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INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM FARMACOLOGIA E BIOQUÍMICA

LISANDRA DUARTE NASCIMENTO

EXTRATO AQUOSO DE *CORIANDRUM SATIVUM* (EACS) ATENUA OS DISTÚRBIOS DE CONDUÇÃO CARDÍACA PELA MELHORA NO REMODELAMENTO VENTRICULAR E MODULAÇÃO DO SISTEMA REDOX E ATIVIDADE DE MMP-2 ATIVA EM RATAS EXPOSTAS AO METILMERCÚRIO

BELÉM-PA

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Documento de Dissertação apresentada ao Programa de Pós-graduação em Farmacologia e Bioquímica do Instituto de Ciências Biológicas da Universidade Federal do Pará como requisito para a obtenção do título de Mestre em Farmacologia e Bioquímica.

BELÉM-PA

2023

Dados Internacionais de Catalogação na Publicação (CIP) de acordo com ISBD
Sistema de Bibliotecas da Universidade Federal do Pará
Gerada automaticamente pelo módulo Ficat, mediante os dados fornecidos pelo(a) autor(a)

D812e Duarte Nascimento, Lisandra.
EXTRATO AQUOSO DE CORIANDRUM SATIVUM (EACS) ATENUA OS DISTÚRBIOS DE CONDUÇÃO CARDÍACA PELA MELHORA NO REMODELAMENTO VENTRICULAR E MODULAÇÃO DO SISTEMA REDOX E ATIVIDADE DE MMP-2 ATIVA EM RATAS EXPOSTAS AO METILMERCÚRIO / Lisandra Duarte Nascimento. — 2023.
x, 64 f. : il. color.

Orientador(a): Prof. Dr. Alejandro Ferraz do Prado
Coorientação: Profª. Dra. Keuri Eleutério Rodrigues
Dissertação (Mestrado) - Universidade Federal do Pará, Instituto de Ciências Biológicas, Programa de Pós-graduação em Farmacologia e Bioquímica, Belém, 2023.

1. Metilmercúrio. 2. Coriandrum. 3. Coração. I. Título.

CDD 615.92566309811

DEDICATÓRIA/AGRADECIMENTOS

Primeiramente, agradeço a Deus pelo dom da vida, pelas graças alcançadas, pela oportunidade e pelas pessoas incríveis que encontro diariamente pelo caminho.

A minha família, pelo apoio, por me incentivarem todos os dias, por serem meu maior exemplo de força e de fé. Em especial, dedico este trabalho a minha avó, Maria Helena Duarte, minha mãe e padrasto, Lucia Duarte e Fabio Salgado, e meu pai, Mauricio Nascimento, que me estimularam a iniciar e terminar essa jornada acadêmica. Obrigada por todo amor e carinho.

Ao meu noivo, Otavio Albuquerque, que me incentivou a voltar para a academia depois de iniciar a carreira profissional, que me acompanha nos estudos, nos desesperos e nas vitórias.

Aos meus amigos, em especial, Scarllet, Ângela, Ingrid, Célio, Paulo, Pascoal, Adriana, Linda, Rakhel e tantos outros, pelos momentos de descontração e de alívio no meio das dificuldades impostas pela dupla jornada: estudo e trabalho.

Aos meus colegas de laboratório que me ensinaram tantas coisas e eu só posso agradecer o tempo gasto a me ensinar.

Ao meu orientador, Dr. Alejandro, por me aceitar e não desistir de mim. Obrigada pela confiança e desculpa pelos estresses que posso ter causado.

À minha co-orientadora, Dra. Keuri, pelo aprendizado constante, por toda contribuição no desenvolvimento deste trabalho e no meu desenvolvimento pessoal.

À todos os mestres que colaboraram com a realização deste estudo. Obrigada prof. Moises Hamoy, Prof Rafael Lima, Prof Sandro Percário e seu aluno Everton Varela e Profa Criatiane Maia

Ao programa, Farmabio, ao ICB e à Universidade Federal do Pará pela oportunidade.

E a todos que encontrei nesta caminhada e que tornaram este trabalho possível.

RESUMO

A exposição humana ao metilmercúrio (MeHg) resulta no aumento de desenvolvimento de doenças cardiovasculares, possivelmente associado ao estresse oxidativo e alterações epigenéticas em proteínas, como as metaloproteinases da matriz (MMPs). O *Coriandrum sativum,* popularmente conhecido como coentro, é uma planta amplamente utilizada na culinária que possui atividade antioxidante e potencial efeito cardioprotetor. Desta forma, buscou-se identificar se o Extrato Aquoso de *Coriandrum sativum* (EACS) é capaz de proteger contra os efeitos deletérios do MeHg no coração de ratas intoxicadas no período gestacional e lactacional pela modulação no sistema redox e sobre as MMPs. Realizamos eletrocardiograma, morfometria, avaliação do sistema redox, e atividade de MMP-2 no coração. O grupo MeHg induziu redução da frequência cardíaca bloqueio atrioventricular de primeiro grau e distúrbio de condução ventricular, associados a diminuição do diâmetro do miócito, espessura da parede do VE, do septo interventricular e aumento no conteúdo de colágeno, desbalanço redox, demonstrado pelo aumento dos níveis de nitrito e peroxidação lipídica, com redução de GSH, CAT e SOD, e elevação da atividade de MMP-2 ativa. O EACS em animais expostos ao MeHg inibiu significativamente as alterações elétricas induzidas pelo metal. Simultaneamente, estes animais mantiveram as medidas morfométricas (Diâmetro miócito; espessura da parede do ventrículo esquerdo; espessura da parede do septo; conteúdo de colágeno), as medidas do sistema redox, e atividade de MMP-2 ativa similar ao Grupo Controle. Concluímos que o EACS foi capaz de atenuar os distúrbios de condução cardíaca pela melhora no remodelamento ventricular, redução de fibrose e modulação de MMP-2 ativa, através da manutenção do estado redox, bloqueando os efeitos deletérios do MeHg no coração.

Palavras-chave: Mercúrio, Remodelamento cardíaco, Fibrose, Coentro e Antioxidante

ABSTRACT

Human exposure to methylmercury (MeHg) results in increased development of cardiovascular disease, possibly associated with oxidative stress and epigenetic changes in proteins such as matrix metalloproteinases (MMPs). *Coriandrum sativum*, popularly known as coriander, is a plant widely used in cooking with antioxidant activity and potential cardioprotective effects. Thus, we sought to identify whether the *Coriandrum sativum* Aqueous Extract (CSAE) can protect against the deleterious effects of MeHg in the heart of intoxicated rats during the gestational and lactational period by modulating the redox system and on the MMPs. We performed electrocardiography, morphometry, assessment of the redox system, and MMP-2 activity in the heart. The MeHg group induced a reduction in heart rate, first-degree atrioventricular block, and ventricular conduction disorder, associated with a decrease in myocyte diameter, LV wall thickness, interventricular septum, and an increase in collagen content, redox imbalance, demonstrated by the increase in nitrite levels and lipid peroxidation, with reduction of GSH, CAT and SOD, and elevation of active MMP-2 activity. CSAE in animals exposed to MeHg significantly inhibited the electrical changes induced by the metal. Simultaneously, these animals maintained morphometric measurements (myocyte diameter; left ventricular wall thickness; septal wall thickness; collagen content), redox system measurements, and active MMP-2 activity like the Control Group. We conclude that CSAE attenuated cardiac conduction disorders by improving ventricular remodeling, reducing fibrosis, and modulating active MMP-2, by maintaining the redox state, blocking the deleterious effects of MeHg on the heart.

Keywords: Mercury, Cardiac remodeling, Fibrosis, Coriander and Antioxidant

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LISTA DE SIGLAS E SÍMBOLOS

µmol	Micromol
ACE	Enzima Conversona de Angiotensina
AHA	American Heart Association / Associação Americana do Coração
ATP	Trifosfato de Adenosina
BNP	Peptídeo natriurético tipo B
BPM	Batimentos por minute
CaCl2	Cloreto de Cálcio
CAT	Catalase
CONCEA	Conselho Nacional de Controle de Experimentação Animal
CSAE / EACS	Extrato Aquoso De *Coriandrum Sativum*
DTNB	Ácido 5,5-ditiobis-2-nitrobenzóico
ECG	Eletrocardiograma
ECM	Matrix Extracelular
EF	Fração de Ejeção
ERO / ROS	Espécies Reativas de Oxigênio
FN	Fibronectina
g	Grama
GSH	Glutationa Reduzida
HE	Hematoxilina-Eosina
HFiEF	Fração de Ejeção Intermediária
HFpEF	Fração de Ejeção Preservada
HFrEF	Redução da Fração de Ejeção
Hg	Mercúrio
Hg(II) / Hg^{+2}	Íon Mercuroso
Hg^{0}	Mercúrio Metálico
IC / HF	Insuficiência Cardíaca
KCl	Cloreto de Potássio
kDa	Quilodaltons
MDA	Melonildialdeido
MeHg	Metilmercúrio
mg	Miligrama
mL	Microlitro
MMP-2	Metaloproteinases da Matriz 2

mRNA	Ácido ribonucleico mensageiro
ms	Milissegundo
NEM	n- Etilmaleimida
NTT-MMP-2	Isoforma N-terminal truncada de Metaloproteinases da Matriz 2
$ONOO^-$	Peróxinitrito
PBS	Tampão Salino
PMSF	Fenil-metil-sulfonil fluoreto
RAAS	Sistema Renina-Angiotensina-aldosterona
RNS	Espécies reativas de nitrogênio
SAR	Relação estrutura-atividade
SDS-PAGE	Eletroforese em gel de poliacrilamida com dodecil sulfato de sódio
SEM	Erro Médio Padrão
SOD	Superóxido Desmutase
TBARS	Espécies reativas ao ácido tiobarbitúrico
TGF-β	Fator de crescimento transformador beta
TIMP	Inibidor Tecidual de Metaloproteinases
TNB	Ácido nitrobenzóico
U	Unidade internacional
US	Estados Unidos da América
UVB	Radiação ultravioleta tipo B
VE / LV	Ventrículo Esquerdo
ρmol	Picomol

SUMÁRIO

1. VISÃO INTEGRADORA DO PROBLEMA

O mercúrio (Hg) é um composto químico de grande preocupação em relação à saúde pública devido à sua toxicidade e alta afinidade pelos diversos órgãos e tecidos humanos (BERNHOFT et al., 2012; ZHAO et al., 2022). Encontrado naturalmente no ambiente em menor proporção, sua principal fonte de emissão envolve atividades antropogênicas, como a liberação de resíduos industriais e mineração, resultando na liberação de uma grande quantidade do metal para o meio ambiente (DRISCOLL et al., 2013; BISHOP et al., 2020). Neste sentido, em 2013, foi assinado o tratado internacional de redução da liberação de mercúrio no ambiente, a Convenção de Minamata (UNEP, 2019).

Apesar dos esforços internacionais, estudos demonstraram níveis alarmantes de contaminação em diversas localidades (GUNDERSEN et al., 2023; DE SOUZA-ARAUJO et al., 2022; ARAUJO et al., 2021). Atualmente, a mineração possui o status de maior fonte global de liberação antropogênica de mercúrio para o meio ambiente, devido ao mau gerenciamento do Hg após a separação do ouro do amálgama, gerando contaminação de leitos de rios, processo usado principalmente em minas de ouro artesanais, como as encontradas nos garimpos da região amazônica (TEIXEIRA et al., 2021; UDODENKO et al., 2022; MALONE et al., 2023).

No meio ambiente, o mercúrio liberado (Hg metálico ou Hg^0) é volatilizado e oxidado à forma inorgânica Hg^{+2}, sendo depositado em ecossistemas de água doce. O Hg (II) em ambiente aquático pode ser reduzido, sendo então volatilizado para a atmosfera, e uma pequena porção de Hg (II) é metilada e convertida na forma tóxica de metilmercúrio (MeHg), um processo mediado por bactérias redutoras de ferro e sulfato (CLARKSON, 2002; MASON et al., 2012). O metilmercúrio possui alta capacidade de acumular ao longo da cadeia alimentar aquática, processo denominado biomagnificação, tornando a ingestão de peixes um dos principais meios de exposição humana, particularmente para comunidades com altos níveis de consumo de peixes, como as populações ribeirinhas (DA SILVA et al., 2020). Estudos na Amazônia demonstram elevadas concentrações de mercúrio em comunidades ribeirinhas e tradicionais (COSTA JUNIOR et al., 2018; LINO et al., 2018; VASCONCELLOS et al., 2021), o que é motivo de preocupação devido aos riscos de toxicidade nessas populações, em especial os grupos mais vulneráveis, como mulheres em idade fértil e crianças.

O consumo de peixe contaminado ocasiona repercussões extensas no organismo humano. Em mulheres em idade fértil, é conhecido o seu papel como desregulador endócrino, podendo induzir à infertilidade feminina (BJØRKLUND et al., 2019; MAEDA et al., 2019). Em grávidas, estudos demonstraram correlação entre os níveis de mercúrio e a ocorrência de aborto, parto prematuro e problemas de desenvolvimento em bebês, especialmente no desenvolvimento neurocomportamental (BURCH et al., 2014; CHEN & DONG, 2022). A nível cardiovascular, embriões expostos ao MeHg apresentaram alterações morfológicas, celulares e hemodinâmicas no coração (RONCONI-KRÜGER et al., 2022).

Os mecanismos envolvidos no desenvolvimento de doenças cardiovasculares em adultos não estão completamente elucidados. É incontestável o papel do desbalanço redox, ocasionado pela elevação de espécies reativas de oxigênio (ERO), associada à diminuição nas defesas antioxidantes, em especial o GSH, na toxicidade induzida pelo metilmercúrio no coração (GROTTO et al., 2009; GHIZONI et al., 2017). Em cardiomiócitos, a exposição ao metilmercúrio ocasiona declínio na produção de ATP, levando a uma incapacidade das mitocôndrias de satisfazer as demandas de energia na célula, fator contribuinte para o papel contrátil dos cardiomiócitos (TRUONG et al., 2015). Adicionalmente, estudos relatam a influência da disfunção mitocondrial na toxicidade cardíaca, através do aumento dos níveis de Bax e Bak mitocondriais, que promovem a formação de poros na membrana mitocondrial, liberando citocromo c da mitocôndria e desencadeando o processo apoptótico (SATO et al., 2020; SOKOLOWSKI et al., 2011).

No aspecto hemodinâmico, autores descrevem o papel da exposição ao mercúrio no comprometimento da contratilidade miocárdica, atenuação da sensibilidade do barorreflexo, indução do desequilíbrio simpato-vagal e distúrbio na atividade do marcapasso do nódulo sinusal (JINDAL et al., 2011; SIMÕES et al., 2016). Corroborando com esses achados, Santos e colaboradores demonstraram a capacidade do MeHg de induzir alteração na condução elétrica cardíaca, através do prolongamento do tempo de repolarização ventricular e aumento da dispersão da repolarização ventricular (SANTOS-RUYBAL et al., 2020).

Combinado com os efeitos elétricos no coração, autores descreveram a influência do metilmercúrio em alterações na pressão arterial (WILLDEMANN et al., 2015; SILVA et al., 2021). Wildermann e colaboradores apresentaram outro potencial mecanismo de alteração da pressão, através da influência do MeHg sobre o sistema renina-angiotensina (WILDEMANN et al., 2016).

Diversos mecanismos foram descritos para a toxicidade do MeHg, entretanto, o mecanismo mais conhecido e descrito envolve o estresse oxidativo como consequência do aumento de ERO (PEREIRA et al., 2020; LI et al., 2021). Este aumento está associado à modulação da expressão e atividade de MMP-2, enzima relatada em processos patológicos cardiovasculares (JACOB-FERREIRA et al., 2009; VITORINO et al., 2023). Além dos desfechos cardíacos, a alteração dos níveis de MMP foi relacionada com desfechos inesperados no parto, tendo influência no processo gestacional (AU et al., 2016).

O período gestacional e lactacional é marcado por mudanças no organismo materno para adaptá-lo às necessidades dessas fases. No sistema cardiovascular, observam-se modificações hemodinâmicas que, associadas às alterações neuro-hormonais, metabólicas e ao estilo de vida prévio, podem contribuir para as patologias cardiovasculares em mulheres grávidas (HAMEED et al., 2007; LIU et al., 2014). Agregado a esses fatores, a literatura demonstra aumento da pressão arterial sistêmica e do risco de pré-eclâmpsia quando níveis elevados de Hg são detectados (WELL et al., 2017; WANG et al., 2022).

Em gestantes, a suplementação dietética com agentes antioxidantes poderia prevenir os efeitos deletérios da exposição ao mercúrio. Desta forma, em nosso trabalho, estamos investigando se a suplementação com *Coriandrum sativum* apresenta benefícios como alimento funcional, principalmente devido a sua capacidade antioxidante (PANJWANI, MISHRA, & BANJI, 2010). A análise fitoquímica das folhas de *Coriandrum sativum* demonstrou altos teores de substâncias fenólicas e flavonoides, resultando em maiores níveis de atividade antioxidante nesta porção (WANGENSTEEN et al., 2004; ERGUN, 2022; WEI et al., 2019). Além disso, a administração de extrato de coentro apresentou potencial efeito cardioprotetor pela ação no controle hemodinâmico, morfológico e celular no coração (PATEL et al., 2012; DHYANI et al., 2020). Em estudo anterior, foi observado que o extrato etanólico da folha de *Coriandrum sativum* possui atividade inibitória sobre a MMP-1 (HWANG et al., 2014). Em relação ao

coração, ainda não foi investigado se os efeitos cardioprotetores do *Coriandrum sativum* estão relacionados a modulação da atividade de MMP-2. Assim, levantamos a hipótese de que a suplementação dietética com extrato aquoso de folhas de *Coriandrum sativum* (EACS) previne a cardiotoxicidade induzida pelo metilmercúrio no coração de ratas durante a gestação e lactação devido aos seus efeitos antioxidantes e inibitórios sobre a MMP-2.

O objetivo deste estudo foi avaliar o efeito do extrato aquoso de *Coriandrum sativum* sobre o coração de ratas expostas ao metilmercúrio durante o período gestacional e lactacional. Isso foi feito através da avaliação morfométrica, níveis de colágeno, atividade elétrica cardíaca, análise do sistema redox e quantificação da atividade das MMP-2 no coração das ratas expostas ao metilmercúrio na ausência e na presença do cotratamento com o extrato aquoso de *Coriandrum sativum*.

O estudo está dividido em dois capítulos. No capítulo 1, abordamos a influência da MMP-2 sobre as alterações morfológicas e funcionais cardíacas, assim como compilamos os achados que mostram a MMP-2 como possível alvo terapêutico na disfunção cardíaca. A MMP-2, pertence ao grupo das gelatinases, e é expressa constitutivamente em células cardíacas. Um modelo de doença cardíaca induzida em animais experimentais demonstrou intensa produção de espécies reativas e estresse oxidativo, sendo este o principal fator relacionado a modulação da atividade da MMP-2. Estudos demonstraram que o aumento da atividade de MMP-2 leva à proteólise de várias proteínas do citoesqueleto, envolvidas na contratilidade miocárdica. Corroborando com dados experimentais, a literatura demonstrou uma relação entre a MMP-2 e alterações eletrocardiográficas. Assim, é demonstrado o potencial da MMP-2 como biomarcador de dano cardíaco em diferentes processos patológicos (BATISTA-LÓPEZ et al., 2013; GONÇALVES et al., 2022).

Levando em consideração o papel da MMP-2 em alterações cardíacas e que o metilmercúrio é capaz de modular a atividade de MMPs diretamente ou pela indução de estresse oxidativo, neste trabalha investigamos o potencial papel antioxidante e cardioprotetor do *Coriandrum sativum*. O capítulo 2 apresenta os resultados benéficos da suplementação com o EACS na morfometria, estresse oxidativo, modulação de MMP-2 e condução elétrica cardíaca de ratas gestantes e lactantes expostas ao metilmercúrio.

2. ARTIGO 1:

Metaloproteinase de Matriz 2 como alvo farmacológico na insuficiência cardíaca

pharmaceuticals

MDPI

Review

Matrix Metalloproteinase 2 as a Pharmacological Target in Heart Failure

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Citation: Gonçalves, P.R.; Nascimento, L.D.; Gerlach, R.F.; Rodrigues, K.E.; Prado, A.F. Matrix Metalloproteinase 2 as a Pharmacological Target in Heart Failure. Pharmaceuticals **2022**, 15, 920. https://doi.org/10.3390/ph15080920

Received: 3 June 2022

Accepted: 11 July 2022

Published: 25 July 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract: Heart failure (HF) is na acute or chronic clinical syndrome that results in a decrease in cardiac output and an increase in intracardiac pressure at rest or upon exertion. The pathophysiology of HF is heterogeneous and results from an initial harmful event in the heart that promotes neurohormonal changes such as autonomic dysfunction and activation of the renin-angiotensin-aldosterone system, endothelial dysfunction, and inflammation. Cardiac remodeling occurs, which is associated with degradation and disorganized synthesis of extracellular

matrix (ECM) components that are controlled by ECM metalloproteinases (MMPs). MMP-2 is part of this group of proteases, hich are classified as gelatinases and are constituents of the heart. MMP-2 is considered a biomarker of patients with HF with reduced ejection fraction (HFrEF) or preserved ejection fraction (HFpEF). The role of MMP-2 in the development of cardiac injury and dysfunction has clearly been demonstrated in animal models of cardiac ischemia, transgenic models that overexpress MMP-2, and knockout models for this protease. New research to minimize cardiac structural and functional alterations using non-selective and selective inhibitors for MMP-2 demonstrates that this protease could be used as a possible pharmacological target in the treatment of HF.

Keywords: MMP-2 inhibitor; cardiac dysfunction; ischemia

1. Introduction

HF is highly prevalent, affecting approximately 26 million people worldwide Every year, with high rates of hospitalization and death [1]. According to the annual report on cardiovascular disease of the American Heart Association (AHA), the lifetime risk of developing HF is high, ranging from 20 to 45% after the age of 45 years. Estimates indicate that six million American adults aged 20 years or older have HF [2,3]. In Europe, the prevalence of HF is around 17.2 cases per 1000 individuals. It is an important public health problem with an average number of hospitalizations of 2671 per million people [4]. Furthermore, it is one of the most expensive syndromes in the US and Europe, consuming around 1–2% of the overall healthcare budget [5]. Global spending on CI is around US $108 billion per year [6].

HF is an acute or chronic clinical syndrome that results in a decrease in cardiac output and an increase in intracardiac pressure at rest or upon exertion. In this condition, the heart is unable to pump enough blood to meet the metabolic needs of tissue [7]. HF can be determined according to the left ventricle (LV) ejection fraction (EF), characterized as the percentage of blood ejected from the LV with each systole. HF can be classified as reduced ejection fraction (HFrEF), HF with intermediate ejection fraction (HFiEF) or

HF with preserved ejection fraction (HFpEF). Patients with HFrEF have a left ventricular ejection fraction <40%, with inadequate stroke volume and cardiac output as the primary manifestation. Patients with HFpEF have a left ventricular ejection fraction _50%, with impaired left ventricular relaxation. Patients with EF ranging from 41% to 49% were classified as HFiEF, presenting clinical characteristics similar to the population with HFpEF (Table 1). The New York Heart Association (NYHA) HF classification system, which stratifies the patient into classes I–IV, is based on the symptoms presented by the patient and the level of tolerated physical activity (Table 2) [8,9]. The etiology of HF stems from several conditions, and the leading causes are hypertension, valvular diseases, genetic cardiomyopathies, myocarditis, extracardiac diseases, and ischemia [10,11] (Figure 1).

The pathophysiology of HF is complex and results from an initial harmful event in the heart. The event can occur acutely (such as ischemic events, valvular diseases, and viral and bacterial myocarditis) or chronically (in arterial hypertension, genetic cardiomyopathies, and extracardiac diseases) and promotes functional and structural changes that compromise both systolic and diastolic blood pumping [7,9,12]. Diastolic dysfunction occurs due to structural changes resulting from fibrosis, promoting increased stiffness, decreased cardiac compliance, and hypertrophic cardiac remodeling, which causes an increase in LV filling pressure [12]. Systolic and diastolic electrical and mechanical asynchronies are related to the extent of diastolic dysfunction and exercise tolerance. Neurohormonal changes such as autonomic dysfunction and activation of the renin-angiotensin-aldosterone system are also implicated, as are endothelial dysfunction and inflammation. This makes HF heterogeneous and creates difficulties in choosing the therapeutic approach [13] (Figure 1).

In HF, cardiac remodeling occurs with degradation and disorganized synthesis of extracellular matrix (ECM) components. The ECM content is divided into fibrillar components (collagen, elastin, and reticular) and non-fibrillar components (glycoproteins and proteoglycans), which are responsible for tissue resistance and elasticity. Furthermore, their breakdown promotes functional changes. ECM metalloproteinases (MMPs) are proteases specialized in controlling the content of ECM [14–16] (Figure 1).

Table 1. Definition of HF, according to left ventricular ejection fraction.

Classification	Left Ventricle Ejection Fraction (LVEF)	Main Cardiaco Alterations (Ecodoppler)
HFrEF	<40%	Structural change and systolic dysfunction
HFpEF	≥50%	Structural change and diastolic dysfunction
HFiEF	41% to 49%	Structural change and diastolic dysfunction

HFrEF: heart failure with reduced ejection fraction; HFpEF: heart failure with preserved ejection fraction; HFiEF: heart failure with intermediate ejection fraction.

Table 2. New York Heart Association (NYHA) classification of heart failure based on symptoms and level of tolerated physical activity.

Class	General Description	Patient Symptoms
I	Asymptomatic	No limitation of physical activity; regular physical activity does not cause undue fatigue, palpitation and dyspnea.
II	Mild symptoms	Slight limitation of physical activity; comfortable at rest; activity results in fatigue, palpitation and dyspnea.
III	Moderate symptoms	Marked limitation of physical activity; comfortable at rest; regular exercise causes fatigue, palpitation and dyspnea.
IV	Severe symptoms	Unable to perform any physical activity without discomfort; HF symptoms at rest; if any physical activity is performed, the pain increases.

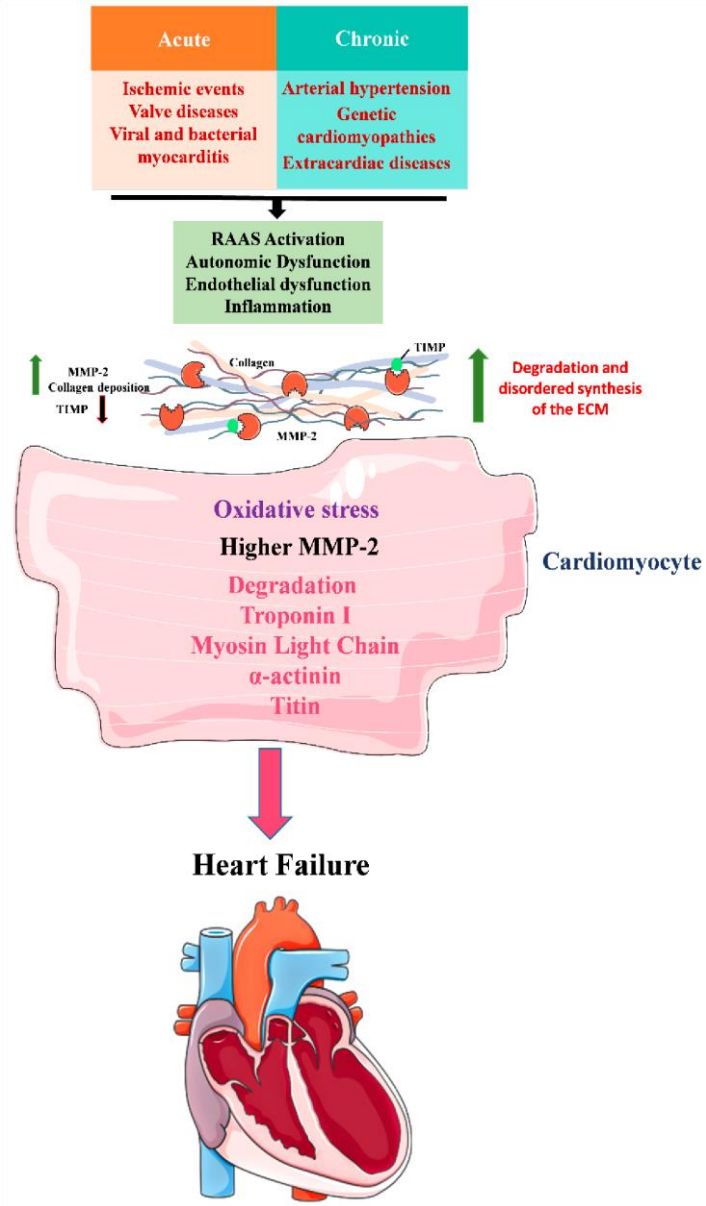

Figure 1. Cardiac remodeling in HF. HF occurs after an acute or chronic harmful event. The conditions that trigger this disease are hypertension, valvular diseases, genetic cardiomyopathies, myocarditis, extracardiac diseases and ischemia, generating autonomic dysfunction and activation of the reninangiotensin-aldosterone system (RAAS), endothelial dysfunction and inflammation. In addition, the degradation and disordered synthesis of the extracellular matrix (ECM) occurs due to increased activity of MMP-2 and decreased activity of endogenous tissue inhibitors (TIMP), leading to collagen deposition and oxidative stress, causing degradation of components of the contractile apparatus, troponin I, light chain myosin, alpha-actinin and titin, promoting structural and functional changes; image elements from smart.server.com.

MMP-2 is a gelatinase constitutive of the heart that is considered a biomarker of patients with HFrEF and HFpEF. MMP-2 can digest components of the contractile apparatus, such as troponin I and light chain myosin 1, which contributes to the reduction in cardiac contractility [17]. At the transcriptional level,

MMP-2 expression is controlled by transcription factors [18]. At the post-transcriptional level, MMP-2 activity and expression are regulated by inflammatory stimuli, oxidative stress, and alteration of the renin-angiotensinaldosterone (RAAS) axis [19–22]. There is also a class of endogenous tissue inhibitors of metalloproteinase (TIMPs) that participate in the control of MMPs, and the balance between MMPs and TIMPs plays an essential role in the pathophysiology of heart disease [23,24] (Figure 1).

MMP-2 can be produced and secreted in the heart by cardiomyocytes, fibroblasts, endothelial cells and inflammatory cells present during the progression of HF. Although present in greater volume, cardiomyocytes are in smaller numbers than non-myocyte cells, which comprise 70% of the cells in cardiac tissue, most of which include fibroblasts. Cardiac fibroblasts maintain cardiac structural integrity by controlling cardiac extracellular matrix content. In addition, fibroblasts surround cardiomyocytes, and the myocyte function depends on the fibroblast. In pathological processes, cytokines and growth factors alter the fibroblast phenotype by increasing the secretion of ECM proteins that lead to fibrosis [25–27]. Fibroblasts and myofibroblasts are the central MMP-secreting cells in the heart and are indicated as a therapeutic target for the treatment of myocardial infarction, hypertension and HF [26].

Before introducing neurohormonal therapies in treating patients with HF, more than a third of deaths were attributed to sudden cardiac death. However, evidence-based clinical trials using neurohormonal treatments in patients not using a defibrillator showed a reduction in the rate of premature death. Furthermore, in addition to current pharmacological therapies, resynchronization devices, cardioverter-defibrillators and ventricular assist devices drastically reduced the risk of death. Indeed, HFrEF shows a different trajectory in response to drug therapy and cardiac resynchronization devices compared to HFpEF. However, both patient groups have benefited from available treatments, showing improvements in myocyte function, normalization of action potential duration, and improvement in mitochondrial energy metabolism. However, an unacceptable number of patients suffer impairment of functional capacity, low quality of life and early death due to HF. Thus, therapies that can stop or minimize the progression of HF continue to be challenging [8,9,28].

In this mini-review, we will highlight the participation of MMP-2 in cardiac alterations related to HF. Next, we will present some MMP inhibitors used in pre-clinical and clinical trials as a pharmacological tool for treating various diseases. Then we will address the use of MMP-2 inhibitors as an alternative treatment for HF in animal and human models. Finally, we will briefly explain the possible use of MMP-2 inhibitors and new technologies as an adjuvant treatment associated with standard therapy and their impact on the progression of HF.

2. Matrix Metalloproteinase 2 (MMP-2)

MMPs are a family of proteases specialized in degrading ECM components, which have a highly homologous protein structure. Most have four basic domains: signal peptide, pro-peptide, catalytic, and hemopexin-like domains [29]. Based on their substrate affinity and structural organization, MMPs are commonly classified as collagenases, gelatinases, stromelysins, matrilysins, membrane MMPs, and others [30]. MMP-2 belongs to a group of gelatinases that have a unique catalytic domain among MMPs composed of a triple repeat of type II fibronectin, which forms a collagen affinity domain. This allows the binding and degradation of type IV collagen and denatured collagen (gelatin) [29] (Figure 2).

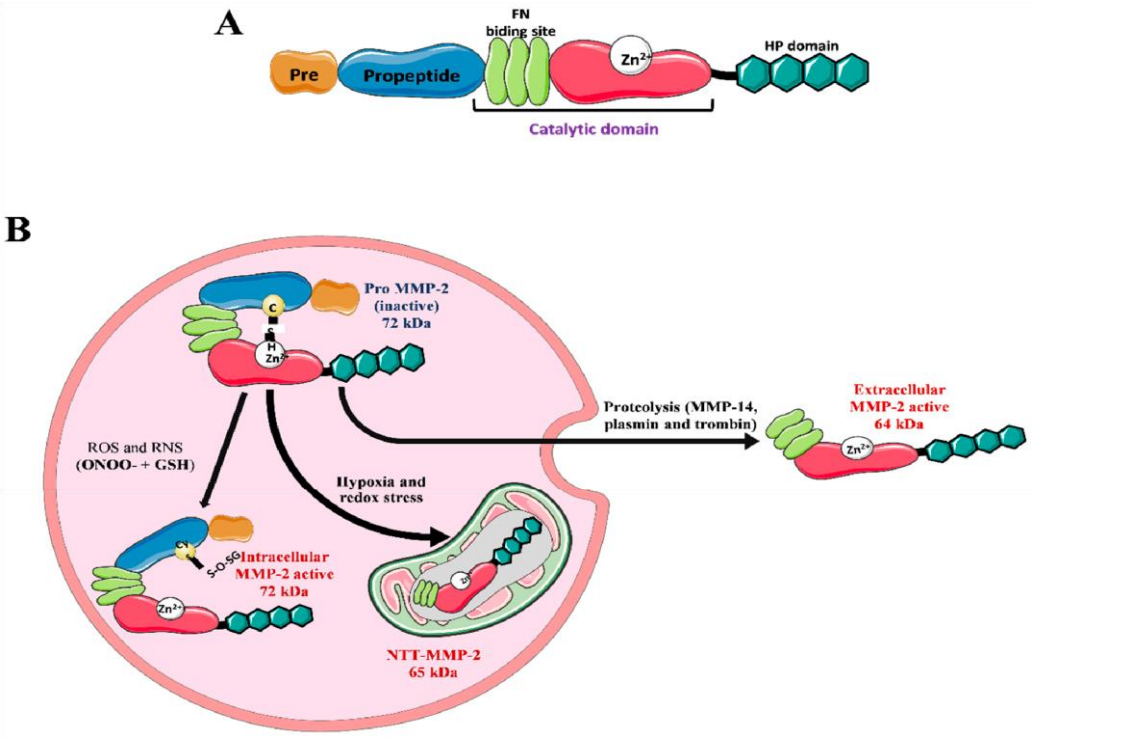

Figure 2. Structure, activation and isoforms of MMP-2. (**A**) MMP-2 has in its structure a signal peptide (Pre), a propeptide (Pro), a catalytic domain (having a zinc ion and three fibronectin

repeats that confer affinity to collagen) and hemopexin (HP). (**B**) The inactive isoform of MMP-2 has a molecular size of 72 kDa (pro-MMP-2). Inactivity is guaranteed by a cysteine residue in the propeptide domain, which binds to Zn^{2+} in the catalytic domain, preventing the binding and proteolysis of substrates. The active intracellular isoform of MMP-2 has a molecular size of 72 kDa and occurs when $ONOO^-$ or GSH reacts with $ONOO^-$. The reaction product binds to the cysteine residue of the propeptide, which prevents it from complexing with the Zn^{2+} atom in the catalytic domain, allowing the catalytic domain to interact with substrates. The 65 kDa NTT-MMP-2 is constitutively active, formed under conditions of hypoxia and oxidative stress, and leads to activation of alternative MMP-2 promoters that do not translate the first 77 amino acids. This isoform is not secreted into the extracellular environment, found in mitochondria and cytosol. The extracellular isoform of MMP-2 with a molecular size of 72 kDa, activated by the proteolytic removal of the propeptide domain by MMP-14, thrombin and plasmin, produces an active isoform of 64 kDa. FN: fibronectin GSH: reduced glutathione; $ONOO^-$: peroxynitrite; ROS: reactive oxygen species; RNS: reactive nitrogen species; NTT-MMP-2: truncated N-terminal isoform of MMP-2; image elements from smart.server.com.

MMP-2 is encoded by a 27-kb gene that has 13 exons and 12 introns located on chromosome 16. The gene has consensus sequences for the transcription factors AP-2 and SpI [31]. The gene is transcribed into a 3.1-kb mRNA [32], which is translated into a 660-residue protein that contains a 29-residue signal peptide. This peptide is responsible for translocating MMP-2 to the endoplasmic reticulum, followed by secretion into the extracellular medium, giving rise to a latent enzyme of about 72 kDa [29,33] (Figure 2).

The absence of catalytic activity of the enzyme is maintained by the interaction between a sulfhydryl bond between a cysteine residue present in the pro-peptide and zinc at the catalytic site [34]. In the enzymatic activation, the catalytic site is exposed, which can occur through proteolysis of the propeptide by other proteases (MMP-2, plasmin, and thrombin) or through the interruption of the sulfhydryl bond by reactive species [35,36]. In proteolytic activation, an MMP-2 with a molecular size of 64 kDa is formed [29]. In the process of activation by reactive species, the molecular size of 72 Kda is maintained due to the permanence of the pro-peptide [36]. MMP-2 is expressed in most body tissues and modulates several physiological processes, such as cell migration, angiogenesis, and wound healing [29] (Figure 2). However, increased expression and activity of MMP-2 is involved in cardiovascular diseases such as atherosclerosis, aneurysm, hypertension, and HF [37,38].

3. Role of MMP-2 in HF

The knowledge of specific biomarkers used in the clinic to determine pathological states, define diagnoses or even prognoses are of great value since the symptoms of HF are often not pathognomonic, making diagnosis difficult. The main biomarkers to diagnose HF are the natriuretic peptides BNP and NT-pro-BNP [9,39]. However, BNP can be altered due to several factors such as kidney disorders, advanced age, obesity, diabetes, sepsis, Cushing's syndrome and hyperthyroidism. Thus, the search for biomarkers that can help in the diagnosis and prognosis of HF becomes imperative.

MMP-2 can be considered a biomarker of HF, as higher plasma MMP-2 levels were found in patients with congestive HF, resulting from different etiologies (acute myocardial infarction, dilated cardiomyopathy and valvular disease). Higher levels of MMP-2 are correlated with patients with a worse prognosis for HF (NYHA class II–IV) [40], as well as an increased risk of death or hospitalizations for HF [41]. MMP-2 and TIMP-1 are higher in the plasma of patients with acute HF [42]. MMP-2 is considered the best biomarker among the other proteases in the ECM, as its levels varied only slightly during a temporal evaluation [43].

MMP-2 has also been considered a biomarker of LV remodeling in patients with HF who have suffered an acute myocardial infarction, with extensive areas of injury and decreased ejection fraction [44]. A study that evaluated control patients (who did not have cardiovascular disease), patients with LV hypertrophy without HF, and patients with diastolic HF and LV hypertrophy, showed that the dosage of MMP-2 and procollagen III N-terminal propeptide (PIIINP) together, were shown to be biomarkers that predict HFpEF better than NT-proBNP dosing alone [45]. MMP-2, MMP-9 and TIMP-1 have also been recognized as highly valuable biomarkers for predicting the risk of death in patients with HF [46].

In addition to being a biomarker, the participation of MMP-2 in the development of HF was suggested due to its role in the degradation of components of the myocardial matrix and the regulation of the fibrotic process, contributing to progressive dilation of the cardiac chambers, reduction in heart compliance and driving problems. In this context, MMP-2 is a key protease in the maladaptive remodeling process of the heart [20,40,41].

The role of MMP-2 in developing cardiac injury and dysfunction was demonstrated in a transgenic model of MMP-2 overexpression. Interestingly, this model showed that an increase in MMP-2 levels in the heart is sufficient to induce ventricular dysfunction, with myocyte hypertrophy, contractile protein lysis and cardiac fibrosis, even without a pathological process [47]. Furthermore, when subjected to ischemia and reperfusion injury, those animals that overexpress MMP-2 have higher infarction areas, lipid peroxidation and cardiac dysfunction compared to normal animals [48].

For a better understanding of the involvement of MMP-2 in pathological processes that lead to HF, the hearts of rats that suffered ischemia and reperfusion were evaluated, and there was an increase in the production of reactive species, including peroxynitrite ($ONOO^-$) associated with MMP activation, before being secreted into the extracellular environment [36,49]. As a result, MMP-2 promoted proteolysis of contractile machinery proteins, including titin [50], troponin I [51], myosin light chain [52,53] and alpha-actinin [54]. In this experimental model, the disruption of the cell cytoskeleton by MMP-2 is involved in the decrease in myocardial contractility, with oxidative stress being the main factor related to the increase in MMP-2 activity. Thus, decreasing the oxidative stress or inhibiting the catalytic activity of MMP-2 may be a therapeutic target.

The redox imbalance in cardiomyocytes during ischemia also leads to the activation of alternative MMP-2 promoters, producing an N-Terminal Truncated isoform called NTTMMP-2, constitutively active and present in mitochondria, altering energy metabolism and mitochondrial function and activation of the innate immune response [33]. In addition, overexpression of NTT-MMP-2 in mouse hearts results in LV hypertrophy, intense inflammatory cell infiltration, cardiomyocyte apoptosis and cardiac dysfunction [55].

Studies with knockout mice for MMP-2 were performed to confirm the participation of MMP-2 in HF after acute myocardial infarction. The absence of MMP-2 did not change the infarct area. However, the animals showed less LV dilation and increased survival than wild animals [56]. MMP-2 deletion was also beneficial in mice subjected to increased cardiac preload, in which they showed decreased myocyte hypertrophy and improved fibrosis and cardiac dysfunction [57].

On the other hand, inhibition of MMP-2 at below baseline levels can become an issue [58]. This was demonstrated in a preclinical study using MMP-$2^{-/-}$ mice with cardiac overexpression of TNF-α showing decreased survival, LV contractile dysfunction, and increased infiltration of inflammatory cells of the myocardium [59]. Another study with *MMP-*$2^{-/-}$ mice infused with angiotensin II showed that MMP-2 deletion did not affect the severity of hypertension but caused cardiac hypertrophy to develop earlier and to a greater extent than in wild-type animals [60]. Furthermore, clinical studies have shown that patients with loss of MMP-2 function due to mutations in the *MMP-2* gene are predisposed to a complex multisystem syndrome involving abnormalities of cardiac development [61,62].

The manipulation of MMP-2 genes helped us to confirm its participation in the pathophysiology of HF. However, when we analyzed the studies mentioned above, we realized that an exacerbated increase in activity or expression and the complete deletion of this protease is harmful during HF's evolution. In this way, the ideal would be to modulate the levels and the activity of MMP-2 to prevent functional dysfunction caused by the remodeling of the heart. Therefore, the use of molecules that can inhibit MMP-2 may work as an effective treatment for the progression of HF.

4. The Development of Inhibitors for MMPs and Their Use as a Pharmacological Tool in Disease

The understanding of the role of MMPs in the pathophysiology of cardiovascular diseases, neurodegenerative disorders such as Parkinson's and Alzheimer's and cancer raised the hypothesis of the importance of regulating these prostheses as a way to stop changes in physiological processes such as angiogenesis, tissue remodeling, healing, migration cell, activation of signaling molecules and immunity [63]. Table 3 summarizes the different MMP inhibitors and their characteristics.

MMPs have endogenous inhibitors, including α2-macroglobulin, a protease secreted by the liver, that binds to MMPs in plasma, preventing them from degrading their substrates. Tissue MMPs are inactivated by TIMPs, with four members that make up this family: TIMP-1 to 4. TIMPs can inhibit all MMPs, but

with different specificities for each one of them [64]. TIMPs have been used as a therapeutic tool in several diseases to modulate MMPs. However, without favorable results, possibly because they share similar pathways without directly interfering with each other's role [63].

Table 3. Development of MMP inhibitors and their characteristics.

Class of MMP Inhibitors	Inhibitor (Alternative Names)	Characteristics
Endogenous inhibitors	α2-macroglobulin and TIMPs	It traps MMPs in the plasma, preventing them from degrading their substrates. It inhibits tissue MMPs and has four members: TIMP-1 to 4. TIMPs can inhibit all MMPs, but with different specificities.
Hydroxamate-based inhibitors	Batimastat and Marimastat	They are designed to mimic the natural peptide substrate (collagen) of MMPs. It targets the catalytic site of MMPs.
The new generation of hydroxamate-based inhibitors	Cipemastat and MMI-166	They were developed with a sulfonamide and a zinc-binding hydroxamate group, in addition to the substitution of an aryl group, generating a compound with more specificity. It targets the catalytic site of MMPs.
Non-hydroxamate inhibitors	Rebimastat and Tanomastat	They were designed with various peptidomimetics and non-mimetics, not limited to mimicking the substrate of MMPs. It targets the catalytic site of MMPs.
Inhibitors targeting alternative binding sites	BMS-275291 and specific MMP-13 inhibitor (provided by Pfizer, Ann Arbor, MI, USA)	Highly selective, unlike previous MMP inhibitors, because it does not bind to catalytic zinc ion and is not competitive for substrate binding. They target alternative, less conserved binding sites.

The first class of synthetic molecules used as inhibitors of MMPs was based on hydroxamate. These compounds were designed to mimic the natural peptide substrate (collagen) of MMPs associated with a group that can chelate the Zn^{2+} ion of the catalytic site, thus favoring broad-spectrum inhibition [65,66]. An example of this type of drug was batimastat, which could inhibit MMP-9 and slow the growth of solid tumors in preclinical trials [67]. In a double-blind prospective clinical trial, this drug was used to inhibit MMPs, as an adjuvant treatment in patients with small-cell lung cancer. However, it did not improve survival, decreasing the quality of life of patients who used it [68]. Another example of a hydroxamate-based drug was marimastat, a structural analog of batimastat, which showed favorable results in most preclinical studies in tumor models, thus serving as a basis for clinical trials [69]. A series of phase I, phase II and phase III tests were performed against solid metastatic tumors, with significant depletions in the increase in tumor markers being observed. However, in phase III studies, patients showed musculoskeletal toxicity, which may be associated with inhibition of ADAM and ADAMTS family members [66,70–72]. This class of hydroxamate-based inhibitors was able to inhibit several MMPs, including MMP-1, MMP-2, MMP-7 and MMP-9, showing benefits in preclinical trials but failed in clinical trials mainly due to their nonspecificity promoting combined inhibition of several MMPs resulting in musculoskeletal syndrome (arthralgia, myalgia and tendinitis) [63,66]. In addition, these drugs may have been introduced too late to modify a pathological condition, which could explain their failure in clinical trials. Therefore, first-generation hydroxamate-based molecules were discontinued after clinical trials failed.

However, a new generation of hydroxamate-based molecules was developed, presenting a sulfonamide and a zinc hydroxamate linking group and substituting an aryl group, generating a compound with more specificity to minimize the adverse effects of the previous generation triggered. Its development uses structure-activity relationship analysis (SAR), which helps identify molecular substructures related to the presence or absence of biological activity [63]. Cipemastat, used in treating patients with rheumatoid arthritis and osteoarthritis, is an example of this class. It was able to inhibit MMP-1, MMP-3 and MMP-9 more selectively. However, it did not prevent the progression of joint damage [66]. MMI-166 is selective for MMP-2, MMP-9 and MMP-14 and

decreases the cellular invasion of cervical carcinoma in vitro. However, there was no suppression of the proliferation of tumor cells [66,73]. A recurring limitation in the use of these inhibitors was the premature metabolism suffered by the drug, leading to the loss of the hydroxamate group that binds with zinc. Despite the difficulties encountered in the therapeutic use of this new generation of inhibitors, there is still significant interest in developing drugs derived from hydroxamic acid, as these compounds are the most potent inhibitors of MMP available to date [63].

The search for molecules with lower metabolic lability and more stable bonds to the Zn^{2+} ion of the catalytic site led to the development of compounds derived from phosphoric acid, hydantoins and carboxylates, usually called non-hydroxamate MMP inhibitors. Next-generation MMP inhibitors have been designed with a variety of peptidomimetics and non-mimetics, not limited to mimicking their substrates [63]. One of the first non-hydroxamate MMP inhibitors developed was Rebimastat, a broad-spectrum inhibitor containing a thiol group that binds to zinc. Phase II clinical trials in early-stage breast cancer and a phase III study in lung carcinoma using this compound as adjuvant therapy discontinued treatment because patients experienced arthralgia consistent with MMP inhibitor-induced toxicity [74,75]. Another tanomastat inhibitor showed good tolerability and variable efficacy that depended on the timing of administration concerning disease progression [76,77]. Several biphenyl sulfonamide carboxylate-based MMP inhibitors have been designed to treat osteoarthritis by inhibiting MMP-13.

Substances such as polyphenols, flavonoids and carotenoids, obtained from natural products, can inhibit MMPs, with photoprotective and antioxidant properties. For example, *P. leucotomos* inhibits the expression of MMPs in epidermal keratinocytes and fibroblasts and stimulates TGF-β in skin fibroblasts by decreasing lipid peroxidation and oxidative stress [78,79]. Xanthohumol directly inhibits MMP-1, MMP-3 and MMP-9 while increasing the expression of collagen types I, III and V, fibrillin-1 and 2 in dermal fibroblasts [80]. Lutein prevents photoaging by inhibiting MMPs and oxidative stress, reducing epidermal hyperproliferation, expanding mutant keratinocytes, and mast cell infiltration in response to solar radiation [81,82]. The photoprotective activity presented by these compounds is probably associated with a decrease in the degradation of collagen fibers by MMP-1 and MMP-2 and of elastin fibers by MMP-2 and MMP-9 as well as by stimulating TGF-β in fibroblasts, which inhibits MMP-1 and

stimulates collagen production [78]. Tetracycline antibiotics, such as doxycycline and minocycline, have an innate inhibitory capacity for MMPs [63,66].

To reduce off-target effects and to avoid broad inhibition of MMPs, due to their high structural homology, current inhibitors are being designed to target alternative, less conserved binding sites. Using crystallography and X-rays combined with computational methods allows the modeling of drug-protein interactions with inhibitors that bind to other sites. In addition, combining techniques with computational prediction revealed hidden sites in the structure of MMPs that can be explored for the rational design of new molecular effectors and therapeutic agents [63,66]

5. Use of Nonspecific and Specific Inhibitors for MMP-2 in HF

Preclinical studies demonstrate that a therapeutic approach to MMP-2 inhibition may be a promising strategy for treating patients with HF. ONO-4817 a selective inhibitor, has shown beneficial results in an ischemia and reperfusion model, improving contractile dysfunction, associated with decreased MMP-2 activity and titin proteolysis [50]. In addition, it showed promising results in attenuating LV remodeling and myocardial fibrosis in mice treated with doxorubicin, a drug used in cancer patients that is cardiotoxic and leads to HF, by increasing oxidative stress and MMP-2 activity [83]. Collectively these studies support the hypothesis that inhibiting MMPs by selectively using ONO-4817 has therapeutic potential, as this compound selectively inhibits MMP2, significantly decreasing the extent of lesions and disease severity. Furthermore, it cannot inhibit MMP-1, which has been associated with adverse effects triggered by hydroxamate-based inhibitors.

Antibiotics of the tetracycline class, such as doxycycline, which has an innate inhibitory capacity for MMPs, with greater specificity for MMP-2, MMP-9 and MMP-8, have also been used in models of cardiac injury. In preclinical trials, doxycycline prevented the conversion of concentric hypertrophy to eccentric hypertrophy of the LV during hypertension. This effect was associated with decreased MMP-2 activity and reduced troponin I and dystrophin proteolysis, thus improving the mechanical stability of cardiomyocytes and the contractile function [84]. On the other hand, doxycycline could not reduce scar thinning and

compensatory LV hypertrophy, despite having decreased MMP-2 and MMP-9 activity in a model of acute myocardial infarction with LV dysfunction. These findings draw attention to the non-selective inhibition of MMPs in the initial healing phase after IM [85]. Doxycycline is an antibiotic capable of inhibiting MMPs at subantimicrobial doses and is currently the only FDA-approved MMP inhibitor for the treatment of periodontal disease [86]. In addition, it shows benefits in other conditions such as abdominal aortic aneurysm [87], arterial hypertension [88–92] and acute myocardial infarction [93].

Clinical studies that evaluated the effects of doxycycline on HF showed results dependent on the dose used and the etiology. For example, patients with acute myocardial infarction (40% of patients with HFrEF) were treated with doxycycline 100 mg as adjunctive therapy. They improved diastolic function and decreased the infarct area [94]. On the other hand, two randomized clinical trials that evaluated the effects of adjuvant treatment with doxycycline at a dose of 20 mg in patients with coronary artery disease and atherosclerosis showed no improvement in cardiac dysfunction parameters and sudden death outcomes [95,96]. The pathophysiological processes that trigger the damage and cardiac remodeling are closely correlated with the therapeutic response and the dose used since the preferential inhibition of specific MMPs are associated with the dose of doxycycline used as well time of therapy instituted.

The adverse effects of non-selective MMP inhibition are reinforced by the clinical study in patients with acute myocardial infarction and HFpEF, which evaluated the impact of the inhibitor PG-116800 (oral MMP inhibitor with an affinity for MMP-2, MMP-3, MMP-8, MMP-9, MMP-13 and MMP-14 and low affinity for MMP-1 and MMP-7). The PG-116800treated group showed no improvement in heart function and death rates compared to the placebo. Unfortunately, this study was discontinued due to the development of musculoskeletal toxicity with no apparent benefit following administration of PG-116800 [97]. Therefore, we emphasize the importance of using inhibitors as an adjuvant therapy with greater specificity and fewer off-target effects.

In this way, using more selective inhibitors aimed at binding to alternative, less conserved sites can be a therapeutic strategy in HF, minimizing the adverse effects of nonspecific inhibitors. For example, TISAM, an N-sulfonylamino acid derivative, which selectively inhibits MMP-2, was used in a model of acute

myocardial infarction, improving survival rate, preventing cardiac rupture and delaying post-infarction remodeling. These benefits were associated with decreased MMP-2 activity and macrophage infiltration into cardiac tissue [56].

Using chemical modeling to inhibit MMP-2 selectively, the inhibitors MMPI-1154, MMPI-1260 and MMPI-1248 were developed. MMPI-1154 and MMPI-1260 showed efficacy in reducing infarct size associated with decreased MMP-2 activity [98,99]. However, despite the potential of the TISAM, MMPI-1154 and MMPI-1260, these compounds have not been tested in other preclinical, experimental models of HF and clinical studies have not been conducted.

The selective inhibition of MMP-2 was also evaluated with siRNA technology. MMP-2 deletion in cardiomyocytes isolated from adult rats undergoing ischemia and reperfusion injury prevented contractile dysfunction associated with decreased MLC1/2 degradation [100]. The encapsulation of siRNA for MMP-2 in a hydrogel to improve cell penetration was also investigated in an in vivo acute infarction model. Positive effects on heart hemodynamics were observed, where the reduction in MMP-2 in cardiomyocytes led to the maintenance of cardiac output and ejection fraction [101]. Together, the studies that used compounds or technology of selective inhibition of MMP-2 reinforce that this protease can be a therapeutic target in the treatment of HF.

Clinical studies evaluating the effects of statins on MMP-2 inhibition (atorvastatin, rosuvastatin and pravastatin) in patients with HFrEF after acute myocardial infarction showed decreased serum MMP-2 levels associated with a reduction in the number of deaths and hospital readmission [102–104]. These studies did not assess whether these drugs directly inhibit MMP-2, but statins are known to decrease inflammation and oxidative stress [105,106], which may be related to the results found in reduced serum levels of MMP-2.

Drugs used in the treatment of hypertension, such as verapamil, carvedilol and trimetazidine, have shown positive effects on cardiac function and remodeling associated with decreased activity and expression of MMP-2 [107–109]. Low-dose carvedilol is cardioprotective and inhibits MMP-2 activity in an ischemia/reperfusion model [109]. Verapamil has been shown to reduce MMP-2 activity by decreasing oxidative stress and calpain-1 that regulates MMP-2 activity in a model of HF induced by hypertension [108]. At the same time, trimetazidine has reduced MMP-2 expression by decreasing oxidative stress in

an animal model of myocardial infarction [107]. Table 4 summarizes the non-selective and selective MMP-2 inhibitors evaluated in preclinical and clinical studies of HF.

It is evident that MMP-2 plays an essential role in cardiac injury and HF development. Preclinical studies better explain the pathophysiological mechanisms involving extracellular matrix proteins. Clinical evidence indicates that selective inhibition of MMPs is optimal, as non-selective inhibition with MMP-inhibiting compounds is associated with loss of response and adverse reactions, possibly through inhibition of MMPs essential for body homeostasis. It is noteworthy that more clinical studies involving selective and nonselective MMP-2 compounds are needed, as the use of inhibitors for MMP-2 has shown promise as adjuvant therapy for HF in preclinical models. In addition, the use of drugs already used in the clinic can be an alternative for inhibiting MMP-2, having the advantage of safety in its use.

Table 4. Non-selective and selective MMP-2 inhibitors were evaluated in preclinical and clinical studies of HF.

Non-Selective Inhibitor	Species	Disease	Comments	References
Doxycycline	Rats	Renovascular hypertension with HF	Prevented the conversion of concentric hypertrophy to eccentric hypertrophy in the LV, associated with decreased MMP-2 activity and reduced troponin I and dystrophin proteolysis	[84]
Doxycycline	Mice	Model of acute myocardial infarction with HF	It has not reduced scar thinning and compensatory LV hypertrophy, despite having decreased MMP-2 and MMP-9 activity	[85]

Doxycycline (Adjuvant therapy)	Humans	Acute myocardial infarction (40% of patients with HFrEF)	Improved diastolic function and reduced infarct área	[94]
Doxycycline (Adjuvant therapy)	Humans	Coronary artery disease and atherosclerosis	There was no improvement in cardiac dysfunction parameters and sudden death outcomes	[95,96]
PG-116800 (Adjuvant therapy)	Humans	Acute myocardial infarction (HFpEF) with HF	No improvement in heart function and death rates Development of musculoskeletal toxicity	[97]
MMP-2 selective inhibitor				
ONO-4817	Mice	Ischemia and reperfusion model with HF	Shown to improve contractile dysfunction associated with decreased MMP-2 activity and titin proteolysis	[50]
ONO-4817	Mice	Model of doxorubicin-induced cardiotoxicity	Attenuated LV remodeling and myocardial fibrosis	[83]
TISAM	Mice	Model of acute myocardial infarction with HF	It improved survival rate by preventing cardiac rupture and delaying post-infarction remodeling	[56]
MMPI-1154, MMPI-1260 and MMPI-1248 (Chemical Modeling)	Mice	Model of acute myocardial infarction with HF	They showed inhibitory activity on MMP-2, associated with a reduction in the infarct área	[98,99]

siRNA for MMP-2	Mice	Ischemia and reperfusion model with HF	It prevented contractile dysfunction associated with decreased degradation of MLC1/2	[100]
Hydrogel encapsulated siRNA for MMP-2	Mice	Model of acute myocardial infarction	Improved cardiac output and ejection fraction	[101]
Statins (Atorvastatin, Rosuvastatin and Pravastatin)	Humans	Acute myocardial infarction (HFrEF)	Decreased serum MMP-2 levels are associated with a reduced number of deaths and hospital readmission	[102–104]
Antihypertensive drugs (Verapamil, Carvedilol and Trimethazine)	Mice and rats	Ischemia/reperfusion model; Model of HF induced by hypertension and Myocardial Infarction Model	Positive effects on cardiac function and remodeling associated with decreased activity and expression of MMP-2	[107–109]

Selective inhibitors for MMP-2 tested in a multitude of diseases may be a plausible alternative in the treatment of HF, but the safety profile, possible adverse effects, and clinical results must be taken into account [110–115]. In addition, as a future perspective, the use of computational modeling as a tool can help predict the behavior of a molecule in non-living systems. Besides that, structure-activity relationship (SAR) analysis helps identify molecular substructures related to the presence or absence of biological activity. Finally, genomic engineering, such as clustered regularly interspaced short palindromic repeat (CRISPR), has a potential future in treating cardiovascular diseases, including HF.

6. Conclusions

Modulation of MMP-2 by non-selective or specific inhibitors has the potential to provide new directions for studying the mechanisms underlying various heart diseases, including HF. In addition, it has potential as a therapeutic tool for clinical practice and could have a significant impact on the development of new approaches to protect against cardiac remodeling and dysfunction in HF.

Author Contributions: Writing—original draft preparation, P.R.G., L.D.N., R.F.G., K.E.R. and A.F.P.; writing—review and editing, R.F.G., K.E.R. and A.F.P.; supervision, K.E.R. and A.F.P.; project administration, A.F.P.; funding acquisition, R.F.G. and A.F.P. All authors have read and agreed to the published version of the manuscript.

Funding: Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP Grant number 2014-23888-0), Conselho Nacional de Desenvolvimento científico e Tecnológico, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES)—Finance Code 001. The APC payment was supported by Pró-reitoria de Pesquisa e Pós-Graduação (PROPESP) from Federal University of Pará (UFPA).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

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

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Artigo 2: Coentro (Coriandrum Sativum L.) apresenta atividade antioxidante e efeito inibitório sobre MMP-2 no coração de ratas intoxicadas com metilmercúrio durante a gestação e lactação

Coriander (*Coriandrum Sativum* L.) antioxidant activity and inhibitory effect on MMP-2 in the heart of rats poisoned with methylmercury during pregnancy and lactation

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Abstract

Human contamination by MeHg increases cardiovascular disease risk, possibly associated with oxidative stress and epigenetic alterations in proteins. Coriander (*Coriandrum sativum* L), a plant widely used in world cuisine, has antioxidant activity. Our study evaluated the effect of *Coriandrum sativum* Aqueous Extract (CSAE) in the heart of rats intoxicated with MeHg during pregnancy and lactation. Primiparous rats were divided into four groups: Control, MeHg, CSAE and MeHg + CSAE, intoxicated and contracted for 21 days. We performed morphological analysis, redox system, MMP-2 activity in the heart and electrocardiogram. The MeHg group showed increased nitrite levels and reduced GSH, CAT and SOD. We were associated with decreased myocyte diameter, LV wall thickness, interventricular septum, increased collagen content, and MMP-2 activity. There was a reduction in heart rate, followed by a first-degree atrioventricular block and ventricular conduction disturb, compared to the Control and CSAE group. The

MeHg + CSAE group maintained the measurements of the redox system, morphometric measurements, collagen content and MMP-2 activity similar to the Control and CSAE group. CSAE treatment attenuated conduction disturbances in the heart when ced to the MeHg group. Coriander maintained the redox system and modulated MMP-2, thus preventing cardiac remodeling, attenuating cardiac conduction disturbances, and blocking the deleterious effects of MeHg on the heart.

Palavras-chave: methylmercury, oxidative stress, cardiac remodeling.

1. Introduction

Mercury (Hg) is an environmental pollutant, which in its biogeochemical cycle, is transformed into a more toxic organic form, methylmercury (MeHg), which bioaccumulates in food webs through trophic magnification [1, 2]. Elevated levels of MeHg in carnivorous fish are one of the primary sources of human exposure to this metal [3, 4].

Throughout history, episodes of human poisoning by MeHg from consuming contaminated food, as in Minamata in Japan, gained enormous repercussions due to the large number of people who developed severe neurological disorders or died [5, 6]. In addition, pregnant women in the region who eat contaminated fish have had children with extensive brain damage [7, 8].

The gastrointestinal tract rapidly absorbs MeHg ingested in food due to its liposolubility and readily crosses cell membranes and the transplacental barrier [9, 10]. The repercussions on the cardiovascular system caused by exposure to MeHg in human populations have been investigated [11-13]. A prospective study in Finland suggested a possible correlation between mercury accumulation in the body from contaminated fish and an increased risk of acute myocardial infarction, coronary disease and other types of cardiovascular diseases with MeHg. In addition, the authors suggested that the harmful effects of MeHg on the cardiovascular system could be associated with oxidative stress [14].

The deregulation of the redox balance by suppressing the antioxidant system and increasing reactive species is one of the primary mechanisms associated with the harmful effects promoted by MeHg, which triggers lipid peroxidation, altering the permeability of membranes [15-17]. In addition, MeHg

promotes protein denaturation and enzyme inactivation, triggering epigenetic changes [18].

Another mechanism that may be associated with the adverse effects of exposure to MeHg is the activation of matrix metalloproteinases (MMPs) [19, 20]. MMPs are a family of zinc-dependent endopeptidases that control the synthesis and degradation of extracellular matrix (ECM) components, acting directly on intracellular substrates [21]. Reactive species such as MeHg can activate these enzymes, disrupting the interaction of a propeptide domain cysteine residue with catalytic zinc within the enzyme's active site [19, 21].

MMP-2 is extensively investigated in cardiovascular diseases because the increase in its activity is closely related to heart remodeling. In addition, it has also been suggested that there is a correlation between increased MMP-2 activity and plasma mercury concentrations as a possible mechanism that could increase susceptibility to cardiovascular disease, showing a possible causal relationship between mercury exposure and increased susceptibility to cardiovascular disease [22].

The gestational and lactation period comprises a critical moment for the cardiovascular system, as hemodynamic changes promote an increase in the heart's workload, resulting in changes in the oxidative state. Therefore, exposure to methylmercury during this phase is of great concern due to possible unfavorable cardiovascular outcomes that can be fatal [23-26].

Introducing foods during pregnancy that can help prevent oxidative damage and its harmful effects is an alternative that has been gaining strength. Within this context, Coriander (*Coriandrum sativum* L.) stands out, a plant widely used in world cuisine, originating in regions of Europe and Asia [27].

Coriander has been shown to have antioxidant and cardioprotective activity, improving left ventricular function, baroreflex sensitivity associated with modulation of endothelin receptors and attenuation of lipid peroxidation in a model of heart failure [27, 28]. In addition, Coriander has flavonoids with inhibitory activity on the angiotensin-converting enzyme (ACE), capable of modulating blood pressure [29]. It also modulated MMPs and removed inorganic and organic mercury from plant roots [30, 31].

Thus, this study raises the hypothesis that the Aqueous Extract of *Coriandrum sativum* (CSAE) can prevent the remodeling of the heart of rats

exposed to methylmercury by modulating the redox state and MMP-2. Therefore, our objective was to evaluate the effect of CSAE on oxidative stress and cardiac remodeling in rats exposed to methylmercury during pregnancy and lactation.

2. Materials and methods

2.1 Animals and Experimental Design

Wistar rats (Rattus norvegicus) primiparous with two months of age weighing approximately 200g, obtained from the Central bioterium of Evandro Chagas Institute and stored in the bioterium of the Laboratory of Pharmacology and Toxicology of Natural Products in the Federal University of Para. The animals were kept on a 12-hour light/dark control system, controlled temperature (22°C), and free access to water and feed. This study was carried out according to the guidelines of Ethical Principles of the National Council for the Control of Animal Experimentation (CONCEA) (CEPAE-UFPA 3610090519. Furthermore, *Coriandrum sativum* Aqueous Extract (CSAE) was prepared according to Rodrigues et al., 2019 [32].

The rats were randomized into four groups according to the experimental protocol: 1) Control group (n = 8): received water; 2) MeHg group (n = 9): received MeHg diluted in drinking water (40µg/mL); 3) CSAE group (n=6): received CSAE by gavage (45µg/mL); 4) MeHg + CSAE group (n=10): received MeHg diluted in drinking water (40µg/mL) and CSAE by gavage (45µg/mL). Intoxication with methylmercury and co-treatment with CSAE occurred for 21 days, from the 14th gestational day to the 14th lactational day.

2.2 Assessment of oxidative stress and the antioxidant system

2.2.1 Nitrite levels

Nitrite levels were quantified in duplicate using heart extract. For that, 100µL of the sample was used, which was mixed with Griess' reagent [33], forming a pink-colored product that was measured in a microplate reader at the absorption length of 540nm. Nitrite levels were calculated using a standard nitrite curve (2 – 100 µM). Results were expressed in µmol/mg of protein.

2.2.2 Lipid peroxidation

This assay measures substances that react when in contact with thiobarbituric acid (TBARS), considered products of lipid peroxidation [34]. Briefly, 10µL of the supernatant collected from the samples was incubated with 10 mM thiobarbituric acid and heated at 94°C for 60 min. Next, n-butyl alcohol was added to the resulting supernatant, vortexed and centrifuged. The reaction result was read in a microplate reader (BIO-RAD Model 450 Microplate Reader) at 535 nm. We used a standard curve of malonaldehyde in concentrations ranging from (2-20 ρmol/mL). The result being expressed in ρmol/mg of protein.2.5.1. Peroxidação lipídica

2.2.3 SOD activity

SOD activity was measured by reacting heart samples with hypoxanthine and NBT [35]. Absorbance was measured at a wavelength of 470 nm, and results were expressed as U/mg of protein.

2.2.4 Catalase activity

Catalase enzyme activity was evaluated in the heart extract by measuring formaldehyde levels. First, the sample was exposed to methanol, which produced formaldehyde in the presence of hydrogen peroxide (H_2O_2) [36]. Next, the colorimetric reaction product (purple) was obtained after adding the Purpald® reagent (4-amino-3-hydrazino-5-mercapto-1, 2, 4-triazole). The plate was measured after 20 minutes of reaction in a microplate reader at a length absorbance of 540 nm. Catalase activity was calculated using a standard formaldehyde curve (5 to 75 µM). The results are expressed in U/mg of protein.

2.2.5 Assessing reduced glutathione levels in the heart

The method used is based on the ability of glutathione to reduce 5,5-dithiobis-2-nitrobenzoic acid (DTNB) to nitrobenzoic acid (TNB) [37]. For the assay, we used a standard curve of reduced glutathione (3-50 µM). The results are expressed in ρmol/mg of protein.

2.3 Heart collection for histological and biochemical analysis

One week after the experimental protocol, the rats were euthanized, with a preparation based on ketamine chloride (90 mg/kg) and xylazine chloride (10

mg/kg), and anthropometric measurements were performed. Subsequently, we opened the sternum, removed the heart, and placed it in cold saline buffer (PBS) pH: 7.4. Then, the heart was immersed in 50μM KCl solution to maintain diastole. The heart was dried and weighed, then dissected and divided. The upper part was used for biochemical measurements, and the lower was used for histological analyses.

2.4 Assessment of cardiac morphology, myocyte diameter and interstitial collagen content

Paraffin-embedded hearts were cut transversely on a microtome and stained with hematoxylin and eosin (HE), and picrosirius. Cardiac sections of 5 μm stained with HE were used to evaluate the thickness of the left ventricle wall and the interventricular septum. Then, slides were photographed using a Leica M205 coupled to a Leica DFC 450 camera, where the scale was measured to calibrate the scale option in the Image J software. Then, the interventricular septum and left ventricular wall were measured in 5 different parts of the slice, obtaining the average of each slice.

Sections of 5 μm stained with HE were photographed at 400x magnification under a white light microscope (Zeiss) to measure the myocyte diameter. First, the Image J program opened the images containing a scale to calibrate the scale. Then, the myocyte diameter was obtained in the fiber regions where the nucleus was apparent, and it was possible to observe the beginning and end of the muscle fiber. Next, ten animal images were captured from different parts of the left ventricle, where ten myocytes were quantified, totaling 100 myocytes per animal.

Interstitial collagen content was quantified using 7 μm paraffin sections stained with Picrosirius and photographers at 400x magnification under a polarized light microscope (Zeiss). Ten random fields/slides from the left ventricle of each animal were photographed. Two identical images were opened side by side in the Image J program for quantification. First, an image was transformed into 16 bits. Then the threshold option was selected, where the collagen marked area was adjusted, using the other open image as a basis.

2.5 MMP-2 Activity by Gel Zymography

The metalloproteinase activity was evaluated using 10% polyacrylamide gel containing 1% of gelatin [38]. The heart was macerated in a tissue crusher in the presence of dry ice and homogenized in 300µL of buffer containing 50 mM Tris, 10 mM CaCl2 and protease inhibitors (1 mM phenanthroline, 1 mM PMSF and 1 mM NEM). Then, the homogenate was centrifuged at 10,000rpm at 4°C, and the supernatant was collected for protein dosage using the BCA Protein Kit (Thermo). For the assay, 20 µg of proteins were mixed with buffer containing 125mM Tris; 4% SDS; 20% glycerol and 0.001% bromophenol blue, then applied to a 10% SDS-PAGE gel containing 1% gelatin. After the electrophoresis run, the gels were incubated with triton X-100 solution for 60 minutes for renaturation, followed by incubation in 50 mM tris buffer containing 10 mM CaCl2 in an oven at 37°C for 16 hours. The gels were stained in a coomassie blue solution for 24h, followed by incubation in a decolorizing solution to visualize the white bands, where the gelatin was degraded. Gels were photographed, and white bands were quantified using the Image J program.

To assess whether Coriander directly inhibits MMP-2, we performed zymograms where fetal bovine serum was used as a source of MMP-2, which was applied in 3 points (channels) of the gel. After electrophoresis, the gels were submitted to two 30 min baths with Triton X-100 (2% v/v, Sigma) to remove the SDS from the gel. Then, the gels were divided into three parts. One part was used as a control and was incubated in Tris solution (50 mmol/L) containing $CaCl_2$ (10 mmol/L), pH 7.4 for 18h at 37ºC to allow degradation of the gelatin present in the gels. The other two parts were incubated with coriander extract at 20 and 200 µg/mL concentrations. After 18h, the gels were stained, bleached and photographed to quantify the MMP-2 activity as previously described.

2.6 Electrocardiography

One week after the experimental protocol, the ECG was performed in the rats (n=8) per group. Before recording, the animals were anesthetized with tribromoethanol 15 mL/kg and shaved in the thoracic region. Then, the rat was placed inside a Faraday cage, and three electrodes were fixed. The first electrode was set on the animal's hind paw, the second reference electrode on the right shoulder and the third electrode on the opposite side, to the left of the xiphoid process, in lead DII. All electrodes were made of silver measuring 7x3 mm and

connected directly to an acquisition system. A differential amplifier was performed the acquisition of the record with a high-impedance AC input (Grass Technologies, P511), adjusted with filtering of 0.3 Hz (high-pass) and 300 Hz (low-pass). The records were monitored with an oscilloscope (Protek, 6510) and continuously digitized at 10 kHz by a computer equipped with the Axoscope 9.0 software (Axon Instruments, USA). Each animal was recorded for 5 minutes for each animal, and the files were saved and stored for later analysis. The records were analyzed using the "LabChart v.7.3.8" interface software (ADInstruments, Colorado Springs, CO, USA). A portion of the 180-second electrocardiographic tracing was randomly selected, which contains a sequence of R-R intervals used to calculate the heart rate (bpm), QRS complex and the PR, QT and QTc segments [39-42].

2.7 Statistical analysis

The results obtained were analyzed in the statistical program GraphPad Prism ® 8.0 software (GraphPad Software, San Diego, CA, USA). Initially, the data were evaluated for normality using the Shapiro-Wilk test. The data were considered normal. Therefore, the two-way ANOVA test was used to assess the statistical differences, followed by the Tukey test, considering $p<0.05$ statistically significant. Results were expressed as mean ± standard error of the mean (SEM).

3. Results

3.1 Coriander prevented redox imbalance in the heart

We evaluated the nitrite and MDA levels in the rats' left ventricles to verify an increase in reactive species and possible oxidative damage that could contribute to changes in cardiac morphology. We observed enhanced nitrite and MDA levels in the MeHg group compared to the control group ($p > 0.05$, Table 1). On the other hand, the MeHg + CSAE group had nitrite and MDA levels similar to the CSAE and control groups ($p > 0.05$, Table 1).

Next, we decided to evaluate the antioxidant system to investigate whether oxidative damage was related to antioxidant system depletion. Thus, we assessed the levels of GSH, which represents the first line of defense against

xenobiotics poisoning and evaluated the enzymatic system by measuring SOD and CAT activity.

Our results showed that the MeHg group had a decrease in GSH levels, SOD and CAT activity in the heart compared to the control group and EACS ($p < 0.05$, Table 1). Meanwhile, the MeHg + CSAE group maintained GSH levels and SOD and CAT activity similar to the Control and EACS groups ($p > 0.05$. Table 1).

Table 1. Values of nitrite, MDA, glutathione levels and SOD and catalase activity in the left ventricle of the control, CSAE, MeHg and MeHg + CSAE groups.

	Control	**CSAE**	**MeHg**	**MeHg + CSAE**
Nitrite (µmol/ mg de protein)	1.3 ± 0.2	1.2 ± 0.2	4.3 ± 0.5*	1.2 ± 0.1#
MDA (ρg/mg de proteín)	0.3 ± 0.02	0.2 ± 0.02	0.7 ± 0.05*	0.3 ± 0.02#
Glutathione (ρmol/mg de protein)	1.7 ± 0.2	1.7 ± 0.1	0.9 ± 0.1*	1.8 ± 0.2#
SOD (U/mg de protein)	21 ± 1	22 ± 1	11 ± 1*	20 ± 1#
Catalase (U/ mg de protein)	24 ± 2	26 ± 1	8 ± 1*	26 ± 2#

CSAE (*Coriandrum sativum* Aqueous Extract). Values were expressed as mean ± SEM (n=5-10). * $p > 0.05$ compared to the control group and # * $p > 0.05$ compared to the MeHg group (two-way ANOVA followed by Tukey test).

3.2 Coriander prevented MeHg-induced cardiac atrophy

After identifying a decline in the antioxidant system, with an increase in reactive species and lipid peroxidation in the heart of rats poisoned with MeHg, Coriander was able to protect against such alterations. We decided to evaluate cardiac morphometry to assess possible alterations triggered by oxidative stress generated by MeHg. The first parameter we considered was the anthropometric measurements associated with heart measurements. Our results showed that rats intoxicated with MeHg reduced body and heart weight compared to Control and CSAE. However, the heart and body weight ratio showed no statistical difference ($p > 0.05$. Table 2). The MeHg + CSAE group maintained measurements similar to those of the Control and EACS groups ($p > 0.05$. Table 2). In morphometric measurements, the MeHg group decreased the thickness of the left ventricular wall and interventricular septum, accompanied by

disorganization of cardiac fibers and a decrease in the diameter of cardiomyocytes to the control group and EACS ($p < 0.05$, Figure 1, A-E). CSAE treatment prevented MeHg-induced thinning of the left ventricular wall and interventricular septum. In addition, it improved structural disorganization of cardiac fibers and cardiomyocyte atrophy compared to the MeHg group ($p < 0.05$, Figure 1, A-E), maintaining measures similar to the Control and CSAE group ($p > 0.05$, Figure 1, A-E).

Table 2. Body weight, heart weight and heart/body weight ratio of the control, CSAE, MeHg and MeHg + CSAE experimental groups.

	Control	**CSAE**	**MeHg**	**MeHg + CSAE**
Body weight (g)	242 ± 8	239 ± 6	160 ± 8*	226 ± 7$^{\#}$
Heart weight (mg)	901 ± 27	930 ± 26	608 ± 40*	826 ± 31$^{\#}$
Heart Weight/Body Weight (mg/g)	3.7 ± 0.2	3.9 ± 0.2	3.7 ± 0.1	3.8 ± 0.1

CSAE (*Coriandrum sativum* Aqueous Extract). Values were expressed as mean ± SEM (n=10). * $p > 0.05$ compared to the control group and # * $p > 0.05$ compared to the MeHg group (two-way ANOVA followed by Tukey test).

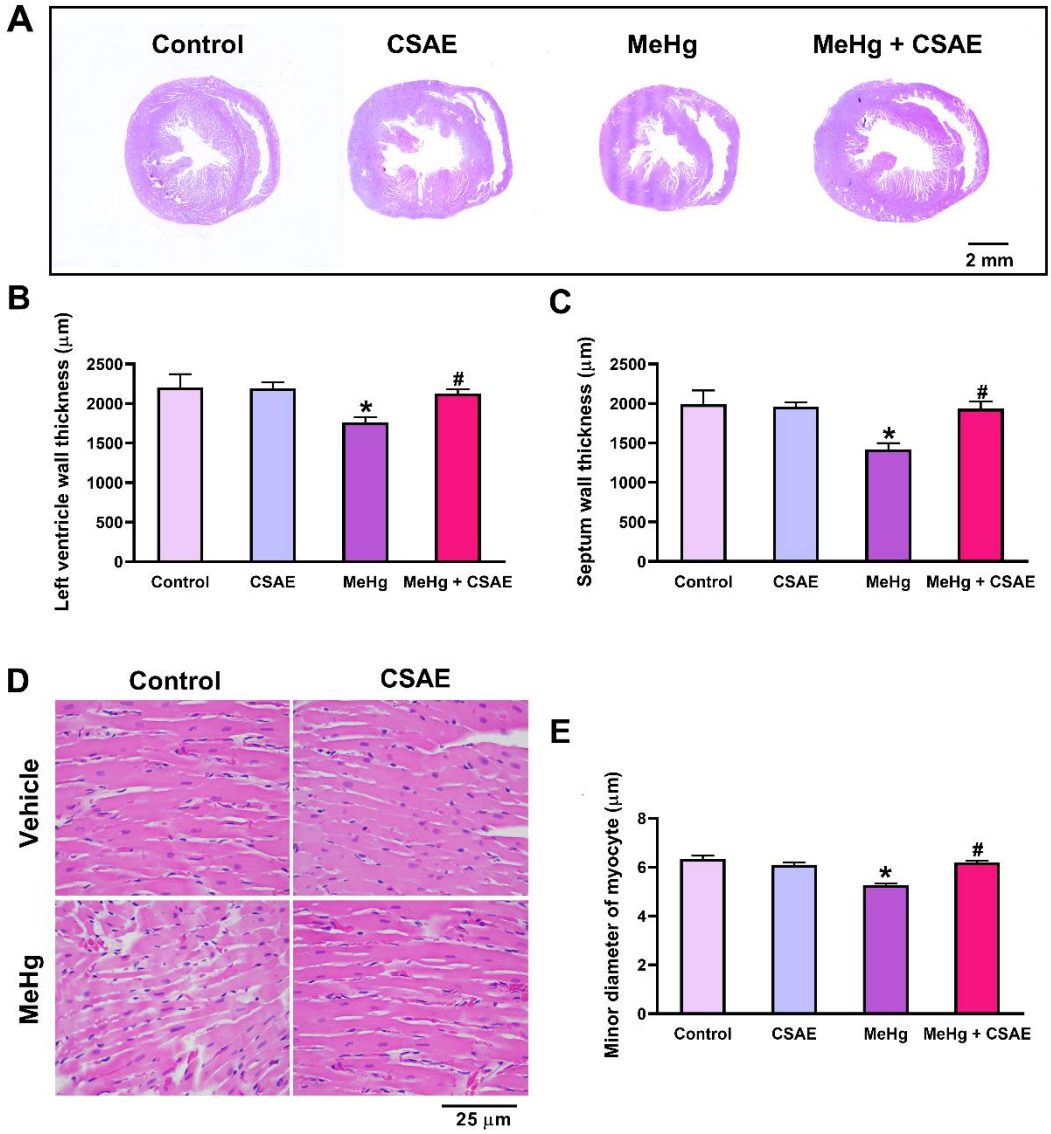

Figure 1: Treatment with CSAE prevents atrophy of cardiomyocytes and thinning of the left ventricular wall and interventricular septum in rats intoxicated with MeHg during pregnancy and lactation. **(A)** White light microscope photomicrograph of the different experimental groups of cardiac sections of rats during the gestational and lactational period stained with hematoxylin and eosin. **(B)** Quantification of left ventricular wall diameter. **(C)** Quantification of the diameter of the intraventricular septum. Values were expressed as mean ± SEM. (**D**) White light microscope photomicrograph of the different experimental groups of cardiac sections from rats stained with hematoxylin and eosin during the gestational and lactational period. CSAE treatment prevented MeHg-induced myocyte atrophy. (**E**) Quantification of myocyte diameter. * $p < 0.05$ compared to the control group; #, $p < 0.05$ compared to the MeHg group (two-way ANOVA followed by Tukey test).

3.3 Coriander prevented MeHg-induced heart fibrosis

We investigated whether MeHg poisoning would promote increased interstitial collagen content and whether Coriander could prevent this increase. Thus, our results demonstrated an increase in the interstitial collagen content in the left ventricle of rats poisoned with MeHg, indicative of fibrosis (3.7 ± 0.3 versus control: 2.0 ± 0.2), which was avoided by treatment with CSAE (2.7 ± 0.2) maintaining values similar to the Control and CSAE groups (control: 2.0 ± 0.2; CSAE: 2.2 ± 0.1, $p > 0.05$, Figure 2).

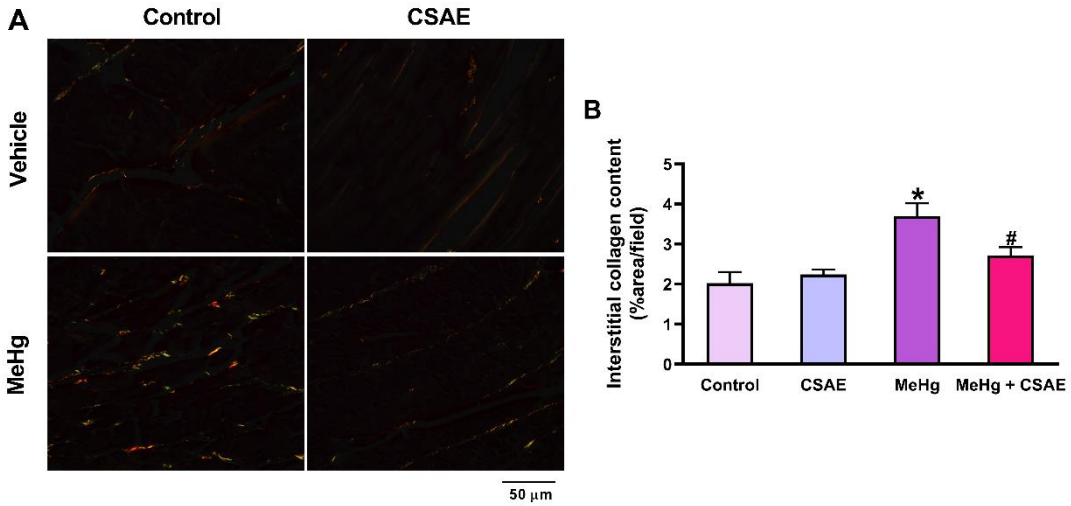

Figure 2: Treatment with CSAE prevents cardiac fibrosis in the left ventricle of rats intoxicated with MeHg during pregnancy and lactation. (**A**) Photomicrograph of the polarized light microscope of different experimental groups representing heart sections of rats during the gestational and lactational period stained with picrosirius red. (**B**) Quantification of interstitial collagen content. Values were expressed as mean ± SEM. * $p < 0.05$ compared to the control group; #, $p < 0.05$ compared MeHg (two-way ANOVA followed by Tukey test).

3.4 Coriander prevented MeHg-induced MMP-2 activation in the heart

Considering that MeHg and Coriander can modulate the activity of MMPs and that these proteases are involved in processes of cardiomyocyte injury and cardiac fibrosis, we decided to evaluate the activity of MMP-2 in the heart after intoxication with MeHg and treatment with Coriander, as well as evaluating whether the CSAE can modulate MMP-2 activity. Our results showed that rats

poisoned with MeHg showed increased MMP-2 activity of 75 kDa, 72 kDa and 64 kDa in the heart compared to Control and EACS animals ($p < 0.05$, Figure 3). Interestingly, the MeHg + CSAE group decreased 64 kDa MMP-2 activity compared to the MeHg group ($p < 0.05$, Figure 3), showing no differences in 75 kDa and 72 kDa MMP-2 activity compared to the MeHg group ($p > 0.05$, Figure 3). Furthermore, there were no differences in MMP-2 activity between the control and CSAE groups ($p > 0.05$, Figure 3). On the other hand, the results of EACS activity on MMP-2 demonstrated a reduction in MMP-2 activity of 48% at the 20 µg/mL concentration and 100% at the 200 µg/mL concentration ($p < 0.05$, Figure 3, E-F).

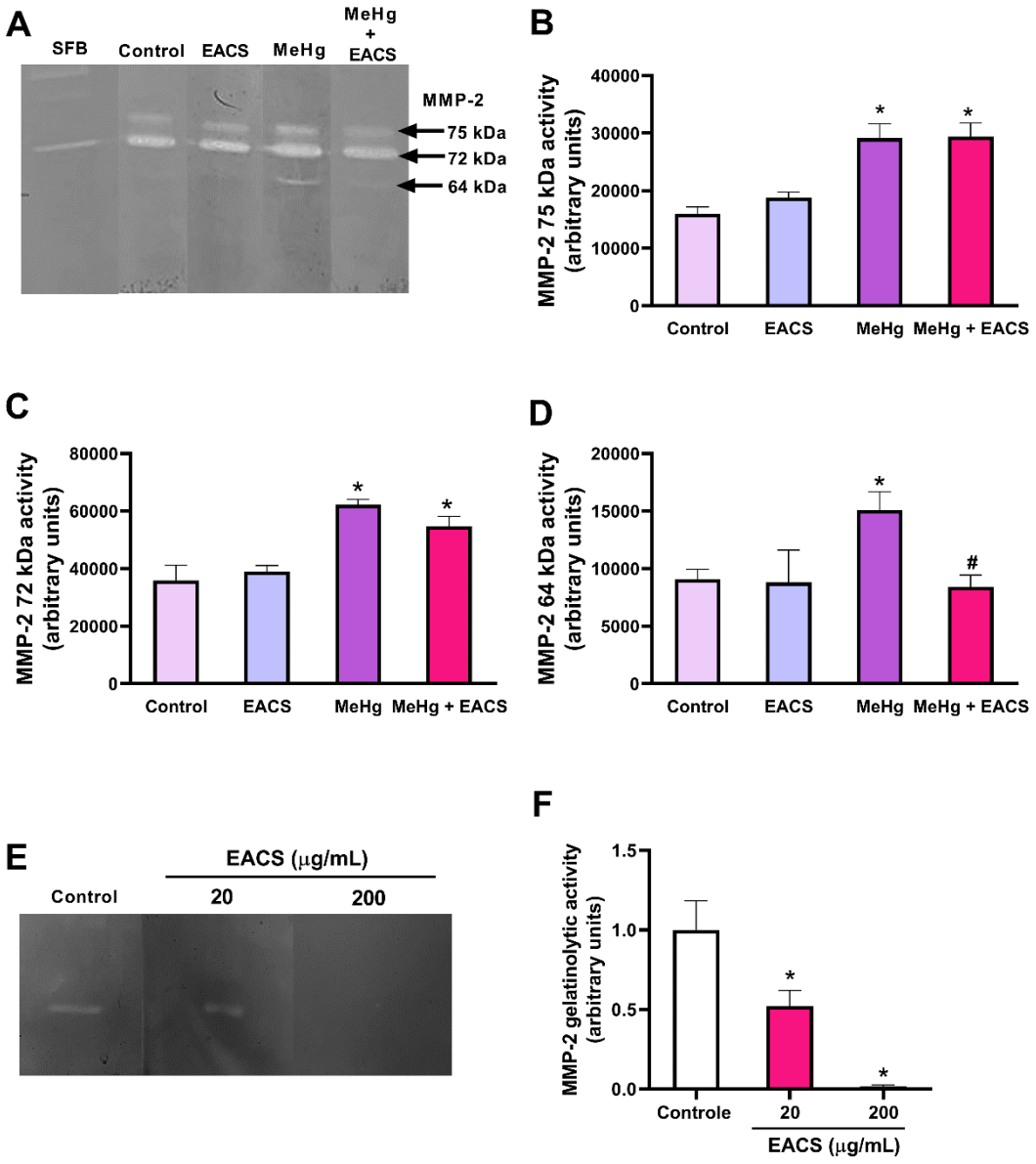

Figure 3: Treatment with CSAE prevents MeHg-induced increases in MMP-2 activity in the left ventricle of rats intoxicated with MeHg during pregnancy and lactation. **(A)** Representative gelatin zymogram of left ventricle extracts (**B**) Quantification of MMP-2 75 kDa activity **(C)** Quantification of MMP-2 72 kDa activity **(D)** Quantification of MMP-2 64 kDa activity **(E)** Representative gelatin zymogram in absence or presence of EACS (**F**) Quantification of MMP-2 activity in absence and presence of EACS. Values were expressed as mean ± SEM (n=10). * $p > 0.05$ compared to the control group and # * $p > 0.05$ compared to the MeHg group (two-way ANOVA followed by Tukey test).

3.5 Coriander prevents changes in cardiac electrical conduction induced by MeHg

After identifying atrophy and fibrosis in the heart of rats poisoned with MeHg, Coriander was able to protect against such alterations. Next, we decided to evaluate cardiac electrical conduction to assess possible changes triggered by

oxidative stress generated by MeHg, and whether Coriander could minimize these potential changes. In our results, we found that rats poisoned with MeHg showed a reduction in heart rate (306 ± 10 versus 434 ± 16 control, $p < 0.05$), an increase in the PR interval (63 ± 3 ms versus 46 ± 1 ms) and an increase in the Qtc interval (85 ± 1 ms versus 62 ± 1 ms). The MeHg + EACS group had heart rates within normal limits (HR:416 ± 8 bpm, $p < 0.05$) and prevented changes PR interval (35 ± 1, $p < 0.05$) and QTc interval (63 ± 1 ms, $p < 0.05$), when compared with the MeHg group. There were no electrocardiographic differences between the control and EACS groups (Table 3, $p > 0.05$).

Table 3. Electrocardiographic parameters of rats with their respective treatments.

	Control	CSAE	MeHg	MeHg + CSAE
Heart rate (bpm)	434 ± 16	428 ± 14	306 ± 10*	416 ± 8#
PR (ms)	46 ± 1	44 ± 1	63 ± 3*	47 ± 1#
QRS (ms)	34 ± 2	35 ± 2	47 ± 1*	35 ± 1#
QT (ms)	63 ± 1	64 ± 1	78 ± 1*	64 ± 1#
QTc (ms)	62 ± 1	63 ± 1	85 ± 1*	63 ± 1#

bpm (beats per minute), ms (milliseconds). Values were expressed as mean ± SEM (n=8). * $p > 0.05$ compared to the control group and # * $p > 0.05$ compared to the MeHg group (two-way ANOVA followed by Tukey test).

4. Discussion

This study was a pioneer in showing the effects of MeHg on the remodeling and alteration of electrical conduction in the heart in an animal model during the gestational and lactation period. In addition, it is the first to demonstrate the cardioprotective effect of coriander against mercury poisoning in a model of pregnancy and lactation. Several studies have shown evidence of MeHg cardiotoxicity [11, 14, 26, 43-46], including at non-neurotoxic doses in animal and human models [43]. Here, we show that oral coriander supplementation in rats during pregnancy and breastfeeding prevented the morphological and electrocardiographic changes induced by MeHg by maintaining the redox state and modulating MMP-2 activity in the heart.

Mercury poisoning is a global health problem affecting both developed and developing countries. Ingestion of contaminated fish is humans' most frequent

source of MeHg contamination [4, 47, 48]. Pregnant women are encouraged to eat fish because it is rich in nutrients and low in fat. However, fish consumption during pregnancy and lactation exposes the mother and her offspring to MeHg poisoning [49]. Population studies demonstrate a correlation between high mercury levels in the body and increased blood pressure, cardiac arrhythmia and increased risk of myocardial infarction [14, 50-55]. Although the mechanisms involved in the cardiotoxicity of mercury are not fully understood, evidence shows that MeHg can change the redox state by depleting GSH, inactivating thiol enzymes, SOD, catalase, glutathione peroxidase and by increasing the production of reactive species [56-58]. In line with previous studies, our results showed a redox imbalance in the heart of rats intoxicated with MeHg, due to increased nitrite levels, decreased GSH levels and SOD and CAT activity, resulting in lipid peroxidation.

The use of supplementation during the gestational period is frequent, and consuming foods that contain antioxidant bioactive compounds can be an adjuvant therapeutic strategy [59-61]. In our research, treating female rats with coriander prevented cardiac redox imbalance by maintaining the antioxidant system. Antioxidant compounds have been described in coriander [28]. Rodrigues et al. showed that the aqueous extract of coriander leaves has polyphenols, anthocyanins, and flavonoids with antioxidant potential similar to vitamin C [32]. Coriander also demonstrated antioxidant and cardioprotective effects in an isoproterenol-induced infarction model [62].

Our results showed that the intoxication of rats with 40 µg/mL of MeHg for 21 days induced body and heart weight loss. These results align with other studies demonstrating that high MeHg doses reduce body weight [43, 63]. However, when we evaluated the ratio between heart weight and body weight, the rats poisoned with MeHg maintained values similar to the other groups, suggesting that the decrease in heart weight is related to the reduction in body weight. However, it was clear that MeHg could promote cardiac remodeling, generating atrophy by decreasing the myocyte diameter, the thickness of the left ventricular wall, and the interventricular septum, accompanied by the disorganization of cardiac fibers. Interestingly, Nishimura et al. observed MeHg dose-dependent cardiac morphology and function changes. In their research, intoxication with 100 µg/mL (neurotoxic dose) alters body weight. Still, it seems

to have little effect on the morphology and function of the left ventricle, despite an increase in response to stress and apoptosis. While the dose of 10 µg/mL does not change body weight but induces hypertrophy and cardiac dysfunction [43]. We emphasize that this study used male rats as a model, and our research used females during pregnancy and lactation, presenting a completely different physiology. Because the period used in our study, which corresponds to the last gestational trimester and the lactation period, represents a critical moment for the cardiovascular system, resulting in changes in the oxidative state due to the increase in the heart's workload due to hemodynamic changes [25, 30].

Changes in the redox state observed in the heart were accompanied by histopathological changes, evidenced by disorganization and disruption of cardiac fibers, cardiomyocyte atrophy and interstitial fibrosis. These results align with previous findings, where disorganization and disruption of atrial myofibrils and papillary muscle were observed in isolated hearts incubated with MeHg [44]. In addition, Nishimura et al., 2019 also found interstitial fibrosis and increased expression of pro-fibrotic genes in MeHg-intoxicated rat Hearts [43].

MeHg-triggered cardiac fibrosis induction mechanisms still need to be fully understood. However, here, we demonstrate increased MMP-2 activity in the heart of MeHg-intoxicated rats. MMP-2 is one of the main MMPs involved in cardiac remodeling and dysfunction under pathological conditions [64]. MMP-2 can be expressed in cardiomyocytes and cardiac fibroblasts, and when expressed, it is secreted into the extracellular medium in the inactive 72 kDa isoform. MeHg can activate MMP-2 by disrupting the sulfhydryl bond between the cysteine present in the propeptide and the zinc in the catalytic site, generating an active MMP-2 of 72 kDa, which subsequently removes the propeptide by autolysis, generating an MMP-2. 2 of 64 kDa active [19, 20]. Increased MMP-2 activity may activate pro-fibrotic pathways by increasing TGF-β1 expression and activity [65-69]. Studies with MMP-2 inhibitors and MMP-2 gene knockout tools show improvements in cardiac fibrosis [68, 70, 71]. Our study was pioneering in demonstrating an increase in MMP-2 in the heart of rats intoxicated with MeHg, suggesting it as a possible mechanism associated with atrophy and fibrosis presented by our model. Coriander prevented these events from occurring in the face of MeHg poisoning. Thus, we investigated whether coriander would have direct inhibitory activity on MMP-2. Our result confirmed the modulation of MMP-

2 by coriander, corroborating with previous findings that showed that coriander was able to negatively modulate MMPs and reduce ROS in a murine model subjected to type B ultraviolet radiation (UVB), known to be responsible for premature aging [30]. Increased plasma MMP-2 and MMP-9 activity were observed in a sample population from the Amazon region, which showed elevated mercury levels due to a diet rich in contaminated fish [22]. A positive correlation was demonstrated between mercury levels in the MMP-2/TIMP-2 ratio, increasing the gelatinolytic activity, which is related as a possible mechanism of cardiovascular toxicity of MeHg. It has even been shown that polymorphisms in the MMP-2 gene are associated with changes in gelatinolytic activity in mercury poisoning [72].

Collagen deposition in the myocardium is considered a poor prognosis in cardiomyopathies and alters cardiac electrical conduction leading to arrhythmias [73-77]. We observed that rats poisoned with MeHg had fibrosis associated with reduced heart rate, followed by first-degree atrioventricular block (PR interval alteration) and ventricular conduction disturbance (increase in QTc interval). In accordance with our results, previous findings also observed an increase in the QTc interval and prolongation of the T interval in rats intoxicated with 3 mg/kg MeHg for 28 days [78]. Furthermore, a clinical study also found an association between increased levels of mercury in the body and heart rate variability[51] . Our study demonstrated that coriander treatment improved electrical conduction in the heart of rats poisoned with MeHg. A possible explanation for this would be the change in the phenotype of fibroblasts generated by MeHg, which are the most abundant population of cells in the heart and are closely related to cardiomyocytes, contributing to the process of electrical conduction and myocardial contraction [79, 80]. Another explanation would be altering mitochondrial function and calcium and potassium channels [78]. Since this change was not evaluated in our study, being a limitation presented by our research, the findings presented in our study are of great relevance for understanding the cardiac repercussions of MeHg poisoning in pregnant and lactating women as well as using coriander as a possible adjuvant therapeutic tool that can be used against xenobiotic poisoning.

In conclusion, supplementation with coriander in rats during the gestational and lactational period has cardioprotective effects by preventing morphological

and electrocardiographic changes induced by MeHg by improving oxidative stress and MMP-2 activity in the heart.

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5. CONCLUSÕES INTEGRADORAS

Em nossa revisão abordamos a influência do aumento da atividade e expressão de MMP-2 sobre a fisiopatologia de doenças que afetam o coração, e que a redução de sua atividade, a partir do uso de inibidores contribui para minimizar processo de remodelação e alterações de função. Somado a isso, verificamos que a exposição ao MeHg promoveu aumento da atividade de MMP-2 no coração de ratas durante o período gestacional e locacional, associado a remodelamento ventricular, alterações na condução cardíaca e estado redox, e que a suplementação, a partir, do coentro bloqueou os efeitos deletérios provocados pelo MeHg, impedindo os distúrbios de condução cardíaca, remodelamento e modulando MMP-2, mantendo o estado redox.

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7. COMPROVANTE DE SUBMISSÃO/ACEITE DE ARTIGO CIENTÍFICO

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x, 64 f. : il. color.

Orientador(a): Prof. Dr. Alejandro Ferraz do Prado
Coorientação: Profª. Dra. Keuri Eleutério Rodrigues
Dissertação (Mestrado) - Universidade Federal do Pará, Instituto de Ciências Biológicas, Programa de Pós-graduação em Farmacologia e Bioquímica, Belém, 2023.

1. Metilmercúrio. 2. Coriandrum. 3. Coração. I. Título.

CDD 615.92566309811
