**UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"**

**Instituto de Biociências – Câmpus de Botucatu**

**Programa de Pós-graduação em Farmacologia e Biotecnologia**

# Efeitos do sildenafil na hipertensão arterial induzida pela intoxicação com chumbo em ratos

**Ediléia de Souza Paula Caetano**

**Botucatu -SP**

**2022**

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# Efeitos do sildenafil na hipertensão arterial induzida pela intoxicação com chumbo em ratos

Tese de Doutorado apresentada ao Instituto de Biociências, Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus de Botucatu, para obtenção do título de Doutora em Farmacologia e Biotecnologia.

**Orientador:**
Prof. Dr. Carlos Alan Candido Dias Junior

# Ficha catalográfica

FICHA CATALOGRÁFICA ELABORADA PELA SEÇÃO TÉC. AQUIS. TRATAMENTO DA INFORM.
DIVISÃO TÉCNICA DE BIBLIOTECA E DOCUMENTAÇÃO - CÂMPUS DE BOTUCATU - UNESP
BIBLIOTECÁRIA RESPONSÁVEL: ROSANGELA APARECIDA LOBO-CRB 8/7500

Caetano, Edileia de Souza Paula.
Efeitos do sildenafil na hipertensão arterial induzida pela intoxicação com chumbo em ratos / Edileia de Souza Paula Caetano. - Botucatu, 2022

Tese (doutorado) - Universidade Estadual Paulista "Júlio de Mesquita Filho", Instituto de Biociências de Botucatu
Orientador: Carlos Alan Candido Dias Junior
Coorientador: Gabriela Palma Zochio
Capes: 21004005

1. Chumbo. 2. Citrato de sildenafila. 3. Endotélio. 4. Hipertensão. 5. Óxido nítrico.

Palavras-chave: Chumbo; Citrato de sildenafil; Disfunção endotelial; Hipertensão; Oxido nítrico.

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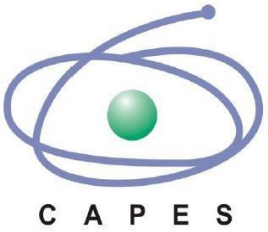
***Financiamento***




***(Bolsa capes de doutorado: Período de vigência 03/2019 – 08/2022)***


***Proc. 2018/24484-0***
***(Auxilio a pesquisa regular: Prof. Dr. Carlos Alan Candido Dias Junior)***

C


***Proc****. 305091/2021-2*
***(Auxilio a pesquisa : Prof. Dr. Carlos Alan Candido Dias Junior)***

# *Dedicatória*

*Aos meus pais,* ***Osvaldo e Terezinha***

*De quem Deus me deu a honra de ser filha, meus exemplos e meus maiores incentivadores*
*Meu amor e minha gratidão por vocês serão eternos.*

# *AGRADECIMENTOS*

... a **Deus**, por me guiar e estar sempre presente em minha vida,

... a minha amada avó **Ricarda** (in memoriam), por ter me incentivado e acreditado nos meus sonhos,

... a minha irmã **Edilaine**, por seu apoio e companheirismo,

... a meu eterno e fiel companheiro **Guilherme**, por sempre acreditar na minha capacidade, pelo incentivo, pela paciência e compreensão,

... as companhias de quatro patas: caninos **Jade** e **Big** e aos felinos **Pudim** e **Kobe Maria**, que me deram a honra de compartilhar suas jornadas e por todo amor e afago em todos esses anos,

... ao meu orientador **Prof. Dr. Carlos Alan Candido Dias Junior**, pela oportunidade, amizade, ensinamentos, carinho e o comprometimento que possibilitaram meu crescimento científico,

... ao **Prof. Dr. Erick José Ramo da Silva,** pela imensa colaboração durante todo o período do doutorado,

... a **Prof. Dra. Valéria Cristina Sandrim**, pela amizade e apoio durante todo operíodo do doutorado,

...aos integrantes e ex-integrantes do Laboratório de Farmacologia Cardiovascular, **Maria Luiza, Nara, Laísla, Filipi, Erick, Cristal, Carol Rosa, Zé, Regina, Letícia, Gabriela,** pela grande amizade, por compartilhar conhecimentos, sonhos e até frustrações nos experimentos. Desejo todo sucesso do mundo pra vocês. Podem contar comigo sempre!!

... aos amigos dos laboratórios vizinhos, **Sara, Priscila, Tainá, Mariane, Carol, André** e todos os outros pela amizade, compartilhamento de equipamentos, vidrarias, reagentes e experimentos realizados em conjunto.

...aos amigos do cafezinho, das compras no supermercado, dos churrasquinhos, da ciência e da vida, **Alan, Natalia Tamiris, Alexandre** e minha amada comadre **Noêmia**, vocês fazem a minha vida mais bonita.

... aos professores **Dra. Valéria Sandrim, Dra. Arielle Cristina Arena** e **Dr. Alaor aparecido Almeida**, pelas contribuições na banca de qualificação,

...a todos os técnicos e funcionários do departamento de farmacologia pelo auxilio profissional, **Paulão, Luiz, Cris, Janete, Giovana** e principalmente ao **Hélio** pela grande ajuda nas análises bioquímicas e na padronização das novas metodologias.

...a todos grandes amigos que fiz nesses 4 anos e meio morando em Botucatu,

...aos funcionários da Seção de Pós Graduação do IBB, pela disposição em resolver vários problemas e esclarecer nossas dúvidas,

...ao Instituto de Biociências de Botucatu e a todos professores do departamento de Farmacologia, por todo suporte dado para a realização desse trabalho,

...a CAPES, FAPESP e CNPq pelo auxílio financeiro.

***"Apenas uma flor entre os fios de grama entrelaçados"***

*(Guilherme Caetano)*

***"Não te desamparem a benignidade e a fidelidade; ata-as ao teu pescoço; escreve-as na tábua do teu coração.***
***E acharás graça e bom entendimento aos olhos de Deus e do homem."***

*(Provérbios 3:3,4)*

## *Prefácio*

A tese "Efeitos do sildenafil na hipertensão arterial induzida pela intoxicação com chumbo em ratos", foi dividida em três partes.

A primeira parte refere-se a uma introdução sobre as características do chumbo, a hipertensão arterial causada pela intoxicação com o chumbo, o estresse oxidativo e os tratamentos convencionais na intoxicação com chumbo. Além disso, teve o objetivo de descrever os principais aspectos farmacológicos do citrato de sildenafil como possíveis estratégias terapêuticas baseadas no estudo da via NO- GMPc.

A segunda parte inclui dados do artigo *"Low lead levels-induced hypertension and endothelial dysfunction in rats are attenuated by sildenafil: evidence of pleiotropic antioxidant effects"* onde se é possível observar os efeitos do sildenafil na hipertensão arterial induzida por baixos níveis de chumbo em que o efeito do sildenafil está associado à sua propriedade antioxidante e não diretamente a produção do NO.

A terceira parte apresenta uma breve discussão sobre os achados do artigo e a quinta parte descreve considerações finais da tese, concluindo os principais achados no trabalho desenvolvido durante o doutorado.

Durante a execução desse projeto, outras atividades foram desenvolvidas com o objetivo de enriquecer a formação acadêmica.

## Disciplinas cursadas

| Disciplinas | Ano | Conceito | Freq | Créditos | Carga Horária |
|---|---|---|---|---|---|
| Bases e Atualizações em Farmacologia e Biotecnologia | 1º / 2018 | A | 100% | 3 | 45 |
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| Metabolismo Energético | 1º / 2019 | A | 100% | 4 | 60 |
| Biologia Molecular | 2º / 2019 | A | 93,3% | 4 | 60 |
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| Exploração alternativa de carreiras na ciência | 1º / 2021 | A | 86,7% | 1 | 15 |
| Conceitos gerais em Estatística aplicada a modelos biológicos | 2º / 2021 | A | 100% | 2 | 30 |
| **Total de geral créditos** | | | | 38 | 570 |

## Artigos publicados

**-SOUZA-PAULA, E**. ; POLONIO, L. C.; ZOCHIO, G. P.; da SILVA, K. P.; KUSHIMA, HÉLIO; Dias-Junior, Carlos A. Anticontractile effect of perivascular adipose tissue but not of endothelium is enhanced by hydrogen sulfide stimulation in hypertensive pregnant rat aortae. JOURNAL OF CARDIOVASCULAR PHARMACOLOGY JCR, v. Publish Ahead of Print, p. press-press, 2020.

## Resumos em evento

21 Encontro Nacional de Biomedicina (ENBM), 2018, Botucatu. ENBM, 2018.

**SOUZA-PAULA, E.** ; CHIMINI, J. S. ; POLONIO, L. C. ; NASCIMENTO, R. A. ; Dias-Junior, C. A. . Sildenafil reduz a hipertensão em ratos intoxicados com chumbo por sete dias .

21 Encontro Nacional de Biomedicina (ENBM), 2018, Botucatu. ENBM, 2018.

POLONIO, L. C. ; Possomato-VIEIRA, J. S. ; CHIMINI, J. S. ; Dias-Junior CA ; **SOUZA-PAULA, E**. . Perda do efeito anti-contrátil mediada pelo sulfeto de hidrogênio derivado do tecido adiposo aórtico na hipertensão gestacional.

50th Brazilian Congress of Pharmacology and Experimental Therapeutics (SBFTE), 2018, Ribeirão Preto. SBFTE, 2018.

**SOUZA-PAULA, E**. ; CHIMINI, J. S. ; NASCIMENTO, R. A. ; DIAS-JUNIOR, CARLOS ALAN CANDIDO . Sildenafil reduces hypertension in seven-day lead-treated rats.

XVII Jornada de Toxicologia, 2019, Botucatu. XVII Jornada de Toxicologia, 2019.

**SOUZA-PAULA**, E. ; TOZZATO, G. P. ; POLONIO, L. C. ; Dias-Junior CA . Sildenafil e Atorvastatina reduz a hipertensão em ratos intoxicados com chumbo por sete dias.

XXIII Simposio brasileiro de fisiologia cardiovascular ,Araraquara 2019

**SOUZA-PAULA**, E. ; TOZZATO, G. P. ; POLONIO, L. C. ; Dias-Junior CA. Sildenafil reduz a hipertensão em ratos intoxicados com chumbo por sete dias.

22 Encontro Nacional de Biomedicina (ENBM), 2019, Botucatu, SP. 22 Encontro Nacional de Biomedicina (ENBM), 2019.

RIZZATI, O. ; **SOUZA-PAULA, E**. ; Dias-Junior, Carlos A. . Hipertensão associada ao diabetes compromete a função vascular em ratos.

51th Brazilian Congress of Pharmacology and Experimental Therapeutics (SBFTE), 2019, Maceio, AL.

POLONIO, L. C. ; ZOCHIO, G. P. ; **SOUZA-PAULA, E.** ; Dias-Junior, Carlos A. . Anticontractile effect of perivascular adipose tissue is enhanced by stimulated hydrogen sulfide formation and is ATP-sensitive potassium channel-dependent in hypertensive pregnant rats.

51th Brazilian Congress of Pharmacology and Experimental Therapeutics (SBFTE), 2019, Maceio, AL

**SOUZA-PAULA, E**. ; POLONIO, L. C. ; ZOCHIO-TOZZATO, G. P. ; Dias-Junior, Carlos A. . Atorvastatin and sildenafil improve seven-day lead exposed rats hypertension.

51th Brazilian Congress of Pharmacology and Experimental Therapeutics (SBFTE), 2019, Maceio, AL.

SOUZA, C. R. R. ; **SOUZA-PAULA, E.** ; Dias Junior CA . Isoflurane presents a trend in enhancing nitric oxide-dependent vasodilation response.

XXXII Congresso de Iniciação Científica da Unesp, 2020, Sao Paulo. CIC Unesp 2020, 2020.

FERREIRA, T. R. ; SOUZA, C. R. R. ; **SOUZA-PAULA, E.** ; Dias-Junior CA . Investigação em cultura de células endoteliais após a anestesia com isoflurano em ratas prenhes.

XXXII Congresso de Iniciação Científica da Unesp, 2020, Sao Paulo. CIC Unesp 2020, 2020.

SOUZA, C. R. R. ; **SOUZA-PAULA, E**. ; Dias-Junior CA . Avaliação dos parâmetros hemodinâmicos e vasculares após anestesia com isoflurano em ratas prenhes.

23 Encontro Nacional de Biomedicina (ENBM), 2020, Botucatu. ENBM-2020, 2020.

FERREIRA, T. R. ; SOUZA, C. R. R. ; VIANA-MATTIOLI, S. ; RIZZATI, O. ; MACEDO, E. E. P. ; ZOCHIO, G. P. ; **SOUZA-PAULA, E.** ; SANDRIM, V. C. ; Dias-Junior CA . Avaliação do estresse oxidativo e da formação de óxido nítrico em células endoteliais humanas incubadas com plasma de ratas após anestesia com isoflurano.

V Simpósio de Biologia Vascular, 2021, Online. Disfunção endotelial, 2021.

PEREIRA-MACEDO, E. E.; ZOCHIO, G. P. ; **PAULA, E. S.** ; DIAS-JUNIOR, C. A. . Evaluation of cardiovascular repercussions in first generation adult descending rats in an experimental model of gestational hypertension.

V Simpósio de Biologia Vascular, 2021, Virtual. Disfunção endotelial, 2021.

BOZONI, F. T.; PEREIRA-MACEDO, E. E.; **PAULA, E. S**. Evaluation of Cardiovascular repercussions in adult virgin and pregnant rats, descendants of first generation, of rats with experimentally induced gestational hypertension.

## Prêmios

**2021** Menção honrosa do trabalho com tema "Evaluation of cardiovascular repercussions in first generation adult descending rats in an experimental model of gestational hypertension, V Simposio de Biologia Vascular.

**2021** Prêmio de melhor trabalho com tema "Evaluation of cardiovascular repercussions in first generation adult descending rats in an experimental model of gestational hypertension, V Simposio de Biologia Vascular.

## Aulas na Graduação

-Estágio de docência realizado na disciplina de Farmacologia no 1º semestre de 2020

-Estágio de docência realizado na disciplina de Farmacologia no 2º semestre de 2021

# Lista de abreviaturas e siglas

ANOVA – Análise de Variância

BH4 – Tetrahidrobiopterina

CYP3A4 – Citocromo P450 3A4

eNOS – Óxido nítrico sintase endotelial

ROs / ERO – Espécies reativas de oxigênio

GCs – Guanilato ciclase solúvel

GMPc – Guanosina monofosfato cíclico

HUVECS – Células endoteliais da veia umbilical humana

Pb- Chumbo

SHAM- Controle submetido a gavagem e injeção

intraperitonial inertes

$O2^{-}$ – Superóxido

$ONOO^{-}$ - Peroxinitrito

PDE – Fosfodiesterase

SOD-Superoxido dismutase

FRAP- Capacidade antioxidante

NOx- Nitrato+Nitrito

SRAA – Sistema renina angiotensina aldosterona

TBARS – Substâncias reativas ao ácido tiobarbitúrico

iPDE5 – Inibidores da fosfodiesterase 5

L-NAME - N(G)-Nitro-L-arginine methyl ester

MTT - Brometo de [3-(4,5-dimetiltiazol-2yl)-2,5-difenil tetrazolium]

NADPH - Fosfato de dinucleótido de nicotinamida e adenina

NO – Óxido nítrico

$NO^{-2}$ – Nitrito

$NO^{-3}$ – Nitrato

NOS – Óxido nítrico sintase

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## Sumário

## Resumo

O chumbo é um poluente ambiental capaz de causar distúrbios cardiovasculares como a hipertensão arterial. Esse fenômeno pode ser explicado pelo aumento da formação de espécies reativas de oxigênio (ERO), redução da biodisponibilidade do óxido nítrico (NO) ou aumento da reatividade vascular a agentes constritores. O sildenafil é um inibidor da fosfodiesterase atualmente utilizado para o tratamento da disfunção erétil. Além disso, inovou os tratamentos de crises hipertensivas, hipertensão pulmonar e isquemia miocárdica por inibição da fosfodiesterase 5 (PDE5). Essa inibição promove o acúmulo de monofosfato cíclico de guanosina (GMPc). A produção de GMPc resulta da ativação da guanilato ciclase solúvel pelo NO produzido nas células endoteliais. Esses mecanismos induzem o relaxamento do músculo liso como resultado da diminuição dos níveis intracelulares de cálcio. Para avaliarmos os efeitos do sildenafil na hipertensão induzida por chumbo em ratos, ratos Wistar aproximadamente 90 dias de vida cujo peso corporal variava entre 250 e 400g, foram distribuídos em três grupos experimentais: chumbo+Sildenafil (Pb+Sildenafil), chumbo (Pb) e controle (Sham). Os animais dos grupos Pb+Sildenafil e Pb receberam por via intraperitoneal (i.p) acetato de chumbo 8 µg/100g de peso corporal/dia no primeiro dia do protocolo. A intoxicação foi mantida com acetato de chumbo 0,1 µg/100g de peso corporal/dia por sete dias subsequentes. O grupo Pb+Sildenafil e Sildenafil recebeu 15 mg/kg/dia por gavagem. O grupo Sham recebeu ainda acetato de sódio i.p. nas mesmas concentrações de acetato de chumbo em relação aos outros grupos e água por gavagem 0,5 ml/kg/dia. A pressão arterial sistólica (PAS) foi mensurada por pletismografia de cauda diariamente até o oitavo dia, quando os animais foram mortos por exsanguinação. O sangue foi coletado para determinação das concentrações de chumbo no sangue total realizado por espectrometria de absorção atômica, O plasma dos ratos foi armazenado para dosagens dos níveis plasmáticos de nitrito+nitrato, capacidade antioxidante (FRAP), peroxidação lipídica (TBARS) e atividade da superóxido dismutase (SOD). Além disso, o plasma dos ratos foi incubado com células endoteliais de cordão umbilical humano (HUVECs ) para avaliar a produção de NO endotelial, de nitrito+nitrato e a formação de ERO intracelular. O Sildenafil preveniu a hipertensão induzida por baixas concentrações de chumbo e reestabeleceu vasodilatação endotélio-NO-dependente, reduziu a formação de ERO, aumentou a atividade da superóxido dismutase (SOD) e melhorou os níveis dos metabólitos de NO. No entanto, não foram encontradas alterações na detecção direta de NO intra-HUVECs, mostrando que os efeitos fornecidos pelo sildenafil na hipertensão induzida por chumbo em ratos está relacionado as propriedades antioxidantes desta droga que protegem contra a inativação do NO mediada por ERO, prevenindo assim a hipertensão e a disfunção endotelial causadas pela intoxicação por chumbo.

**Palavras-chaves: Hipertensão, citrato de sildenafil, oxido nítrico, chumbo, disfunção endotelial.**

## Abstract

Lead is an environmental pollutant capable of causing cardiovascular disorders such as high blood pressure. This phenomenon can be explained by the increased formation of reactive oxygen species (ROS), reduced nitric oxide (NO) bioavailability and increased vascular reactivity to constricting agents. Sildenafil is a phosphodiesterase inhibitor currently used for the treatment of erectile dysfunction. In addition, it innovated the treatment of hypertensive crises, pulmonary hypertension and myocardial ischemia by inhibiting phosphodiesterase 5 (PDE5). This inhibition promotes the accumulation of cyclic guanosine monophosphate (cGMP). The production of cGMP results from the activation of soluble guanylate cyclase by NO produced in endothelial cells. These mechanisms induce smooth muscle relaxation as a result of decreased intracellular calcium levels. To evaluate the effects of sildenafil on lead-induced hypertension in rats, male Wistar rats approximately 90 days of life whose body weight ranged from 250 at 400g were divided into three experimental groups: Lead+Sildenafil (Pb+Sildenafil), lead (Pb) and control (Sham). The animals in the Pb+Sildenafil and Pb groups received intraperitoneally (i.p) lead acetate 8µg/100g body weight/day on the first day of the protocol. Intoxication was maintained with lead acetate 0.1µg/100g body weight/day for seven subsequent days. The Pb+Sildenafil and Sildenafil group received 15mg/kg/day by gavage. The Sham group also received sodium acetate i.p. in the same concentrations of lead acetate in the other groups and water by gavage 0.5 ml/Kg/day. Systolic blood pressure (SBP) was measured by tail cuff plethysmography every day until the eighth day, when the animals were killed by exsanguination. Blood was collected for the determination of lead concentrations in whole blood performed by atomic absorption spectrometry. The plasma of the rats was stored for measurements of plasma levels of nitrite+nitrate, antioxidant capacity (FRAP), lipid peroxidation (TBARS), superoxide dismutase (SOD). In addition, plasma from rats was incubated with human umbilical cord endothelial cells (HUVECS) to assess endothelial NO, nitrite+nitrate production and intracellular ROS formation. Sildenafil prevented hypertension induced by low lead levels and impaired NO-dependent vasodilation, reduced ROS formation, increased superoxide dismutase (SOD) activity, and improved levels of NO metabolites. However, no changes were found in the direct detection of NO in HUVECs, showing that the effects provided by sildenafil on lead-induced hypertension in rats is related to the antioxidant properties of this drug that protect against ROS-mediated NO inactivation, thus preventing hypertension and endothelial dysfunction caused by lead intoxication.

**Keywords: Hypertension, sildenafil citrate, nitric oxide, lead, endothelium disfunsion.**

# 1-Introdução

## 1.1 Chumbo

O chumbo é um metal pesado, de massa atômica 207,2, sólido, maleável e com ponto de fusão de 327 °C, bastante baixo para um metal, facilitando seu uso industrial disseminado e sua manipulação em setores de baixa tecnologia. Pode apresentar-se na forma de sais inorgânicos, compreendendo uma extensa gama de óxidos utilizados como pigmentos (PbO; $PbO_2$; Pb304; Pb203), carbonato de Pb, sulfato, cromatos, arsenato, cloreto e silicato. Os compostos orgânicos de importância industrial, incluem o chumbo tetrametila e o chumbo tetraetila, o acetado de chumbo, o ftalato de chumbo, o salicilato de chumbo, etc. Cerca de 40% do chumbo é utilizado na forma de metal, 25% como ligas com outros metais e 35% como compostos na indústria química (TARCHER, 1992).

O chumbo é relativamente bem absorvido pela via inalatória, dependendo do tamanho das partículas inaladas, podendo haver taxas de absorção que vão de 25-30% a 60%. Pela pele, somente os compostos orgânicos de chumbo são absorvidos de forma importante. A absorção pelo trato gastrointestinal é de cerca de 10% no adulto, sendo bem maior na criança, podendo atingir até 50% (PIOMELLI *et al.*, 1984).

O chumbo absorvido é distribuído a partir do plasma, onde encontramos apenas 1% do chumbo do sangue total, os outros 99% estando ligados aos eritrócitos. Atravessa bem as membranas celulares, distribuindo-se rapidamente para tecidos moles, onde podemos encontrar as maiores concentrações no fígado e nos rins, atravessa barreira hematoencefálica e placenta com certa facilidade, e grande proporção do chumbo absorvido incorpora-se ao esqueleto (cerca de 90% da carga corpórea) onde se deposita na forma de fosfato insolúvel (BARRY, 1975).

Baixos níveis de chumbo que varia entre 8 e 35 µg/dl no sangue têm sido associados a distúrbios cardiovasculares, incluindo hipertensão, disfunção endotelial, disfunção erétil e

prejuízo da função túbulo intersticial renal (GONÇALVES-RIZZI *et al.*, 2016; NASCIMENTO *et al.*, 2015; POSSOMATO-VIEIRA *et al.*, 2018). No Brasil, a NR-7 (Portaria n.º 24, de 29/12/94), estabelece os Valores de Referência (VR), isto é, os níveis máximos de chumbo em pessoas não ocupacional mente expostas e os Índices Biológicos Máximos Permitidos (IBMP) em trabalhadores expostos; para a presença de chumbo no sangue os valores são de 40 µg/dl para pessoas não expostas ocupacional mente e de 60 µg/dl para trabalhadores ocupacionais mente expostos ao metal (SCHIFER; JUNIOR; MONTANO, 2005).

Apesar de a terapia de quelação estar disponível para reduzir os níveis de chumbo no sangue total, a remoção total de chumbo do sangue e de outros tecidos ainda é um desafio, colocando assim a intoxicação ou exposição ao chumbo em risco elevado de consequências adversas para a saúde.

### 1.2 Ação do chumbo na hipertensão

Parece que o chumbo afeta o sistema cardiovascular principalmente por interagir com o sistema renina-angiotensina, além disso, parece provocar o desbalanço entre substâncias vasodilatadoras e vasoconstritoras nas artérias e arteríolas através do prejuízo da função do endotélio vascular, podendo também alterar a elasticidade dos vasos sanguíneos através de interações com as proteínas de matriz extracelular (GONÇALVES-RIZZI *et al.*, 2016; NASCIMENTO *et al.*, 2015; SIMÕES *et al.*, 2011).

### 1.3 Estresse oxidativo relacionado ao chumbo

O estresse oxidativo relacionado ao chumbo pode desempenhar um papel, pelo menos em parte, na patogênese da hipertensão induzida pela toxicidade do chumbo (AHAMED; SIDDIQUI, 2007), devido à inativação do NO (oxido nítrico) através da interação do mesmo com as espécies reativas de oxigênio (ERO) levando a formação de $ONOO^-$ (peroxinitrito) (VAZIRI, 2008). Assim, aumentos na pressão arterial com reduções concomitantes na

biodisponibilidade de NO têm sido associados ao estresse oxidativo induzido pelo chumbo, relacionado a maior vasoconstrição, resposta vaso relaxante endotélio-dependente prejudicada e hipertensão induzida pelo chumbo (GRIZZO; CORDELLINI, 2008; MARQUES *et al.*, 2001).

Parece que o chumbo afeta o sistema cardiovascular principalmente por interagir com o sistema renina-angiotensina, além disso, parece provocar o desbalanço entre substâncias vasodilatadoras e vasoconstritoras nas artérias e arteríolas através do prejuízo da função do endotélio vascular, podendo também alterar a elasticidade dos vasos sanguíneos através de interações com as proteínas de matriz extracelular (GONÇALVES-RIZZI *et al.*, 2016; NASCIMENTO *et al.*, 2015; SIMÕES *et al.*, 2011).

**1.4 Tratamentos na intoxicação por chumbo**

As drogas indicadas para o tratamento da intoxicação pelo chumbo são os medicamentos quelantes, que tem a capacidade química de ligar-se aos elementos metálicos em geral, de preferência formando complexos estáveis e excretáveis pela urina, as drogas indicadas e que apresentam bons resultados são o ácido etileriodiaminotetracético cálcico dissódico (EDTACaNa2), o ácido dimercaptopropanol (BAL ou dimercaprol), o ácido dimercaptosuccínico (DIVISA), e a penicilamina (Cuprimine). O tratamento é realizado em ciclos de 5 dias consecutivos, com intervalos de dez a quinze dias entre ciclos (PIOMELLI *et al.*, 1984; PORRU; ALESSIO, 1996).

No entanto, essas drogas exercem pouco ou nenhum efeito sobre o estresse oxidativo relacionado ao chumbo, embora existam potenciais candidatos para mitigar os danos relacionados ao chumbo causados pelo estresse oxidativo, efeitos limitados para atenuar as elevações da pressão arterial e, concomitantemente, preservar a sinalização do NO foram encontrados com a administração desses candidatos em modelos de hipertensão induzida por chumbo (DING; VAZIRI; GONICK, 1998; MALVEZZI *et al.*, 2001; MARQUES *et al.*, 2001),

faz se então necessário a utilização de uma droga que exerça efeito efetivo sobre a hipertensão arterial, como também efeito sobre o estresse oxidativo, para haver uma melhor resposta terapêutica a farmacoterapia combinada com os agentes quelantes.

### 1.5 Sildenafil como uma alternativa para o tratamento de disfunção vascular

Em busca do tratamento para a hipertensão e diminuição dos danos do estresse oxidativo causados por baixos níveis de chumbo, um agente alternativo, o sildenafil, revelou efeitos protetores contra a hipertensão em humanos, apresentando vasodilatação seguida de diminuição da pressão arterial sistólica, possivelmente relacionado ao NO (DISHY *et al.*, 2001; MAHMUD; HENNESSY; FEELY, 2001). Estudos recentes mostraram que o tratamento com sildenafil também protege contra o estresse oxidativo, inibindo a formação de ROS em voluntários homens saudáveis e em ratos (LAXMI *et al.*, 2019; PERK *et al.*, 2008). É importante ressaltar que em ratos intoxicados com acetato de chumbo foi encontrado estresse oxidativo relacionado ao chumbo caracterizado por aumentos nos níveis de MDA (malonaldeído) e reduções nos metabólitos do NO (nitrito/nitrato) mas nenhum resultado relacionado à formação direta do NO foi relatado em tratamentos com sildenafil (ABDOLLAHI *et al.*, 2003; SENBEL; HELMY, 2013). Esses achados dão suporte a hipótese de que o sildenafil pode fornecer efeitos protetores nos distúrbios cardiovasculares relacionados ao chumbo, particularmente nos distúrbios envolvendo comprometimento do NO.

O Sildenafil foi o primeiro inibidor seletivo de fosdiesterase5 (iPDE5) aprovado para o tratamento da disfunção erétil em 1998. É um potente inibidor de PDE5, com alta seletividade (> 1000 vezes) para PDE5 em relação à PDE2, PDE3 e PDE4 e seletividade moderada (>80 vezes) sobre PDE1. O sildenafil, no entanto, é aproximadamente 10 vezes apenas mais potente para PDE5 do que para PDE6, encontrada nos fotorreceptores da retina humana. (GUPTA; KOVAR; MEIBOHM, 2005).

## 1.6 Mecanismo de ação dos iPDEs

A ativação do sGC pôr NO, que leva ao acúmulo de $GMP_C$, requer a presença de um grupo prostético heme. O heme de sGC é um grupo de anéis de cinco membros contendo átomos de nitrogênio em torno de um átomo de ferro, que pode ser encontrado na forma reduzida (Fe2+) ou na forma oxidada ($Fe^3$+). Os mecanismos propostos para relaxamento mediado por cGMP incluem: (1) Via NO-cGMP dependente de endotélio inibição da geração de inositol-1,4,5-trifosfato; (2) aumento da extrusão citoplasmática de $Ca^2$+; (3) desfosforilação da quinase de cadeia leve de miosina; (4) inibição do influxo de $Ca^2$+; (5) ativação da proteína quinase; (6) estimulação da mebrana $Ca^2$+ ATPase; e (7) abertura dos canais de potássio. A remoção do grupo heme ou sua oxidação leva a uma forma da enzima insensível ao NO (KHALIL, 2016).

Há 11 tipos de enzimas fosfodiesterase (PDEs), todas com função de degradação de monofosfato de adenosina cíclico (AMPc) para monofosfato de adenosina (AMP), e, de GMPc para monofosfato de guanosina (GMP). As PDEs estão amplamente distribuídas no organismo, com atividade variável em diferentes tecidos. A PDE5 é encontrada na musculatura lisa do corpo cavernoso, no músculo esquelético, no músculo liso visceral e vascular, nos tecidos cerebelar e pancreático, nas plaquetas, nos rins e nos pulmões. A PDE1 é encontrada no coração, a PDE6 na retina e a PDE11 no músculo esquelético (H. P RANG,2018; KHALIL, 2016).

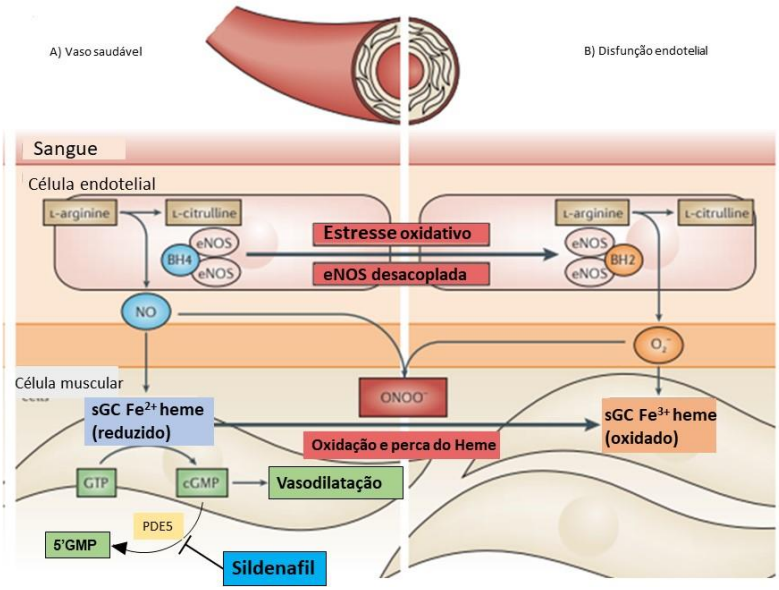


**Figura 1** | Sinalização vascular do óxido nítrico na saúde e na doença e a ação do sildenafil. A) Em um vaso sanguíneo saudável, o óxido nítrico (NO) e l-citrulina são gerados a partir de l-arginina pela sintase endotelial de óxido nítrico (eNOS) e cofatores (por exemplo,tetrahidrobiopterina (BH4)) no endotélio em resposta ao estresse de cisalhamento e outros estímulos. O NO se difunde para o células musculares lisas subjacentes e se liga ao heme reduzido ($Fe^{2+}$) na guanilil ciclase solúvel (sGC), que ativa a enzima. Isso gera monofosfato de guanosina cíclico (cGMP), levando à vasodilatação.B) disfunção endotelial é uma característica de várias doenças cardiovasculares. Um evento central nesse processo é a reduzida formação e biodisponibilidade do NO, que é causada pelo estresse oxidativo e desacoplamento da eNOS. Quando o cofator BH4 é oxidado à dihidrobiopterina (BH2), a eNOS pode se desacoplar e, em vez de produzir NO, gera superóxido ($O2^{-}$),que por sua vez pode reagir com NO para gerar peroxinitrito ($ONOO^{-}$), limitando ainda mais a biodisponibilidade de NO. Além disso, espécies reativas de oxigênio e nitrogênio podem oxidar o grupo heme em sGC (de $Fe^{2+}$ para $Fe^{3+}$), tornando-o insensível à ativação pelo NO ( Reproduzido e adaptado deLUNDBERG; GLADWIN; WEITZBERG, 2015) .

## 1.7 Farmacocinética do sildenafil

O sildenafil é relativamente lipofílico com uma base fraca central na amina terciária piperazina (pKa = 6,5), resultando em ionização apenas parcial em pH fisiológico. Após administração oral (doses entre 25- 100 mg), o sildenafil é rapidamente absorvido, atingindo o pico de concentração plasmática no intervalo de 30-120 minutos e tempo de meia vida (*t1/2*) de eliminação é cerca de 3 – 5 horas (GUPTA; KOVAR; MEIBOHM, 2005; H. P RANG, 2018).

A biodisponibilidade oral média do sildenafil é aproximadamente entre 38% a 41%. O sildenafil é extensamente metabolizado, não sendo detectado nenhum traço de droga inalterado na urina ou nas fezes, os metabólitos são predominantemente excretados nas fezes entre73% e 88% e em menor extensão na urina entre 6% e 15% (GUPTA; KOVAR; MEIBOHM, 2005).

O sildenafil é metabolizado principalmente pela isoenzima CYP3A4 do citocromo P-450 (CYP) e por um menor grau CYP2C9. As vias metabólicas para sildenafil são bastante complexas, com 16 metabólitos diferentes sendo isolados. As principais vias de metabolismo são N-desmetilação, oxidação e hidroxilação (H. P RANG, 2018) .

Os efeitos adversos do sildenafil estão relacionados a baixa seletividade em relação a PDE6, sendo a causa de anormalidades da visão de cores observadas com altas doses de sildenafil, podem ser observados ainda efeitos adversos relacionados a vasodilatação de outros leitos vasculares; esses efeitos incluem hipotensão, rubor, cefaleia e congestão nasal (H. P RANG, 2018).

# *2- Capítulo 1*

# Low lead levels-induced hypertension and endothelial dysfunction in rats are attenuated by sildenafil: evidence of pleiotropic antioxidant effects

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## Highlights

Lead intoxication for 7 days increases blood pressure in rats.

Lead-induced hypertension is attenuated by sildenafil.

Sildenafil prevents ROS formation in lead-induced hypertension.

Sildenafil protects against endothelial dysfunction caused by lead.

Oxidative stress and NO imbalance caused by lead is reversed by sildenafil treatment.

Low lead levels-induced intoxication may be a cardiovascular risk factor.

**Abstract**

Sildenafil, the first available drug that revolutionized erectile dysfunction treatment, has been shown nitric oxide (NO)-independent pleiotropic effects. Lead intoxication reduces NO bioavailability and increases reactive oxygen species (ROS); however, mechanisms involved are unclear. Therefore, we examined the sildenafil effects in the oxidative stress, reductions of NO, hypertension and endothelial dysfunction caused by lead intoxication. Rats were distributed into three groups: Pb (lead-intoxicated rats), Pb+Sildenafil (lead-intoxicated rats and treated with sildenafil) and Sham (non-lead-intoxicated rats). ROS levels, NO metabolites and NO levels in human umbilical vein endothelial cells (HUVECs) were also evaluated. Sildenafil prevents hypertension and impaired NO-dependent vasodilation, reduces ROS formation, increases superoxide dismutase (SOD) activity in plasma and enhances NO metabolites in plasma and in HUVECs supernatants, while no changes were found in direct intra-HUVECs NO detection. In conclusion, pleiotropic effects of sildenafil may involve antioxidant properties that protect against ROS-mediated NO inactivation, thus preventing hypertension and endothelial dysfunction caused by lead intoxication.

**Keywords:** sildenafil; lead-induced hypertension; oxidative stress; nitric oxide; rats.

## 1. Introduction

Low lead levels have been associated with cardiovascular disturbs including hypertension (Gambelunghe et al., 2016; Simões et al., 2011; Zheutlin et al., 2018),

endothelial dysfunction (Khalil-Manesh et al., 1993), erectile dysfunction (Senbel and Helmy, 2013), and impaired renal tubulointerstitial function (Roncal et al., 2007). Despite chelation therapy is available to reduce whole blood lead levels (ACMT AACT, 2015; Goyer et al., 1995), total removal of lead from blood and other tissues is still a challenge (Saxena and Flora, 2004), thus placing intoxication or exposure to lead at significant elevated risk for adverse consequences for health (Lamas et al., 2016; Vaziri and Khan, 2007). Hence, studies have been carried out to assess the relationship between hypertension and oxidative stress caused by low levels of circulating lead in the blood (Ahamed and Siddiqui, 2007; Roy and Kordas, 2016).

Previous studies have examined lipid peroxidation of cell membranes, measuring the concentrations of final product of oxidative stress, the malondialdehyde (MDA) levels (Cabral et al., 2012; Roy and Kordas, 2016), and, using probes for detecting reactive oxygen species (ROS) generation (Wu et al., 2018). Furthermore, investigators have also assessed the cellular formation of nitric oxide (NO) and ROS in lead-treated endothelial cells (Vaziri and Ding, 2001; Wu et al., 2018). However, the underlying relationship among hypertension, endothelial dysfunction, impaired NO bioavailability, and oxidative stress caused by lead are still unclear.

Lead-related oxidative stress may play a role, at least in part, on the pathogenesis of lead toxicity-induced hypertension (Ahamed and Siddiqui, 2007) because of ROS-mediated inactivation of the NO, an important endothelium-derived relaxing factor (Vaziri, 2008). Accordingly, increases in arterial blood pressure with concomitant reductions in NO bioavailability have been associated with lead-induced oxidative stress, which were related to greater vasoconstriction, impaired vasorelaxation response endothelium-dependent and lead-induced hypertension (Grizzo and Cordellini, 2008; Marques et al., 2001). However, although there are

potential candidates to mitigate lead-related damages caused by oxidative stress, limited effects for attenuating the elevations in blood pressure and concomitantly preserving the NO signaling have been found with administration of these candidates in lead-induced hypertension models (Ding et al., 1998; Malvezzi et al., 2001; Marques et al., 2001).

In search of the treatment for hypertension and oxidative stress damages caused by low lead levels, an alternative agent, sildenafil, has already revealed protective effects against hypertension in humans, showing vasodilation followed by decreases in systolic blood pressure (Mahmud et al., 2001), possibly related to NO (Dishy et al., 2001). This lines up with reports that sildenafil treatment also protects against oxidative stress by inhibiting ROS formation in healthy men volunteers (Perk et al., 2008) and in male rats (Laxmi et al., 2019). Importantly, in lead acetate-intoxicated rats were found lead-related oxidative stress (Abdollahi et al., 2003; Senbel and Helmy, 2013), in which authors have found increases in MDA levels and reductions in metabolites of NO (nitrite/nitrate) but no result related to direct NO formation was reported to the treatments with sildenafil (Abdollahi et al., 2003; Senbel and Helmy, 2013). Together, these previous findings support the hypothesis that sildenafil may provide protective effects in lead-related cardiovascular disturbs, particularly the disorders involving NO impairment.

Therefore, we aimed to examine the sildenafil effects on hypertension and oxidative stress induced by low lead levels in rats. Furthermore, we assessed endothelial function in aorta segments, ROS and NO levels in endothelial cells incubated with plasma of rats that received lead acetate and treatment with (or without) sildenafil.

## 2.Methods

### 2.1. Animals and treatments

Wistar male rats (3 months of age) weighing between 250 ± 50g were distributed three experimental groups. Cages were used to place the animals in a room with a controlled temperature in a light-dark cycle (12 hours) and their access to water and food was free. For research purposes, care was guaranteed in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments. For animal experimentation, all procedures were forwarded to our Institutional Ethics Committee and were approved (Biosciences Institute of Botucatu, State University of Sao Paulo, protocol n° 1081/ 2018).

Rats of lead-intoxicated groups received intraperitoneal (i.p.) injections of 8 µg/100 g of lead acetate as first dose ($Pb(C_2H_3O_2)_2$, 100% purity, Merck, USA), and 0.1 µg/100 g once a day, and daily treatment with vehicle (water; Pb group) or sildenafil citrate (15 mg/kg/day; Pb+Sildenafil group) by gavage for 7 days (from the first to the seventh day). The Sham group received i.p. injections of 8 µg/100 g of sodium acetate ($Na(C_2H_3O_2) + 3H_2O$, 99% purity, J. T. Baker, Canada), and 0.1 µg/100g once a day and daily treatment with vehicle (water) by gavage for 7 days (from the first to the seventh day). Previously published studies were used for the lead acetate intoxication protocol (Dursun et al., 2005; Fiorim et al., 2011; Gonçalves-Rizzi et al., 2016; Possomato-Vieira et al., 2018). On the eighth day, 24 hours after each treatment, the animals were anesthetized between 2-4% and euthanized by exsanguination. Thoracotomy was performed, and the descending thoracic aorta was removed for vascular experiments. Tubes containing lyophilized heparin were used to collect whole

blood (Vacutainer BD, Oxford UK) through which it was possible to separate the plasma and access lead concentrations. Plasma was stored in the freezer at −80ºC until biochemical analyses and *in vitro* assays.

### 2.1.2. Tail-cuff plethysmography

Systolic blood pressure measurements were assessed by tail-cuff plethysmography method (catalog # EFF 306, Insight, Ribeirao Preto, SP, Brazil). Conscious animals were housed for 10 minutes in a heated box (Insight, Ribeirão Preto-SP, Brazil) in a quiet place, where they were subjected to several cycles of cuff inflation-deflation by a trained blind operator. The mean systolic blood pressure was obtained through 3 measurements and recorded from the first to the seventh day of the experimental protocol, as previously described in detail by other authors using rodents (Fiorim et al., 2011). One hour after each injection of lead acetate or sodium acetate, systolic blood pressure measurements were taken.

## 2.2. Assessment of whole blood lead concentrations

For the detection of lead concentrations in the blood, previously established protocols were used (Zhou et al., 2002). After seven days of intoxication, whole blood collected from different groups was measured by duplicate atomic absorption spectrometry performed in a graphite oven. (GF-AAS; Varian Spectrometer AA 220G, Agilent Technologies, Palo Alto, CA, USA). Initially the blood samples were diluted in the proportion of 1+49 containing a diluent solution with 0.5% (v/v) double distilled $HNO_3$, 25µg/L Rh and 0.005% (v/v) Triton X-100. For calibration it was performed against the matrix correspondence. The detection limit of the method was 0.5µg/L. Blood lead concentrations were expressed in µg/dL.

### 2.3. Vascular reactivity

Vascular segments of rat thoracic artery were cleaned carefully and placed in 10 ml of previously prepared Krebs-Henseleit solution) kept at pH 7.4 (Glucose 11.1; NaCl 130; $CaCl_2$ 1.6; KCl 4.7; $KH_2PO_4$ 1.2; $MgSO_4$ 1.2; $NaHCO_3$ 15, in mmol/L continuously bubbled with a mixture of 95% $O_2$ and 5% $CO_2$ at 37°C. These segments were connected between a hook seated at the bottom of the bath and another hook connected to the FORT10 Isometric Force Transducer connected to the Transbridge 4M Transducer Amplifier (WPI, Sarasota, FL, USA) connected to a PC-based MP100 system and analyzed offline using AcqKnowledge software version 3.5.7 (Biopac Systems Inc., Goleta, CA, USA). The aortic artery segments were stretched under 1.5g of basal tension and allowed to equilibrate for 45 min, after this the segments were tension-contraction stimulated with 96 mM KCl as determined by preliminary curves. When the tissue reached maximum contraction in response to KCl, washing with Krebs' solution was continued for 3 times within a period of 15 minutes each. Changes in aorta tension were recorded. Aortic rings were stimulated with increasing concentrations of phenylephrine Phe, $10^{-9}$ to $10^{-4}$ M. Seeking to investigate endothelial function, vascular tissues were pre-contracted with Phe at a concentration of $10^{-6}$ M followed by increasing concentrations of acetylcholine ACh, $10^{-9}$ to $10^{-4}$ M. And to investigate the endothelium-dependent NO participation in vascular relaxation, concentration-response curves to ACh were performed, obtained in the presence of of Nω-nitro-L-arginine methyl ester (L-NAME, $3 \times 10^{-4}$ M), added in the last 30 -minutes of the stabilization period (Chimini et al., 2019; Raffetto et al., 2019). Phe contraction-response curves were constructed and maximum responses were recorded. Phe-induced contraction curves were constructed to assess ACh-induced relaxation in the presence or absence of L-NAME and were expressed as the % relaxation to Phe-

induced contraction. Non-linear regression (variable slope) of the obtained concentration-effect curves revealed the maximal response ($R_{max}$) and negative logarithm of the concentration that evoked 50% of the maximal response ($pEC_{50}$), as previously described (Raffetto et al., 2019).

### 2.4. Determination of Plasmatic Nitrite/Nitrate (Total NO$x$)

Plasma concentrations of total NOx were obtained by the Griess reaction as previously determined (Possomato-Vieira et al., 2018). Briefly, the plasma of the animals was incubated with a solution of vanadium III chloride under agitation at 37°C for a period of 3 hours. After this incubation, the other reagents were added to read the absorbance (535nm) in a spectrophotometer (Synergy 4, BioTek,Winooski, VT) a standard curve of increasing nitrite concentrations was used for comparisons (1.56–100 $\mu$M). Total NOx levels in plasma were expressed in $\mu$mol/L.

### 2.5. Plasma antioxidant capacity

The ferric reducing ability of plasma (FRAP) method consist in reducing ferric-tripyridyl triazine (FeIII-TPTZ) by antioxidants of sample that forms the ferrous-tripyridyl triazine (FeII-TPTZa), a blue color substance (Benzie IF et al ;1996). The FRAP reagent was formulated using acetate buffer (300 mmol/L), TPTZ/HCl (10mmol/L) and ferric chloride (20mmol/L) solution. In a 96-well microplate, 10µL of plasma samples and 290µL of FRAP solution were added. Iron (II) sulfate solutions (range 0.0625–4 mmol) were added and the microplate was incubated for four minutes. The absorbance was read at 593nm by spectrophotometer (Synergy 4, BIOTEK, Winooski, VT). The data were expressed in µmol/L (Benzie and Strain, 1996).

### 2.6. Antioxidant enzyme activity

To measure the superoxide dismutase (SOD) activity, the inhibition of the reaction of the superoxide radical with pyrogallol by spectrophotometry at 420 nm was used as a basis. For its quantification, a unit of SOD activity (U) is used, which is defined as the quantification of the enzyme that was able to inhibit 50% of the autoxidation of pyrogallol, in this way these results were expressed in U/mg protein/minute (Marklund , 1985).

### 2.7. Cell culture

Endothelial cells (HUVECs), strain CRL 2873 (American Type Culture Collection, ATCC, Manassas, Virginia, USA), were cultured (at 37 °C) in 5% $CO_2$ in DMEM medium and supplemented with 10 % (v/v) fetal bovine serum (Gibco CA, USA) up to reach 70-80% confluence. After that, the HUVECs were replanted in 96-well microplates (Jet Biofil) and allowed to reach the necessary confluence, then culture medium was carefully withdrawn and the HUVECs were washed-out with phosphate-buffered saline to withdraw traces of serum bovine fetal. HUVECs were then incubated (at 37°C) for 24 h in 5% $CO_2$ with 10% (v/v) plasma of each group.

#### 2.7.1. Determination of Nitrite/Nitrate (Total NO$x$) in cells Culture Supernatant

NOx concentrations in HUVECs supernatants were measured in duplicate using Griess reagents (Rocha-Penha et al., 2017). In a 96-well microplate, 50$\mu$L of samples were incubated (in the dark for ten minutes) with 50$\mu$L of sulfanilamide (1%) and phosphoric acid (5%) solution. Then added 50 $\mu$L of N-(1-Naphthyl)-ethylenediamine dihydrochloride (0.1%) solution and followed by ten-minute incubation. Microplate was spectrophotometrically read at 540 nm (Synergy 4 spectrophotometer, BioTek,

Winooski, VT). Sodium nitrite (S2252, Sigma, St. Louis, MO, USA) solutions (0.46–29.5 $\mu$mol/L) were used to generate the standard curve and evaluate the readings. NOx measurements were expressed in $\mu$mol/L.

### 2.8. Presto Blue viability assay

To assess cell viability, Presto Blue Cell Viability Reagent (Invitrogen, Thermo Fisher Scientific, CA) was used. Briefly, this reagent is converted emitting fluorescence by reduction into resorufin by mitochondrial enzymes of metabolically active cells (Cinegaglia et al., 2020). In a 96-well dark side microplate, HUVECs were seeded (Synergy 4, BioTek, VT, USA) (~1×104/well) and incubated with treatments for 24 h. Then supernatant was withdrawn, and each well was washed-out once with phosphate-buffered saline, and, added 90µL of medium and 10µL of Presto Blue Cell Viability reagent. Microplate reader (Synergy 4 spectrophotometer, BioTek, VT, USA) was used to read the fluorescence. This method requires excitation at 560nm and emission at 590nm, as previously described (Viana-Mattioli et al., 2020).

### 2.9. Determination of oxidative stress in plasma and endothelial cells culture

#### 2.9.1. Determination of Lipid Peroxidation in plasma

Plasma lipid peroxide levels were determined by measuring thio-barbituric acid-reactive substances (TBARS), as previously described (Possomato-Vieira et al., 2018). Briefly, distilled water (100 µl), sodium dodecyl sulfate (SDS, 50 µl, 8.1%) and and thiobarbituric acid (TBA, 375 µl, 0.8%) were diluted in acetic acid (375 µl, 20%, pH 3.5) in test tubes and plasma sample (100 µl) was added. In a water bath, test tubes were incubated (at 95°C for one hour) and centrifuged at 1792 g (for ten minutes). In a 96-well microplate, aliquot (200 µl) of each sample was transferred. This

method uses malondialdehyde solution (of known concentration) to evaluate the malondialdehyde formed by sample, which resulted of colorimetric reaction with TBA, and that was read (at 532 nm) in a spectrophotometer (Synergy 4, BIOTEK, Winooski, VT). Lipid peroxide levels were expressed in malondialdehyde (nmol/mL).

### 2.9.2. Intracellular ROS assay

This method quantifies the fluorescence emission of alkoxyl, hydroxyl and peroxyl radicals, and, peroxynitrite and hydrogen peroxide, intracellularly released after 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA, Cayman Chemical) is added, as previously described (Wang and Joseph, 1999. In a dark side 96-well microplate, HUVECs (~1×104/well) were incubated (for 24 hour) with the treatments (Synergy 4, BioTek, VT, USA). Then supernatants were collected and HUVECs incubation (for 30 minutes) with DCFH-DA (25μM) diluted in phosphate-buffered saline was performed. Using microplate reader (Synergy 4, BioTek, VT, USA) with excitation at 502 nm and emission 523 nm, fluorescence was read.

## 2.10. Intracellular measurement of NO Level

To assess intracellular NO production, a fluorescent probe (2-(3,6-diacetyloxy-4,5-diamino-9H-xanten-9-yl)-benzoic acid, DAF-2 diacetate, Cayman, MI, USA) was used. A microplate reader (Synergy 4, BioTek, VT, USA) was used to measure (fivefold) the fluorescence intensity at 485–520 nm, as previously described (Cinegaglia et al., 2020).

## 2.11. Data analysis and statistics

The results are expressed as means ± SD. A Shapiro–Wilk test was applied to verify normality of data distribution, and comparisons between groups were assessed

by two-way analysis of variance (ANOVA) or one-way ANOVA followed by Tukey's test (GraphPad Prism® 8.0, San Diego, CA, USA). A probability value $P < 0.05$ was considered significant.

## 3.Results

### 3.1. Whole-blood lead levels

When we evaluated the levels of lead in the whole blood of rats that were not intoxicated (Sham group), they did not present detectable concentrations. However, these concentrations in the lead poisoned groups were 23.25±1.9 µg/dL in Pb group, and 21.50±3.3 µg/dL in Pb+Sildenafil group (both *p< 0.05, Figure 1), i.e., treatment with sildenafil was unable to produce any effect on lead toxicokinetics.

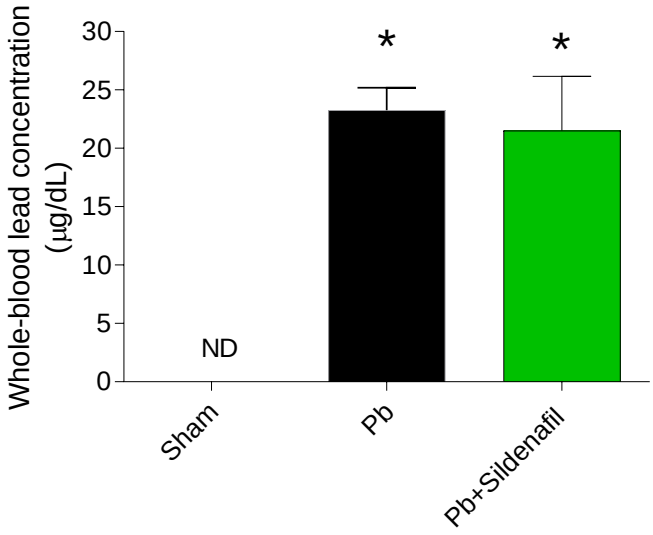


**Fig.1.** Whole-blood lead concentrations of Sham (non-lead-intoxicated group) and Pb or Pb+Sildenafil (lead-intoxicated groups for 7 days). Values represent mean ± SD Nondetectable levels (ND), n=8-10 per group. *P<0.05 versus Sham group.

### 3.2. A single daily dose of Sildenafil shows antihypertensive effects in groups receiving lead.

Systolic blood pressure values were similar in all experimental groups at baseline, and showed no significant changes in Sham group throughout the study period. However, significant increases in systolic blood pressure were observed on 6$^{th}$ and 7t$^{h}$ days of lead intoxication (Pb group, 151 ±3 and 153 ±3 mmHg, *$p$<0.05, Figure 2), while sildenafil was able to prevent the lead-induced increase in systolic blood pressure in the 6$^{th}$ and 7$^{th}$ days.

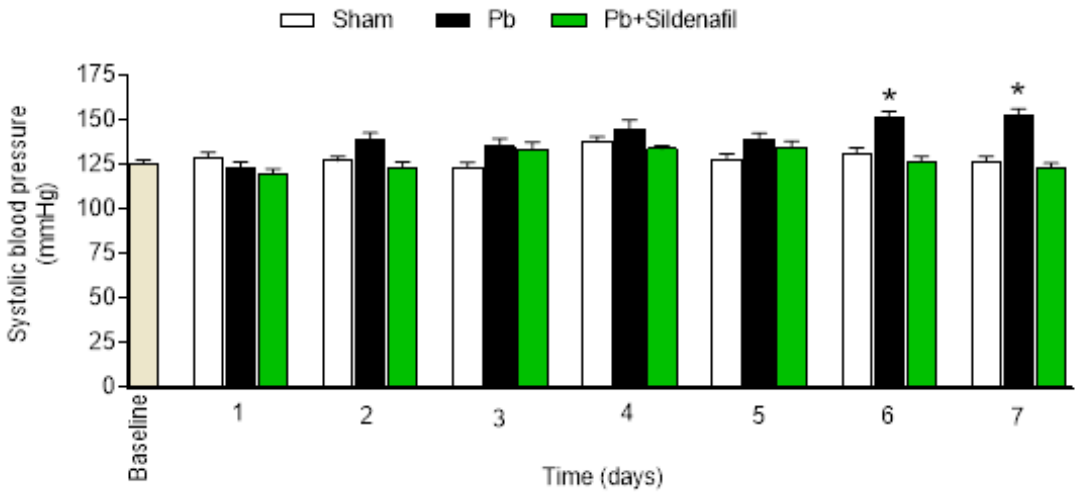


**Fig.2.** Systolic blood pressure of Sham (non-lead-intoxicated group) and Pb or Pb+Sildenafil (lead-intoxicated groups for 7 days). Values represent mean ± SD. n= 8-10. *P<0.05 versus Sham group.

### 3.3. Sildenafil prevents both decreases in NO metabolites and increases in lipid peroxidation in plasma of lead-intoxicated animals

Total NOx levels were increased in Pb+Sildenafil animals (35 ± 4µmol/L) compared with Pb group (26 ± 2 µmol/L, $^{\&\#}p< 0.05$; Figure 3B). In addition, plasma MDA levels were greater in the Pb group (56 ± 1 nmol/L) compared with Sham and Pb+Sildenafil animals (51 ± 1 and 49± 4 nmol/L, respectively, $^{\&\#}p< 0.05$; Figure 3A).

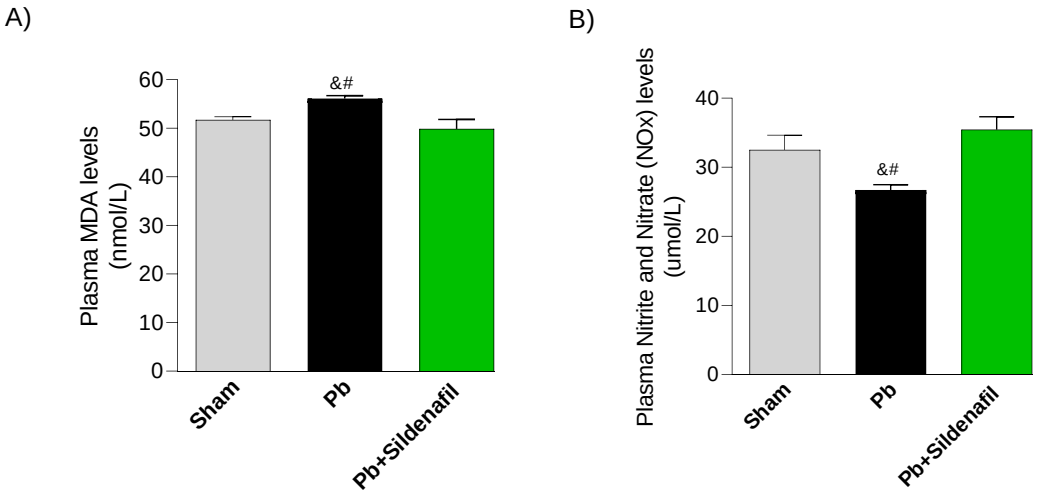


**Fig.3.**

Biochemical markers of NO and oxidative stress in plasma. Sham (non-lead-intoxicated group) and Pb or Pb+Sildenafil (lead-intoxicated groups for 7 days). MDA levels (A) NOx levels (B). Values represent mean ± SD (n=8-10). $^{\&}P < 0.05$ versus Pb+Sildenafil group. $^{\#}P < 0.05$ versus Sham group.

### 3.4. Sildenafil induces antioxidants effects

Decreases in plasma antioxidant capacity were found in Pb animals (614± 115 µmol/L) compared with Sham group (1932 ± 431 µmol/L, $^{\#}p< 0.05$; Figure 4).

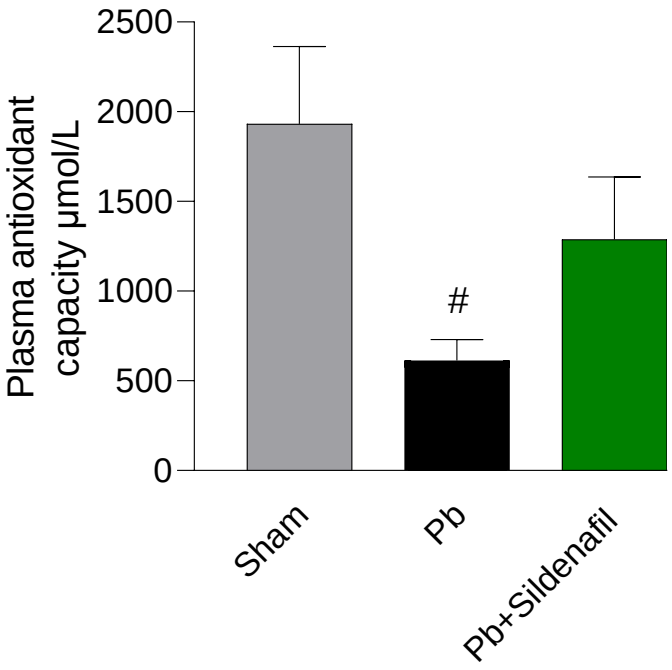


**Fig.4.** Plasma antioxidant capacity of Sham (non-lead-intoxicated group) and Pb or Pb+Sildenafil (lead-intoxicated groups for 7 days). Values represent mean ± SEM (n= 8-10). $^{\#}P < 0.05$ versus Sham group**.**

In addition, SOD activity was greater in the Pb+Sildenafil group (0,06 ± 0,005 U/mg protein/minute) compared with Pb animals (0,05± 0,003 U/mg protein/minute, *$p$< 0.05; Figure 5).

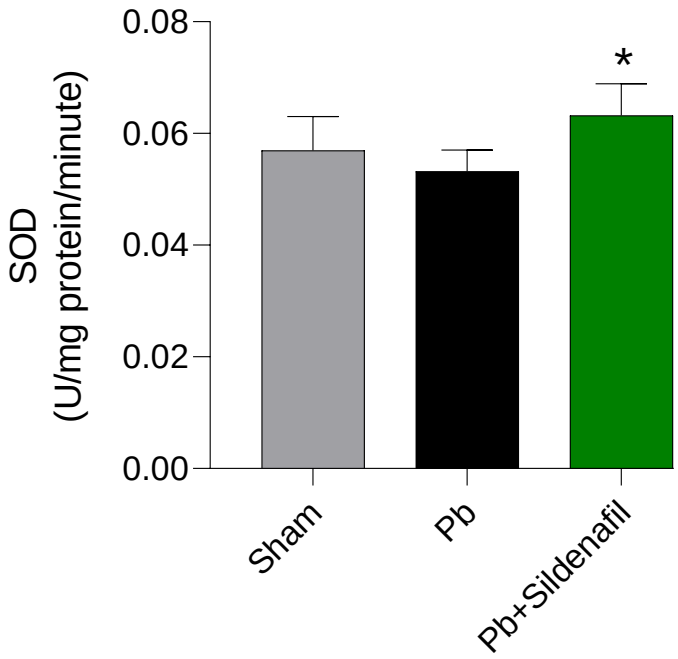


**Fig.5.** Superoxide dismutase activity of Sham (non-lead-intoxicated group) and Pb or Pb+Sildenafil (lead-intoxicated groups for 7 days). Values represent mean ± SD (n= 8-10). $^{*}P < 0.05$ versus Pb group.

**3.5 Sildenafil was able to prevent endothelium-dependent acetylcholine-induced vasodilation and decreased phenylephrine hyperreactivity in the rat model of lead-induced hypertension.**

To evaluate the vascular effects in the different groups, reactivity experiments were performed by which we directly evaluate the responses between the three different groups of animals. The relaxing responses provoked by ACh (which triggers the release of NO by endothelial cells) were tested in rings with the endothelium intact (+E) and pre-contracted with Phe. Differences were observed in $R_{max}$ and $pEC_{50}$ for ACh-induced relaxation in Pb compared to Pb+Sildenafil and Sham groups ($^{\&\#}p<0.05$,

Figure 6A and Table 1). Blockade of NO synthase (NOS) using L-NAME inhibited the ACh-induced relaxation in all groups (Figure 6B).

Regarding the maximum response $R_{max}$ to Phe-induced contraction among the three experimental groups, no differences were observed in aortic rings with preserved endothelium (+E) (Figure 6C and Table 1). Also, no differences in maximum response $R_{max}$ to Phe-induced contraction were observed between Pb and Sham group in endothelium denuded aortic rings; however, reductions in $R_{max}$ were reached in Pb+Sildenafil versus Pb and Sham groups ($^{\#}$*$p<0.05$, Figure 6D and Table 1). Differences in $pEC_{50}$ by Phe-induced contraction were observed in Pb compared with Pb+Sildenafil and Sham groups in aortic rings with (+E) endothelium and in Sham compared with Pb+Sildenafil and Pb groups endothelium denuded (-E) an aortic rings ($^{\&\#}p<0.05$, Table 1).

KCl-induced contractions were not different among the experimental groups in endothelium intact (+E) or denuded (-E) aortic rings (Figures 6E and 6F).

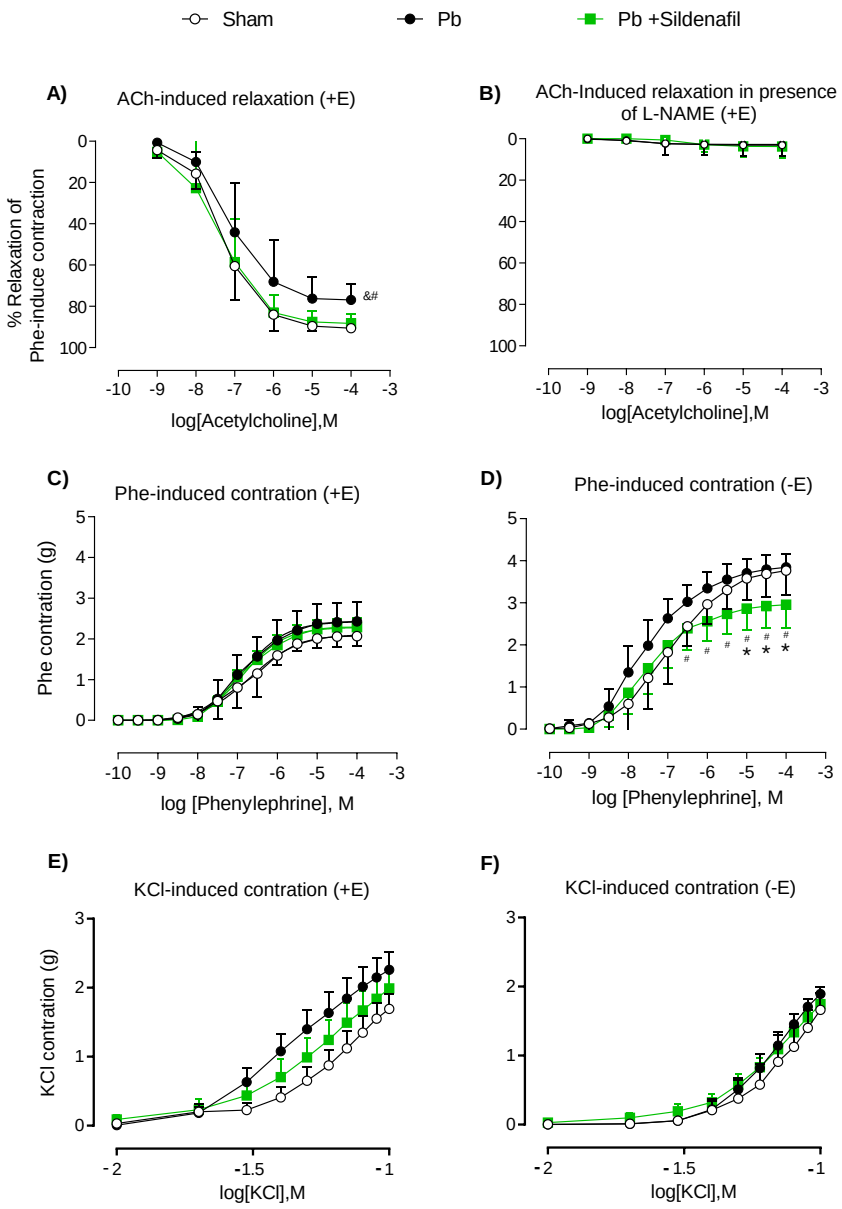


**Fig.6.** Vascular reactivity of thoracic aorta rings with (+E) or without (-E) endothelium of Sham (non-lead-intoxicated group) and Pb or Pb+Sildenafil (lead-intoxicated groups for 7 days). Acetylcholine (ACh)-induced relaxation (A), ACh-induced relaxation in presence of L-NAME (B), Phenylephrine (Phe)-induced contraction in aorta +E (C) and aorta –E (D), Potassium chloride (KCl)-induced contraction in aorta +E (E) and aorta –E (F). Values represent mean ± SD (n= 8-10). $^{\#}P < 0.05$ versus Sham group. *P < 0.05 versus Pb group. $^{\&}P < 0.05$ versus Pb+Sildenafil group.

**Table 1.** Maximal response ($R_{max}$) and negative logarithm of the concentration that evoked 50% of the maximal response ($pEC_{50}$) for acetylcholine (ACh) and phenylephrine (Phe) in thoracic aorta rings, with (+E) or without endothelium (-E) of Sham (non-lead-intoxicated group) and Pb or Pb+Sildenafil (lead-intoxicated groups for 7 days). Data represents mean ± SD (n= 8-10). $^{\#}P < 0.05$ versus Sham group. *P < 0.05 versus Pb group. $^{\&}P < 0.05$ versus Pb+Sildenafil group.

| | **Sham** | **Pb** | **Pb+Sildenafil** |
|---|---|---|---|
| **ACh $R_{max}$ (%)** | | | |
| **+E** | 90.8 ± 1 | 76.8± 7.5$^{\&\#}$ | 86.8±3.9 |
| **ACh $pEC_{50}$ (-log M)** | | | |
| **+E** | 7.2± 0.3 | 7.7 ± 0.2$^{\#}$ | 7.4 ± 0.1 |
| **Phe $R_{max}$ (g)** | | | |
| **+E** | 2.1 ± 0.2 | 2.4 ± 0.4 | 2.3 ± 0.2 |
| **-E** | 4.0 ± 0.2 | 3.9 ± 0.3 | 2.9 ± 0.4$^{\#}$ |
| **Phe $pEC_{50}$ (-log M)** | | | |
| **+E** | 6.9± 0.4 | 6.6 ± 0.1$^{\&\#}$ | 6.8 ± 0.1 |
| **-E** | 6.9 ± 0.3$^{\&*}$ | 7.5 ± 0.3 | 7.5 ± 0.3 |

### 3.6 Protective effects of sildenafil in HUVECs viability, NO metabolites and ROS production

HUVECs culture experiments showed increases in viability after incubation with plasma of Sham and Pb+Sildenafil groups compared with Pb group (38 ± 9, 22± 10 and 0.5 ±9.9 % of viability, respectively, $^{\&\#}p< 0.05$; Figure 7).

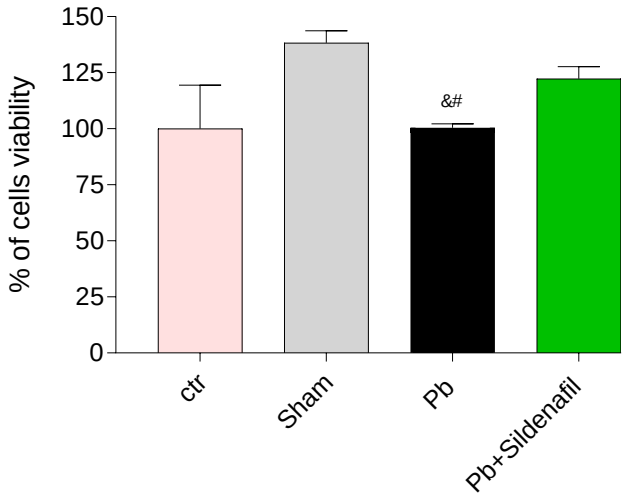


**Fig.7.** Cell viability of Sham (non-lead-intoxicated group) and Pb or Pb+Sildenafil (lead-intoxicated groups for 7 days). Values represent mean ± SD (n= 8-10). $^{\&}P < 0.05$ versus Pb+Sildenafil group. $^{\#}P < 0.05$ versus Sham group**.**

NOx from cells supernatant after plasma incubation was increased in Pb+Sildenafil group (263± 26µmol/L) compared with the Pb and Sham groups (216 ± 4 and 207 ± 11 µmol/L, respectively, $^{*}p< 0.05$; Figure 8B).

Increases in ROS levels were observed in HUVECs incubated with plasma of Pb group, while incubation of HUVECs with plasma of Sham group or Pb+Sildenafil group showed lower ROS levels compared with Pb group ($^{\&\#}p< 0.05$; Figure 8A).

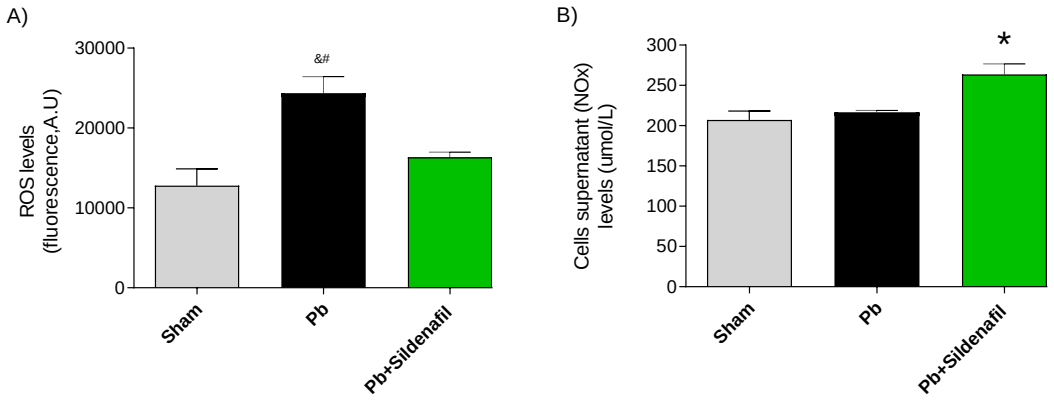


**Fig.8.**

Intracellular levels of reactive oxygen species (ROS, A) and Nitrite + Nitrate levels in the cell supernatant (B) of human umbilical vein endothelial cells incubated for 24 hours with 5% (v/v) plasma samples of Sham (non-lead- intoxicated group) and Pb or Pb+Sildenafil (lead- intoxicated groups for 7 days). Values represent mean ± SD (n= 8-10). *P < 0.05 versus Pb group. $^{\&}$P < 0.05 versus Pb+Sildenafil group. $^{\#}$P < 0.05 versus Sham group.

### 3.7 Decreased NO levels in HUVECs cells incubated with plasma of lead-intoxicated animals

Increases in NO fluorescence signal were found in HUVECs incubated with plasma of Sham but not of Pb and Pb+Sildenafil groups (*$p$< 0.05; Figure 9).

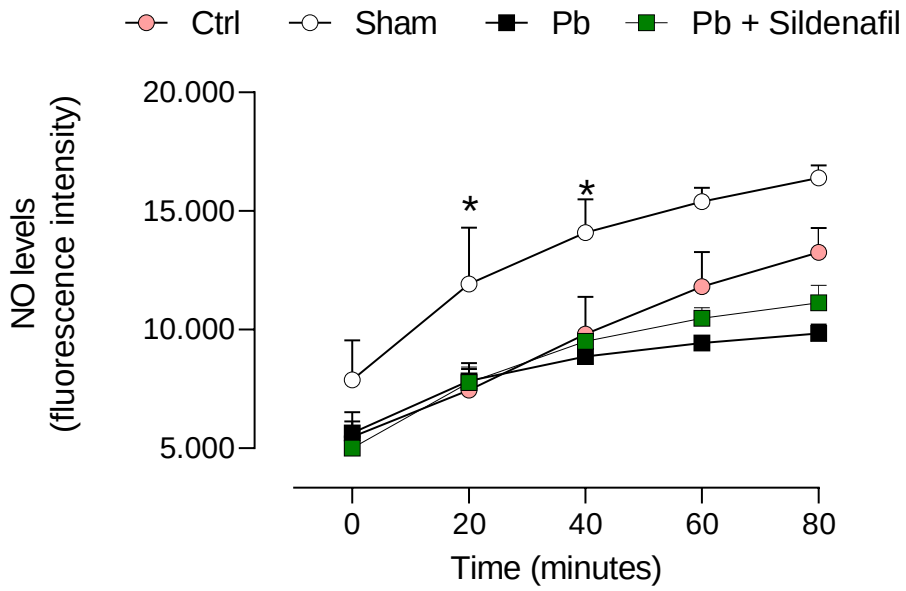


**Fig.9.** Direct quantification of intracellular NO levels in human umbilical vein endothelial cells incubated for 24 hours with DMEM Ctl group or 10% (v/v) plasma samples of Sham (non-lead- intoxicated group) and Pb or Pb+Sildenafil (lead-intoxicated groups for 7 days). Values represent mean ± SD (n= 8-10). *P < 0.05 versus Pb, Pb+Sildenafil and Ctl group.

## 4. Discussion

In this we study showed that sildenafil attenuated hypertension caused by short-term administration of low lead concentrations in rats. Also, sildenafil reduced ROS formation with concomitant increases in SOD activity and NO metabolites. These data, taken together, suggest that sildenafil effects on lead-induced hypertension may be related to its antioxidant properties. Moreover, sildenafil treatment protects against impaired endothelium-dependent vasodilation caused by lead, thus re-establishing NO-dependent vasorelaxation.

Earlier studies have found that lead levels in blood with the range of 9 to 37µg/dL were associated with hypertension during acute intoxication with lead in rats (Gonçalves-Rizzi et al., 2016; Nascimento et al., 2015; Possomato-Vieira et al., 2018; Silveira et al., 2014). In this study, the hypertension found in rats was induced by lead in which whole blood lead concentrations were approximately 23±2µg/dl.

The mechanisms explaining how lead causes hypertension are not totally clear. Lead-related toxicity has been shown to interfere with the function of vascular and endothelial cells through increases in oxidative stress, which is manifested as a significant elevation of the MDA levels and reductions of antioxidant status followed by decreases in bioavailability of the potent vasodilator, NO (Gonçalves-Rizzi et al., 2016; Silveira et al., 2014; Zheutlin et al., 2018). This supports the hypothesis that oxidative stress is a key trigger to the functional and biochemical disorders found in lead-induced hypertension.

We found that lead caused hypertension in rats and that was associated with increases in MDA levels and reductions in NO metabolites in plasma. The increases in ROS formation in the endothelium and vascular smooth muscle may inactivate NO, decreasing NO bioavailability which was observed in lead-induced hypertension (Vaziri et al., 1999). Thus, reducing ROS in lead-induced hypertension is an important strategy to preserve NO bioactivity in the vasculature. In this regard, our results show that sildenafil treatment attenuated hypertension and blunts both increases in MDA levels and reductions in NO metabolites in plasma of rats intoxicated with lead. This is consistent with reports that sildenafil reversed the lead-induced anti-erectile effect in rats (Senbel and Helmy, 2013).

Therefore, in order to understand the oxidative stress and NO imbalance involved in lead-induced hypertension, we examined the NO metabolites (levels of NOx) in HUVECs culture supernatants that reflect the intracellular levels of NO in endothelial cells. In our hands, sildenafil protected against the reduction of circulating NO caused by lead, and sildenafil also showed an effect in increasing NO in endothelial cells supernatant incubated with plasma from lead-intoxicated rats and treated with sildenafil (Pb+Sildenafil group, Figure 8). Although we found these protective effects with sildenafil treatment, it should be taken into account that the levels of NOx, ie NO metabolites nitrite + nitrate, may not reflect direct intracellular NO formation in endothelial cells. For this reason, we then examined whether the reductions of NOx levels in plasma caused by lead intoxication in rats that received (or not) sildenafil could be confirmed by the assessment of intracellular NO levels in endothelial cells. In this experimental setup, there were increases in intracellular formation of the NO (over experimental time) in HUVECs incubated with plasma from non-lead-intoxicated animals (Sham group, Figure 9), while no significant increases were found in endothelial cells incubated with plasma of lead-intoxicated rats and treated (or not) with sildenafil (Pb and Pb+Sildenafil groups, Figure 9).

Thus, our present results suggest that the effects by which sildenafil enhances the bioavailability of NO may be related to the reduction of oxidative stress but not with the direct formation of NO, in accordance with previously observed (Guimarães et al, 2013; Laxmi et al., 2019), since sildenafil showed greater SOD activity in the Pb+Sildenafil group and prevented the decreases in plasma antioxidant capacity (Figures 4 and 5).

To further investigate the effects of lead intoxication and sildenafil treatment in NO-dependent vasodilation, vascular function experiments have been performed. We have found that sildenafil restored NO-dependent acetylcholine-induced vasodilation in the Pb+Sildenafil group, which was similar to that found in the Sham group in endothelium intact aortic rings, while in the presence of NO synthase inhibitor, L-NAME, the relaxation was inhibited. However, the Pb group presented impaired responses in acetylcholine-induced vasodilation in $R_{max}$ and $pEC_{50}$. These present results are corroborated by Rodriguez-Miguelez et al., (2018), which showed that sildenafil treatment enhanced endothelial function in cystic fibrosis patients (Rodriguez-Miguelez et al., 2018). Thereby, while lead impairs endothelium-dependent vasodilation, sildenafil may be able to reestablish the vasodilation dependent of NO in the lead-induced hypertension for exerting antioxidant effect, thus preventing NO degradation by oxidative stress, in accordance with results that were previously found in experimental model of hypertension in rats treated with sildenafil (Guimarães et al., 2013).

## 5. Conclusion

In summary, the present data show that sildenafil may be able to reverse oxidative stress and NO imbalance caused by lead-intoxication, since sildenafil decreased the production of ROS and consequently enhanced the circulating levels of NO metabolites. Also, sildenafil treatment protected against impaired endothelium-dependent vasodilation caused by lead-intoxication, thus re-established NO-dependent vasorelaxation and protected against lead intoxication-induced increases in systolic blood pressure, suggesting pleiotropic antioxidant effects provided for sildenafil.

**Funding**

This study was supported by Sao Paulo Research Foundation, Brazil (FAPESP – Finance Code: 2018/24.484-0), National Council for Scientific and Technological Development, Brazil (CNPq- 301370/2018-4) and Coordenacao de Aperfeicoamento de Pessoal de Nivel Superior - Brazil (CAPES – Finance Code 001).

**Authors' contributions**

**Ediléia Souza Paula Caetano**: Conceptualization, Data curation and Formal analysis, Investigation, Methodology, Validation, Roles/Writing - original draft and Writing - review & editing. **Sarah Viana Mattioli**: Data curation and Formal analysis, Investigation, Methodology and Validation. **Maria Luiza Santos da Silva**: Investigation and roles/Writing - original draft. **Laisla Zanetoni Martins**: Roles/Writing - original draft. **Alaor Aparecido Almeida**: Methodology. **Ananda Lini Vieira da Rocha**: Methodology. **Priscila Rezeck Nunes**: Methodology. **Núbia Alves Grandini**: Methodology and Validation.

**Camila Renata Correa**: Methodology and Validation. **Gabriela Palma Zochio**: Investigation and Roles/Writing - original draft. **Carlos A. Dias-Junior**: Conceptualization, Funding acquisition, Investigation, Project administration, Resources and Supervision, Validation, Roles/Writing - original draft and Writing - review & editing.

**Declaration of Competing Interest**

There are no known conflicts of interest associated with this manuscript and there has been no significant financial support for this work that could have influenced its outcome.

Consent for publication: the manuscript has been revised and approved by all named authors.

**Declarations**

Availability of data and material: the data are original, have not been published before and are not currently being considered for publication elsewhere.

**REFERENCES**

Abdollahi, M., Fooladian, F., Emami, B., Zafari, K., Bahreini-Moghadam, A., 2003. Protection by sildenafil and theophylline of lead acetate-induced oxidative stress in rat submandibular gland and saliva. Hum Exp Toxicol 22, 587–592. https://doi.org/10.1191/0960327103ht399oa

Aebi, H., 1974. Catalase, in: Bergmeyer, H.U. (Ed.), Methods of Enzymatic Analysis (Second Edition). Academic Press, pp. 673–684. https://doi.org/10.1016/B978-0-12-091302-2.50032-3

Ahamed, M., Siddiqui, M.K.J., 2007. Low level lead exposure and oxidative stress: current opinions. Clin Chim Acta 383, 57–64. https://doi.org/10.1016/j.cca.2007.04.024

American College of Medical Toxicology, American Academy of Clinical Toxicology, 2015. Antidote shortages in the USA: impact and response. J Med Toxicol 11, 144–146. https://doi.org/10.1007/s13181-013-0372-1

Benzie, I.F.F., Strain, J.J., 1996. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of “Antioxidant Power”: The FRAP Assay. Analytical Biochemistry 239, 70–76. https://doi.org/10.1006/abio.1996.0292

Cabral, M., Dieme, D., Verdin, A., Garçon, G., Fall, M., Bouhsina, S., Dewaele, D., Cazier, F., Tall-Dia, A., Diouf, A., Shirali, P., 2012. Low-level environmental exposure to lead and renal adverse effects: a cross-sectional study in the population of children bordering the Mbeubeuss landfill near Dakar, Senegal. Hum Exp Toxicol 31, 1280–1291. https://doi.org/10.1177/0960327112446815

Chimini, J.S., Possomato-Vieira, J.S., da Silva, M.L.S., Dias-Junior, C.A., 2019. Placental nitric oxide formation and endothelium-dependent vasodilation underlie pravastatin effects against angiogenic imbalance, hypertension in pregnancy and intrauterine growth restriction. Basic Clin Pharmacol Toxicol 124, 385–393. https://doi.org/10.1111/bcpt.13149

Cinegaglia, N., Acosta-Navarro, J., Rainho, C., Antoniazzi, L., Mattioli, S., Pimentel, C., Santos, R.D., Sandrim, V., 2020. Association of Omnivorous and Vegetarian Diets With Antioxidant Defense Mechanisms in Men. J Am Heart Assoc 9, e015576. https://doi.org/10.1161/JAHA.119.015576

Ding, Y., Vaziri, N.D., Gonick, H.C., 1998. Lead-induced hypertension. II. Response to sequential infusions of L-arginine, superoxide dismutase, and nitroprusside. Environ Res 76, 107–113. https://doi.org/10.1006/enrs.1997.3796

Dishy, V., Sofowora, G., Harris, P.A., Kandcer, M., Zhan, F., Wood, A.J., Stein, C.M., 2001. The effect of sildenafil on nitric oxide-mediated vasodilation in healthy men. Clin Pharmacol Ther 70, 270–279. https://doi.org/10.1067/mcp.2001.117995

Dursun, N., Arifoglu, C., Süer, C., Keskinol, L., 2005. Blood pressure relationship to nitric oxide, lipid peroxidation, renal function, and renal blood flow in rats exposed to low lead levels. Biol Trace Elem Res 104, 141–149. https://doi.org/10.1385/BTER:104:2:141

Fiorim, J., Ribeiro Júnior, R.F., Silveira, E.A., Padilha, A.S., Vescovi, M.V.A., de Jesus, H.C., Stefanon, I., Salaices, M., Vassallo, D.V., 2011. Low-level lead exposure increases systolic arterial pressure and endothelium-derived vasodilator factors in rat aortas. PLoS One 6, e17117. https://doi.org/10.1371/journal.pone.0017117

Gambelunghe, A., Sallsten, G., Borné, Y., Forsgard, N., Hedblad, B., Nilsson, P., Fagerberg, B., Engström, G., Barregard, L., 2016. Low-level exposure to lead, blood pressure, and hypertension in a population-based cohort. Environ Res 149, 157–163. https://doi.org/10.1016/j.envres.2016.05.015

Gonçalves-Rizzi, V.H., Nascimento, R.A., Possomato-Vieira, J.S., Dias-Junior, C.A., 2016. Sodium Nitrite Prevents both Reductions in Circulating Nitric Oxide and Hypertension in 7-Day Lead-Treated Rats. Basic Clin Pharmacol Toxicol 118, 225–230. https://doi.org/10.1111/bcpt.12480

Goyer, R.A., Cherian, M.G., Jones, M.M., Reigart, J.R., 1995. Role of chelating agents for prevention, intervention, and treatment of exposures to toxic metals. Environ Health Perspect 103, 1048–1052. https://doi.org/10.1289/ehp.951031048

Grizzo, L.T., Cordellini, S., 2008. Perinatal lead exposure affects nitric oxide and cyclooxygenase pathways in aorta of weaned rats. Toxicol Sci 103, 207–214. https://doi.org/10.1093/toxsci/kfn018

Guimarães, D.A., Rizzi, E., Ceron, C.S., Pinheiro, L.C., Gerlach, R.F., Tanus-Santos, J.E., 2013. Atorvastatin and sildenafil lower blood pressure and improve endothelial dysfunction, but only atorvastatin increases vascular stores of nitric oxide in hypertension. Redox Biol 1, 578–585. https://doi.org/10.1016/j.redox.2013.11.004

Khalil-Manesh, F., Gonick, H.C., Weiler, E.W., Prins, B., Weber, M.A., Purdy, R.E., 1993. Lead-induced hypertension: possible role of endothelial factors. Am J Hypertens 6, 723–729. https://doi.org/10.1093/ajh/6.9.723

Lamas, G.A., Navas-Acien, A., Mark, D.B., Lee, K.L., 2016. Heavy Metals, Cardiovascular Disease, and the Unexpected Benefits of Chelation Therapy. J Am Coll Cardiol 67, 2411–2418. https://doi.org/10.1016/j.jacc.2016.02.066

Laxmi, V., Gupta, R., Bhattacharya, S.K., Ray, A., Gulati, K., 2019. Inhibitory effects of sildenafil and tadalafil on inflammation, oxidative stress and nitrosative stress in animal model of bronchial asthma. Pharmacol Rep 71, 517–521. https://doi.org/10.1016/j.pharep.2019.02.008

Mahmud, A., Hennessy, M., Feely, J., 2001. Effect of sildenafil on blood pressure and arterial wave reflection in treated hypertensive men. J Hum Hypertens 15, 707–713. https://doi.org/10.1038/sj.jhh.1001244

Malvezzi, C.K., Moreira, E.G., Vassilieff, I., Vassilieff, V.S., Cordellini, S., 2001. Effect of L-arginine, dimercaptosuccinic acid (DMSA) and the association of L-arginine and DMSA on tissue lead mobilization and blood pressure level in plumbism. Braz J Med Biol Res 34, 1341–1346. https://doi.org/10.1590/s0100-879x2001001000016

Marklund, S.L., 1985. Product of extracellular-superoxide dismutase catalysis. FEBS Letters 184, 237–239. https://doi.org/10.1016/0014-5793(85)80613-X

Marques, M., Millás, I., Jiménez, A., García-Colis, E., Rodriguez-Feo, J.A., Velasco, S., Barrientos, A., Casado, S., López-Farré, A., 2001. Alteration of the soluble guanylate cyclase system in the vascular wall of lead-induced hypertension in rats. J Am Soc Nephrol 12, 2594–2600. https://doi.org/10.1681/ASN.V12122594

Nascimento, R.A., Mendes, G., Possomato-Vieira, J.S., Gonçalves-Rizzi, V.H., Kushima, H., Delella, F.K., Dias-Junior, C.A., 2015. Metalloproteinase Inhibition Protects against Reductions in Circulating Adrenomedullin during Lead-induced Acute Hypertension. Basic Clin Pharmacol Toxicol 116, 508–515. https://doi.org/10.1111/bcpt.12337

Perk, H., Armagan, A., Naziroğlu, M., Soyupek, S., Hoscan, M.B., Sütcü, R., Ozorak, A., Delibas, N., 2008. Sildenafil citrate as a phosphodiesterase inhibitor has an antioxidant effect in the blood of men. J Clin Pharm Ther 33, 635–640. https://doi.org/10.1111/j.1365-2710.2008.00962.x

Possomato-Vieira, J.S., Gonçalves-Rizzi, V.H., do Nascimento, R.A., Wandekin, R.R., Caldeira-Dias, M., Chimini, J.S., da Silva, M.L.S., Dias-Junior, C.A., 2018. Clinical and Experimental Evidences of Hydrogen Sulfide Involvement in Lead-Induced Hypertension. Biomed Res Int 2018, 4627391. https://doi.org/10.1155/2018/4627391

Raffetto, J.D., Calanni, F., Mattana, P., Khalil, R.A., 2019. Sulodexide promotes arterial relaxation via endothelium-dependent nitric oxide-mediated pathway. Biochem Pharmacol 166, 347–356. https://doi.org/10.1016/j.bcp.2019.04.021

Rocha-Penha, L., Caldeira-Dias, M., Tanus-Santos, J.E., de Carvalho Cavalli, R., Sandrim, V.C., 2017. Myeloperoxidase in Hypertensive Disorders of Pregnancy and Its Relation With Nitric Oxide. Hypertension 69, 1173–1180. https://doi.org/10.1161/HYPERTENSIONAHA.116.08854

Rodriguez-Miguelez, P., Lee, N., Tucker, M.A., Csányi, G., McKie, K.T., Forseen, C., Harris, R.A., 2018. Sildenafil improves vascular endothelial function in patients with cystic fibrosis. Am J Physiol Heart Circ Physiol 315, H1486–H1494. https://doi.org/10.1152/ajpheart.00301.2018

Roncal, C., Mu, W., Reungjui, S., Kim, K.M., Henderson, G.N., Ouyang, X., Nakagawa, T., Johnson, R.J., 2007. Lead, at low levels, accelerates arteriolopathy and tubulointerstitial injury in chronic kidney disease. Am J Physiol Renal Physiol 293, F1391-1396. https://doi.org/10.1152/ajprenal.00216.2007

Roy, A., Kordas, K., 2016. The Relation Between Low-Level Lead Exposure and Oxidative Stress: a Review of the Epidemiological Evidence in Children and Non-Occupationally Exposed Adults. Curr Environ Health Rep 3, 478–492. https://doi.org/10.1007/s40572-016-0115-y

Saxena, G., Flora, S.J.S., 2004. Lead-induced oxidative stress and hematological alterations and their response to combined administration of calcium disodium EDTA with a thiol chelator in rats. J Biochem Mol Toxicol 18, 221–233. https://doi.org/10.1002/jbt.20027

Senbel, A.M., Helmy, M.M., 2013. Lead acetate may cause erectile dysfunction by modulating NO/cGMP pathway in rat corpus cavernosum. Cell Biol Toxicol 29, 355–364. https://doi.org/10.1007/s10565-013-9258-x

Silveira, E.A., Siman, F.D.M., de Oliveira Faria, T., Vescovi, M.V.A., Furieri, L.B., Lizardo, J.H.F., Stefanon, I., Padilha, A.S., Vassallo, D.V., 2014. Low-dose chronic lead exposure increases systolic arterial pressure and vascular reactivity of rat aortas. Free Radic Biol Med 67, 366–376. https://doi.org/10.1016/j.freeradbiomed.2013.11.021

Simões, M.R., Ribeiro Júnior, R.F., Vescovi, M.V.A., de Jesus, H.C., Padilha, A.S., Stefanon, I., Vassallo, D.V., Salaices, M., Fioresi, M., 2011. Acute lead exposure increases arterial pressure: role of the renin-angiotensin system. PLoS One 6, e18730. https://doi.org/10.1371/journal.pone.0018730

Vaziri, N.D., 2008. Mechanisms of lead-induced hypertension and cardiovascular disease. Am J Physiol Heart Circ Physiol 295, H454-465. https://doi.org/10.1152/ajpheart.00158.2008

Vaziri, N.D., Ding, Y., 2001. Effect of lead on nitric oxide synthase expression in coronary endothelial cells: role of superoxide. Hypertension 37, 223–226. https://doi.org/10.1161/01.hyp.37.2.223

Vaziri, N.D., Khan, M., 2007. Interplay of reactive oxygen species and nitric oxide in the pathogenesis of experimental lead-induced hypertension. Clin Exp Pharmacol Physiol 34, 920–925. https://doi.org/10.1111/j.1440-1681.2007.04644.x

Vaziri, N.D., Liang, K., Ding, Y., 1999. Increased nitric oxide inactivation by reactive oxygen species in lead-induced hypertension. Kidney Int 56, 1492–1498. https://doi.org/10.1046/j.1523-1755.1999.00670.x

Viana-Mattioli, S., Cinegaglia, N., Bertozzi-Matheus, M., Bueno-Pereira, T.O., Caldeira-Dias, M., Cavalli, R.C., Sandrim, V.C., 2020. SIRT1-dependent effects of resveratrol and grape juice in an in vitro model of preeclampsia. Biomed Pharmacother 131, 110659. https://doi.org/10.1016/j.biopha.2020.110659

Wang, H., Joseph, J.A., 1999. Quantifying cellular oxidative stress by dichlorofluorescein assay using microplate reader. Free Radic Biol Med 27, 612–616. https://doi.org/10.1016/s0891-5849(99)00107-0

Wu, S., Yang, Y., Li, F., Huang, L., Han, Z., Wang, G., Yu, H., Li, H., 2018. Chelerythrine induced cell death through ROS-dependent ER stress in human prostate cancer cells. Onco Targets Ther 11, 2593–2601. https://doi.org/10.2147/OTT.S157707

Zheutlin, A.R., Hu, H., Weisskopf, M.G., Sparrow, D., Vokonas, P.S., Park, S.K., 2018. Low-Level Cumulative Lead and Resistant Hypertension: A Prospective Study of Men Participating in the Veterans Affairs Normative Aging Study. J Am Heart Assoc 7, e010014. https://doi.org/10.1161/JAHA.118.010014

Zhou, Y., Zanão, R.A., Barbosa Jr, F., Parsons, P.J., Krug, F.J., 2002. Investigations of a W-Rh permanent modifier for the determination of Pb in blood by electrothermal atomic absorption spectrometry. Spectrochimica Acta Part B: Atomic Spectroscopy 57, 1291–1300. https://doi.org/10.1016/S0584-8547(02)00085-X

## 3-Discussão

O chumbo é um metal muito presente nos processos industriais, por ser um material abundante, muito resistente e de fácil manuseio ele barateia os processos e contribui para o avanço da tecnologia; no entanto, esses benefícios primários precisam ser contrabalanceados aos prejuízos que o chumbo causa a saúde humana.

O chumbo exerce efeito nocivo sobre o sistema cardiovascular mesmo em concentrações consideradas índices normais pela legislação laboral vigente, entre os efeitos adversos está a hipertensão. A hipertensão causada por chumbo caracteriza-se como uma hipertensão resistente que segundo a 7ª Diretriz Brasileira de Hipertensão Arterial, da Sociedade Brasileira de Cardiologia é considerada hipertensão resistente, pressão arterial não controlada (> 140/90 mmHg) em uso ≥ 3 anti-hipertensivos ou pressão arterial controlada, ou não em uso ≥ 4 anti-hipertensivos. A hipertensão resistente acontece em cerca 1 a 5% dos quadros hipertensivos, aumentando os riscos de eventos cardiovasculares em até 95% em pacientes portadores desta síndrome (MVB *et al.*, [*s. d.*]).

O presente estudo mostra que o sildenafil atenuou a hipertensão causada pela administração a curto prazo de baixas concentrações de chumbo em ratos. Além disso, o sildenafil reduziu a formação de ERO (espécies reativas de oxigênio) com aumento concomitante dos metabólitos do NO e aumentou a atividade da SOD. Esses dados, em conjunto, sugerem que os efeitos do sildenafil na hipertensão induzida pelo chumbo podem estar relacionados às suas propriedades antioxidantes. Além disso, o tratamento com sildenafil protege contra a vasodilatação endotélio-dependente prejudicada causada pelo chumbo, restabelecendo assim o relaxamento endotélio-NO-dependente.

Nos observamos que em ratos com concentrações de chumbo no sangue total de aproximadamente 23±2 µg/dl houve a presença de hipertensão, os nossos achados concordam

com estudos anteriores que evidenciaram que níveis de chumbo no sangue na faixa de 9 a 37 µg/dL estavam associados à hipertensão durante a exposição aguda por chumbo em ratos (GONÇALVES-RIZZI *et al.*, 2016; NASCIMENTO *et al.*, 2015; POSSOMATO-VIEIRA *et al.*, 2018; SILVEIRA *et al.*, 2014).

Os mecanismos pelos quais o chumbo causa a hipertensão arterial são ainda misteriosos, linhas de pesquisas baseiam-se na interferência do chumbo nas funções das células endoteliais através do estresse oxidativo, que se manifesta como uma elevação significativa dos níveis de MDA e reduções do status antioxidante, seguidas por diminuições na biodisponibilidade do NO, uma vez que, a sinalização de NO é amplamente governada pelo estado redox basal, que determina a biodisponibilidade de NO e a capacidade de resposta de seu principal alvo a sGC. Durante o estado de estresse oxidativo, aumento da atividade da oxidase gera superóxido, que pode reagir com NO formando peroxinitrito, efetivamente extinguindo o NO e reduzindo sua concentração. ERO pode oxidar enzimas NOS via glutationilação e diminuir as razões $BH_4/BH_2$, convertendo a enzima de uma NOS para uma oxidase. Isso apoia a hipótese de que o estresse oxidativo é um gatilho chave para os distúrbios funcionais e bioquímicos encontrados na hipertensão induzida por chumbo (GONÇALVES-RIZZI *et al.*, 2016; LUNDBERG; GLADWIN; WEITZBERG, 2015; SILVEIRA *et al.*, 2014; ZHEUTLIN *et al.*, 2018).

Para melhor compreendermos o estresse oxidativo e o desequilíbrio de NO envolvido na hipertensão induzida por chumbo, examinamos os metabólitos de NO (níveis de NOx) em sobrenadantes de cultura de HUVECs que refletem os níveis intracelulares de NO em células endoteliais uma vez que ao se examinar os metabolitos do NO podemos correlacionar a quantidade desse gás presente no sistema analisado. Constatamos que o sildenafil aumentou os níveis de NOx em sobrenadante de células endoteliais incubadas com plasma de ratos intoxicados por chumbo e tratados com sildenafil. Embora tenhamos encontrado esses efeitos protetores com o tratamento com sildenafil, deve-se considerar que os níveis de NOx, ou seja,

metabólitos de NO nitrito + nitrato, podem não refletir a formação direta de NO intracelular nas células endoteliais. Por esse motivo, examinamos então se as reduções dos níveis de NOx no plasma causadas pela exposição por chumbo em ratos que receberam (ou não) sildenafil poderiam ser confirmadas pela avaliação dos níveis de NO intracelular em células endoteliais. Nesta configuração experimental, houve aumentos na formação intracelular do NO (ao longo do tempo experimental) em HUVECs incubadas com plasma de animais não intoxicados com chumbo, enquanto não foram encontrados aumentos significativos em células endoteliais incubadas apenas com meio de cultura, o que chamamos de grupo controle e nas células endoteliais incubadas com plasma de ratos intoxicados por chumbo e tratados (ou não) com sildenafil. Assim, nossos resultados sugerem que os efeitos pelos quais o sildenafil aumenta a biodisponibilidade do NO podem estar relacionados com a redução do estresse oxidativo, mas não com a formação direta de NO, de acordo com observado anteriormente (GUIMARÃES *et al.*, 2013; LAXMI *et al.*, 2019), visto que o sildenafil apresentou maior atividade na enzima superóxido dismutase no grupo Pb+Sildenafil e preveniu a diminuição da capacidade antioxidante plasmática.

Os estudos realizados em vitro podem não representar o que ocorre no organismo completo na exposição por chumbo, uma vez que são conduzidos em condições controladas, podendo não mimetizar com precisão a ação do metabolismo, por esse motivo analisamos os efeitos da exposição por chumbo e tratamento com sildenafil na vasodilatação dependente de NO, por análise direta da função vascular em experimento realizado em banho de órgãos isolados.

Em anéis de aorta torácica com endotélio intacto, o sildenafil restaurou a vasodilatação induzida por acetilcolina dependente de NO, no grupo Pb+Sildenafil, ao ponto de se equiparar ao relaxamento induzido por acetilcolina dependente de NO encontrado no grupo Sham, enquanto na presença do inibidor da sintase de NO, L-NAME, o relaxamento foi inibido. No entanto, o grupo Pb apresentou respostas prejudicadas na vasodilatação induzida pela

acetilcolina em Emax e pEC50. Nossos achados concordam com o descrito por Rodriguez-Miguelez et al., (2018), mostrando que o tratamento com sildenafil melhorou a função endotelial vascular em pacientes com fibrose cística (RODRIGUEZ-MIGUELEZ *et al.*, 2018) Desta forma, enquanto o chumbo exerce um efeito prejudicial sobre o endotélio vascular, o sildenafil mostrou-se capaz de reestabelecer a vasodilatação dependente de NO, na hipertensão induzida por chumbo por exercer efeito antioxidante, evitando assim a degradação do NO pelo estresse oxidativo, acordando com os dados descritos por Guimarães et al., em modelo experimental de hipertensão em ratos tratados com sildenafil(GUIMARÃES *et al.*, 2013).

## 4- Conclusão

Nossos achados mostraram que o sildenafil tem ação sobre o estresse oxidativo, podendo reverter o desequilíbrio na biodisponibilidade do NO causado pela exposição com chumbo, uma vez que, o sildenafil aumentou a atividade da SOD, diminuiu a produção de ROS e consequentemente aumentou os níveis circulantes dos metabolitos do NO. O Sildenafil também protegeu o endotélio vascular do prejuízo causado pela exposição com chumbo, reestabelecendo a vasodilatação endotélio-NO-dependente e impedindo o aumento da pressão arterial sistólica. Constatamos que o sildenafil exerce propriedades antioxidantes capazes de reestabelecer o equilíbrio redox na exposição com baixos níveis de chumbo. Entretanto, estudos clínicos são necessários para demonstrar a eficácia e segurança do sildenafil como tratamento alternativo nos quadros de hipertensão resistente.

## 5- Referências bibliográficas


ABDOLLAHI, M.; FOOLADIAN, F.; EMAMI, B.; ZAFARI, K.; BAHREINI-MOGHADAM, A. Protection by sildenafil and theophylline of lead acetate-induced oxidative stress in rat submandibular gland and saliva. **Human & Experimental Toxicology**, v. 22, n. 11, p. 587–592, nov. 2003. https://doi.org/10.1191/0960327103ht399oa.

AHAMED, M.; SIDDIQUI, M. K. J. Low level lead exposure and oxidative stress: current opinions. **Clinica Chimica Acta; International Journal of Clinical Chemistry**, v. 383, n. 1–2, p. 57–64, ago. 2007. https://doi.org/10.1016/j.cca.2007.04.024.

BARRY, P. S. A comparison of concentrations of lead in human tissues. **British Journal of Industrial Medicine**, v. 32, n. 2, p. 119–139, maio 1975. https://doi.org/10.1136/oem.32.2.119.

DING, Y.; VAZIRI, N. D.; GONICK, H. C. Lead-induced hypertension. II. Response to sequential infusions of L-arginine, superoxide dismutase, and nitroprusside. **Environmental Research**, v. 76, n. 2, p. 107–113, fev. 1998. https://doi.org/10.1006/enrs.1997.3796.

DISHY, V.; SOFOWORA, G.; HARRIS, P. A.; KANDCER, M.; ZHAN, F.; WOOD, A. J.; STEIN, C. M. The effect of sildenafil on nitric oxide-mediated vasodilation in healthy men. **Clinical Pharmacology and Therapeutics**, v. 70, n. 3, p. 270–279, set. 2001. https://doi.org/10.1067/mcp.2001.117995.

GONÇALVES-RIZZI, V. H.; NASCIMENTO, R. A.; POSSOMATO-VIEIRA, J. S.; DIAS-JUNIOR, C. A. Sodium Nitrite Prevents both Reductions in Circulating Nitric Oxide and Hypertension in 7-Day Lead-Treated Rats. **Basic & Clinical Pharmacology & Toxicology**, v. 118, n. 3, p. 225–230, mar. 2016. https://doi.org/10.1111/bcpt.12480.

GRIZZO, L. T.; CORDELLINI, S. Perinatal lead exposure affects nitric oxide and cyclooxygenase pathways in aorta of weaned rats. **Toxicological Sciences: An Official Journal of the Society of Toxicology**, v. 103, n. 1, p. 207–214, maio 2008. https://doi.org/10.1093/toxsci/kfn018.

GUIMARÃES, D. A.; RIZZI, E.; CERON, C. S.; PINHEIRO, L. C.; GERLACH, R. F.; TANUS-SANTOS, J. E. Atorvastatin and sildenafil lower blood pressure and improve endothelial dysfunction, but only atorvastatin increases vascular stores of nitric oxide in hypertension. **Redox Biology**, v. 1, p. 578–585, 2013. https://doi.org/10.1016/j.redox.2013.11.004.

GUPTA, M.; KOVAR, A.; MEIBOHM, B. The Clinical Pharmacokinetics of Phosphodiesterase-5 Inhibitors for Erectile Dysfunction. **The Journal of Clinical Pharmacology**, v. 45, n. 9, p. 987–1003, set. 2005. https://doi.org/10.1177/0091270005276847.

H. P RANG, JAMES RITTER, YOON KONG LOKE, DAVID J. MACEWAN, ROD J. FLOWER, GRAEME HENDERSON. **RANG AND DALE'S Pharmacology**. 9. ed. [*S. l.*: *s. n.*], 2018.

KHALIL, R. A. Advances in Pharmacology - Endothelium.pdf. 2016. **Google Docs**. Disponível em:

https://drive.google.com/file/u/0/d/1c0LDENhoDkc1xZ8bLVRb2P679CNJvFNr/view?usp=drive_web&usp=embed_facebook. Acesso em: 25 jul. 2022.

LAXMI, V.; GUPTA, R.; BHATTACHARYA, S. K.; RAY, A.; GULATI, K. Inhibitory effects of sildenafil and tadalafil on inflammation, oxidative stress and nitrosative stress in animal model of bronchial asthma. **Pharmacological reports: PR**, v. 71, n. 3, p. 517–521, jun. 2019. https://doi.org/10.1016/j.pharep.2019.02.008.

LUNDBERG, J. O.; GLADWIN, M. T.; WEITZBERG, E. Strategies to increase nitric oxide signalling in cardiovascular disease. **Nature Reviews Drug Discovery**, v. 14, n. 9, p. 623–641, set. 2015. https://doi.org/10.1038/nrd4623.

MAHMUD, A.; HENNESSY, M.; FEELY, J. Effect of sildenafil on blood pressure and arterial wave reflection in treated hypertensive men. **Journal of Human Hypertension**, v. 15, n. 10, p. 707–713, out. 2001. https://doi.org/10.1038/sj.jhh.1001244.

MALVEZZI, C. K.; MOREIRA, E. G.; VASSILIEFF, I.; VASSILIEFF, V. S.; CORDELLINI, S. Effect of L-arginine, dimercaptosuccinic acid (DMSA) and the association of L-arginine and DMSA on tissue lead mobilization and blood pressure level in plumbism. **Brazilian Journal of Medical and Biological Research = Revista Brasileira De Pesquisas Medicas E Biologicas**, v. 34, n. 10, p. 1341–1346, out. 2001. https://doi.org/10.1590/s0100-879x2001001000016.

MARQUES, M.; MILLÁS, I.; JIMÉNEZ, A.; GARCÍA-COLIS, E.; RODRIGUEZ-FEO, J. A.; VELASCO, S.; BARRIENTOS, A.; CASADO, S.; LÓPEZ-FARRÉ, A. Alteration of the soluble guanylate cyclase system in the vascular wall of lead-induced hypertension in rats. **Journal of the American Society of Nephrology: JASN**, v. 12, n. 12, p. 2594–2600, dez. 2001. https://doi.org/10.1681/ASN.V12122594.

MVB, M.; WKSB, S.; FL, P.; CIS, R.; AA, B.; MFT, N.; LA, B.; RJS, F.; CE, P.-F.; PCBV, J.; RB, P.; RMS, P.; FC, C. 7ª Diretriz Brasileira de Hipertensão Arterial. , p. 103, [*s. d.*]. .

NASCIMENTO, R. A.; MENDES, G.; POSSOMATO-VIEIRA, J. S.; GONÇALVES-RIZZI, V. H.; KUSHIMA, H.; DELELLA, F. K.; DIAS-JUNIOR, C. A. Metalloproteinase Inhibition Protects against Reductions in Circulating Adrenomedullin during Lead-induced Acute Hypertension. **Basic & Clinical Pharmacology & Toxicology**, v. 116, n. 6, p. 508–515, jun. 2015. https://doi.org/10.1111/bcpt.12337.

PERK, H.; ARMAGAN, A.; NAZIROĞLU, M.; SOYUPEK, S.; HOSCAN, M. B.; SÜTCÜ, R.; OZORAK, A.; DELIBAS, N. Sildenafil citrate as a phosphodiesterase inhibitor has an antioxidant effect in the blood of men. **Journal of Clinical Pharmacy and Therapeutics**, v. 33, n. 6, p. 635–640, dez. 2008. https://doi.org/10.1111/j.1365-2710.2008.00962.x.

PIOMELLI, S.; ROSEN, J. F.; CHISOLM, J. J.; GRAEF, J. W. Management of childhood lead poisoning. **The Journal of Pediatrics**, v. 105, n. 4, p. 523–532, 1 out. 1984. https://doi.org/10.1016/S0022-3476(84)80414-X.

PORRU, S.; ALESSIO, L. The Use of Chelating Agents in Occupational Lead Poisoning. **Occupational Medicine**, v. 46, n. 1, p. 41–48, 1 fev. 1996. https://doi.org/10.1093/occmed/46.1.41.

POSSOMATO-VIEIRA, J. S.; GONÇALVES-RIZZI, V. H.; DO NASCIMENTO, R. A.; WANDEKIN, R. R.; CALDEIRA-DIAS, M.; CHIMINI, J. S.; DA SILVA, M. L. S.; DIAS-JUNIOR, C. A. Clinical and Experimental Evidences of Hydrogen Sulfide Involvement in Lead-Induced Hypertension. **BioMed Research International**, v. 2018, p. 4627391, 2018. https://doi.org/10.1155/2018/4627391.

RODRIGUEZ-MIGUELEZ, P.; LEE, N.; TUCKER, M. A.; CSÁNYI, G.; MCKIE, K. T.; FORSEEN, C.; HARRIS, R. A. Sildenafil improves vascular endothelial function in patients with cystic fibrosis. **American Journal of Physiology. Heart and Circulatory Physiology**, v. 315, n. 5, p. H1486–H1494, 1 nov. 2018. https://doi.org/10.1152/ajpheart.00301.2018.

SCHIFER, T. D. S.; JUNIOR, S. B.; MONTANO, M. A. E. ASPECTOS TOXICOLÓGICOS DO CHUMBO. , p. 6, 2005. .

SENBEL, A. M.; HELMY, M. M. Lead acetate may cause erectile dysfunction by modulating NO/cGMP pathway in rat corpus cavernosum. **Cell Biology and Toxicology**, v. 29, n. 5, p. 355–364, out. 2013. https://doi.org/10.1007/s10565-013-9258-x.

SILVEIRA, E. A.; SIMAN, F. D. M.; DE OLIVEIRA FARIA, T.; VESCOVI, M. V. A.; FURIERI, L. B.; LIZARDO, J. H. F.; STEFANON, I.; PADILHA, A. S.; VASSALLO, D. V. Low-dose chronic lead exposure increases systolic arterial pressure and vascular reactivity of rat aortas. **Free Radical Biology & Medicine**, v. 67, p. 366–376, fev. 2014. https://doi.org/10.1016/j.freeradbiomed.2013.11.021.

SIMÕES, M. R.; RIBEIRO JÚNIOR, R. F.; VESCOVI, M. V. A.; DE JESUS, H. C.; PADILHA, A. S.; STEFANON, I.; VASSALLO, D. V.; SALAICES, M.; FIORESI, M. Acute lead exposure increases arterial pressure: role of the renin-angiotensin system. **PloS One**, v. 6, n. 4, p. e18730, 11 abr. 2011. https://doi.org/10.1371/journal.pone.0018730.

TARCHER, A. B. (Org.). **Principles and Practice of Environmental Medicine**. Boston, MA: Springer US, 1992. DOI 10.1007/978-1-4899-2447-6. Disponível em: http://link.springer.com/10.1007/978-1-4899-2447-6. Acesso em: 15 jul. 2022.

VAZIRI, N. D. Mechanisms of lead-induced hypertension and cardiovascular disease. **American Journal of Physiology. Heart and Circulatory Physiology**, v. 295, n. 2, p. H454-465, ago. 2008. https://doi.org/10.1152/ajpheart.00158.2008.

ZHEUTLIN, A. R.; HU, H.; WEISSKOPF, M. G.; SPARROW, D.; VOKONAS, P. S.; PARK, S. K. Low-Level Cumulative Lead and Resistant Hypertension: A Prospective Study of Men Participating in the Veterans Affairs Normative Aging Study. **Journal of the American Heart Association**, v. 7, n. 21, p. e010014, 6 nov. 2018. https://doi.org/10.1161/JAHA.118.010014.