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Avaliação da ação da EGCG, composto do chá-verde, sobre a função endotelial em modelo *in vitro* de Pré-Eclâmpsia

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Avaliação da ação da EGCG, composto do chá-verde, sobre a função endotelial em modelo *in vitro* de Pré-Eclâmpsia

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PREFÁCIO

A dissertação aqui apresentada, intitulada: “Avaliação da ação da EGCG, composto do chá-verde, sobre a função vascular em modelo *in vitro* de Pré-Eclâmpsia” está dividida em 3 partes:

Primeiro, será apresentada uma introdução que aborda as principais informações sobre a Pré-Eclâmpsia: suas características, fisiopatologia, dados epidemiológicos e tratamento, assim como informações sobre a disfunção endotelial e a utilização de polifenóis nesse contexto. Detalharemos, também, a importância do modelo *in vitro* da doença e da aplicação do *shear stress* em nosso trabalho.

Já na segunda parte, o artigo “*EGCG, a green tea compound, increases NO production and has antioxidant action in a static and shear stress in vitro model of preeclampsia.”,* que mostra os potenciais efeitos da EGCG sobre a produção de Óxido Nítrico (NO) e seu potencial antioxidante em um modelo *in vitro* de células estáticas e submetidas ao *shear stress* será apresentado.

Por fim, a terceira parte contém uma discussão sobre o artigo e os dados apresentados, as limitações sobre o nosso estudo e uma conclusão sobre o tema abordado.

Apresento, também, as atividades realizadas durante o período de mestrado que vão além da bancada. Foram disciplinas, cursos e eventos que contribuíram significativamente para minha formação e aprimoramento acadêmico. Essas atividades estão listadas ao final da dissertação.

LISTA DE ABREVIATURAS

EC – Célula Endotelial

EGCG – Epigalocatequina-3-galato

EROS – Espécies Reativas de Oxigênio

FRAP – Potencial Antioxidante de Redução do Ferro (do inglês: Ferric Reducing Antioxidant Power)

GS – Gestante Saudável

L-NAME - Nω-nitro-L-arginina-metil éster

MDA - Malondialdeído

NO – Óxido Nítrico

O2•- - Superóxido

P3IK- Fosfatidillinositol-3-quinase

PE – Pré-Eclâmpsia

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RESUMO

A Pré-Eclâmpsia (PE) é uma doença hipertensiva gestacional caracterizada por importante estresse oxidativo e disfunção endotelial. A PE não tem cura e a busca por tratamentos e prevenção da doença se mostra de grande importância. Os polifenóis, de importante ação antioxidante e vasodilatadora, são substâncias amplamente encontrados em alimentos e bebidas de origem natural. Dentre eles podemos citar a EGCG, composto do chá-verde, pouco investigado no contexto da PE. Dessa forma, o objetivo desse estudo é investigar os efeitos da EGCG na função endotelial *in vitro* e avaliar os potenciais benefícios desse tratamento e suas implicações na PE. Considerando a importância do endotélio e a liberação de fatores prejudiciais na circulação materna, células endoteliais foram incubadas com o plasma de gestante saudável (GS) e PE para avaliação dos efeitos dessas amostras sobre as células. Para aproximar nosso modelo do organismo materno, as células também foram submetidas a uma metodologia que mimetiza a força de cisalhamento do sangue (*shear stress*) sobre o endotélio. Depois, células do grupo PE foram incubadas com a EGCG para avaliação dos níveis de NO, EROS e O2•-, capacidade antioxidante e níveis de MDA. Inibidores da principal via de ação da EGCG, a via PI3K/AKT/eNOS também foram utilizados: L-NAME para inibição da NOS e LY294002 para inibição da proteína PI3K. Nossos resultados apontam que a EGCG recupera os níveis de NO diminuídos no grupo PE. A ação do polifenol é diminuída nos grupos incubados com L-NAME e LY294002, indicando que o aumento de NO pelo fitoquímico se dá pela ativação da via PI3K/Akt/eNOS. Os níveis de EROS são menores no grupo PE quando comparados ao grupo GS e de O2•- similares entre os grupos, enquanto a EGCG não altera esses níveis. Entretanto, o tratamento aumenta a capacidade antioxidante no grupo PE, enquanto atenua os níveis de MDA no mesmo grupo, ações diminuídas com o uso de LY294002. No sobrenadante de células expostas ao *shear stress,* a EGCG aumentou os níveis de nitrito, assim como a capacidade antioxidante e também diminuiu os níveis de MDA, ou seja, sua ação foi mantida nesse modelo experimental. Sugerimos, portanto, que a EGCG atua na função endotelial, sendo um importante alvo terapêutico para a PE.

Palavras-chave: Pré-Eclâmpsia; disfunção endotelial; NO; estresse oxidativo; capacidade antioxidante; EGCG; polifenóis; shear stress; chá-verde

ABSTRACT

Preeclampsia (PE) is a gestacional disease characterized by important oxidative stress and endothelial dysfunction. PE has no cure, and the search for treatment and prevention of the disease are extremely important. In green tea we can find EGCG, a polyphenol with antioxidant and vasodilator properties well described in the context of cardiovascular diseases. However, investigations of these effects on PE are scarce. Therefore, the objective of this study is to investigate the effects of EGCG on endothelial function *in vitro* in order to evaluate the potential benefits of this treatment and its implications in PE. Considering the importance of the endothelium and the release of harmful factors in the maternal circulation, endothelial cells were incubated with plasma from healthy (HP) and PE pregnant women to evaluate the effects of these samples on the cells. Subsequently, cells from the PE group were incubated with EGCG to evaluate the levels of NO, ROS, and O2•-, antioxidant capacity, and MDA levels. Inhibitors of the main EGCG action pathway, the PI3K/AKT/eNOS pathway, were also used: L-NAME for NOS inhibition and LY294002 for PI3K inhibition. To approximate our model to the maternal organism, the cells were also subjected to a methodology that mimics the shear stress on the endothelium. Our results indicate that EGCG recovering NO levels decreased in the PE group and the polyphenol action is reduced in groups incubated with L-NAME and LY294002, indicating that the increase in NO by the phytochemical is due to the activation of the PI3K/Akt/eNOS pathway. The ROS levels are lower in the PE group when compared to the HP group and the O2•- levels are similar between them, while the EGCG does not change these levels. However, the treatment increases the antioxidant capacity in the PE group, while attenuating MDA levels in the same group, actions decreased with the LY294002 inhibitor. In the supernatant of cells exposed to shear stress, EGCG increased nitrite levels, as well as antioxidant capacity, and also decreased MDA levels, meaning that its action was maintained in this experimental model. Therefore, we suggest that EGCG acts on endothelial function, being an important therapeutic target for PE

Keywords: preeclampsia; endothelial dysfunction; NO; oxidative stress; antioxidant capacity; EGCG; polyphenols; green tea

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INTRODUÇÃO

1. Pré-Eclâmpsia: características e fisiopatologia

As doenças hipertensivas gestacionais estão entre as principais causas de complicações na gestação e morte materna no mundo (Gestational Hypertension and Preeclampsia: ACOG Practice Bulletin, Number 222, 2020). Dentre elas, podemos citar a Pré-Eclâmpsia (PE), que atinge entre 2-8% das gestantes, com uma maior prevalência em regiões subdesenvolvidas ("Gestational Hypertension and Preeclampsia: ACOG Practice Bulletin, Number 222", 2020; GIORDANO et al., 2014). Caracterizada pelo aumento da pressão arterial e disfunção de órgãos alvo, a PE ainda pode limitar o crescimento fetal e causar abortamentos ou partos prematuros, além de estar relacionada com o aumento do risco de desenvolvimento de doenças cardiovasculares no futuro dessa gestante (BARROSO et al., 2021; SIDDIQUI; HLADUNEWICH, 2011)

Durante a gestação, o organismo materno é submetido a diversas mudanças e adaptações, inclusive no sistema vascular. No útero, por exemplo, as células do citotrofoblasto invadem as artérias espiraladas para que elas se tornem mais calibrosas e o fluxo sanguíneo para a placenta aumente (BROSENS; ROBERTSON; DIXON, 1967; RANA et al., 2019). Essa invasão e remodelamento são profundos, atingindo as artérias até a altura do miométrio (BROSENS; ROBERTSON; DIXON, 1967; RANA et al., 2019). Entretanto, na PE, essa transformação das células do citotrofoblasto do subtipo "proliferativo" para o "invasivo" é diminuída, resultando na deficiência da invasão citotrofoblástica (RANA et al., 2019). Dessa forma, há uma falha no remodelamento das artérias espiraladas e seu calibre não é aumentado, o que limita o fluxo sanguíneo para a placenta e leva ao desenvolvimento de uma isquemia placentária (ROBERTS et al., 1989; ROBERTS; GAMMILL, 2005).

Essa isquemia placentária é um ponto-chave no desenvolvimento da PE (ROBERTS et al., 1989; ROBERTS; GAMMILL, 2005; OLIVEIRA; KARUMANCHI; SASS, 2010). Em uma gestação normal é comum que haja baixas tensões de oxigênio, seguidas de oxigenação provenientes do fluxo sanguíneo materno (CHIARELLO et al., 2020). Esse processo favorece a alteração do citotrofoblasto do subtipo proliferativo para o invasivo e, consequentemente, a placentação normal (RANA et al., 2019). Entretanto, em gestantes com PE, o que observamos é uma constante hipóxia seguida de reoxigenação causada pela isquemia, que favorece, então, o estresse oxidativo e a liberação de fatores inflamatórios, vasoativos e antiangiogênicos na circulação materna (OLIVEIRA; KARUMANCHI; SASS, 2010; SANDRIM et al., 2008; TAYLOR et al., 1998).

São esses fatores responsáveis pelo desenvolvimento da chamada "síndrome materna" que consiste nos sintomas característicos da doença, como a hipertensão, a proteinúria, a disfunção hepática, o edema pulmonar e problemas neuronais (ROBERTS et al., 1989; ROBERTS; GAMMILL, 2005). Em casos mais graves, a PE ainda pode evoluir para um quadro de Eclâmpsia, caracterizada por convulsões e coma; ou síndrome de HELLP, com hemólise, disfunção renal e baixa contagem de plaquetas ("Gestational Hypertension and Preeclampsia: ACOG Practice Bulletin, Number 222", 2020; PERAÇOLI et al., 2019).

2. Disfunção endotelial

A disfunção endotelial juntamente com a liberação desses fatores na circulação materna, também contribui significativamente para o desenvolvimento da PE e da síndrome materna (POSSOMATO-VIEIRA; KHALIL, 2016; TUZCU et al., 2015). São característica da disfunção endotelial a vasoconstrição, o ambiente pró-inflamatório, o favorecimento da adesão celular, entre outros (GALLO; VOLPE; SAVOIA, 2022;

HADI; CARR; AL SUWAIDI, 2005). O estresse oxidativo nesse contexto também é muito presente (ROCHETTE et al., 2013). Além da diminuição de agentes vasodilatadores e anti-oxidantes, observa-se um aumento de substâncias vasoconstritoras, pró-oxidantes e inflamatórias e é esse ambiente que se observa no organismo de gestante com PE (POSSOMATO-VIEIRA; KHALIL, 2016; TUZCU et al., 2015).

Nesse cenário, uma molécula é especialmente importante: o Óxido Nítrico (NO) (JOHAL et al., 2014; OSOL; KO; MANDALÀ, 2017; ROCHA-PENHA et al., 2017; SELIGMAN et al., 1994). O NO é um vasodilatador importante no controle da pressão arterial e, no organismo de gestantes com PE, se encontra diminuído, o que contribui com a hipertensão observada nessas mulheres (JOHAL et al., 2014; OSOL; KO; MANDALÀ, 2017; ROCHA-PENHA et al., 2017; SELIGMAN et al., 1994). A produção desse vasodilatador se dá pela enzima Óxido Nítrico Sintase Endotelial (eNOS), que converte o substrato L-arginina em NO e L-citrulina (SANKARALINGAM; XU; DAVIDGE, 2010). Essa enzima, sua disfunção e alterações que possam impedir a produção de NO, são, portanto, de grande importância para o estudo das doenças cardiovasculares, principalmente distúrbios hipertensivos. (DAIBER et al., 2019; FÖRSTERMANN; LI, 2011; KUKOR; VALENT; TÓTH, 2000; RYTLEWSKI et al., 2005).

Já o estresse oxidativo, citado anteriormente como um dos pontos-chave para a fisiopatologia da PE, também contribui consideravelmente para a disfunção endotelial e está ainda relacionado com a diminuição da biodisponibilidade de NO (ROCHETTE et al., 2013). O $O_2^{\bullet -}$, por exemplo, é um agente oxidante que sequestra o NO e que gera, com essa reação, peroxinitrito, outro agente oxidante (MITCHELL et al., 2007; POTJE et al., 2017; XIA et al., 1996). Além disso, pode contribuir com a oxidação do BH4, co-

fator da eNOS, necessário para a produção de NO (MITCHELL et al., 2007; POTJE et al., 2017; XIA et al., 1996). Dessa forma, notamos como o estresse oxidativo, a disfunção endotelial e a síndrome materna na PE estão intimamente ligadas.

3. Tratamento e prevenção

Atualmente, a PE não tem cura, e os medicamentos utilizados são para tratar os sintomas da doença (PERAÇOLI et al., 2019). Anti-hipertensivos são prescritos para que a pressão arterial seja controlada até uma idade gestacional segura para o parto ("Gestational Hypertension and Preeclampsia: ACOG Practice Bulletin, Number 222", 2020). No Brasil, os medicamentos mais utilizados para o controle da pressão a longo prazo incluem a metildopa (agonista alfa-adrenérgico) e a hidralazina (mecanismo de ação desconhecido); além de bloqueadores de canal de cálcio, como a nifedipina, para tratamento das crises hipertensivas (PERAÇOLI et al., 2019; SINKEY et al., 2020). Já a aspirina em baixas doses é recomendada como prevenção em pacientes que possuem fatores de risco alto ou moderado para a doença ("Gestational Hypertension and Preeclampsia: ACOG Practice Bulletin, Number 222", 2020; PERAÇOLI et al., 2019; SINKEY et al., 2020).

Dessa forma, a resolução da gestação é considerada a única cura para a PE. Ainda assim, inúmeros estudos apontam que a doença traz problemas a longo prazo para a gestante e feto (BARROSO et al., 2021; SIDDIQUI; HLADUNEWICH, 2011). A PE é, por exemplo, um fator de risco para o desenvolvimento de doenças cardiovasculares para as mulheres, e seus filhos também possuem chances aumentadas de desenvolver hipertensão durante a infância e adolescência (TURBEVILLE; SASSER, 2020).

4. Chá-verde e seus benefícios

Considerando essa problemática, a busca por tratamentos e prevenção da doença é de extrema importância. Considerando a saúde da mãe e do feto, é essencial que os efeitos adversos da substância-alvo sejam mínimos ou ausentes, além de ser uma substância acessível. Por isso, os alimentos de origem natural, ricos em fitoquímicos, despertam o interesse de grupos de pesquisa (BUENO-PEREIRA et al., 2022; CALDEIRA-DIAS et al., 2019; VIANA-MATTIOLI et al., 2020).

Dentre os diversos grupos de fitoquímicos há os polifenóis, cuja ação antioxidante e anti-inflamatória estão bem descritas na literatura (YAMAGATA, 2019; YAMAGATA; TAGAMI; YAMORI, 2015). Divididos em subgrupos: flavonóides (que inclui os flavanóis, as flavanonas, flavonas, antocianinas e flavonóis), ácidos fenólicos, lignanas e estilbenos, os polifenóis estão presente na maioria dos alimentos de origem vegetal, como frutas, especiarias, leguminosas, ervas e chás (MUSSATTO, 2015). Este último foi relacionado com a diminuição do risco de desenvolvimento de aterosclerose e acidente vascular cerebral em populações cujo consumo de chá era frequente, enquanto o consumo de chá-verde por homens japoneses, foi associado com menores níveis de colesterol total e maiores níveis de HDL no plasma desses indivíduos (KIM et al., 2007; LORENZ et al., 2004).

Ao investigar os polifenóis por trás desses efeitos, estudam apontam as epigalocatequina-3-galato (EGCG), principal fitoquímico do chá-verde, como o responsável pelos benefícios da bebida (KIM et al., 2007; LORENZ et al., 2004). *S*ua ação na função vascular já foi descrita e inclui a melhora de parâmetros de disfunção endotelial e a diminuição do estresse oxidativo, devido à sua importante ação antioxidante (LORENZ et al., 2015; REITER; KIM; QUON, 2010; TANG et al., 2006; YAMAGATA, 2020). Além disso, a EGCG é capaz de aumentar os níveis de NO através

da ativação da via PI3K/AKT/eNOS. Ou seja, ativando a proteína fosfatidilinositol-3-quinase (PI3K), que fosforila a proteína AKT, há, também, a fosforilação do resíduo Ser1179 e ativação da enzima eNOS (LORENZ et al., 2004, 2015; ZHANG; ZHANG, 2020). Consequentemente, observamos o aumento da produção de NO (LORENZ et al., 2004, 2015; ZHANG; ZHANG, 2020).

Entretanto, os estudos sobre esse composto no contexto da PE são escassos, apesar de promissores (GAO et al., 2022; SHI et al., 2018; ZHONG et al., 2020). Um estudo clínico, onde cápsulas de EGCG eram consumidas por gestante juntos com a nifedipina, apontou que o composto diminui as doses do medicamento necessária para o controle da crise hipertensiva, assim como o intervalo entre essas doses (SHI et al., 2018). Já estudos *in vitro* apontam que a EGCG melhora parâmetros de disfunção endotelial induzidos por hipóxia, além de inibir a apoptose e diminuir o estresse oxidativo em células endoteliais expostas a hiperhomocisteinemia (ZHANG; ZHANG, 2020; ZHONG et al., 2020).

5. Modelo *in vitro*

Nenhum dos estudos citados acima faz uso do modelo *in vitro* utilizado nesse trabalho. Considerando a importância da disfunção endotelial e a liberação de fatores na circulação materna na fisiopatologia da PE, células endoteliais (EC) foram incubadas com o plasma de gestante saudáveis (GS) e com PE para comparação dos efeitos dessas amostras sobre as células. O grupo PE foi, então, tratado com a EGCG para avaliarmos a ação deste fitoquímico em parâmetros de função endotelial.

A PE é uma doença exclusiva da gestação humana, portanto, a utilização de animais como modelos *in vivo* possui suas limitações. Além disso, a complexidade da fisiopatologia da PE também limita os estudos *in vitro,* o que reforça a necessidade de

metodologias e alternativas que aproximem os modelos disponíveis ao que acontece biologicamente nessas gestantes.

Dessa forma, um diferencial do nosso estudo é a aplicação de uma metodologia a fim de mimetizar a ação mecânica do fluxo sanguíneo unidirecional (*laminar shear stress*) nas células endoteliais. Nos vasos sanguíneos estreitos o fluxo sanguíneo é unidirecional e constante, sendo assim, chamado de "*laminar shear stress".* Esse tipo de *shear stress* é protetor e quando reconhecido por mecanorreceptores da superfície da célula endotelial, esse estímulo mecânico é traduzido em respostas que auxiliam na função endotelial, como regulação da eNOS e aumento da produção de NO, vasodilatação, regulação da coagulação e favorecimento da ação antioxidante (VIANA-MATTIOLI et al., 2023). Entretanto, em bifurcações e ramos de árvores arteriais, o fluxo sanguíneo orbital (*orbital shear stress)* que é bidirecional e menos constante, favorece o estresse oxidativo, a resposta inflamatória, a coagulação, a apoptose e a disfunção endotelial (VIANA-MATTIOLI et al., 2023).

Algumas metodologias que mimetizam o *shear stress* já foram apresentadas na literatura, porém pouco investigadas no cenário da PE. Um estudo evidenciou uma menor liberação de NO mediada por *shear stress* em artérias do miométrio de gestantes com PE quando comparadas a gestantes normotensas (KUBLICKIENE et al., 2000), enquanto estudos *in vitro* apontaram que a aplicação de *shear stress* teve efeito na produção de nitrito, aumentando os níveis dessa substância tanto no sobrenadante de células incubadas com o plasma de PE (BAKER et al., 1996), quanto em células endoteliais da decídua de gestante com PE (ROWE; CAMPBELL; GALLERY, 2003). Esses são os poucos estudos sobre as implicações do *shear stress* na doença.

Portanto, para melhor investigar a ação do *shear stress* sobre as células endoteliais e aproximar nosso modelo do que ocorre fisiologicamente no organismo dessas mulheres,

submetemos as células endoteliais a rotação em um shaker orbital por 72 horas para também avaliar a ação do plasma e da EGCG nessa condição.

Assim, nosso objetivo com o presente trabalho foi investigar os efeitos da EGCG na função endotelial *in vitro*, incluindo a produção de NO, agentes oxidantes e capacidade antioxidante, para dessa forma avaliar os potenciais benefícios desse tratamento e suas implicações na PE. Nossa hipótese é que a EGCG aumenta a disponibilidade do NO através da ativação da eNOS, além de atenuar o estresse oxidativo, atuando sobre a capacidade antioxidante das células.

ARTIGO

EGCG, a green tea compound, increases NO production and has antioxidant action in a static and shear stress *in vitro* model of preeclampsia.

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Abstract: Preeclampsia (PE) is a gestational hypertensive disease characterized by important endothelial dysfunction. In the search for possible treatments for the disease, EGCG, the main compound found in green tea is a promising therapeutic target. EGCG has been shown to activate eNOS, leading to increased NO production and exerting an important antioxidant action. But the specific impact of EGCG in the context of PE remains understudied. Therefore, the aim of this study is to evaluate the effects of EGCG in endothelial function, including NO production and antioxidant activity in an *in vitro* model of PE. Thus, endothelial cells were incubated with plasma from healthy (HP) and preeclamptic (PE) pregnant women, and the latter group was treated with EGCG. Additionally, NOS (L-NAME) and PI3K protein (LY249002) inhibitors were also used. The levels of NO, ROS, and O2•- were evaluated, as well as the antioxidant potential and MDA levels. These investigations were also carried out in a shear stress model, also underexplored in PE. The results show that EGCG increases the NO levels, which were reduced in the PE group. However, this effect was attenuated with the use of L-NAME and LY249002. Although EGCG had no significant impact on ROS and

O2•- levels, it effectively increased the antioxidant capacity of the PE group and decreased MDA levels, actions decreased with the LY294002 inhibitor. In cells subjected to shear stress, EGCG also increased nitrite levels in the PE group and maintained its action on the antioxidant capacity and decrease in MDA levels. This is the first study of the effects of EGCG in this experimental model, as well as the investigation of its effects along with shear stress. Our findings suggest that EGCG improves parameters of endothelial dysfunction in this *in vitro* model of PE, making it a promising target for further study in the search for treatment and prevention of the disease.

Keywords: preeclampsia; endothelial dysfunction; NO; oxidative stress; antioxidant capacity; EGCG; polyphenols; green tea

1. INTRODUCTION

Preeclampsia (PE) is a pregnancy disorder, characterized by hypertension (≥140/90 mmHg) occurring after the 20th week of gestation accompanied by proteinuria and/or target organs dysfunction ("Gestational Hypertension and Preeclampsia: ACOG Practice Bulletin, Number 222," 2020; RANA et al., 2019). PE affects an estimated 2-8% of pregnant women globally, and this percentage is higher in underdeveloped countries ("Gestational Hypertension and Preeclampsia: ACOG Practice Bulletin, Number 222," 2020). PE causes long-term problems for the mother and can restrict fetal growth, in addition to miscarriages or premature births (RANA et al., 2019; SIDDIQUI; HLADUNEWICH, 2011; UKAH et al., 2018). The disease has no cure and the only known solution is the delivery of the placenta after childbirth (RANA et al., 2019; SIDDIQUI; HLADUNEWICH, 2011; UKAH et al., 2018).

Poor remodeling of uteroplacental spiral arterioles, inadequate placentation, and insufficient placental perfusion are known to lead to hypoxia and oxidative stress (ROBERTS et al., 1989; ROBERTS; GAMMILL, 2005; STAFF, 2019). This condition results in the release of oxidant, antiangiogenic, and inflammatory factors into the maternal circulation, causing significant endothelial dysfunction, a crucial factor in the development of maternal syndrome (RANA et al., 2019; SANDRIM et al., 2008; TAYLOR et al., 1998). An important feature of pregnant women with PE is the decrease in Nitric Oxide (NO) bioavailability (SUTTON; GEMMEL; POWERS, 2020). NO is an important vasodilator produced by endothelial nitric oxide synthase (eNOS), which can also become dysfunctional in these women due to factors such as uncoupling or inactivation (LOWE, 2000; SANDRIM et al., 2008; SUTTON; GEMMEL; POWERS, 2020)

At present, research groups look for compounds or drugs that can help in treatment or prevention of PE. The use of antihypertensive drugs to maintain the pregnancy until an appropriate week for the baby's delivery or aspirin for women who have risk factors for developing the disease are currently the only recommendations(BROWN et al., 2018; “Gestational Hypertension and Preeclampsia: ACOG Practice Bulletin, Number 222,” 2020). In this context, researchers have investigated the antihypertensive and anti-inflammatory properties of foods and beverages derived from natural sources, rich in polyphenols (SUGANYA et al., 2016; YAMAGATA, 2019; YAMAGATA; TAGAMI; YAMORI, 2015). Among these beverages are teas, accessible and that generally do not have adverse effects or restrictions (HAYAT et al., 2015; ZHAO et al., 2022)

The main compound in green tea is epigallocatechin-3-gallate (EGCG), which has an important vasodilator and antioxidant properties that can improve endothelial

function. In addition to directly sequestering reactive oxygen species (ROS), EGCG increases the bioavailability of NO through the activation of eNOS via PI3K, and may help in the treatment of several cardiovascular and metabolic diseases (KIM et al., 2007; LORENZ et al., 2004; YAMAGATA, 2020; ZHANG; ZHANG, 2020). However, studies showing the effect of EGCG in PE context are limited, and none using the experimental model of this study (GAO et al., 2022; SHI et al., 2018; ZHONG et al., 2020)

Considering the endothelium role and the release of negative factors into the maternal circulation as the key point for the development of PE, in this study we used an *in vitro* model of endothelial cells (EC) incubated with the plasma of healthy and preeclamptic women (BACHETTI; MORBIDELLI, 2000; CALDEIRA-DIAS et al., 2018, 2019; SANKARALINGAM; XU; DAVIDGE, 2010; VIANA-MATTIOLI et al., 2020). We evaluated the effect of these samples on the cells in the presence or absence of EGCG. To investigate the action mechanism of the treatment, PI3K and NOS inhibitors were also used.

In addition, considering the PE pathophysiology complexity and the limitation of *in vitro* studies in reproducing what occurs in the body of these pregnant women, we employed a methodology that mimics the shear stress force present in the circulatory system (DELA PAZ et al., 2012) to create a model that better simulates the effects of blood flow on the endothelium. As shear stress acts on endothelial function and there are few studies investigating this action in the setting of PE, this *in vitro* methodology becomes another differential of our study.

Thus, the present study aims to assess the impact of EGCG on endothelial function *in vitro*, including the production of NO, oxidizing agents, and antioxidant

response. We investigate the potential benefits of EGCG in this static and shear stress cell culture model and considered its implications for PE.

2. MATERIALS AND METHODS

2.1 Participants selection

Blood samples were collected from healthy pregnant women (n=10) and preeclamptic women (n=10) at the Ribeirão Preto Clinical Hospital (Ribeirão Preto, Brazil) in tubes containing heparin. Samples were centrifuged at room temperature for 10 minutes at 1200 g to obtain plasma. Aliquots of 1000 µL were separated and stored at -80°C until use in incubation with human umbilical vein endothelial cells (HUVECS). The study was approved by the Research Ethics Committee of the Faculty of Medicine of Ribeirão Preto, Brazil (CAAE 37738620.0.0000.5440, October 19, 2020) and by the Research Ethics Committee of the Faculty of Medicine of Botucatu, Sao Paulo – UNESP (reference 4.961.945, September 09, 2021), following the principles of the Declaration of Helsinki. All participants signed the free and informed consent form. Preeclampsia diagnostic criteria were defined by the American College of Obstetricians and Gynecologists (ACOG) and women pregnant with twins, with chronic arterial hypertension, autoimmune, renal or hepatic diseases were excluded from the study. Pregnant women in the GS and PE groups were chosen with similar ages and gestational weeks at the time of collection.

2.2 Cell Culture

Human umbilical vein endothelial cells (HUVECS), lineage EA.hy 926, obtained from the Rio de Janeiro Cell Bank (BCRJ, Duque de Caxias, Rio de Janeiro, Brazil), were used in this study. The cells were cultivated in culture bottles at 37°C with 5% CO2 using a medium supplemented with 10% (v/v) fetal bovine serum (FBS), 50

μg/mL of penicillin, 100 μg/mL of streptomycin and 0.5 μg /mL of amphotericin B until they reached 80-90% confluency. For the experiments the EC were seeded in culture plates in the same medium until reached the necessary confluency. Subsequently, the culture medium was removed and the cells were washed with PBS and incubated in culture medium without FBS and with 10% (v/v) of HP and PE plasma for 24 hours at 37ºC in 5% CO2. In the treated cells, the same procedures were performed, but in the presence or absence of EGCG (Cayman Chemical®, MI, USA) (100 μM) during the last 60 minutes of incubation and L-NAME (Cayman Chemical®, MI, USA) (100 μM), NOS-inhibiting substance, and LY294002 (Cayman Chemical®, MI, USA) (30 μM), a PI3K inhibitor, 30 minutes before incubation with plasma. These procedures were performed prior to all experiments described below.

2.3 Intracelullar NO

Intracellular NO analysis was performed using a reagent that diffuses into cells and reacts with intracellular NO, being deacetylated by esterases and becoming highly fluorescent (KOJIMA et al., 1998). To perform this assay, after plating and incubating the cells, the culture supernatant was removed from the wells, for subsequent addition of PBS (95 μL) and incubation for 30 minutes. Thus, the DAF-FM solution (2,5 μM) (Invitrogen, Thermo Fisher Scientific, California, USA) was added to each well, and readings were taken every 10 minutes for a duration of 1 hour using the Synergy 4 plate reader (BioTek, VT, USA) with 485 nm excitation and 520 nm emission.

2.4 ROS quantification

2',7'-dichlorodihydrofluorescein (DCFH) is a reduced fluorescein reagent used as an indicator of reactive oxygen species in cells. After cleavage of its acetate groups by intracellular esterases and oxidation, this non-fluorescent substance is converted into 2',7'-dichlorofluorescein (DCF), which is read by fluorescence (CROW, 1997). For this,

after plating and incubating the cells, the supernatant from all wells was discarded and 200 µL of DCFH-DA solution (25 µM) (Cayman Chemical®) was added to each well and incubated for 30 minutes at 37ºC. After this period, the supernatant was discarded, and the wells were washed once with PBS. For the reading, 200 µL of PBS were added to each well and were read in the Synergy 4 plate reader (BioTek, VT, USA) with 485 nm excitation and 520 nm emission

2.5 Superoxide quantification

Superoxide quantification is performed using the DHE (Dihydroethidium) probe. When oxidized by the O2•- anion, the probe is converted into 2-hydroxyethidium (2-OH-E+), a fluorescent compound read at 500-530 nm of excitation and 590-620 nm of emission(LAURINDO; FERNANDES; SANTOS, 2008). To perform this assay, after plating and incubating the cells, the supernatant containing the plasma and treatments was discarded for later addition of PBS in the wells. Then, 20 µM of DHE reagent (Sigma-Aldrich, MO, USA) was added to the wells for 30 min at 37°C in 5% CO2. The evaluation of superoxide production was quantified by measuring the fluorescence using the Synergy 4 microplate reader (BioTek, VT, USA) at 518 nm of excitation and 620 nm of emission.

2.6 Antioxidant Capacity

The antioxidant response of the samples was evaluated using the Ferric Reducing Antioxidant Power (FRAP) (ability to reduce iron Fe3+ to Fe2+) in the cell culture supernatant (BENZIE; STRAIN, 1996). For this assay, 80 µL of the samples were pipetted into test tubes containing the working solution consisting of acetate buffer (pH 3.6), 10 mM of 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ), and 20 mM of ferric chloride (FeCl3.6H2O). The tubes were then incubated at 37°C for 15 minutes. After the incubation period, the samples, along with a standard curve of ferrous sulfate

(Fe2SO4.7H2O), were transferred to transparent plates for measurement using the Synergy 4 microplate reader (BioTek, VT, USA) at an absorbance of 593 nm.

2.7 MDA quantification

Malondialdehyde (MDA) is an end product of lipid peroxidation. Therefore, is an indicator of lipid degradation by oxidizing agents, such as reactive oxygen species. For this assay, 500 µL of a pre-homogenized working solution containing thiobarbituric acid (TBARS) (6.7 mg/mL) (BIRD; DRAPER, 1984), trichloroacetic acid (150 mg/mL), and hydrochloric acid (2 M) was combined with 200 µL of the cell culture supernatant in 1.5 mL tubes. The tubes were subsequently shaken and centrifuged at 3500 rpm for 10 minutes. The resulting supernatant was collected and transferred to a water bath, where it was covered with aluminum foil and incubated for 45 minutes. Following incubation, 200 µL of this content was pipetted into transparente plates, and the absorbance was measured using a plate reader at 532 nm and 600 nm for subsequent correction.

2.8 Shear Stress

To assess the impact of shear stress on endothelial cells, EC were cultured and seeded on modified 100 mm culture plates. These plates had a 60 mm plate attached to the center and had been previously sterilized. The culture plates were maintained at 37°C with 5% CO2 until the cells adhered and reached the desired confluence. Subsequently, laminar fluid flow was applied to the culture plates using the AO-330D shaking equipment (Gehaka, São Paulo – SP, Brazil) at a predetermined rotation frequency. This generated a constant shear stress ranging from 6-40 dynes/cm² (equivalent to rotations per second: 199 rpm), which represented physiological conditions. After 48 hours in the shaker, the cells were incubated with the PE plasma and rotated again for 24 hours, totaling 72 hours of rotation. EGCG was added in the last 60 minutes. After this period, the NO production was assessed by detecting nitrite

levels in the culture supernatant. Additionally, an iron reduction and MDA quantification assays (as described above) was conducted to analyze the antioxidant capacity of the supernatant.

2.9 Nitrite supernatant quantification

The measurement of nitrite production serves as an indirect indicator of NO production. This assessment involves the conversion of sulfanilic acid to a diazonium salt through its reaction with nitrite in an acidic solution. The formed salt couples with N-(1-naphthyl)ethylenediamine, resulting in the generation of a colored compound proportional to the nitrite content in the sample. For this evaluation, the Griess Reagent kit (Invitrogen, Thermo Fisher Scientific, California, USA) was employed following the manufacturer's instructions. In a clear 96-well plate, 75 µL of the sample was combined with the necessary reagents (10 µL) for the aforementioned reaction. Additionally, a nitrite standard curve was prepared and measured using a spectrophotometer at an absorbance of 548 nm.

2.10 Statistical analyzes

Replicates of five per group, combined with treatments (plasma, EGCG, and inhibitors), were performed in each experiment. To compare two groups, we performed multiple t-tests or Mann-Whitney test for non-parametric values. When comparing three or more groups we used one-way ANOVA followed by the Dunnet’s multiple comparisons test or Kruskall Wallis test followed by the Dunn’s multiple comparisons test for nonparametric values. Results are expressed in means ± SEM. Statistical analyses were performed using GraphPad Prism (GraphPad Software, San Diego, CA, USA) and for all tests, a p-value ≤ 0.05 (two-tailed) was considered significant.

3. RESULTS

3.1 EGCG increases NO levels in PE group via PI3K and eNOS

EC incubated with PE plasma produced less NO than cells incubated with HP plasma and EGCG treatment recovered these levels in the first group (Fig. 1A). In addition, the inhibitor of NOS, L-NAME and PI3K protein, LY294002, reduced the EGCG action (Fig. 1B).

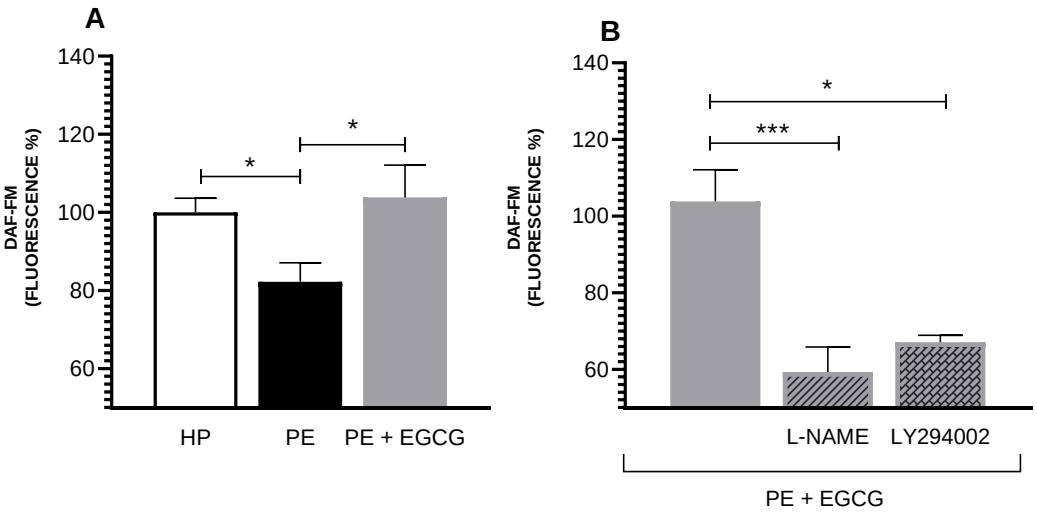

Figure 1. Intracellular Nitric Oxide Levels. NO fluorescence intensity was measured by DAF-FM**.** (A) The PE group had lower NO levels when compared to the HP group (82.26 *vs* 100.00). The EGCG treatment recovered these levels (82.26 *vs* 103.83). (B) By adding L-NAME and LY294002, NOS and PI3K inhibitors respectively, the action of EGCG was reduced (103.83 *vs* 59.32 *vs* 67.23). NO levels are presented in fluorescence percentage based on the HP group. Data are presented as mean ± SEM and comparisons between groups were assessed by Kruskal-Wallis test followed by Dunn's Multiple Comparison Test. *$p < 0.05$; ***$p < 0.001$.

3. 2 EGCG does not alter ROS and O2•- levels

Our data show that the PE group exhibited lower levels of ROS (Fig. 2A) and similar O2•- levels in comparison to the HP group (Fig. 2B), while EGCG treatment did not affect these parameters. L-NAME and LY294002 also did not change the ROS and O2•- values (data not shown).

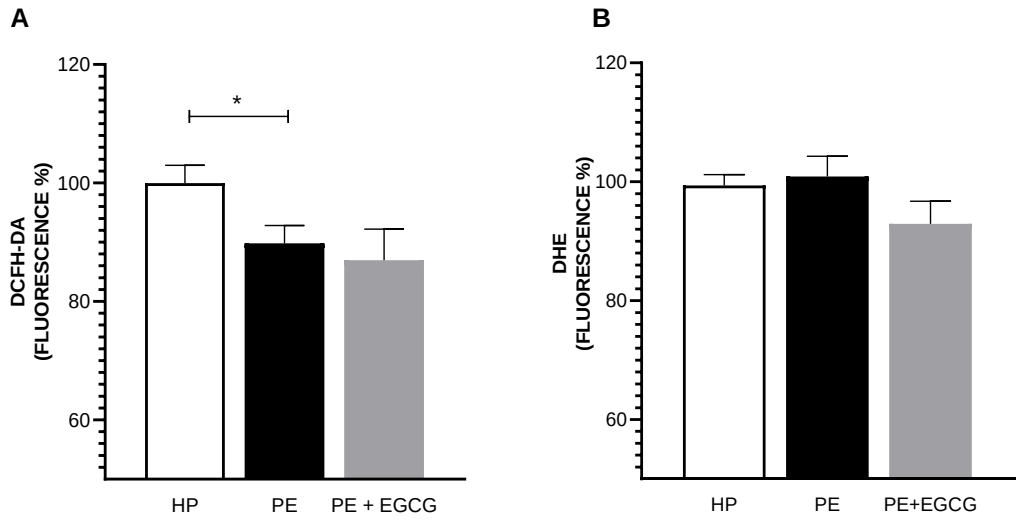

Figure 2. Levels of total ROS and O2•-. Total ROS was measured by DCFH-DA and O2•- by DHE. (**A)** The reactive oxygen species levels in the PE group were lower when compared to HP group (89.83 *vs* 100.00) and the EGCG treatment did not change these levels (89.83 *vs* 86.99). (B) Superoxide levels were similar in all groups (99.36 *vs* 100.87 *vs* 92.93). ROS and O2•- levels are presented in fluorescence percentage based on the HP group. Data are presented as mean ± SEM and comparisons between groups were assessed by (A) One-Way ANOVA test followed by Dunnet's Multiple Comparison Test and (B) Kruskal-Wallis test followed by Dunn's Multiple Comparison Test. *$p < 0.05$.

3.3 EGCG increases antioxidant potential and attenuates lipid peroxidation

The antioxidant potential between the HP and PE group are similar. However, EGCG considerably increases these levels in the PE group (Fig. 3A). The same is observed in MDA levels, showing that the treatment decreases the levels of this lipid peroxidation product (Fig. 3C). L-NAME did not change the action of EGCG in any of the cases, but LY294002 attenuates the phytochemical action in the antioxidant action (Fig. 3B) and, although there was no statistical difference, we observed the same pattern in MDA levels (Fig. 3D).

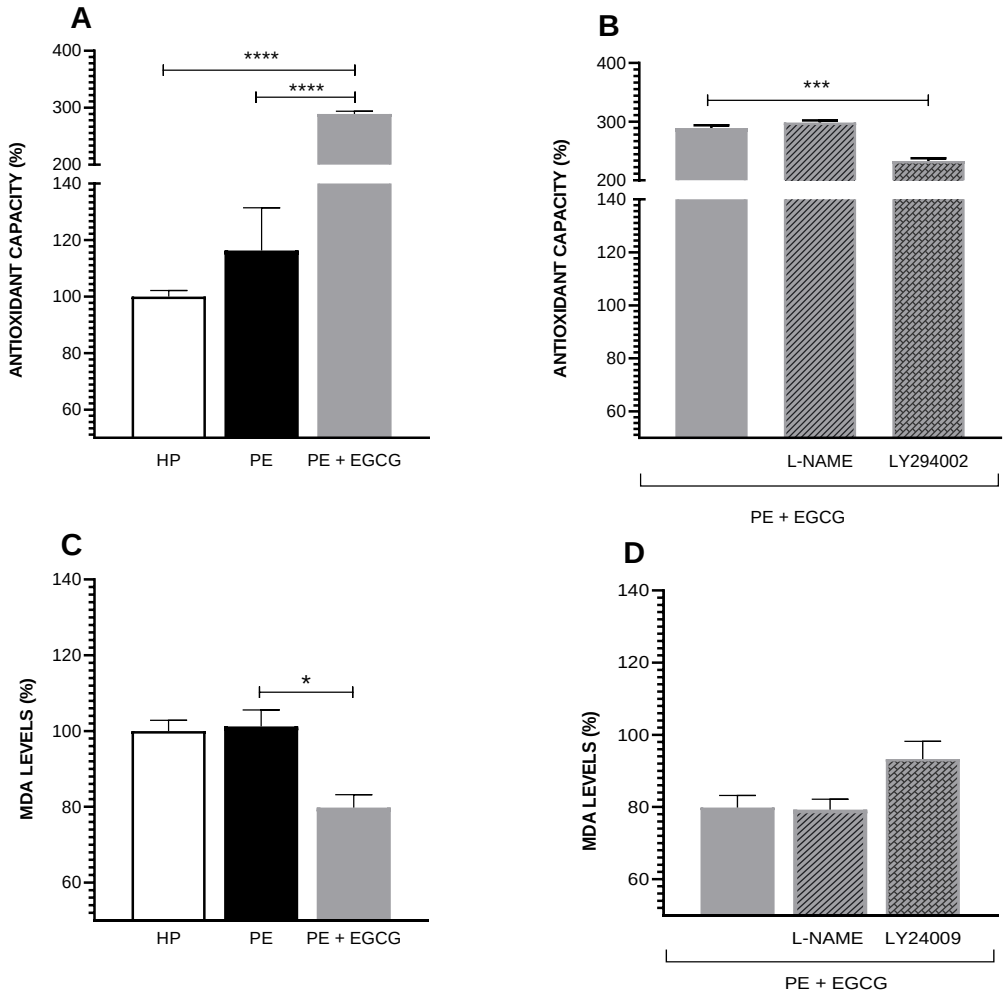

Figure 3. Antioxidant capacity and MDA levels. The antioxidant capacity was measured by FRAP assay and MDA levels by TBARS (A) The antioxidant potential is similar between HP and PE groups (100.00 *vs* 116.31), and the EGCG treatment increases this potential in PE (116.32 *vs* 289.23). (B) LY294002 but not L-NAME attenuated the EGCG action (232.83 *vs* 298.55 *vs* 289.23). The same pattern was observed in the MDA assay: (C) similar levels were observed between HP and PE group (100.00 *vs* 101.42), with the decrease in MDA levels in PE after EGCG treatment (101.42 *vs* 79.83). (D) Although there is no statistical difference, we noticed that LY294002 slightly reduces the EGCG action on this parameter (93.30 *vs* 79.83) The results are presented in absorbance percentage based on the HP group. Data are presented as mean ± SEM and comparisons between groups were assessed by one-way ANOVA test followed by Dunnet's Multiple Comparison Test. *$p < 0.05$; ***$p < 0.001$; **** $p < 0.0001$.

3.4 **EGCG increases nitrite levels and have antioxidant activity in shear stress**

To investigate whether shear stress interferes on our treatment, we evaluated the nitrite levels in the supernatant of cells treated with plasma from PE patients with or without EGCG and subjected to shear stress. Our results showed that EGCG significantly increased nitrite levels compared to the untreated PE group (Fig. 4A).

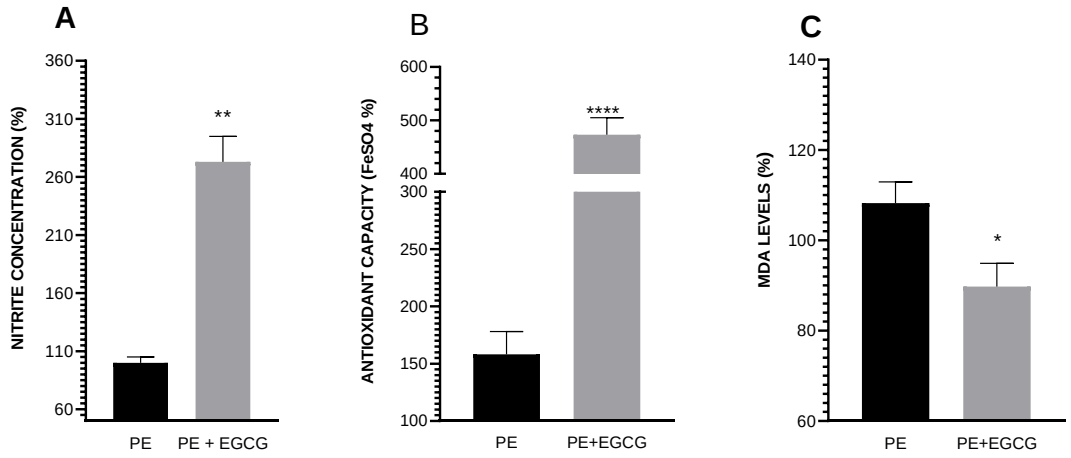

Figure 4. Nitrite levels, antioxidant activity and MDA levels in cell culture supernatant exposed to shear stress. EC were exposed to shear stress for 72 hours, with PE plasma in the last 24 hours and in the presence or absence of EGCG in the last 60 minutes. Then, the supernatant was collected for Griess, FRAP and TBARS assay. (A) The nitrite level was higher in the group treated with EGCG (100.00 *vs* 273.09) (B) Antioxidant capacity was also higher in the same group while (158.28 *vs* 473.22) (C) MDA levels were lower in the EGCG group (108.26 *vs* 89.79). The results are presented in absorbance percentage based on the HP group and data are presented as mean ± SEM. Comparisons between groups were assessed by unpaired t test *$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$.

Regarding the antioxidant action of EGCG, we observed that it remained effective even in cells exposed to shear stress. As shown in figure 4B, the antioxidant capacity of the group treated with EGCG is higher compared to the untreated group. Additionally, the phytochemical also reduced MDA levels in the treated group (Fig. 4C).

4 DISCUSSION

In this work, we observed that EGCG increased NO levels in EC treated with PE plasma. Furthermore, our treatment increasing antioxidant capacity and decreasing MDA levels in this same group. This is one of the few works that investigate the action of EGCG in the PE field and the only one in this *in vitro* model, also considering the action of *shear stress* on cells.

Lower levels of NO in the organisms of pregnant women with PE have already been described in several studies (LOWE, 2000; SELIGMAN et al., 1994; SUTTON; GEMMEL; POWERS, 2020). The decrease of this vasodilator contributes to the characteristic hypertension of the disease (BERNARDI et al., 2008; JOHAL et al., 2014; LOWE, 2000). Our results are in line with these works, showing that endothelial cells incubated with PE plasma have lower levels of intracellular NO compared to cells incubated with HP plasma. Moreover, treatment with EGCG restores these levels in the PE group.

It is known that this phytochemical has an important action on eNOS, the enzyme responsible for the NO production (GAO et al., 2022; LORENZ et al., 2015). By activating the PI3K/Akt/eNOS pathway, leading to the enzyme activation, EGCG is related to increase in NO production and bioavailability (KIM et al., 2007; LORENZ et al., 2004; ZHANG; ZHANG, 2020). To investigate the mechanism of EGCG on this pathway, we used the NOS inhibitor (L-NAME) and the PI3K protein inhibitor (LY294002) with our treatment. The results demonstrated that these inhibitors considerably reduced the action of the phytochemical, demonstrating the importance of this pathway in the EGCG action on the NO bioavailability.

A limited number of studies have explored the mechanism preceding PI3K activation. However, one particular study has proposed the involvement of Src family proteins in this progression (KIM et al., 2007). Upon closer examination, the study reached the conclusion that the Fyn protein, among the eleven members constituting this protein family, assumes an essential role in EGCG-triggered activation of the PI3K/Akt/eNOS pathway, since inhibiting the Fyn protein through siRNA, eNOS phosphorylation was also inhibited. Furthermore, this investigation suggested that the PI3K activation could occur both by direct binding of Fyn in its p85 regulatory domain,

and by phosphorylation of intracellular substrates, mainly Gab-1, which binds and activates PI3K (KIM et al., 2007).

In addition to the NO decrease, some studies point to an increase in free radicals such as ROS and O2• in PE (CHIARELLO et al., 2020; ROBERTS; GAMMILL, 2005; TAYSI et al., 2019). However, these results are controversial and similar levels of oxidative stress markers have already been found in the plasma of pregnant women with PE (BOWEN et al., 2001; GOMES et al., 2013). Likewise, when evaluating ROS and O2•- levels, our results demonstrate that O2•- levels are similar between the HP and PE groups and the total ROS levels are even lower in the PE group when compared to the HP group, while EGCG does not change these levels. In this way, we hypothesize the presence of a compensatory mechanism responding to oxidative stress, since previous findings by our research team showed similar or even increased levels of antioxidant potential in the PE group when compared to the HP group (BUENO-PEREIRA et al., 2022; VIANA-MATTIOLI et al., 2020). This work is in agreement with these results and similar levels of this parameter were found between the two groups. In addition, EGCG considerably increases this response, given its significant antioxidant properties.

Oxidative stress is also responsible for lipid peroxidation, the oxidation of polyunsaturated fatty acids that results in the formation of reactive molecules such as MDA (GUPTA et al., 2009; HUBEL et al., 1989; NEGRE-SALVAYRE et al., 2022). Lipid peroxidation has been associated with the pathogenesis of PE, while increased levels of MDA have been described in the placenta and blood of both the mother and baby (NEGRE-SALVAYRE et al., 2022). Conversely, some studies suggest that lipid peroxidation is not increased in patients with PE (DIEDRICH et al., 2001; GUPTA et al., 2009; REGAN et al., 2001), a finding that aligns with our own analyses. Similar MDA levels between the PE and HP groups were observed, consistent with the other

oxidative stress experiments mentioned earlier. Our treatment, however, considerably reduced the levels of this aldehyde in the PE group.

The L-NAME did not change the EGCG action on the antioxidant activity and MDA levels. However, the use of LY294002 decreased the phytochemical effects. When researching alternative pathways to PI3K/AKT/eNOS that could be involved in the EGCG antioxidant effects, we found some studies that report the action of the nuclear factor Nrf2 (LI et al., 2018; NA et al., 2008; ZHANG et al., 2021). Some groups point out that EGCG can activate this factor directly or via PI3K, which would explain the decrease in the EGCG antioxidant action after the use of LY294002 (NA et al., 2008; ZHANG et al., 2021). After activation, Nrf2, in the nucleus, binds to the antioxidant response element (ARE) making the transcription of several antioxidant genes (LI et al., 2018). Nevertheless, further investigations regarding the role of EGCG in this pathway should be performed. An overview of all the mechanisms discussed is presented in Figure 5.

PE is a disease exclusive to human pregnancy; therefore, the use of animals as experimental models has its limitations. In addition, because it is a multifactorial disease and has a complex pathophysiology, *in vitro* models also do not represent the real disease environment. For example, in blood vessels shear stress modulates arterial function in endothelial cells. The action of laminar shear stress, unidirectional and constant, has a protective effect on cells, regulating eNOS and coagulation, promoting vasodilation and favoring antioxidant action (VIANA-MATTIOLI et al., 2023).

Despite the limited number, studies indicate that the action of shear stress interferes in PE context. One study showed a lower release of NO mediated by shear stress in myometrial arteries of pregnant women with PE when compared to normotensive pregnant women (KUBLICKIENE et al., 2000), while *in vitro* studies

pointed out that the application of shear stress had an effect on the production of nitrite, increasing the levels of this substance both in the supernatant of cells incubated with PE plasma (BAKER et al., 1996) and in endothelial cells from the decidua of pregnant women with PE (ROWE; CAMPBELL; GALLERY, 2003)

With the goal of replicating the effects of shear stress experienced by our blood vessels, we applied a rotational force to simulated unidirectional blood flow and the resulting mechanical stress on endothelial cells (DELA PAZ et al., 2012; GOMES et al., 2020). We observed that EGCG exhibited consistent effects even under these conditions, increasing nitrite levels in the supernatant of cells incubated with PE plasma (at levels even higher than the increase of NO in the static culture) and the antioxidant capacity of the PE group treated with EGCG, while decreasing MDA levels in the same group.

Nowadays, several research groups have focused on plant-derived and natural compounds for treating and preventing PE (BUENO-PEREIRA et al., 2022; CALDEIRA-DIAS et al., 2019; VIANA-MATTIOLI et al., 2020). EGCG, the primary compound found in green tea, has demonstrated beneficial properties in the context of cardiovascular and metabolic diseases (LIU et al., 2017; YAMAGATA, 2020; YAMAGATA et al., 2015). Despite limited research on the action of EGCG in PE, the existing studies show promising results (GAO et al., 2022). In one study, the administration of EGCG capsules to PE patients using hydralazine, an antihypertensive drug for controlling hypertensive crises, reduced the necessary doses of the drug, as well as the interval between doses (SHI et al., 2018). Additionally, an *in vitro* study of EC exposed to hypoxia, demonstrated that EGCG improved endothelial dysfunction parameters and the anti-angiogenic state (ZHONG et al., 2020).

Some limitations of the study include the concentration of EGCG used *in vitro*, higher than that available in the pregnant women organism if they consumed EGCG. Regarding this, a study showed that 1 µM of the phytochemical was responsible for causing vasodilation in the aortic rings of rats (LORENZ et al., 2004), in addition to the clinical study that showed that EGCG capsules consumed by PE pregnant women had an effect in clinical practice (SHI et al., 2018). Furthermore, the consumption of teas during pregnancy should be considered with caution. Some teas, including green tea, have caffeine and are not recommended during pregnancy. A link with green tea and premature births has already been reported (SEBASTIANI et al., 2022). This, however, does not exclude the isolated EGCG beneficial and safe effects.

In summary, our results indicate that both in static culture and in cells subjected to shear stress, EGCG improves parameters of endothelial function and attenuates oxidative stress in cells exposed to the plasma of PE pregnant women.

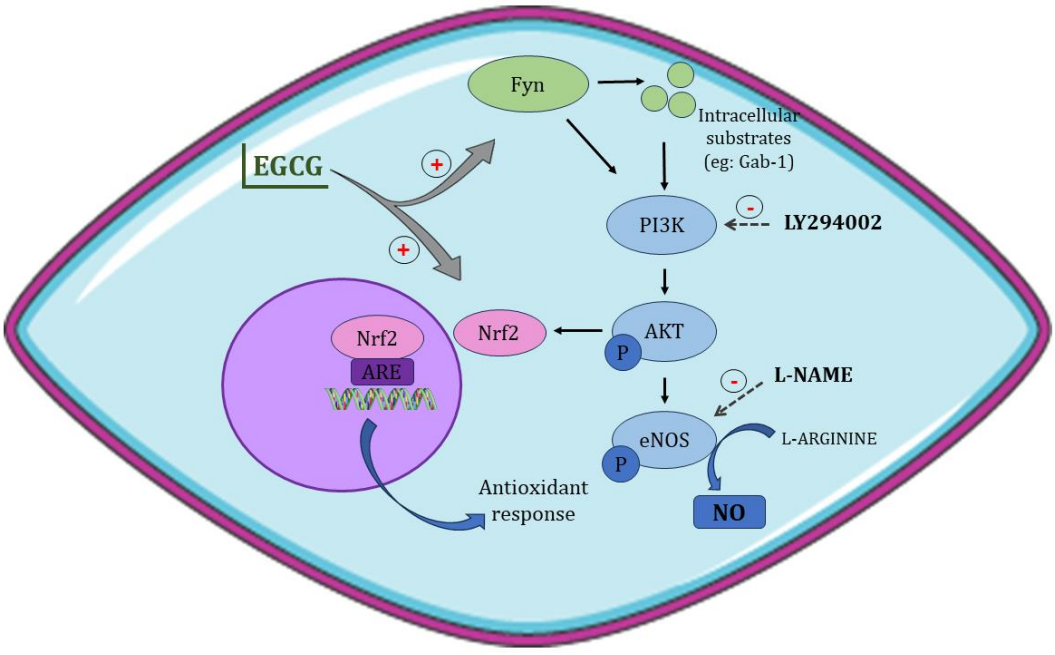

Figure 5. Action mechanisms of EGCG on endothelial cell. By triggering the Fyn protein activation, EGCG initiates the PI3K/Akt/eNOS pathway. This cascade involves a series of phosphorylation events, starting with Akt phosphorylation and culminating in eNOS activation. Consequently, this sequence leads to an increase in NO production. Furthermore, EGCG also triggers the activation of the Nrf2 factor,

influencing the antioxidant response. This is achieved through the interaction of Nrf2 with ARE, which subsequently stimulates the synthesis of numerous antioxidant enzymes

CONCLUSION

Our analyze show that EGCG increases NO levels through the activation of the PI3K/AKT/eNOS pathway, enhances antioxidant activity, and reduces lipid peroxidation in cells incubated with PE plasma. These beneficial effects are also observed in cells exposed to shear stress. Thus, we suggest that EGCG acts on endothelial function, offering potential therapeutic insights for PE treatment.

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Interest conflicts

Authors have no conflicts of interest to declare.

REFERENCES

BACHETTI, T.; MORBIDELLI, L. Endothelial cells in culture: A model for studying vascular functions. **Pharmacological Research**, 2000.

BAKER, P. N. et al. Mechanical stress eliminates the effects of plasma from patients with preeclampsia on endothelial cells. **American Journal of Obstetrics and Gynecology**, v. 174, n. 2, p. 730–736, fev. 1996.

BENZIE, I. F. F.; STRAIN, J. J. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of “Antioxidant Power”: The FRAP Assay. **Analytical Biochemistry**, v. 239, n. 1, p. 70–76, jul. 1996.

BERNARDI, F. et al. Plasma nitric oxide, endothelin-1, arginase and superoxide dismutase in pre-eclamptic women. **Journal of Obstetrics and Gynaecology Research**, 2008.

BIRD, R. P.; DRAPER, H. H. [35] Comparative studies on different methods of malonaldehyde determination. In: [s.l: s.n.]. p. 299–305.

BOWEN, R. S. et al. Oxidative stress in pre-eclampsia. **Acta Obstetricia et Gynecologica Scandinavica**, v. 80, n. 8, p. 719–725, ago. 2001.

BROWN, M. A. et al. **Hypertensive disorders of pregnancy: ISSHP classification, diagnosis, and management recommendations for international practice**. **Hypertension**, 2018.

BUENO-PEREIRA, T. O. et al. Markers of Endothelial Dysfunction Are Attenuated by Resveratrol in Preeclampsia. **Antioxidants**, v. 11, n. 11, p. 2111, 26 out. 2022.

CALDEIRA-DIAS, M. et al. Preeclamptic plasma stimulates the expression of miRNAs, leading to a decrease in endothelin-1 production in endothelial cells. **Pregnancy Hypertension**, 2018.

CALDEIRA-DIAS, M. et al. Resveratrol improves endothelial cell markers impaired by plasma incubation from women who subsequently develop preeclampsia. **Hypertension Research**, 2019.

CHIARELLO, D. I. et al. Oxidative stress: Normal pregnancy versus preeclampsia. **Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease**, v. 1866, n. 2, p. 165354, fev. 2020.

CROW, J. P. Dichlorodihydrofluorescein and Dihydrorhodamine 123 Are Sensitive Indicators of Peroxynitritein Vitro:Implications for Intracellular Measurement of Reactive Nitrogen and Oxygen Species. **Nitric Oxide**, v. 1, n. 2, p. 145–157, abr. 1997.

DELA PAZ, N. G. et al. Role of shear-stress-induced VEGF expression in endothelial cell survival. **Journal of Cell Science**, v. 125, n. 4, p. 831–843, 15 fev. 2012.

GAO, X. et al. The Efficacy Mechanism of Epigallocatechin Gallate against Pre-Eclampsia based on Network Pharmacology and Molecular Docking. **Reproductive Sciences**, v. 29, n. 6, p. 1859–1873, 24 jun. 2022.

Gestational Hypertension and Preeclampsia: ACOG Practice Bulletin, Number 222. **Obstetrics and gynecology**, 2020.

GOMES, A. M. et al. Wortmannin targeting phosphatidylinositol 3-kinase suppresses angiogenic factors in shear-stressed endothelial cells. **Journal of Cellular Physiology**, v. 235, n. 6, p. 5256–5269, 19 jun. 2020.

GOMES, H. F. et al. Assessment of oxidative status markers and NO bioavailability in hypertensive disorders of pregnancy. **Journal of Human Hypertension**, v. 27, n. 6, p. 345–348, 10 jun. 2013.

GUPTA, S. et al. Lipid Peroxidation and Antioxidant Status in Preeclampsia. **Obstetrical & Gynecological Survey**, v. 64, n. 11, p. 750–759, nov. 2009.

HAYAT, K. et al. Tea and Its Consumption: Benefits and Risks. **Critical Reviews in Food Science and Nutrition**, v. 55, n. 7, p. 939–954, 7 jun. 2015.

HUBEL, C. A. et al. Lipid peroxidation in pregnancy: New perspectives on preeclampsia. **American Journal of Obstetrics and Gynecology**, v. 161, n. 4, p. 1025–1034, out. 1989.

JOHAL, T. et al. The nitric oxide pathway and possible therapeutic options in pre-eclampsia. **British Journal of Clinical Pharmacology**, 2014.

KIM, J. A. et al. Epigallocatechin gallate, a green tea polyphenol, mediates NO-dependent vasodilation using signaling pathways in vascular endothelium requiring reactive oxygen species and fyn. **Journal of Biological Chemistry**, 2007.

KOJIMA, H. et al. Development of a Fluorescent Indicator for Nitric Oxide Based on the Fluorescein Chromophore. **Chemical and Pharmaceutical Bulletin**, v. 46, n. 2, p. 373–375, 1998.

KUBLICKIENE, K. R. et al. Preeclampsia: Evidence for impaired shear stress–mediated nitric oxide release in uterine circulation. **American Journal of Obstetrics and Gynecology**, v. 183, n. 1, p. 160–166, jul. 2000.

LAURINDO, F. R. M.; FERNANDES, D. C.; SANTOS, C. X. C. Assessment of Superoxide Production and NADPH Oxidase Activity by HPLC Analysis of Dihydroethidium Oxidation Products. In: [s.l: s.n.]. p. 237–260.

LI, H. et al. Neuroprotective effect of phosphocreatine on oxidative stress and mitochondrial dysfunction induced apoptosis in vitro and in vivo: Involvement of dual PI3K/Akt and Nrf2/HO-1 pathways. **Free Radical Biology and Medicine**, v. 120, p. 228–238, maio 2018.

LIU, S. et al. EGCG protects against homocysteine-induced human umbilical vein endothelial cells apoptosis by modulating mitochondrial-dependent apoptotic signaling and PI3K/Akt/eNOS signaling pathways. **Apoptosis**, 2017.

LORENZ, M. et al. A Constituent of Green Tea, Epigallocatechin-3-gallate, Activates Endothelial Nitric Oxide Synthase by a Phosphatidylinositol-3-OH-kinase-, cAMP-dependent Protein Kinase-, and Akt-dependent Pathway and Leads to Endothelial-dependent Vasorelaxation. **Journal of Biological Chemistry**, 2004.

LORENZ, M. et al. Endothelial NO Production Is Mandatory for Epigallocatechin-3-Gallate-induced Vasodilation: Results from eNOS Knockout (eNOS-/-) Mice. **Journal of Cardiovascular Pharmacology**, 2015.

LOWE, D. T. Nitric oxide dysfunction in the pathophysiology of preeclampsia. **Nitric Oxide - Biology and Chemistry**, 2000.

NA, H.-K. et al. (−)-Epigallocatechin gallate induces Nrf2-mediated antioxidant enzyme expression via activation of PI3K and ERK in human mammary epithelial cells. **Archives of Biochemistry and Biophysics**, v. 476, n. 2, p. 171–177, ago. 2008.

NEGRE-SALVAYRE, A. et al. Oxidative stress, lipid peroxidation and premature placental senescence in preeclampsia. **Archives of Biochemistry and Biophysics**, v. 730, p. 109416, nov. 2022.

RANA, S. et al. Preeclampsia: Pathophysiology, Challenges, and Perspectives. **Circulation Research**, 2019.

ROBERTS, J. M. et al. Preeclampsia: An endothelial cell disorder. **American Journal of Obstetrics and Gynecology**, 1989.

ROBERTS, J. M.; GAMMILL, H. S. **Preeclampsia: Recent insights**. **Hypertension**, 2005.

ROWE, J.; CAMPBELL, S.; GALLERY, E. D. M. Nitric Oxide Production by Decidual Endothelial Cells is not Reduced in Preeclampsia. **Hypertension in Pregnancy**, v. 22, n. 1, p. 63–75, 7 jan. 2003.

SANDRIM, V. C. et al. Nitric oxide formation is inversely related to serum levels of antiangiogenic factors soluble fms-like tyrosine kinase-1 and soluble endogline in preeclampsia. **Hypertension**, 2008.

SANKARALINGAM, S.; XU, H.; DAVIDGE, S. T. Arginase contributes to endothelial cell oxidative stress in response to plasma from women with preeclampsia. **Cardiovascular Research**, 2010.

SEBASTIANI, G. et al. Effects of Antioxidant Intake on Fetal Development and Maternal/Neonatal Health during Pregnancy. **Antioxidants**, v. 11, n. 4, p. 648, 28 mar. 2022.

SELIGMAN, S. P. et al. The role of nitric oxide in the pathogenesis of preeclampsia. **American Journal of Obstetrics and Gynecology**, 1994.

SHI, D. D. et al. Epigallocatechin gallate enhances treatment efficacy of oral nifedipine against pregnancy-induced severe pre-eclampsia: A double-blind, randomized and placebo-controlled clinical study. **Journal of Clinical Pharmacy and Therapeutics**, 2018.

SIDDIQUI, N.; HLADUNEWICH, M. **Understanding the Link Between the Placenta and Future Cardiovascular Disease**. **Trends in Cardiovascular Medicine**, 2011.

STAFF, A. C. **The two-stage placental model of preeclampsia: An update**. **Journal of Reproductive Immunology**, 2019.

SUGANYA, N. et al. Reversibility of endothelial dysfunction in diabetes: Role of polyphenols. **British Journal of Nutrition**, 2016.

SUTTON, E. F.; GEMMEL, M.; POWERS, R. W. **Nitric oxide signaling in pregnancy and preeclampsia**. **Nitric Oxide - Biology and Chemistry**, 2020.

TAYLOR, R. et al. Circulating Factors as Markers and Mediators of Endothelial Cell Dysfunction in Preeclampsia. **Seminars in Reproductive Medicine**, v. 16, n. 01, p. 17–31, 15 mar. 1998.

TAYSI, S. et al. Radicals, Oxidative/Nitrosative Stress and Preeclampsia. **Mini-Reviews in Medicinal Chemistry**, v. 19, n. 3, p. 178–193, 11 jan. 2019.

UKAH, U. V. et al. **Prediction of adverse maternal outcomes from pre-eclampsia and other hypertensive disorders of pregnancy: A systematic review**. **Pregnancy Hypertension**, 2018.

VIANA-MATTIOLI, S. et al. SIRT1-dependent effects of resveratrol and grape juice in an in vitro model of preeclampsia. **Biomedicine and Pharmacotherapy**, 2020.

VIANA-MATTIOLI, S. et al. Missing links in preeclampsia cell model systems of endothelial dysfunction. **Trends in Molecular Medicine**, v. 29, n. 7, p. 541–553, jul. 2023.

YAMAGATA, K. et al. Epigallocatechin-3-gallate inhibits VCAM-1 expression and apoptosis induction associated with LC3 expressions in TNFα-stimulated human endothelial cells. **Phytomedicine**, 2015.

YAMAGATA, K. Polyphenols Regulate Endothelial Functions and Reduce the Risk of Cardiovascular Disease. **Current Pharmaceutical Design**, 2019.

YAMAGATA, K. **Protective Effect of Epigallocatechin Gallate on Endothelial Disorders in Atherosclerosis**. **Journal of cardiovascular pharmacology**, 2020.

YAMAGATA, K.; TAGAMI, M.; YAMORI, Y. **Dietary polyphenols regulate endothelial function and prevent cardiovascular disease**. **Nutrition**, 2015.

ZHANG, Q. et al. Activation of Nrf2/HO-1 signaling: An important molecular mechanism of herbal medicine in the treatment of atherosclerosis via the protection of vascular endothelial cells from oxidative stress. **Journal of Advanced Research**, v. 34, p. 43–63, dez. 2021.

ZHANG, Z.; ZHANG, D. (-)-epigallocatechin-3-gallate inhibits eNOS uncoupling and alleviates high glucose-induced dysfunction and apoptosis of human umbilical vein endothelial cells by PI3K/AKT/eNOS pathway. **Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy**, 2020.

ZHAO, T. et al. Green Tea (Camellia sinensis): A Review of Its Phytochemistry, Pharmacology, and Toxicology. **Molecules**, v. 27, n. 12, p. 3909, 18 jun. 2022.

ZHONG, M. et al. Epigallocatechin gallate (EGCG) improves anti-angiogenic state, cell viability, and hypoxia-induced endothelial dysfunction by downregulating high mobility group Box 1 (HMGB1) in preeclampsia. **Medical Science Monitor**, 2020.

DISCUSSÃO

Nossos resultados mostraram que a EGCG atua na função endotelial aumentando os níveis de NO diminuídos no grupo PE, além de aumentar a capacidade antioxidante desse grupo e atenuar os níveis de MDA. O aumento de NO pelo fitoquímico se dá pela ativação da via PI3K/AKT/eNOS, já que inibidores da NOS (L-NAME) e da proteína P3IK (LY249002) atenuam a ação da EGCG. Já sua ação antioxidante não se dá através dessa via, visto que L-NAME não altera a ação do tratamento sobre esse parâmetro, mas LY249002 sim. A aplicação do *shear stress* e a investigação da ação da EGCG nas células submetidas a essa metodologia, permitiu observar que a ação do fitoquímico se manteve, já que o tratamento aumentou os níveis de nitrito no grupo PE, além de manter os níveis de atividade antioxidante aumentados e os de MDA diminuídos nesse grupo.

Outro achado importante desse estudo e de outros trabalhos do nosso grupo, são os níveis similares de EROS, O2•- e potencial antioxidante entre o grupo PE e GS (BUENO-PEREIRA et al., 2022; VIANA-MATTIOLI et al., 2020). Em uma gestação saudável espera-se um aumento de EROS e do estresse oxidativo, porém em gestantes com PE o que se observa é um estresse oxidativo exacerbado (CHIARELLO et al., 2020). Todavia, hipotetizamos a presença de um mecanismo compensatório atuando no organismo dessas gestantes. Com o aumento do estresse oxidativo, a resposta antioxidante no organismo dessas gestantes está aumentada, deixando os níveis de agentes oxidantes similares ao do grupo GS. Entretanto, mais investigações sobre esses parâmetros precisam ser realizadas.

Já que a PE não tem cura, estudos que buscam tratamentos e prevenções para a doença se mostram de extrema importância. A EGCG, composto do chá-verde, é um produto natural e acessível, com propriedades vasodilatadoras e antioxidantes bem

descritas, tendo efeitos benéficos na função vascular (LORENZ et al., 2004; YAMAGATA, 2020; ZHANG; ZHANG, 2020). Entretanto, os estudos dos benefícios da EGCG no contexto da PE são escassos (GAO et al., 2022; SHI et al., 2018; ZHONG et al., 2020). Por isso, o trabalho aqui apresentado e os resultados obtidos nesse estudo, contribuem para as investigações da EGCG como alvo terapêutico para a doença.

Algumas limitações, entretanto, precisam ser consideradas. A concentração da droga, por exemplo, é maior nesse estudo *in vitro* do que a quantidade obtida caso as gestantes fizessem uso do fitoquímico. Entretanto, um estudo mostrou que 1 μM de EGCG foi o suficiente para provocar a vasodilatação em anéis aórticos de ratos (LORENZ et al., 2004) e o estudo clínico da associação da EGCG com a nifedipina já citado nesse trabalho, mostrou que o tratamento tem efeitos benéficos na prática clínica (SHI et al., 2018)

O consumo de chás durante a gestante também deve ser considerado com cautela. Por possuir cafeína, o consumo de chá-verde não é recomendado para as gestantes, enquanto alguns estudos associam o consumo de chá com nascimentos prematuros (SEBASTIANI et al., 2022). Isso, entretanto, não excluí os efeitos benéficos e seguros da EGCG que pode ser encontrada de forma isolada.

Além disso, algumas dificuldades metodológicas precisam ser abordadas. A análise indireta dos níveis de NO é feita através do ensaio de nitrito no sobrenadante. No entanto, essa abordagem possui suas limitações. Alguns estudos apontam maiores níveis de nitrito no plasma de gestantes com PE (ACAUAN FILHO et al., 2016; EGERMAN et al., 1999; NOORBAKHSH; KIANPOUR; NEMATBAKHSH, 2013), o que pode interferir nos resultados da avaliação desse parâmetro. Assim, é recomendável uma avaliação direta do NO, apesar de sua rápida degradação e conversão em nitrito e nitrato. No contexto da cultura de células estáticas, a avaliação direta era simples

usando o ensaio DAF-FM em placas de 96 poços e leitura em fluorímetro. No entanto, a transferência das células das placas de petri modificadas para placas de 96 poços, após aplicação do *shear stress*, gerou interferências, já que as células endoteliais são aderentes e essa transferência exigiria que o protocolo da sonda fosse realizado nessas células em suspensão. Dessa forma, a solução encontrada foi avaliar os níveis de nitrito no sobrenadante de culturas de células submetidas ao *shear stress* como uma medida indireta de NO.

A utilização de sondas para avaliação de EROS também é muito discutida na literatura, já que a detecção de espécies específicas ou de forma isolada é um desafio. Um estudo que investiga as limitações dessas sondas, mostra que o DCFH-DA, por exemplo, pode interagir com diversas espécies, como H2O2, radical hidroxila, espécies reativas formadas da decomposição do peroxinitrito, entre outros (KALYANARAMAN et al., 2012). Dessa forma, utilizamos essa sonda para investigar a produção de EROS total. Limitações semelhantes de inespecificidade são relatadas no ensaio DHE, por isso, técnicas de confirmação mais específicas, como o HPLC ou a utilização de inibidores de espécies que possam interferir nos resultados precisam ser realizadas para melhor investigação desses parâmetros (KALYANARAMAN et al., 2012).

A inespecificidade também precisa ser considerada na avaliação dos níveis de MDA, já que o TBARS não reage unicamente com esse composto. Outros aldeídos, açúcares, aminoácidos, entre outros, podem interferir na reação final ou leitura (GOMES et al., 2013). Entretanto, devido essas limitações e desafios, tivemos a cautela de quantificar mais de um parâmetro de estresse oxidativo, obtendo resultados que se complementam e nos trazem alguma visão do efeito das nossas amostras sobre as células e ação antioxidante da EGCG.

CONCLUSÃO

Concluímos, portanto, que a EGCG aumenta os níveis de NO através da ativação da via PI3K/AKT/eNOS, além de favorecer a atividade antioxidante e reduzir a peroxidação lipídica em células incubadas com plasma PE. Esses efeitos benéficos também são observados em células expostas ao estresse de cisalhamento. Sugerimos, portanto, que a EGCG atua na função endotelial, sendo um importante alvo terapêutico para a pré-eclâmpsia.

CRÉDITOS E DISCIPLINAS

1. Disciplinas Cursadas

Disciplina	Conceito	Frequência	Créditos	Carga Horária
Bases e Atualizações em Farmacologia e Biotecnologia	A	100%	3	45
Exploração alternativa de carreiras na ciência	A	100%	1	15
Bases e Atualizações em Farmacologia e Biotecnologia	A	100%	3	45
Conceitos gerais em Estatística aplicada a modelos biológicos	B	100%	2	30
Farmacologia do Endotélio Vascular	A	100%	4	60
Pré-eclâmpsia: da bancada ao leito	A	100%	4	60
Cultura Celular Animal	A	97,8%	3	45

2. Artigos Publicados

Bertozzi-Matheus M, Bueno-Pereira TO, Viana-Mattioli S, Carlström M, Cavalli RC, Sandrim VC. Different profiles of circulating arginase 2 in subtypes of preeclampsia pregnant women. Clin Biochem. 2021 Jun;92:25-33. doi: 10.1016/j.clinbiochem.2021.03.002. Epub 2021 Mar 11. PMID: 33713637.

Bueno-Pereira TO, **Bertozzi-Matheus M**, Zampieri GM, Abbade JF, Cavalli RC, Nunes PR, Sandrim VC. Markers of Endothelial Dysfunction Are Attenuated by Resveratrol in Preeclampsia. Antioxidants (Basel). 2022 Oct 26;11(11):2111. doi: 10.3390/antiox11112111. PMID: 36358483; PMCID: PMC9686533.

Bueno-Pereira TO, Nunes PR, **Matheus MB**, Vieira da Rocha AL, Sandrim VC. Nebivolol Increases Nitric Oxide Synthase via β3 Adrenergic Receptor in Endothelial Cells Following Exposure to Plasma from Preeclamptic Patients. Cells. 2022 Mar 4;11(5):883. doi: 10.3390/cells11050883. PMID: 35269505; PMCID: PMC8909669.

3. Eventos científicos

X Simpósio de Farmacologia e Biotecnologia da UNESP, 2021

Encontro Nacional de Biomedicina (ENBM), 2021

XI Simpósio de Farmacologia e Biotecnologia da UNESP, 2022

XXVII Simpósio de Brasileiro de Fisiologia Cardiovascular, 2023

4. **Resumos em eventos**

PR, Nunes; TO, Bueno-Pereira; **MB, Matheu**s; ALV. Rocha; V. Sandrim. Glibenclamide increases nitric oxide production in huvec cultured with plasma from pregnant women with preeclampsia, 2022

Bueno-Pereira TO; **Bertozzi-Matheus M**; Nunes PR; Rocha ALV; Sansrim VC. Nebivolol mitigates endothelial dysfunction in a model of preeclampsia, 2022

Bertozzi-Matheus M; Bueno-Pereira TO; Nunes PR; Rocha ALV; Sandrim VC. EGCG, composto do chá-verde, aumenta a produção de Óxido Nítrico e a resposta antioxidante em modelo *in vitro* de Pré-Eclâmpsia, 2023

5. **Cursos**

Curso Farmacogenética/Farmacogenômica da Sociedade Brasileira de Farmacologia e Terapêutica Experimental (SBFTE), 2021

REFERÊNCIAS

ACAUAN FILHO, B. J. et al. Serum nitrate and NOx levels in preeclampsia are higher than in normal pregnancy. **Hypertension in Pregnancy**, v. 35, n. 2, p. 226–233, 2 abr. 2016.

BACHETTI, T.; MORBIDELLI, L. Endothelial cells in culture: A model for studying vascular functions. **Pharmacological Research**, 2000.

BAKER, P. N. et al. Mechanical stress eliminates the effects of plasma from patients with preeclampsia on endothelial cells. **American Journal of Obstetrics and Gynecology**, v. 174, n. 2, p. 730–736, fev. 1996.

BARROSO, W. K. S. et al. Diretrizes Brasileiras de Hipertensão Arterial – 2020. **Arquivos Brasileiros de Cardiologia**, v. 116, n. 3, p. 516–658, 3 mar. 2021.

BENZIE, I. F. F.; STRAIN, J. J. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of “Antioxidant Power”: The FRAP Assay. **Analytical Biochemistry**, v. 239, n. 1, p. 70–76, jul. 1996.

BERNARDI, F. et al. Plasma nitric oxide, endothelin-1, arginase and superoxide dismutase in pre-eclamptic women. **Journal of Obstetrics and Gynaecology Research**, 2008.

BIRD, R. P.; DRAPER, H. H. [35] Comparative studies on different methods of malonaldehyde determination. Em: [s.l: s.n.]. p. 299–305.

BROSENS, I.; ROBERTSON, W. B.; DIXON, H. G. The physiological response of the vessels of the placental bed to normal pregnancy. **The Journal of Pathology and Bacteriology**, v. 93, n. 2, p. 569–579, abr. 1967.

BROWN, M. A. et al. **Hypertensive disorders of pregnancy: ISSHP classification, diagnosis, and management recommendations for international practice**. **Hypertension**, 2018.

BUENO-PEREIRA, T. O. et al. Markers of Endothelial Dysfunction Are Attenuated by Resveratrol in Preeclampsia. **Antioxidants**, v. 11, n. 11, p. 2111, 26 out. 2022.

CALDEIRA-DIAS, M. et al. Preeclamptic plasma stimulates the expression of miRNAs, leading to a decrease in endothelin-1 production in endothelial cells. **Pregnancy Hypertension**, 2018.

CALDEIRA-DIAS, M. et al. Resveratrol improves endothelial cell markers impaired by plasma incubation from women who subsequently develop preeclampsia. **Hypertension Research**, 2019.

CHIARELLO, D. I. et al. Oxidative stress: Normal pregnancy versus preeclampsia. **Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease**, v. 1866, n. 2, p. 165354, fev. 2020.

CROW, J. P. Dichlorodihydrofluorescein and Dihydrorhodamine 123 Are Sensitive Indicators of Peroxynitritein Vitro:Implications for Intracellular Measurement of Reactive Nitrogen and Oxygen Species. **Nitric Oxide**, v. 1, n. 2, p. 145–157, abr. 1997.

DAIBER, A. et al. **New therapeutic implications of endothelial nitric oxide synthase (eNOS) function/dysfunction in cardiovascular disease**. **International Journal of Molecular Sciences**, 2019.

DELA PAZ, N. G. et al. Role of shear-stress-induced VEGF expression in endothelial cell survival. **Journal of Cell Science**, v. 125, n. 4, p. 831–843, 15 fev. 2012.

DIEDRICH, F. et al. Lipid hydroperoxides and free radical scavenging enzyme activities in preeclampsia and HELLP (hemolysis, elevated liver enzymes, and low platelet count) syndrome: No evidence for circulating primary products of lipid peroxidation. **American Journal of Obstetrics and Gynecology**, v. 185, n. 1, p. 166–172, jul. 2001.

EGERMAN, R. S. et al. Neuropeptide Y and nitrite levels in preeclamptic and normotensive gravid women. **American Journal of Obstetrics and Gynecology**, v. 181, n. 4, p. 921–923, out. 1999.

FÖRSTERMANN, U.; LI, H. **Therapeutic effect of enhancing endothelial nitric oxide synthase (eNOS) expression and preventing eNOS uncoupling**. **British Journal of Pharmacology**, 2011.

GALLO, G.; VOLPE, M.; SAVOIA, C. Endothelial Dysfunction in Hypertension: Current Concepts and Clinical Implications. **Frontiers in Medicine**, v. 8, 20 jan. 2022.

GAO, X. et al. The Efficacy Mechanism of Epigallocatechin Gallate against Pre-Eclampsia based on Network Pharmacology and Molecular Docking. **Reproductive Sciences**, v. 29, n. 6, p. 1859–1873, 24 jun. 2022.

Gestational Hypertension and Preeclampsia: ACOG Practice Bulletin, Number 222. **Obstetrics and gynecology**, 2020.

GIORDANO, J. C. et al. The Burden of Eclampsia: Results from a Multicenter Study on Surveillance of Severe Maternal Morbidity in Brazil. **PLoS ONE**, v. 9, n. 5, p. e97401, 13 maio 2014.

GOMES, A. M. et al. Wortmannin targeting phosphatidylinositol 3-kinase suppresses angiogenic factors in shear-stressed endothelial cells. **Journal of Cellular Physiology**, v. 235, n. 6, p. 5256–5269, 19 jun. 2020.

GOMES, H. F. et al. Assessment of oxidative status markers and NO bioavailability in hypertensive disorders of pregnancy. **Journal of Human Hypertension**, v. 27, n. 6, p. 345–348, 10 jun. 2013.

GUPTA, S. et al. Lipid Peroxidation and Antioxidant Status in Preeclampsia. **Obstetrical & Gynecological Survey**, v. 64, n. 11, p. 750–759, nov. 2009.

HADI, H. A. R.; CARR, C. S.; AL SUWAIDI, J. Endothelial dysfunction: cardiovascular risk factors, therapy, and outcome. **Vascular health and risk management**, v. 1, n. 3, p. 183–98, 2005.

HAYAT, K. et al. Tea and Its Consumption: Benefits and Risks. **Critical Reviews in Food Science and Nutrition**, v. 55, n. 7, p. 939–954, 7 jun. 2015.

HUBEL, C. A. et al. Lipid peroxidation in pregnancy: New perspectives on preeclampsia. **American Journal of Obstetrics and Gynecology**, v. 161, n. 4, p. 1025–1034, out. 1989.

JOHAL, T. et al. The nitric oxide pathway and possible therapeutic options in pre-eclampsia. **British Journal of Clinical Pharmacology**, 2014.

KALYANARAMAN, B. et al. **Measuring reactive oxygen and nitrogen species with fluorescent probes: Challenges and limitations**. **Free Radical Biology and Medicine**Elsevier Inc., , 1 jan. 2012.

KIM, J. A. et al. Epigallocatechin gallate, a green tea polyphenol, mediates NO-dependent vasodilation using signaling pathways in vascular endothelium requiring reactive oxygen species and fyn. **Journal of Biological Chemistry**, 2007.

KOJIMA, H. et al. Development of a Fluorescent Indicator for Nitric Oxide Based on the Fluorescein Chromophore. **Chemical and Pharmaceutical Bulletin**, v. 46, n. 2, p. 373–375, 1998.

KUBLICKIENE, K. R. et al. Preeclampsia: Evidence for impaired shear stress–mediated nitric oxide release in uterine circulation. **American Journal of Obstetrics and Gynecology**, v. 183, n. 1, p. 160–166, jul. 2000.

KUKOR, Z.; VALENT, S.; TÓTH, M. Regulation of nitric oxide synthase activity by tetrahydrobiopterin in human placentae from normal and pre-eclamptic pregnancies. **Placenta**, 2000.

LAURINDO, F. R. M.; FERNANDES, D. C.; SANTOS, C. X. C. Assessment of Superoxide Production and NADPH Oxidase Activity by HPLC Analysis of Dihydroethidium Oxidation Products. Em: [s.l: s.n.]. p. 237–260.

LIU, S. et al. EGCG protects against homocysteine-induced human umbilical vein endothelial cells apoptosis by modulating mitochondrial-dependent apoptotic signaling and PI3K/Akt/eNOS signaling pathways. **Apoptosis**, 2017.

LORENZ, M. et al. A Constituent of Green Tea, Epigallocatechin-3-gallate, Activates Endothelial Nitric Oxide Synthase by a Phosphatidylinositol-3-OH-kinase-, cAMP-dependent Protein Kinase-, and Akt-dependent Pathway and Leads to Endothelial-dependent Vasorelaxation. **Journal of Biological Chemistry**, 2004.

LORENZ, M. et al. Endothelial NO Production Is Mandatory for Epigallocatechin-3-Gallate-induced Vasodilation: Results from eNOS Knockout (eNOS-/-) Mice. **Journal of Cardiovascular Pharmacology**, 2015.

LOWE, D. T. Nitric oxide dysfunction in the pathophysiology of preeclampsia. **Nitric Oxide - Biology and Chemistry**, 2000.

MITCHELL, B. M. et al. Uncoupled Endothelial Nitric Oxide Synthase and Oxidative Stress in a Rat Model of Pregnancy-Induced Hypertension. **American Journal of Hypertension**, 2007.

MUSSATTO, S. I. Generating Biomedical Polyphenolic Compounds from Spent Coffee or Silverskin. Em: **Coffee in Health and Disease Prevention**. [s.l.] Elsevier, 2015. p. 93–106.

NEGRE-SALVAYRE, A. et al. Oxidative stress, lipid peroxidation and premature placental senescence in preeclampsia. **Archives of Biochemistry and Biophysics**, v. 730, p. 109416, nov. 2022.

NOORBAKHSH, M.; KIANPOUR, M.; NEMATBAKHSH, M. Serum Levels of Asymmetric Dimethylarginine, Vascular Endothelial Growth Factor, and Nitric Oxide Metabolite Levels in Preeclampsia Patients. **ISRN Obstetrics and Gynecology**, v. 2013, p. 1–5, 11 set. 2013.

OLIVEIRA, L. G. DE; KARUMANCHI, A.; SASS, N. Pré-eclâmpsia: estresse oxidativo, inflamação e disfunção endotelial. **Revista Brasileira de Ginecologia e Obstetrícia**, v. 32, n. 12, p. 609–616, dez. 2010.

OSOL, G.; KO, N. L.; MANDALÀ, M. Altered Endothelial Nitric Oxide Signaling as a Paradigm for Maternal Vascular Maladaptation in Preeclampsia. **Current Hypertension Reports**, v. 19, n. 10, p. 82, 23 out. 2017.

PERAÇOLI, J. C. et al. Pre-eclampsia/Eclampsia. **Revista Brasileira de Ginecologia e Obstetricia**, 2019.

POSSOMATO-VIEIRA, J. S.; KHALIL, R. A. Mechanisms of Endothelial Dysfunction in Hypertensive Pregnancy and Preeclampsia. Em: **Advances in Pharmacology**. [s.l: s.n.].

POTJE, S. R. et al. Nitric oxide donor [Ru(terpy)(bdq)NO]3+ induces uncoupling and phosphorylation of endothelial nitric oxide synthase promoting oxidant production. **Free Radical Biology and Medicine**, 2017.

RANA, S. et al. Preeclampsia: Pathophysiology, Challenges, and Perspectives. **Circulation Research**, 2019.

REGAN, C. L. et al. No evidence for lipid peroxidation in severe preeclampsia. **American Journal of Obstetrics and Gynecology**, v. 185, n. 3, p. 572–578, set. 2001.

REITER, C. E. N.; KIM, J. A.; QUON, M. J. Green tea polyphenol epigallocatechin gallate reduces endothelin-1 expression and secretion in vascular endothelial cells: Roles for AMP-activated protein kinase, Akt, and FOXO1. **Endocrinology**, 2010.

ROBERTS, J. M. et al. Preeclampsia: An endothelial cell disorder. **American Journal of Obstetrics and Gynecology**, 1989.

ROBERTS, J. M.; GAMMILL, H. S. **Preeclampsia: Recent insights**. **Hypertension**, 2005.

ROCHA-PENHA, L. et al. Myeloperoxidase in Hypertensive Disorders of Pregnancy and Its Relation with Nitric Oxide. **Hypertension**, 2017.

ROCHETTE, L. et al. **Nitric oxide synthase inhibition and oxidative stress in cardiovascular diseases: Possible therapeutic targets? Pharmacology and Therapeutics**, 2013.

ROWE, J.; CAMPBELL, S.; GALLERY, E. D. M. Nitric Oxide Production by Decidual Endothelial Cells is not Reduced in Preeclampsia. **Hypertension in Pregnancy**, v. 22, n. 1, p. 63–75, 7 jan. 2003.

RYTLEWSKI, K. et al. Effects of prolonged oral supplementation with L-arginine on blood pressure and nitric oxide synthesis in preeclampsia. **European Journal of Clinical Investigation**, 2005.

SANDRIM, V. C. et al. Nitric oxide formation is inversely related to serum levels of antiangiogenic factors soluble fms-like tyrosine kinase-1 and soluble endogline in preeclampsia. **Hypertension**, 2008.

SANKARALINGAM, S.; XU, H.; DAVIDGE, S. T. Arginase contributes to endothelial cell oxidative stress in response to plasma from women with preeclampsia. **Cardiovascular Research**, 2010.

SEBASTIANI, G. et al. Effects of Antioxidant Intake on Fetal Development and Maternal/Neonatal Health during Pregnancy. **Antioxidants**, v. 11, n. 4, p. 648, 28 mar. 2022.

SELIGMAN, S. P. et al. The role of nitric oxide in the pathogenesis of preeclampsia. **American Journal of Obstetrics and Gynecology**, 1994.

SHI, D. D. et al. Epigallocatechin gallate enhances treatment efficacy of oral nifedipine against pregnancy-induced severe pre-eclampsia: A double-blind, randomized and placebo-controlled clinical study. **Journal of Clinical Pharmacy and Therapeutics**, 2018.

SIDDIQUI, N.; HLADUNEWICH, M. **Understanding the Link Between the Placenta and Future Cardiovascular Disease**. **Trends in Cardiovascular Medicine**, 2011.

SINKEY, R. G. et al. Prevention, Diagnosis, and Management of Hypertensive Disorders of Pregnancy: a Comparison of International Guidelines. **Current Hypertension Reports**, v. 22, n. 9, p. 66, 27 set. 2020.

STAFF, A. C. **The two-stage placental model of preeclampsia: An update**. **Journal of Reproductive Immunology**, 2019.

SUGANYA, N. et al. Reversibility of endothelial dysfunction in diabetes: Role of polyphenols. **British Journal of Nutrition**, 2016.

SUTTON, E. F.; GEMMEL, M.; POWERS, R. W. **Nitric oxide signaling in pregnancy and preeclampsia**. **Nitric Oxide - Biology and Chemistry**, 2020.

TANG, W. J. et al. Epigallocatechin gallate preserves endothelial function by reducing the endogenous nitric oxide synthase inhibitor level. **Canadian Journal of Physiology and Pharmacology**, 2006.

TAYLOR, R. et al. Circulating Factors as Markers and Mediators of Endothelial Cell Dysfunction in Preeclampsia. **Seminars in Reproductive Medicine**, v. 16, n. 01, p. 17–31, 15 mar. 1998.

TAYSI, S. et al. Radicals, Oxidative/Nitrosative Stress and Preeclampsia. **Mini-Reviews in Medicinal Chemistry**, v. 19, n. 3, p. 178–193, 11 jan. 2019.

TURBEVILLE, H. R.; SASSER, J. M. Preeclampsia beyond pregnancy: long-term consequences for mother and child. **American Journal of Physiology-Renal Physiology**, v. 318, n. 6, p. F1315–F1326, 1 jun. 2020.

TUZCU, Z. B. et al. **Circulating endothelial cell number and markers of endothelial dysfunction in previously preeclamptic women**. American Journal of Obstetrics and Gynecology. **Anais**...2015.

UKAH, U. V. et al. **Prediction of adverse maternal outcomes from pre-eclampsia and other hypertensive disorders of pregnancy: A systematic review**. **Pregnancy Hypertension**, 2018.

VIANA-MATTIOLI, S. et al. SIRT1-dependent effects of resveratrol and grape juice in an in vitro model of preeclampsia. **Biomedicine and Pharmacotherapy**, 2020.

VIANA-MATTIOLI, S. et al. Missing links in preeclampsia cell model systems of endothelial dysfunction. **Trends in Molecular Medicine**, v. 29, n. 7, p. 541–553, jul. 2023.

XIA, Y. et al. Nitric oxide synthase generates Superoxide and nitric oxide in arginine-depleted cells leading to peroxynitrite-mediated cellular injury. **Proceedings of the National Academy of Sciences of the United States of America**, 1996.

YAMAGATA, K. et al. Epigallocatechin-3-gallate inhibits VCAM-1 expression and apoptosis induction associated with LC3 expressions in TNFα-stimulated human endothelial cells. **Phytomedicine**, 2015.

YAMAGATA, K. Polyphenols Regulate Endothelial Functions and Reduce the Risk of Cardiovascular Disease. **Current Pharmaceutical Design**, 2019.

YAMAGATA, K. **Protective Effect of Epigallocatechin Gallate on Endothelial Disorders in Atherosclerosis**. **Journal of cardiovascular pharmacology**, 2020.

YAMAGATA, K.; TAGAMI, M.; YAMORI, Y. **Dietary polyphenols regulate endothelial function and prevent cardiovascular disease**. **Nutrition**, 2015.

ZHANG, Z.; ZHANG, D. (-)-epigallocatechin-3-gallate inhibits eNOS uncoupling and alleviates high glucose-induced dysfunction and apoptosis of human umbilical vein endothelial cells by PI3K/AKT/eNOS pathway. **Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy**, 2020.

ZHAO, T. et al. Green Tea (Camellia sinensis): A Review of Its Phytochemistry, Pharmacology, and Toxicology. **Molecules**, v. 27, n. 12, p. 3909, 18 jun. 2022.

ZHONG, M. et al. Epigallocatechin gallate (EGCG) improves anti-angiogenic state, cell viability, and hypoxia-induced endothelial dysfunction by downregulating high mobility group Box 1 (HMGB1) in preeclampsia. **Medical Science Monitor**, 2020.