genomic sites; however, in the presence of CP, TGFβ-induced Smad3 recruitment is largely compromised while VDR binding to these sites increases almost 10-fold. CP promotes VDR occupancy at all three major VDR/Smad3 co-binding sites in the *Col1a1* gene among these sites [10]. Therefore, CP has been shown to inhibit the effects of TGF-β on HSCs, as well as its pro-fibrotic effects.

Furthermore, CP was found to reduce cytoplasmic lipid droplet content and increase extracellular LDH, indicating a loss of membrane integrity, frequently associated with HSCs inactivation or cell death. Consistent with this, morphological analyses revealed that CP caused pronounced changes, including cell rounding, irregular plasma membranes and loss of cytoplasmic projections. These changes are consistent with cytoskeletal disassembly and a shift towards

a non-activated phenotype. Taken together, these findings suggest that CP affects several key features of HSCs activation, including oxidative stress, profibrotic cytokine production, mitochondrial function and cytoskeletal arrangement, resulting in the loss of the activated phenotype. This demonstrates its potential as an antifibrotic adjuvant in chronic schistosomiasis, where parasite clearance alone is insufficient to reverse established fibrosis.

5. CONCLUSION

In summary, CP demonstrated a protective effect on hepatic tissue by reducing oxidative stress markers such as nitric oxide and reactive oxygen species, while modulating mitochondrial function through membrane potential depolarization. Unlike PZQ, which increased superoxide anion production and mitochondrial membrane hyperpolarization, CP promoted controlled cell deactivation or death, as evidenced by lactate dehydrogenase release and decreased lipid droplet accumulation, without inducing significant oxidative damage. These effects were accompanied by reduced macrophage infiltration, diminished deposition of type I and III collagen, and decreased granuloma size, correlating with lower levels of pro-fibrotic and inflammatory cytokines, including IL-12, IL-4, and TGF-β. Collectively, these findings suggest that CP not only alleviates liver and spleen pathology but also modulates key fibrogenic and inflammatory pathways, highlighting its potential as a complementary therapeutic agent in schistosomiasis-associated liver fibrosis.

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5 CONCLUSÃO GERAL

Trans-Chalcona consiste um flavonoide natural com reconhecido potencial biológico, cujas propriedades farmacológicas qualificam como um candidato promissor ao desenvolvimento de novos fármacos. Neste estudo, demonstramos que *trans*-Chalcona apresenta atividade esquistossomicida significativa, especialmente contra formas juvenis de *Schistosoma mansoni*, promovendo alterações no perfil nitrosativo, despolarização da membrana mitocondrial, perda de integridade da membrana celular e danos ao tegumento, culminando em comprometimento na oviposição. Em modelo *in vivo*, o tratamento com *trans*-Chalcona reduziu a carga parasitária e melhorou parâmetros relacionados aos granulomas hepáticos. Assim, diante da crescente preocupação com o surgimento de cepas resistentes e da eficácia observada contra formas jovens de *S. mansoni*, aliada à baixa toxicidade em células saudáveis, *trans*-Chalcona se apresenta como uma alternativa promissora para o tratamento da esquistossomose.

Calcipotriol, por sua vez, é um análogo sintético da vitamina D conhecido por suas propriedades anti-fibróticas e antioxidantes em modelos de fibrose hepática. No contexto da esquistossomose, o tratamento com calcipotriol apresentou efeitos hepatoprotetores, regulando o estresse oxidativo e o infiltrado inflamatório, o que resultou em melhora na lesão hepática, com redução dos granulomas e da deposição de colágeno. Além disso, o calcipotriol atuou diretamente sobre células estreladas hepáticas de forma mais eficaz que o tratamento padrão e reduzindo níveis de citocinas pró-fibróticas. Embora não tenha impactado a carga parasitária, seus efeitos protetores sobre o fígado indicam um potencial terapêutico importante em formas avançadas ou graves da esquistossomose.

Assim, os resultados obtidos reforçam a relevância da investigação de abordagens terapêuticas combinadas ou complementares, que atuem tanto diretamente sobre *S. mansoni* quanto aos danos induzidos durante a infecção, abrindo novas perspectivas para um tratamento mais eficaz e abrangente da esquistossomose.

Universidade
Estadual de Londrina

COMISSÃO DE ÉTICA NO USO DE ANIMAIS

OF. CIRC. CEUA Nº 078/2021

Londrina, 26 de agosto de 2021.

Prezado(a) professor(a),

Certificamos que o projeto intitulado: **"Avaliação *in vitro e in vivo* da ação da trans-chalcona no modelo experimental da esquitossomose mansônica"** protocolo CEUA n° **020.2021** sob a responsabilidade de **Wander Rogério Pavanelli** que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem) para fins de pesquisa científica (ou ensino), encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovado pela Comissão de Ética no Uso de Animais da Universidade Estadual de Londrina (CEUA/UEL) em **25/08/2021**.

Este projeto tem por objetivo avaliar a ação esquistossomicida da trans-chalcona *in vitro* sobre esquistossômulos e vermes adultos de *S. mansoni* (cepa BH) e no modelo *in vivo*, quanto à ação esquistossomicida, imunomoduladora e alterações hepáticas histológicas. **Grau de invasividade: G3.**

Finalidade	( ) Ensino (X) Pesquisa científica
Vigência da autorização	31/08/2021 a 30/08/2023
Espécie/ linhagem/ raça	Camundongo isogênico/ BALB/c
N° de animais	**95**
Peso/ Idade	25 g
Sexo	Machos ou Fêmeas
Origem	Biotério da FIOCRUZ - Curitiba/PR
Amostras a serem coletadas	Sangue, estômago, intestino, fígado e baço.

Cumpre orientar que caso pretendam-se quaisquer alterações no protocolo experimental aprovado, deve-se submeter o novo protocolo à apreciação da CEUA/UEL anteriormente à execução das modificações.

Em cumprimento às exigências do CONCEA, em até 30 dias da finalização do projeto de pesquisa ou extensão envolvendo o uso de animais (verificar período de vigência expresso neste ofício), é necessário encaminhar relatório da descrição de uso de animais para ceua@uel.br, conforme modelo disponível no site da CEUA: http://www.uel.br/comites/ceua/pages/relatorio-de-projetos.php.

Coloco-me à disposição para quaisquer esclarecimentos que se fizerem necessários. Sem mais para o momento, subscrevo-me, cordialmente,

Profª Drª Maria Fernanda Rodrigues Graciano
Coordenadora da CEUA/UEL

Ilmo.(a) Sr.(a)
Prof. (a) Dr. (a).Wander Rogério Pavanelli
Responsável pelo projeto
C/C para a Chefia do Departamento de Ciências Patológicas/CCB
C/C para a Direção do Centro de Ciências Biológicas/CCB

Universidade
Estadual de Londrina

COMISSÃO DE ÉTICA NO USO DE ANIMAIS

OF. CIRC. CEUA Nº 028/2024

Londrina, 13 de março de 2024.

Prezado (a) professor(a),

Certificamos que o projeto intitulado: **"Avaliação da ação do calcipotriol associado ao praziquantel no modelo experimental de esquistossomose mansônica",** protocolo CEUA n° **007.2024,** sob a responsabilidade de **Wander Rogério Pavanelli,** que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem) para fins de pesquisa científica (ou ensino), 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 da Experimentação Animal (CONCEA), e foi **aprovado** pela Comissão de Ética no Uso de Animais da Universidade Estadual de Londrina (CEUA/UEL), em reunião realizada em **13/03/2024**.

Este projeto tem por objetivo avaliar a ação esquistossomicida e imunomoduladora do Calcipotriol (CP) associado ao Praziquantel (PZQ) no modelo in vivo de esquistossomose mansônica nas fases aguda e crônica da infecção. **Grau de invasividade**: **GI3.**

Finalidade	( ) Ensino (X) Pesquisa científica
Vigência da autorização	01/04/2024 a 31/03/2026
Espécie/ linhagem/ raça	Camundongo isogênico / BALB/c
N° de animais	**152**
Peso/ Idade	20 a 25 g / 6 a 8 semanas
Sexo	Fêmeas
Origem	Biotério Central da Universidade Estadual de Londrina
Amostras a serem coletadas	Plasma, estômago, intestino, fígado e baço

Cumpre orientar que caso pretendam-se quaisquer alterações no protocolo experimental aprovado, deve-se submeter o novo protocolo à apreciação da CEUA/UEL anteriormente à execução das modificações.

Em cumprimento às exigências do CONCEA, em até 30 dias da finalização do projeto de pesquisa ou extensão envolvendo o uso de animais (verificar período de vigência expresso neste ofício), é necessário encaminhar relatório da descrição de uso de animais para ceua@uel.br, conforme modelo disponível no site da CEUA: http://www.uel.br/comites/ceua/pages/relatorio-de-projetos.php.

Coloco-me à disposição, para quaisquer esclarecimentos que se fizerem necessários. Sem mais para o momento, subscrevo-me, cordialmente,

Profª Drª Patricia Chimin Perandini
Coordenadora da CEUA/UEL

Ilmo.(a) Sr.(a) Prof. (a) Dr. (a) Wander Rogério Pavanelli
Responsável pelo projeto
C/C para a Chefia do Departamento de Ciências Patológicas /CCB
C/C para a Direção do Centro de Ciências Biológicas /CCB
C/C para a Direção do Biotério Central/CCB