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Programa de Pós-Graduação em Ciências da Saúde: Ginecologia e Obstetrícia

Efeitos citoprotetores de secreções obtidas da lagarta *Lonomia obliqua* sobre células-tronco endometriais humanas: perspectivas de aplicação na síndrome de aborto gestacional recorrente

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SUMÁRIO

LISTA DE ABREVIATURAS

Bcl-2 – B-cell lymphoma 2

CIT – Centro de Informações Toxicológicas

ERK – cinases reguladas por sinal extracelular

ESCs – células-tronco endometriais

hESCs – células-tronco endometriais humanas

NADPH – fosfato de dinucleótido de nicotinamida e adenina

PGR – perda gestacional recorrente

LISTA DE FIGURAS

RESUMO

Introdução. A perda gestacional recorrente (PGR) está associada à morbidade física e psicológica grave, para a qual, quando se excluem causas anatômicas e genéticas, não há opções de tratamento. A fisiopatologia envolve a deficiência nas capacidades de proliferação e migração das células do estroma endometrial (hESCs), prejudicando a implantação e o desenvolvimento do embrião. Por isso, encontrar novas moléculas citoprotetoras capazes de estimular a proliferação de hESCs pode ser interessante. Uma vez que os venenos de animais são fontes ricas em moléculas bioativas, neste trabalho objetivamos caracterizar os efeitos citoprotetores do veneno da *Lonomia obliqua* em hESCs. **Metodologia.** hESCs foram isoladas de biópsias endometriais humanas frescas e caracterizadas de acordo com protocolos padrão. Em seguida, os efeitos das secreções venenosas de *L. obliqua* sobre a viabilidade, proliferação e migração celular foram determinados. Os componentes do veneno envolvidos nos efeitos de proliferação celular também foram identificados por métodos cromatográficos clássicos e análises proteômicas. **Resultados.** O veneno de L. *obliqua* induz a proliferação, viabilidade e migração de hESC de uma maneira dependente da dose, tanto na presença como nas condições de privação de soro. Por cromatografia de troca iônica foi obtida uma fração enriquecida em componentes citoprotetores desprovidos de efeito hemotóxico. A análise proteômica do veneno identificou pelo menos seis classes de proteínas com propriedades citoprotetoras potenciais (hemolinas, lipocalinas, hemocianinas, proteínas antivirais, peptídeos antimicrobianos e inibidores de protease). O mecanismo envolvido na citoproteção parece estar relacionado à regulação negativa da geração do ânion superóxido, aumento das enzimas antioxidantes, produção de óxido nítrico e ativação de vias de proliferação dependentes de ERK.

Conclusões. A viabilidade, proliferação e migração de hESC induzidas por veneno de L. *obliqua* ocorreram principalmente por proteção contra dano oxidativo e ativação da via dependente de ERK. Assim, esta é uma ferramenta farmacológica promissora para compreender os mecanismos subjacentes da deficiência de hESC em PGR.

Palavras-chave: células endometriais, *Lonomia*, citoproteção, análise proteômica, perda gestacional recorrente

ABSTRACT

Background. Recurrent pregnancy loss (RPL) is associated with severe physical and psychological morbidity, for which there is no treatment options when excluded anatomic and genetic causes. The pathophysiology involves deficiency in proliferation and migration capacities of endometrial stromal cells (hESCs) impairing embryo implantation and development. Thus, finding new cytoprotective molecules able to stimulate hESCs proliferation could be interesting. Since animal venoms are rich source of bioactive molecules, in this work we aimed to characterize the cytoprotective effects of *Lonomia obliqua* venom on hESCs. **Methodology.** hESCs were isolated from fresh human endometrial biopsies and characterized according standard protocols. Then the effects of *L. obliqua* venomous secretions on cell viability, proliferation and migration were determined. Venom components involved in cell enhancing effects were also identified by classical chromatographic methods and proteomic analysis. **Results.** *L. obliqua* venom induced hESC proliferation, viability and migration in a dose-dependent manner both in the presence and serum deprivation conditions. By ion-exchange chromatography was obtained one fraction enriched in cytoprotective components devoid of hemotoxic effect. Venom proteomic analysis identified at least six protein classes with potential cytoprotective properties (hemolins, lipocalins, hemocyannins, antiviral proteins, antimicrobial peptides and protease inhibitors). The mechanism involved in cryoprotection seems to be related to downregulation of superoxide anion generation, increase in antioxidant enzymes, nitric oxide production and activation of ERK-dependent pathways of proliferation. **Conclusions.** *L. obliqua* venom-induced hESC viability, proliferation and migration occurred mainly by protection against oxidative damage and ERK-dependent pathway activation.

Thus, this is a promising pharmacological tool to understand underlying mechanisms of hESC deficiency in RPL.

Keywords: Endometrial cell, *Lonomia*, cytoprotective, proteomic analysis, recurrent pregnancy loss

INTRODUÇÃO

O endométrio é um tecido que apresenta alta regeneração e diferenciação, como pode ser visto no ciclo menstrual, na gestação e no crescimento endometrial pós-menopausa em mulheres com terapia de reposição hormonal (Prianishnikov, 1978). O conceito de que a regeneração endometrial é mediada por células-tronco residentes no endométrio foi proposto em 1978 por Prianishnikov (Prianishnikov, 1978). As células-tronco endometriais (ESCs) presentes na camada basal apresentam diferenciação coordenada pela receptividade hormonal, já que essas células geralmente são encontradas em tecidos que apresentam alta taxa de divisão e regeneração (Chan, 2004).

A primeira evidência da presença de ESCs foi somente em 2004. Chan *et al* isolou células-tronco adultas de tecidos originados de histerectomias (Chan, 2004). Desde então, essas células têm recebido atenção e se tornaram foco de estudo por serem células-tronco adultas de alta clonogenicidade, fácil diferenciação e de fácil acesso.

A vasculogênese e a angiogênese são evidentes no endométrio durante o ciclo menstrual e implantação. Durante a vasculogênese a formação capilar primária se deve a diferenciação "*in situ*" da hemangiogênese a partir de células tronco (Bashiri, 2018; Demir, 2010). Múltiplas alterações endometriais fazem parte deste processo desde molecular até a estrutura tissular passando necessariamente pela neovascularização ou angiogênese.

Em 2016, foi estabelecida uma associação entre as células-tronco residentes no endométrio e o abortamento de repetição (Lucas, 2016). O endométrio, na perda gestacional recorrente, gera uma resposta inflamatória prolongada da decídua, o que prolonga a janela de receptividade endometrial, aumentando a taxa de

implantação nesse período e diminuindo, consequentemente, a seleção embrionária. Isso pode levar ao abortamento por implantação de um embrião com baixa capacidade de desenvolvimento ou por implantação de um embrião viável fora da janela de receptividade endometrial (Lucas, 2016).

O estímulo ovariano leva a alterações regulatórias nos níveis de células NK uterinas que devem expressar fator de crescimento vascular endotelial (VEGF). O desenvolvimento vascular endometrial inapropriado pode estar relacionado a abortamento devido a angiogênese alterada (Lash, 2012). Desta forma, a sincronização do desenvolvimento embrionário e da diferenciação celular endometrial estão relacionadas à perspectiva de sucesso do processo reprodutivo.

Venenos animais são amplamente estudados para obtenção de moléculas com potencial biológico para tratamento de doenças (Bon, 2000). O veneno da lagarta *Lonomia obliqua* é conhecido por causar síndrome hemorrágica e apresentar ação nefrotóxica, sendo responsável por acidentes principalmente em áreas rurais do RS, SC, PR, SP e RJ (Pinto, 2006). Esse veneno já foi utilizado em fibroblastos e demonstrou aumento da sobrevivência e da viabilidade celular por diminuição da apoptose mesmo em células com privação de nutrientes (Boscha, 2016).

REVISÃO DE LITERATURA

1. Estratégias para localizar e selecionar as informações

Foi realizada uma busca eletrônica de artigos durante o mês de outubro de 2020, indexados em 03 (três) bases de dados: PubMed, Scielo e LILACS. Utilizaram-

se os seguintes descritores: 1) stem cells 2) endometrial stem cells 3) *Lonomia obliqua* 4) recurrent pregnancy loss.

Ao concluir a busca, os artigos foram selecionados a partir da leitura prévia de seus resumos. Aqueles que não contemplavam o propósito do estudo foram excluídos. Foram selecionadas as publicações que iam ao encontro dos objetivos propostos pelo estudo. Deu-se preferência para artigos publicados nos últimos 15 anos, assim como livros em meio físico e virtual com o objetivo de sustentar a questão de pesquisa. No total, foram utilizados 22 artigos. Também foram utilizados o Guideline da Sociedade Europeia de Reprodução Humana e Embriologia e o site oficial do CIT (Centro de Informações Toxicológicas).

Palavra-chave	**Artigos no PUBMED**
“stem cell”	339.752
“endometrial stem cells”	978
“*Lonomia obliqua*”	94
“recurrent pregnancy loss”	3.027
“stem cell” x “*Lonomia obliqua*”	0
“stem cell” x “recurrent pregnancy loss”	41
“endometrial stem cell” x “recurrent pregnancy loss”	11

2. Mapa conceitual

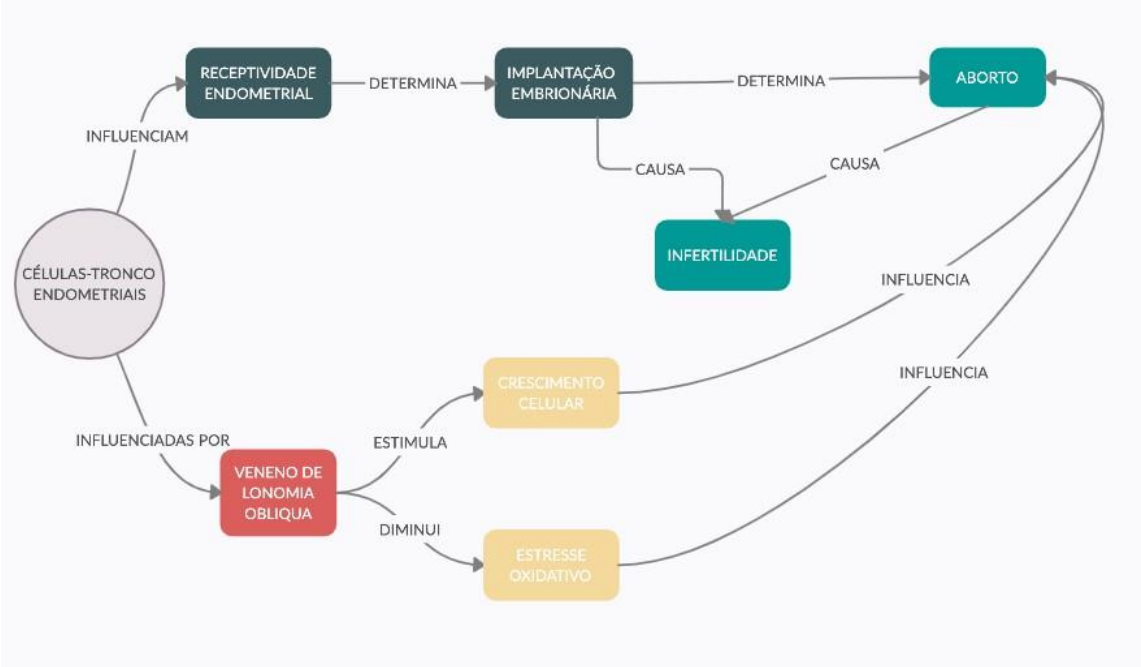

Figura 1. Mapa conceitual.

3. Lonomia obliqua

Os venenos animais são ricos em peptídeos e componentes que podem atuar em diversos organismos. Atualmente, existem seis fármacos derivados de venenos animais aprovados para o tratamento de condições médicas (King, 2011). Desses, o mais conhecido é o captopril, derivado da serpente *Bothrops jararaca*, que é um dos fármacos mais utilizados no tratamento da hipertensão (King, 2011).

A *Lonomia obliqua* é uma lagarta da família dos Lepidópteros que possui veneno nas suas espículas quando em sua fase larval. A *L. obliqua* é encontrada na América do Sul, com maior incidência de acidentes na região sul do Brasil, que é a que reporta mais óbitos também. O Centro de Informações Toxicológicas registrou 1.839 acidentes entre os anos de 1989 até 2005 somente no Rio

Grande do Sul (Ministério, 2009; CIT, 2008). O maior número de acidentes ocorre entre os meses de novembro a abril.

Figura 2. **A e B.** Lagarta Lonomia obliqua. **C.** Colônia de lagartas sobre o tronco de uma árvore. Fonte: CIT/SC.

Os acidentes com lagartas dessa família podem ter gravidades variáveis, desde urticárias leves até morte (Diaz, 2005). No caso da *L. obliqua*, o acidente ocorre quando a vítima encosta na lagarta e entra em contato com as espículas, que quebram e liberam o veneno ali armazenado (Veiga, 2001). O contato com as secreções da lagarta provoca reação imediata local com ardor intenso, hiperemia, prurido, edema e bolhas sanguinolentas. As manifestações locais são seguidas por sintomas gerais e inespecíficos, como cefaleia, mal-estar, náusea, vômitos, mialgia (Kowacs, 2006). Após, inicia-se o quadro hemorrágico com distúrbios de coagulação e sangramentos: gengivorragia, equimose,

sangramento de feridas recentes ou já cicatrizadas, hemorragias intra-articulares, hematêmese, sangramento pulmonar e cerebral, que pode evoluir para hipotensão, falência renal aguda e morte (Berger, 2019). O tratamento se dá pelo uso do soro antilonômico, produzido pelo Instituto Butantan desde 1996.

Os achados laboratoriais indicam prolongamento do tempo de coagulação (tempo de protrombina, tempo de tromboplastina parcialmente ativada e tempo de trombina). Também podem ser encontradas quedas nos níveis de fibrinogênio, de precalicreína, de proteína C e dos fatores de coagulação V e XIII (Arocha-Piñango, 2003).

O veneno da *L. obliqua* possui moléculas que interferem em diferentes sistemas, alterando a fisiologia celular, e que atuam para a defesa do animal contra predadores. O veneno é constituído por princípios ativos de natureza proteica ou peptídica com diferentes atividades tóxicas. Nas cerdas da lagarta foram encontradas proteínas com homologia a lipocalinas, lectinas, serpinas, cisteíno-proteinases e inibidores de proteases (Veiga, 2001; Ricci-Silva, 2008).

4. Células-tronco

Células-tronco são células autorrenováveis com capacidade de diferenciação em outros tipos celulares. A utilização das células-tronco na área científica se dá pela plasticidade dessas células. Elas podem ser divididas de acordo com sua capacidade de diferenciação: células-tronco totipotentes são originadas de zigotos e podem gerar todos os tipos celulares, incluindo os anexos extraplacentários. As pluripotentes são derivadas de blastocistos e não geram tecidos extra-placentários. As células-tronco multipotentes derivam diversos

tipos celulares de uma mesma camada germinativa e são as mais utilizadas por aspectos éticos e facilidade de obtenção (Vassena, 2015).

Até o momento, não existe um marcador específico para a caracterização de células-tronco. Por isso, em 2006, a Sociedade Internacional de Terapia Celular determinou os critérios mínimos para a sua classificação. Para ser utilizada como terapia, essa célula deve apresentar aderência ao plástico em cultura *in vitro*, expressão e não-expressão de marcadores e capacidade de diferenciação em tecidos da mesoderme (Dominici, 2006).

Uma característica importante das células-tronco é a divisão assimétrica, que é a geração de duas células-filhas diferentes na divisão celular – uma diferenciada e uma "tronco" (Watt, 2000). Isso permite que o tecido mantenha seu pool de células-tronco e também gere novas células especializadas do tecido.

A realização de pesquisas utilizando células-tronco adultas é cada vez mais abundante, visto que a utilização de células-tronco embrionárias humanas é proibida em vários países e envolve uma série de questões éticas. As células-tronco adultas geralmente são originadas da medula óssea, do tecido adiposo e do cordão umbilical. Porém, pela dificuldade de obtenção de células nesses tecidos e por necessitarem de procedimentos invasivos para obtenção, iniciou-se uma busca por novas fontes de células-tronco. O endométrio foi pesquisado por apresentar alta taxa de renovação e regeneração. Em 2004, células-tronco foram isoladas do endométrio de pacientes submetidas à histerectomia pela primeira vez (Chan, 2004; Dulak, 2015).

As células-tronco adultas expressam grande número de moléculas bioativas como moléculas de adesão, proteínas de matriz extracelular, citocinas e

receptores para fatores de crescimento (Huss, 2000; Bobis, 2006). Essas moléculas atuam modulando a resposta inflamatória, a angiogênese e a mitose das células envolvidas no processo de reparação tecidual (Meirelles, 2009; Sobhani, 2017).

5. O endométrio

O endométrio é o epitélio que reveste a cavidade uterina e é dividido em duas camadas: a superficial funcional e a basal. A camada superficial contém as glândulas e é a que prolifera, secreta e descama durante o ciclo menstrual. O propósito dessa camada é preparar o endométrio para a implantação do blastocisto. Já a camada basal é mantida durante os ciclos menstruais e são as células residentes ali que são responsáveis pela regeneração e constituição da nova camada funcional após a descamação (Kierszenbaum, 2016).

O crescimento e a descamação do endométrio são respostas aos hormônios sexuais femininos, estrogênio e progesterona, produzidos pelo ovário. Durante a fase proliferativa do ciclo, há crescimento dos folículos ovarianos e consequentemente aumento da secreção do estrogênio. Como resultado do aumento da concentração de estrogênio, há proliferação do endométrio (Kierszenbaum, 2016).

Após a ovulação, há aumento da secreção de progesterona, que inicia a compactação da mucosa, impedindo seu crescimento. As glândulas aumentam em tamanho e tortuosidade, aumentando sua secreção (Prianishnikov, 1978).

A implantação do blastocisto geralmente ocorre entre os dias 20-21 do ciclo. O endométrio apresenta edema do estroma e aumento da permeabilidade capilar por conta da ação das prostaglandinas. Logo após a implantação, células da

decídua – tecido que inicia a formação da placenta – já podem ser identificadas

no entorno dos vasos sanguíneos. Essas células se originam das células-tronco do estroma endometrial e são importantes para a regulação da invasão do trofoblasto (Pinto, 2006).

A diferenciação das células-tronco do endométrio em células da decídua é um processo indispensável para que a gestação ocorra. Quando o blastocisto invade o endométrio e promove essa diferenciação, o trofoblasto induz o remodelamento das artérias espiraladas com aumento da sua luz para a melhor vascularização do útero gravídico (Demir, 2010). A incapacidade de invasão trofoblástica ou da diferenciação das células-tronco em decídua pode levar ao abortamento.

6. Perda gestacional recorrente e células-tronco endometriais

O aborto é uma das situações mais frequentes da gestação, acometendo de 12-15% das mulheres. A síndrome da perda gestacional recorrente (PGR) é caracterizada por dois ou mais abortamentos espontâneos, consecutivos ou não, com idade gestacional de até 24 semanas. A estimativa é que afete de 2 a 5% da população (El Hachem, 2017), porém este número é incerto, pois os critérios variam entre os países. A PGR pode ser divida em primária, quando não há nascidos vivos prévios, e secundária, quando há gravidez prévia com idade gestacional maior que 24 semanas (ESHRE, 2018).

A etiologia da perda gestacional pode ser devida a inúmeros fatores, como defeitos uterinos, síndrome antifosfolipídeo, fatores endócrinos, genéticos, ambientais e, em algumas pacientes, até mesmo a combinação desses fatores (ESHRE, 2018). Os defeitos uterinos atingem em torno de 19% das pacientes com PGR e incluem os anatômicos, como útero bicorno ou septado, fibroses,

pólipos ou aderências causadas por cirurgias ou síndrome de Asherman (Jaslow, 2010).

As células-tronco residentes do endométrio são essenciais para a regeneração endometrial após a descamação no ciclo menstrual. Além disso, atuam como sensores identificando proteases presentes nos embriões e dão suporte para a implantação de embriões viáveis (Jaslow, 2010).

O endométrio está receptivo para o embrião apenas em alguns dias do ciclo, período conhecido como janela de implantação. A diminuição da capacidade receptiva do endométrio pode causar subfertilidade e falha de implantação. Porém, o aumento da receptividade endometrial leva ao abortamento. Isso acontece porque quando a janela de implantação se estende, o endométrio perde a capacidade de seleção embrionária; assim, embriões com menor capacidade de crescimento conseguem implantar, mas não mantêm a gestação (Brosens, 2014; Lucas, 2016).

Lucas *et. al* demonstrou uma associação entre a presença de células-tronco no endométrio e o abortamento de repetição. Pacientes com histórico de repetidos abortos possuíam diminuição da quantidade de células com capacidade de clonogenicidade, que é a habilidade de duplicação celular e formação de colônias. Além disso, um menor número de células-tronco no endométrio foi associado com aumento de células senescentes, citocinas pró-inflamatórias, metaloproteinases de matriz e fatores de crescimento (Lucas, 2016).

As células-tronco presentes no endométrio ao redor do local de implantação do blastocisto aumentam a sua proliferação e a diferenciação em células da

decídua. Esse processo pode acontecer sem a presença de um embrião, mas aumenta sua intensidade na presença de um (Cha, 2012).

Pacientes com síndrome de Asherman – uma síndrome que causa destruição do endométrio por curetagens agressivas ou endometrite – ou com endométrio fino apresentam alterações do ciclo menstrual, como amenorreia, infertilidade e perda gestacional recorrente (Yu, 2008). Apesar do tratamento com células-tronco ser promissor para desordens endometriais relacionadas à infertilidade, ainda não há nenhuma terapia efetiva descrita (Vassena, 2015).

7. Veneno de *Lonomia obliqua* e células-tronco

O veneno da *L. obliqua* já foi testado em fibroblastos (células presentes no tecido conjuntivo) e foi capaz de aumentar a proliferação e migração celular, mesmo em privação de soro, estado em que as células geralmente entram em apoptose. Em privação de nutrientes, as células liberam grandes quantidades de espécies reativas de oxigênio, que causam dano e morte celular (Bernardi, 2019; Boscha, 2016).

Assim, o veneno da *L. obliqua* pode melhorar a resposta das células-tronco presentes no endométrio aumentando a proliferação e a migração celular e diminuindo a quantidade de espécies reativas de oxigênio presentes no endométrio.

Apesar das toxinas hemo e nefrotóxicas, o veneno de Lonomia obliqua também possui em sua composição pelo menos uma proteína anti-apoptótica além de duas classes de proteínas potencialmente citoprotetoras, as lipocalinas e as hemolinas (Veiga, 2001; Vieira et al., 2010). Essas classes de proteínas são ligantes de compostos pró-oxidantes e participam ativamente do processo de

regeneração e remodelamento tecidual nas diferentes fases de metamorfose da

lagarta. Algumas dessas moléculas foram capazes de estimular a cicatrização em modelo experimental de lesão cutânea e aumentar significativamente a proliferação e migração de fibroblastos em cultura (Bosch e, 2016; Sato, 2016). O mecanismo envolvido parece ser a ativação de vias controladas por ERK e PI3K/AKT e a redução da expressão de proteínas pró-apoptóticas como Bcl-2, Bax e caspase-3 (Carrijo-Carvalho et al., 2012; Bosch, 2016).

JUSTIFICATIVA

As células-tronco endometriais apresentam características semelhantes às células-tronco da medula óssea: clonogenicidade, multipotência, habilidade de reconstituir estroma *in vivo* e expressão de marcadores que as diferenciam de células hematopoiéticas. Porém, são de mais fácil obtenção e podem ser utilizadas para entendimento de desordens endometriais, como a perda gestacional recorrente. Identificar moléculas que melhoram a receptividade endometrial pode permitir seu uso em pacientes que apresentam perda gestacional recorrente. Neste sentido, tem se idealizado vários tratamentos visando a melhora da implantação e, igualmente, evitando abortamento com base na melhora da viabilidade celular.

HIPÓTESE

Hipótese alternativa

A fração utilizada do veneno de *L. obliqua* tem efeito benéfico na atividade celular das células-tronco endometriais.

Hipótese nula

A fração utilizada do veneno de *L. obliqua* não apresenta efeito benéfico na atividade celular das células-tronco endometriais.

OBJETIVOS

Objetivo Geral:

Identificar novas moléculas tipo lipocalinas e hemolinas no veneno da taturana *Lonomia obliqua* e investigar os seus efeitos sobre a proliferação e migração de células tronco endometriais humanas (hESC).

Objetivos Secundários:

- Realizar isolamento, cultura e caracterização de hESC;
- Analisar os efeitos do veneno e suas frações sobre a proliferação, a migração e a viabilidade das hESC na presença e ausência de soro fetal bovino;
- Caracterizar os componentes do veneno da L. *obliqua* envolvidos no efeito citoproteror;
- Analisar os efeitos do veneo e suas frações sobre espécies reativas de oxigênio;
- Caracterizar por análise proteômica os componentes do veneno e da hemolinfa da L. *obliqua*;
- Analisar efeitos do veneno e suas frações sobre a via da ERK;
- Analisar mecanismos envolvidos na citoproteção do veneno da L. *obliqua*.

REFERÊNCIAS

Arocha-Piñango CL, Guerrero B. Síndrome hemorrágico producido por contacto con orugas. Estudios clínicos y experimentales. Revisión [Hemorrhagic syndrome induced by caterpillars. Clinical and experimental studies. Review]. Invest Clin. 2003 Jun; 44(2): 155-63.

Bashiri A, Halper KI, Orvieto R. Recurrent Implantation Failure-update overview on etiology, diagnosis, treatment and future directions. Reprod Biol Endocrinol. 2018 Dec 5; 16(1): 121.

Berger M, de Moraes JA, Beys-da-Silva WO, Santi L, Terraciano PB, Driemeier D, Cirne-Lima EO, Passos EP, Vieira MAR, Barja-Fidalgo TC, Guimarães JA. Renal and vascular effects of kallikrein inhibition in a model of Lonomia obliqua venom-induced acute kidney injury. PLoS Negl Trop Dis. 2019; 13(2): e0007197.

Bernardi L, Pinto AFM, Mendes E, Yates JR, Lamers ML. Lonomia obliqua bristle extract modulates Rac1 activation, membrane dynamics and cell adhesion properties. Toxicon. 2019; 162: 32-39.

Bobis S, Jarocha D, Majka M. Mesenchymal stem cells: characteristics and clinical applications. Folia Histochem Cytobiol. 2006; 44(4): 215-30.

Bon C. The natural toxins. Biochimie. 2000; 82: 791-792.

Boscha RV, Alvarez-Flores, MP, Maria DA,Chudzinski-Tavassi AM. Hemolin triggers cell survival on fibroblasts in response to serum deprivation by inhibition of apoptosis. Biomedicine & Pharmacotherapy. 2016; 82: 537-546.

Bosch RV, Alvarez-Flores MP, Maria DA, Chudzinski-Tavassi AM. Hemolin triggers cell survival on fibroblasts in response to serum deprivation by inhibition of apoptosis. Biomed Pharmacother. 2016 Aug; 82: 537-46.

Brosens JJ, et al. Uterine selection of human embryos at implantation. Sci Rep. 2014; 4: 3894.

Carrijo-Carvalho LC, Maria DA, Ventura JS, Morais KL, Melo RL, Rodrigues CJ, Chudzinski-Tavassi AM. A lipocalin-derived Peptide modulating fibroblasts and extracellular matrix proteins. J Toxicol. 2012; 2012: 325-250.

Cha J, Sun X, Dey SK. Mechanisms of implantation: strategies for successful pregnancy. Nat Med. 2012; 18(12): 1754-67.

Chan RW, Schwab KE, Gargett CE. Clonogenicity of human endometrial epithelial and stromal cells. Biol Reprod. 2004; 70(6): 1738-50.

Demir R, Yaba A, Huppertz B. Vasculogenesis and angiogenesis in the endometrium during menstrual cycle and implantation. Acta Histochem. 2010; 112(3): 203-14.

Diaz JH. The evolving global epidemiology, syndromic classification, management, and prevention of caterpillar envenoming. Am J Trop Med Hyg. 2005;72(3):347-57.

Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini FC, Krause DS, Deans RJ, Keating A, Prockop DJ, Horwitz EM. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006; 8(4): 315-317.

Dulak J, Szade K, Szade A, Nowak W, Józkowicz A. Adult stem cells: hopes and hypes of regenerative medicine. Acta Biochim Pol. 2015; 62(3): 329-37.

El Hachem H, Crepaux V, May-Panloup P, Descamps P, Legendre G, Bouet PE. Recurrent pregnancy loss: current perspectives. Int J Womens Health. 2017; 9: 331-345.

Huss R. Isolation of primary and immortalized CD34-hematopoietic and mesenchymal stem cells from various sources. Stem Cells. 2000; 18(1): 1-9.

Jaslow CR, Carney JL, Kutteh WH. Diagnostic factors identified in 1020 women with two versus three or more recurrent pregnancy losses. Fertil Steril. 2010; 93(4): 1234-43.

Kierszenbaum AL, Tres LL. Histologia e Biologia Celular: uma introdução à patologia. Elsevier. 4th edition, 2016. Rio de Janeiro, Brazil. Disponível em: https://www.evolution.com.br.

King GF. Venoms as a platform for human drugs: translating toxins into therapeutics. Expert Opin Biol Ther. 2011; 11(11):1469-84.

Kowacs PA, Cardoso J, Entres M, Novak EM, Werneck LC. Fatal intracerebral hemorrhage secondary to Lonomia obliqua caterpillar envenoming: case report. Arq. Neuro-Psiquiatr. [Internet]. 2006; 64(4): 1030-1032.

Lash GE, Innes BA, Drury JA, Robson SC, Quenby S, Bulmer JN. Localization of angiogenic growth factors and their receptors in the human endometrium throughout the menstrual cycle and in recurrent miscarriage. Human Reproduction. 2012; 27(1): 183–195.

Lucas ES, Dyer NP, Fishwick K, Ott S, Brosens JJ. Success after failure: the role of endometrial stem cells in recurrent miscarriage. Reproduction. 2016; 152(5): 159-66.

Meirelles LS, Fontes AM, Covas DT, Caplain AI. Mechanisms involved in the therapeutic properties of mesenchymal stem cells. Cytokine & Growth Factor Reviews. 2009; 20, 419-427

Núcleo de Comunicação Secretaria de Vigilância em Saúde. Situação Epidemiológica das Zoonoses de Interesse à Saúde Pública. 1st edn, 2009. Ministério da Saúde, Brasília, Brazil.

Pinto AF, Berger M, Reck J Jr, Terra RM, Guimarães JA. Lonomia obliqua venom: In vivo effects and molecular aspects associated with the hemorrhagic syndrome. Toxicon. 2010;56(7):1103-12.

Pinto AF, Silva KR, Guimarães JA. Proteases from Lonomia obliqua venomous secretions: comparison of procoagulant, fibrin(ogen)olytic and amidolytic activities. Toxicon. 2006; 47(1): 113-21.

Prianishnikov VA. On the concept of stem cell and a model of functional-morphological structure of the endometrium. Contraception. 1978; 18(3): 213-23.

Ricci-Silva ME, Valente RH, Leon IR, Tambourgi DV, Ramos OH, Perales, J, Chudzinski-Tavassi AM. Immunochemical and proteomic Technologies as tools for unravelling toxins involved in envenoming by accidental contact with *Lonomia obliqua* caterpillars. Toxicon. 2008; 51: 1017-1028.

Sato, AC et al. Exploring the in vivo wound healing effects of a recombinant hemolin from the caterpillar Lonomia obliqua. J. Venom. Anim. Toxins incl. Trop. Dis [online]. 2016; 22(36).

Sobhani A, Khanlarkhani N, Baazm M, Mohammadzadeh F, Najafi A, Mehdinejadiani S, Sargolzaei Aval F. Multipotent Stem Cell and Current Application. Acta Med Iran. 2017; 55(1): 6-23.

The ESHRE Guideline Group on RPL. ESHRE guideline: recurrent pregnancy loss, Human Reproduction Open. Issue 2, 2018.

Toxicovigilância: Toxicologia Clínica. Centro de Informação Toxicológica do Rio Grande do Sul (CIT). 2008.

Vassena R, Eguizabal C, Heindryckx B, Sermon K, Simon C, van Pelt AM, Veiga A, Zambelli F; ESHRE special interest group Stem Cells. Stem cells in reproductive medicine: ready for the patient? Hum Reprod. 2015; 30(9): 2014-21.

Veiga ABG, Blochtein B, Guimarães JA.Structures involved in production, secretion and injection of the venom produced by the caterpillar Lonomia obliqua (Lepidoptera, Saturniidae). Toxicon. 2001; 39(9): 1343-1351.

Vieira HL, Pereira AC, Peixoto CC, Moraes RH, Alves PM, Mendonça RZ. Improvement of recombinant protein production by an anti-apoptotic protein from hemolymph of Lonomia obliqua. Cytotechnology. 2010 Dec; 62(6): 547-55.

Watt FM, Hogan BL. Out of Eden: stem cells and their niches. Science. 2000; 287(5457): 1427-30.

Yu D, Li TC, Xia E, Huang X, Liu Y, Peng X. Factors affecting reproductive outcome of hysteroscopic adhesiolysis for Asherman's syndrome. Fertil Steril. 2008; 89(3): 715-22.

Effects of *Lonomia obliqua* venom components on human endometrial stromal cells: A potential source for new cytoprotective biomolecules against recurrent pregnancy loss

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Abstract

Background. Recurrent pregnancy loss (RPL) is associated with severe physical and psychological morbidity, for which there is no treatment options. The pathophysiology involves deficiency in proliferation and migration capacities of endometrial stromal cells (hESCs) impairing embryo implantation and development. Thus, finding new cytoprotective molecules able to stimulate hESCs proliferation could be interesting. Since animal venoms are rich source of bioactive molecules, in this work we aimed to characterize the cytoprotective effects of *Lonomia obliqua* venom on hESCs. **Methodology.** hESCs were isolated from fresh human endometrial biopsies and characterized according standard protocols. Then the effects of *L. obliqua* venomous secretions on cell viability, proliferation and migration were determined. Venom components involved in cell enhancing effects were also identified by classical chromatographic methods and proteomic analysis. **Results.** *L. obliqua* venom induced hESC proliferation, viability and migration in a dose-dependent manner both in the presence and serum deprivation conditions. By ion-exchange chromatography was obtained one fraction enriched in cytoprotective components devoid of hemotoxic effect. Venom proteomic analysis identified at least six protein classes with potential cytoprotective properties (hemolins, lipocalins, hemocyannins, antiviral proteins, antimicrobial peptides and protease inhibitors). The mechanism involved in cryoprotection seems to be related to downregulation of superoxide anion generation, increase in antioxidant enzymes, nitric oxide production and activation of ERK-dependent pathways of proliferation. **Conclusions.** *L. obliqua* venom-induced hESC viability, proliferation and migration occurred mainly by protection against oxidative damage and ERK-dependent pathway activation. Thus, this is a promising pharmacological tool to understand underlying mechanisms of hESC deficiency in RPL.

Keywords: Endometrial cell, Lonomia, cytoprotective, proteomic analysis, recurrent pregnancy loss

1. Introduction

Given the fast proliferation and the short self-renew cycle of human endometrial cells, as well as their ability to differentiate into different cell types, the idea that the endometrium contains stromal stem cells was first studied in 1978 [1]. However, it was only in 2004 that the first study really demonstrated the presence of endometrial stromal (stem) cells (hESC) in this organ [2].

In 2016, Lucas *et al* found that there is a link between hESCs and reproductive failure [3], mainly involving cells that are not able to self-renovate, proliferate and differentiate properly when a given stimuli is triggered [4]. Women who do not have a significant number of hESCs may developed embryo implantation failure associated with defective decidua formation, when endometrial fibroblasts and hESCs transform into specialized epithelioid cells [5]. Once this happens, women may experience implantation failure and pregnancy loss. In physiological conditions, endometrium growth is synched with embryo development to guarantee implantation success within the limited two-to-four-day-implantation window. This also acts like a protection mechanism against embryos with deficient development [6]. Implantation outside this window can lead to miscarriage. Thus, an adequate population and functional number of stem cells in the endometrium are vital to right endometrial development and embryo recognition [7].

Pregnant women who have undergone recurrent cycles of miscarriage, may developed a syndrome called recurrent pregnancy loss (RPL). RPL affects 2-5 % of couples in reproductive age [8] being defined as three or more spontaneous miscarriages in the first 20 weeks of gestation and can be divided into primary loss, when a woman has had no live-birth; and secondary loss, when the woman has had at least one child [9]. Since proliferation, differentiation and a proper mechanism of self-renew in hESCs are essential functions for embryo implantation, searching for cytoprotective molecules that stimulate cell proliferation and protective pathways is of therapeutic interest.

In this context animal venoms are rich sources of bioactive molecules [10]. Despite its known toxic effects, animal venoms also have protective components such as pro-proliferative molecules, growth factor-like, anti-apoptotic, anti-oxidant, L-aminoacid oxidases and protease inhibitors which are potential pharmacological tools. Particularly arthropods such as caterpillars express different classes of cytoprotective molecules throughout their developmental stages that participate in tissue regeneration and reconstruction during metamorphosis process [11]. *Lonomia obliqua* is a stinging

caterpillar found mainly in tropical and subtropical areas of Argentina, Venezuela and Brazil. In most cases the accidental contact with their spines (technically called spicules or bristles) cause local inflammatory reactions such as contact dermatitis. Severe cases of systemic envenomation can evolve to a hemorrhagic syndrome, kidney injury and even can be fatal in some conditions (for review see [12]). Since 1997 the Butantan Institute in São Paulo, Brazil has been producing an antivenom serum, which significantly attenuates the severe outcomes of this type of envenomation [13]. Currently, accidents with *L. obliqua* are commonly registered in rural areas of southern Brazil, mainly in the states of Rio Grande do Sul, Santa Catarina and Paraná [12].

Here in this work we reported the identification of at least six classes of bioactive compounds with potential cytoprotective activity through proteomic analysis. It was also demonstrated for the first time that *L. obliqua* venom and its components can increase hESC proliferation, viability and migration, protecting cells from oxidative damage in a mechanism dependent from ERK activation signaling pathway.

2. Materials and methods

2.1 Reagents

Dulbecco's modified Eagle's medium (DMEM), Ham's Nutrient Mixture F12, fetal calf serum (FCS), penicillin/streptomycin, EDTA and trypsin were from Life Technologies (Carlsbad, CA, USA). Vanadium (III) chloride (VCl_3), sulfanilamide (SULF), *N*-(1-naphthyl) ethylenediamine dihydrochloride (NEDD), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and PD98059, were purchased from Sigma-Aldrich (Saint Louis, MO, USA). BCA protein assay kit was obtained from Pierce Biotechnology (Rockford, IL). All other chemicals were of analytical grade and were commercially available.

2.2 Venom component identification and analysis

2.2.1 L. obliqua secretion samples

L. obliqua caterpillars were kindly provided by the Centro de Informações Toxicológicas (CIT), Porto Alegre, Rio Grande do Sul, Brazil. The specimens were collected in rural endemic areas of counties from Rio Grande do Sul and Santa Catarina states in Brazil where usually the accident occurs. Two different secretions were used in this work as previously described [14]. One of them was obtained by cutting bristles at the base of each scoli, macerating them in cold phosphate-buffered saline (PBS), pH 7.4,

and centrifuging at 9,600x*g* for 20 min. The supernatant obtained following this procedure was designated as ***L****onomia* ***o****bliqua* **B**ristle **E**xtract (LOBE). Another secretion (hemolymph) was obtained by cutting the caterpillar´s abdominal and anal prolegs, collecting the expelled fluid with a syringe and centrifuging at 9,600x*g* for 20 min. The supernatant was designated as ***L****onomia* ***o****bliqua* **H**emolymph (LOH). Protein concentrations of LOBE and LOH samples were determined using a BCA assay kit (Pierce, Rockford, USA), and the aliquots were stored at -80 ºC untiluse.

2.2.2 Protein fractionation

The protein mixture containing in LOBE extracts was fractionated in a FPLC system (Amersham Biosciences, Uppsala, Sweden) by ion-exchange liquid chromatography using a Hi-Trap Q column (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA). LOBE samples (50 mg) were applied in the equilibration buffer (20 mM Tris-HCl, pH 7.4) at a flow rate of 0.25 mL/min. Column retained proteins were eluted by a linear NaCl gradient from 0 to 1 M in the equilibrium buffer at a flow rate of 0.5 mL/min. Eluted proteins were monitored at 280 nm and fractions of 1 mL were collected for the experiments of cell viability, toxicity and coagulation assays as described in details below. All chromatographic fractions corresponding to protein peaks were pooled and analyzed by gradient polyacrilamide-gel- electrophoresis (SDS-PAGE 5-20 %) under non-reducing conditions.

2.2.3 In vitro coagulation assay

The procoagulant activity of Hi-Trap Q-derived fractions was determined by recalcification time assay, according to [15]. Briefly, chromatographic fractions (10 µL) were incubated with human citrated plasma (50 µL) for 5 min at 37 ºC in 50 mM Tris-HCl, pH 7.4 containing 150 mM NaCl. Then, coagulation was triggered by the addition of 10 mM $CaCl_2$ and the kinetics of clotting formation was monitored at 650 nm in a microplate reader spectrophotometer spectraMAX 190 (Molecular Devices, Sunnyvale, CA, USA). The recalcification time was calculated as the time necessary to reach an absorbance value of 0.05.

2.2.4 Proteomic analysis

The protein composition of LOBE and LOH samples was identified by mass spectrometry analysis in 3 technical replicates for each extract. Samples containing 100

µg of protein was reduced with 5 mM tris-2-carboxyethyl-phosphine (TCEP) at room temperature for 20 min and alkylated with 10 mM iodoacetamide at room temperature in the dark for 20 min. Afterwards, the proteins were digested with 2 µg of trypsin (Promega, Madison, WI) by incubation at 37°C for 16 h. Formic acid was added to a final concentration of 5%. The samples were centrifuged at 14,000 rpm for 20 min, and the supernatant was collected and stored at –80°C. A mixture of protein digest was pressure loaded into a MudPIT column consisting of the following: 250 µm i.d. capillary packed with 2.5 cm of 5-µm Partisphere strong cation exchanger (SCX) (Whatman, Clifton, NJ), followed by 2 cm of 3-µm Aqua C18 reversed phase (RP) (Phenomenex, Ventura, CA) with a 1 µm frit. The column was washed with buffer containing 95% water, 5% acetonitrile, and 0.1% formic acid. After washing, a 100 µm i.d. capillary with a 5 µm pulled tip packed with 11 cm of 3-µm Aqua C18 resin (Phenomenex, Ventura, CA) was attached via a union according to previous work [16]. The entire split-column was placed in line with an Agilent 1100 quaternary HPLC (Palo Alto, CA), and the sample was analyzed using a modified 11-step separation as previously described [17]. The buffer solutions used were 5% acetonitrile and 0.1% formic acid (Buffer A); 80% acetonitrile and 0.1% formic acid (Buffer B); and 500 mM ammonium acetate, 5% acetonitrile and 0.1% formic acid (Buffer C). Step 1 consisted of a 70 min gradient from 0% to 100% (v/v) buffer B. Steps 2–10 had a similar profile with the following changes: 5 min in 100% (v/v) buffer A, 3 min in X % (v/v) buffer C, a 6 min gradient from 0% to 15% (v/v) buffer B, and a 85 min gradient from 15% to 100% (v/v) buffer B. The 3 min buffer C percentages (X) were 10% to 100% (v/v) for the 10-step analysis. As peptides eluted from the microcapillary column, they were electrosprayed directly into an LTQ-XL (Thermo Finnigan) with the application of a distal 2.4 kV spray voltage. Full MS spectra were acquired in profile mode, with a mass range of 300–2000 *m/z*, and 10 data dependent MS/MS spectra at 35% normalized collision energy were repeated continuously throughout each step of the multidimensional separation. Application of mass spectrometer scan functions and HPLC solvent gradients were controlled by the Xcalibur data system. MS/MS spectra were analyzed using the following software analysis protocol. Protein identification and quantification analysis were done with Integrated Proteomics Pipeline (IP2, Integrated Proteomics Applications, Inc. www.integratedproteomics.com/). Tandem mass spectra were extracted into ms2 files from raw files using RawExtract 1.9.9 [18] and were searched using ProLuCID algorithm [19]. The MS/MS spectra that remained after filtering were searched with the ProLuCID

algorithm against the EBI-IPI_rat_3.30_06-28-2007 concatenated to a decoy database in which the sequence for each entry in the original database was reversed [20]. Searches were performed with cysteine carbamidomethylation as a fixed modification. The ProLuCID results were assembled and filtered using the DTASelect program [21] using the following two SEQUEST defined parameters to achieve a false discovery rate of 1%: the cross-correlation score (XCorr) and normalized difference in cross-correlation scores (DeltaCN). The following parameters were used to filter the peptide candidates: -p 1 -y 1 --trypstat --fpf 0.01 --dm. In addition, we used 50 ppm as the precursor tolerance, 600 ppm as the fragment mass tolerance, and 3 as the number of isotopic peaks, and unlimited missed cleavages were allowed. Searches were performed against a customized database containing sequences from insects, arachnids, amphibians and reptile proteins deposited in the NCBI database (http://www.ncbi.nlm.nih.gov/). Protein identifications were accepted if they contained at least 2 identified peptides. Relative quantifications were based on number of spectral counts. False positive rate was calculated at 2 % for both samples based on a reversed sequence database.

2.3 Human endometrial stromal (hESC) cell experiments

2.3.1 hESC isolation and culture

Endometrial tissue samples were harvested from seven consenting healthy patients undergoing biopsy for *in vitro* fertilization procedures at Hospital de Clínicas de Porto Alegre, RS, Brazil. Patients were invited to enroll this study at the time of their medical appointment and signed the consent form to donate a fragment of the endometrial biopsy and provide their anthropometric data. The protocol followed all recommendations of the Brazilian National Health Council (resolution 466/12) and was approved by the local ethics committee in human research under the number 17-0180. Tissue samples were collected and immediately fragmented into small pieces, macerated and incubated in Hank's balanced saline solution (10 mL), containing 1% penicillin-streptomycin and type 1-collagenase (0.5 %) at 37 °C for 20 min. During this time, tissue was homogenized twice and then centrifuged at 500x*g* for 10 min. Cell pellet was suspended in DMEM low glucose/Ham's Nutrient Mixture F12 supplemented with 10 % fetal bovine serum (FBS) and 1 % penicillin-streptomycin. The cells were kept in a 6-well-plate at 37 °C in a humidified 5 % CO_2 atmosphere. Once cells reached 80 % confluence, they were transferred and maintained in standard cell culture bottles until the experiments.

2.3.2 hESC characterization

The human endometrial stromal cells (hESC) isolated from all seven patients were characterized by their adhesiveness, fibroblastoid shape and ability to *in vitro* differentiate into adipocytes, osteocytes and chondrocytes following standard protocols [22]. To analyze general aspects of cell morphology hESCs were also stained using Giemsa solution, visualized and photographed in a Nikon Eclipse TE 2000-U optical microscope. The immunophenotypic identification for cell markers such as CD105, CD73, CD90, CD44, CD45, CD34, CD11b, CD19 and HLA-DR were performed (BD Stemflow hMSC Analysis Kit, BD Biosciences) using FACSCanto II (BD Biosciences) and FlowJo software (FlowJo LLC). Related isotype antibodies were used as control markers.

2.3.3 Cell viability assays

hESC viability was assessed by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Cells ($3x10^5$) were seeded overnight in 96-well plates using a DMEM medium containing 10 % FBS. Once they reached 80 % confluence, the cells were treated with five different concentrations (0.001, 0.01, 0.1, 1.0 and 10 μg/mL) of LOBE or LOH for 24 hours in the presence of DMEM plus 10 % FBS. Control cells were exposed to the same amounts of phosphate buffered saline (PBS). After this period, medium and treatments were removed and MTT (0.5 mg/mL) solution was added to each well followed by incubation for 4 h. Next, formazan crystals were solubilized in dimethyl sulfoxide (100 μL) and the absorbance was measured at 540 nm. The results were expressed as fold increase to control (cells treated with PBS). In order to understand the participation of venom components on increased hESC viability, the same experiment was performed using Hi Trap-Q-derived fractions and also with LOBE peptides (low-molecular weight fraction <3 kDa), LOBE denatured proteins (boiled LOBE) and high-molecular weight LOBE fraction >14 kDa (dialyzed LOBE).

2.3.4 Cell proliferation assays

hESCs ($3x10^5$) were seeded overnight in 96-well plates using a DMEM medium containing 10 % FBS and received the treatments with different concentrations (0.001, 0.01, 0.1, 1.0 and 10 μg/mL) of LOBE or LOH, as described above. Then, after 24 h incubation cell proliferation was estimated by two different methods: trypan-blue exclusion cell counts and sulforhodamine B (SRB) assay. For trypan-blue cell counts,

viable hESCs were stained and manually counted in a hemocytometer. The SRB method is based on the ability of the protein dye SRB to bind electrostatically and pH dependent on protein basic amino acid residues; thus, the amount of bound dye can be used as a linear function with cell number and cellular protein content [23]. For SRB assay, hESCs were washed in PBS, fixed in trichloroacetic acid and stained with 0.4 % SRB solution. Unbound dye was removed with 1 % acetic acid wash and 100 µL of 10 mM Tris base solution (pH 10.5) was added to each well to solubilize the protein-bound dye. The absorbance was measured at 565 nm and results were expressed as fold increase to control (cells treated with PBS). Additionally, cell proliferation was also estimated by measuring the immunocontent of Ki67. In this case, proteins from hESCs were separated by SDS-PAGE 12 % under reducing conditions, transferred onto nitrocellulose membranes, incubated with anti-human Ki67 antibody (Sigma Aldrich, Saint Louis, MO, USA) and revealed using the colorimetric kit Opti-4CN (Bio-Rad, Hércules, CA, USA). Protein expression levels were normalized with β-actin and quantified using Image J software (https://imagej.nih.gov/ij/).

2.3.5 Cellular toxicity assays

Cell toxicity was estimated by lactate dehydrogenase (LDH) release to the culture media. For this purpose, hESCs were seeded and treated with different concentrations of Hi Trap-Q-derived chromatographic fractions, LOBE or LOH as described above. LDH activity was measured in cell culture media using the *In Vitro* Toxicology Assay Kit (Sigma Aldrich, Saint Louis, MO, USA) following the manufacturer's instructions.

2.3.6 Cell migration assays

Cell migration was evaluated by wound healing assay following a protocol already established [24]. Briefly, hESCs on the 96-well culture plates were incubated approximately 80 % confluent and then made a scratch with yellow tip. Next, cells were treated with LOBE (0.001, 0.01, 0.1, 1.0 and 10 µg/mL) in DMEM containing 10 % FCS and incubated at 37 °C in a 5 % CO_2 atmosphere for 8 h. Microscopic photographs were captured at the beginning (0 h) and after 8 h using a Nikon Eclipse TE 2000-U optical microscope. Clear areas were measured and compared using Image J software and the results were expressed as percent values of wound repaired area (% migration).

2.3.7 Cytoprotective assays during serum starvation

The increasing in hESC viability was also analyzed during nutrient deprivation conditions. Cells ($3x10^5$) were seeded overnight in 96-well plates using a DMEM medium containing 10 % FBS. Once they reached 80 % confluence, the cells were washed twice in PBS and then treated with LOBE (0.1 μg/mL) for 24 hours in the presence of DMEM plus 10 % FBS, 1 % FBS or in the absence of FBS (-FBS). In another set of experiments, hESCs were treated with LOBE in FBS absence for 24, 48 and 72 hours. In both cases, after the incubation period cellular viability was measured by MTT assay as described above.

2.3.8 Cytoprotective assays during oxidative insult

LOBE-induced cytoprotective effects were studied during an oxidative insult triggered by hydrogen peroxide. For this purpose, hESCs on the 96-well culture plates were previously incubated with LOBE (0.01, 0.1 and 1 μg/mL) and treated with hydrogen peroxide (500 μM) for 24 h. Control cells were treated with PBS and received or not the same dose of hydrogen peroxide. After the incubation period cellular viability was measured by MTT assay as described above.

2.3.9 Superoxide anion, nitric oxide and catalase measurements

Superoxide anion, nitrate and nitrite levels and catalase activity were determined in hESC homogenates previously treated with LOBE (0.1 μg/mL) in the absence of FBS. Total nitrate and nitrite levels were determined as an indication of nitric oxide (NO) production according to Griess method [25]. The superoxide production was estimated based on NBT (nitroblue tetrazolium) reduction assay [26]. Briefly, cell homogenates (20 μL) were mixed with 0.1 % NBT solution and incubated in 96-well plates at 37 °C for 1 h. The aqueous mixtures were removed carefully from the wells, and the formazan precipitates were solubilized by adding 2 M KOH and dimethyl sulfoxide (DMSO). The absorbance was measured at 600 nm and the results are expressed as NBT reduction (OD at 600 nm) per mg of protein in homogenates. Catalase activity was also assayed following the decrease in absorbance values at 240 nm due to H_2O_2 decomposition. The reaction medium contained cell extracts, 10 mM potassium phosphate buffer, pH 7.0, 0.1 % Triton X-100 and 20 mM H_2O_2. The kinetics of absorbance decrease was followed by 10 min on the microplate reader spectrophotometer (SpectraMAX 190, Molecular

Devices, Sunnyvale, CA, USA). Catalase activity was expressed as mOD/min per mg of protein presented in each well.

2.3.10 Involvement of ERK in LOBE-induced cytoprotection

The potential involvement of extracellular signal regulated kinase (ERK) pathway was investigated. In this case, hESCs ($3x10^5$) were seeded overnight in 96-well plates and after reached confluence, cells were pretreated or not by 30 min with an ERK inhibitor, PD98059 (10 µM) (Sigma Aldrich, Saint Louis, MO, USA). LOBE treatment (0.1 µg/mL) was then administered in DMEM medium containing 10 % FBS and hESC viability was estimated by MTT assay after 24 h incubation.

2.4 Statistical analysis

Data analyses were performed using GraphPad Prism software (GraphPad Software Inc., San Diego, CA, USA). All data are presented as the means ± SE of at least three replicates. Significant differences were analyzed by one-way ANOVA followed by an unpaired t-test with a Bonferroni's correction for multiple comparisons. P-values < 0.05 were considered to be significant.

3. Results

3.1 Isolation, culture and characterization of human endometrial stromal cells (hESCs)

Endometrial tissue samples were obtained from seven healthy donors undergoing biopsy for *in vitro* fertilization procedures at Hospital de Clínicas de Porto Alegre, RS, Brazil and were used to isolate hESCs using classic protocols of tissue dissociation with type 1-collagenase. The hESCs in culture showed adhesiveness properties, presented a fusiform fibroblastoid morphology and ability to *in vitro* differentiate into adipocytes, osteocytes and chondrocytes **(Fig 1A-D)**. Cells demonstrated positive glycosaminoglycan production after 12 days in culture, as indicated by Alcian blue staining **(Fig 1B)**. Under osteogenic differentiating conditions, cells showed a calcium-rich mineralized matrix which was evident after 14 days in culture, as indicated by Alizarin Red S staining **(Fig 1C)**. Similarly, the formation of intracellular lipid vacuoles was observed after 21 days, as was confirmed by Oil Red staining **(Fig. 1D)**. The expression of cell surface markers was also characterized by flow cytometry. hESCs were positive for mesenchymal markers (CD105, CD90 and CD73) and negative for hematopoietic markers (CD45 and CD11), indicating that isolated cells have potential for multilineage differentiation **(Fig. 1E)**.

3.2 Effects of L. obliqua venom on hESC viability, proliferation and migration

The effects of *L. obliqua* bristle extract (LOBE) on hESC viability were measured by MTT assay for all seven isolates obtained from different healthy donors **(Table 1)**. In general, LOBE increased dose-dependently hESCs viability in a concentration range varying from 0.001 to 0.1 µg/mL, independently of the cell isolation bath. For some cell isolates (patient ID 1, 3, 4, 6 and 7) it was observed a slightly reduction in hESC viability at highest LOBE concentrations (10 µg/mL) **(Table 1)**.

Cell proliferation also significantly increased in the presence of LOBE for a wide range concentration (varying from 0.001 to 10 µg/mL) **(Fig. 2A-D)**. Both evaluation methods, sulforhodamine B (SRB) assay **(Fig 2A)** and trypan-blue exclusion cell counts **(Fig. 2B)** indicated similar results. A maximum increase of 3-times fold was noted in the trypan-blue cell counts when hESCs were cultivated in the presence of LOBE at 0.1 µg/mL **(Fig. 2B)**. After 24 h in culture, LOBE did not induced any morphological changes on hESC structures and the stimulation in cell proliferation can clearly visualized on the microscope **(Fig. 2C)**. Confirming these data, we were able to demonstrate that Ki67 expression, a classical intracellular marker of cell proliferation, increased after LOBE treatment **(Fig. 2D)**.

In a similar way to what was seen with LOBE treatments, *L. obliqua* hemolymph (LOH) can also increase hESC viability between doses from 0.001 to 0.1 µg/mL. However, a clear tendency of reducing viability was observed for highest LOH concentrations such as 1 and 10 µg/mL **(Fig 2E)**. To investigate the potential toxic effect of the highest LOBE and LOH doses on hESCs, LDH levels were measured in culture media after 24 h. As depicted in **Fig. 2F**, a slightly increase in LDH release occurred mainly for doses higher than 0.1 µg/mL of both LOBE and LOH treatments.

Next, as LOBE increased cell viability and proliferation without causing a significant cytotoxicity, we investigated it effects on hESC migration through the wound healing assay **(Fig. 3A)**. As shown in **Fig. 3B**, LOBE increases the cell migratory activity by up to 30 % for doses varying from 0.01 to 10 µg/mL after 8 h of incubation.

3.3 Characterization of L. obliqua venom components involved in hESC cytoprotective effect

Animal venoms are complex mixtures composed mainly by proteins and enzymes (high-molecular weight components), but also by low-molecular weight compounds

including organic molecules, amino acids, metal ions and small peptides [10]. In order to identify the type of components involved in LOBE-induced cytoprotection, the effects of different LOBE preparations were tested in hESC viability assay **(Fig 4A)**. Only dialyzed LOBE (containing high-molecular weight components > 14kDa) was able to increase cell viability. Boiled LOBE (containing denatured proteins) and LOBE peptide fraction (containing peptides and other small components < 3kDa) didn't show any effect **(Fig. 4A)**.

Since the main components involved in cytoprotection are proteins and/or enzymes, we decide to fractionate LOBE using anionic-exchange liquid chromatography on a Hi-Trap Q sepharose. As depicted in **Fig. 4B**, it was obtained four main peaks which were eluted after a linear gradient from 0 to 1M of NaCl. The chromatographic fractions corresponding to each peak were pooled and the protein profile was analyzed by SDS-PAGE (5-20%) **(Fig. 4C)**. Then, all chromatographic pools were tested by its ability to increase hESC viability and induce plasma coagulation **(Fig 4D and E)**. As it is possible to observe in **Fig 4D**, the chromatographic pool 2 was the only one able to significantly increase cell viability, while pool 4 caused a reduction **(Fig. 4D)**. On the other hand, pool 4 presented a strong procoagulant activity, while pool 2 did not cause any change in plasma clotting **(Fig. 4E)**. Confirming the initial tests performed with pool 2, this chromatographic fraction increased in a dose-dependent manner hESCs viability after 24 h incubation even at the higher doses of 1 and 10 µg/mL.

In order to identify the potential molecules involved in the cytoprotective action, a mass spectrometry-based proteomic analysis was performed. It was identified a total number of 430 proteins in LOBE **(Table S1)** and 312 proteins in LOH **(Table S2)**. Hemolins, lipocalins and protease inhibitors were the top 3 abundant protein classes in both proteomes (bristle extract and hemolymph) **(Fig. 5)**. However, LOBE was enriched in hemolin (27 %) and lipocalin (25 %) sequences **(Fig. 5A)**, while LOH was enriched in protease inhibitors (56 %) **(Fig. 5B)**. Based on its functional classification and biological activity all proteins with a potential cytoprotective role were grouped and analyzed as shown in **Tables 2** and **3**. These proteins belong to different classes **(Table 2)**. Immunoglobulin-like superfamily and biliverdin-binding proteins, such as hemolins and lipocalins; hemocyanins, which have antiapoptotic activity; antiviral proteins; antimicrobial proteins and small peptides, such as cecropin and attacin C and protease inhibitors, such as serine-protease inhibitors (SERPINS), Kazal-type and TIL-inhibitors superfamilies. For proteins common to bristle and hemolymph, a semi-quantitative

analysis based on spectral counts was also performed to estimate its abundance in each secretion. Lipocalins were highly enriched in bristles (fold change varying from 20 to 166); while hemocyanins (fold change 31), antiviral proteins (fold change 10) and protease inhibitors (fold change varying from 5 to 25) were identified to be more abundant in hemolymph. The abundance of hemolins was similar in both secretions, with a slightly increase in bristles, while antimicrobial proteins were enriched in bristles **(Table 2)**. Lastly an extensive literature review focused on the cytoprotective effects already described for some of these proteins was carried out and the results are detailed in **table 3**.

3.4 Mechanisms involved in LOBE-induced cytoprotection

In the next experiments we investigated whether LOBE could induce hESC cytoprotection even in serum deprivation conditions and how long lasting would be this protection. As showed in **Fig. 6A**, LOBE (0.1 µg/mL) treatment increased hESC viability in the presence of low concentrations of fetal bovine serum (1 % FBS) and even in its complete absence. This effect was long lasting, being significant up to 72 h of incubation with LOBE in serum deprivation conditions **(Fig. 6B)**. Intracellular nitric oxide generation **(Fig. 6C)** seems to be involved in cytoprotection, as well as antioxidant mechanisms, such as a decrease in superoxide anion production **(Fig. 6D)** and an increase in catalase activity **(Fig. 6 E)**. Corroborating these data, LOBE dose-dependently protects hESCs against the oxidative insult caused by hydrogen peroxide treatment **(Fig. 6F)**. Regarding the mechanism associated to LOBE-induced cell viability and proliferation we also demonstrated that pretreatment with an extracellular signal regulated kinase (ERK) inhibitor (PD98059) completely blocked this effect, suggesting a possible role for ERK activation in increasing hESC survival **(Fig. 6G)**.

3. Discussion

The loss of three or more consecutive pregnancies, designated as recurrent pregnancy loss (RPL), is a common distressing disorder for which there is no good treatment options. A third of women are clinically depressed, and one in five have levels of anxiety that are similar to those in psychiatric outpatient populations [27]. The risk factors may include a spectrum of subclinical dysfunctions, ranging from thrombophilia to anatomical, endocrine and immunological factors, but the underlying pathological pathways are not understood [28]. It seems that activation of endometrial progenitor cells,

tissue regeneration during menstruation cycle, and maturation of endometrial stromal cells (hESCs), which differentiate into specialized decidual cells prior and during pregnancy, are important events. Recently, it was demonstrated that RPL is strongly associated with hESC deficiency and increased cellular senescence, which in turn leads to the impairment of decidualization process and defects over consecutive conception cycles [5]. Moreover, hESCs isolated from RLP patient showed aberrant responsiveness to *in vitro* deciduogenic stimuli [8]. According to Lucas *et al* (2016) [5], a possible mechanism of hESCs deficiency and senescence is the intragenic hypomethylation and reduced expression of HMGB2, a protein involved in chromation bending and DNA damage repair.

Once the level of hESC depletion seems to be correlated with the number of miscarriages, we hypothesized that cytoprotective molecules could be useful not only as a therapeutic option, but also as a pharmacological tool to understand underlying mechanisms of RPL-induced hESC deficiency. In this work we described for the first time a potential cytoprotective action of *Lonomia obliqua* venom secretions on hESCs. *Lonomia obliqua* is a venomous caterpillar found mainly in tropical and subtropical areas of Argentina, Venezuela and Brazil. The main toxins are known to have hemotoxic effects with procoagulant activities, such as prothrombin and factor X activators [12,29]. However, as many other lepidopterans, this animal can produce a variety of protective compounds involved in tissue regeneration cycles during metamorphosis and developmental phases. In general, protective compound are found in hemolymph, but in *L. obliqua* they are also found in bristles [11]. Since a specific venom gland is absent in *L. obliqua* [30] both toxic and protective components circulate in hemolymph and are stored in bristles. Indeed, our results indicate that bristle extract (LOBE) and hemolymph (LOH) were able to increase hESC viability and proteomic analysis confirmed the presence of potential cytoprotective proteins in both secretions.

Regarding the direct cytotoxic effect of *L. obliqua* venom on hESCs, surprisingly it was found to be lower compared to the cytotoxic action of other venoms such as those from snakes, for example. LOBE and LOH caused only a slightly decrease in cellular viability with a consequent modest increase in LDH release mainly at the highest doses. One possible reason it would be the apparent absence of metalloproteinases in *L. obliqua* venom. Metalloproteinases are highly cytotoxic enzymes found to be enriched mainly in Viperidae snakes [31], but are also found in brown spider venoms [32]. A previous transcriptomic study wasn't be able to detect any metalloproteinase sequence [11], which

are in accordance to our proteomic data presented here. As mentioned above, the main toxic components of *L. obliqua* venom are those able to induce blood coagulation. Until now two procoagulant enzymes with factor X and prothrombin activating activities has been characterized. These toxins are serine-proteinase-like enzymes with no apparent cytotoxic action [33,34]. Although these enzymes have no described cytotoxic activity, they do not appear to be responsible for the cytoprotective action as well. Using anionic exchange chromatography, we clearly obtained separated fractions, one enriched in cytoprotective molecules without causing any procoagulant effect (pool 2, **Fig. 4**), and another with procoagulant enzymes, but without any cellular enhancing action (pool 4, **Fig. 4**). Differences in protein composition are evident between fractions of pools 2 and 4 as demonstrated by SDS-PAGE analysis, being pool 2 able to increase hESC viability even at higher concentrations up to 10 µg/mL.

Through mass spectrometry-based proteomic analysis we report a total number of 430 and 312 identified proteins in LOBE and LOH, respectively. All these proteins were categorized based on its classification and biological function, generating at least six protein classes (hemolins, lipocalins, hemocyanins, antiviral proteins, antimicrobial proteins and protease inhibitors) with potential cytoprotective roles. Previous works, have already reported the isolation, characterization and production of recombinant molecules with cytoprotective effects belonging to hemocyanins, lipocalins, hemolins and antiviral proteins. For instance, hemocyanins, which are proteins with molecular weight around of 20 kDa, protects V-79 cells (hamster lung fibroblasts) against H_2O_2-induced oxidative damage and increase cell proliferation by blocking apoptosis [35–37]. Lipocalins also have a similar molecular weight and increases viability and proliferation of human endothelial and fibroblasts primary cultures, mainly by anti-apoptotic mechanisms involving downregulation of caspase-3 and Bcl-2 upregulation [33,38,39]. At least one antiviral molecule has been isolated from hemolymph. It is a protein (20 kDa) able to protect cells by inhibit replication of rubella, herpes and picornavirus [40,41]. Hemolins presented a higher molecular weight (around of 45 kDa) and are cell-adhesion molecules belonging to immunoglobulin-like superfamily. *In vivo* the treatment with recombinant hemolin improved healing in a rat model of circular full-thickness skin wounds. The *in vivo* effects were associated with an increased number of activated fibroblasts, proliferation of epithelial cells and reduced local inflammation [42]. *In vitro* hemolin also protects human fibroblasts against apoptosis and oxidative stress [43]. Interestingly, the majority band identified by us in pool 2 fractions from Q-sepharose column has a

molecular weight between 50-40 kDa **(Fig. 4C)**. Moreover, this band was apparently absent in pool 4 fractions which is devoid of cytoprotective activity **(Fig. 4D)**. Thus, we can speculate that pool 2 is probably enriched in hemolins being the main factor driving the protective effects in hESCs. Unlike what was observed for lipocalins, hemocyanins, antiviral proteins and hemolins, antimicrobial peptides and protease inhibitors have not yet been isolated and characterized. Although these protein sequences are abundant in the proteome and most of them have a promising activity [11], its real cytoprotective effects remained to be studied.

Analyzing the mechanism involved in LOBE-induced hESC viability and proliferation, we found that the protection against oxidative damage is an important pathway. LOBE downregulates superoxide anion production in hESCs serum deprivation conditions and also protects against the direct damage triggered by hydrogen peroxide. It seems that activation of endogenous pathways involving upregulation of antioxidant enzymes, such as catalase, and intracellular generation of nitric oxide are contributing mechanisms. In fact, LOBE-induced cellular protection was long-lasting and it was observed even in stressful conditions of total absence of serum nutrients. Interestingly, Heinen *et al*. (2014) [37] and Moraes *et al*. (2017) [44] reported respectively an involvement of cAMP and ERK-dependent signaling in LOBE-induced cell survival mechanisms. Our data confirm the relevance of ERK pathway, since pretreatment with ERK inhibitor, PD98059, blocked significantly the enhancing effect in hESC viability.

4. Conclusions

Besides the physical trauma, recurrent pregnancy loss (RPL) is also associated with considerable psychological morbidity. Unfortunately, there is no treatment options. Since hESC deficiency, senescence and an aberrant responsiveness to deciduogenic cues are strongly involved in RLP we decide to study the role of cytoprotective molecules obtained from *Lonomia obliqua* venomous secretions on hESC proliferation and survival mechanisms. In this work we reported the identification of at least six protein classes with cytoprotective properties through proteomic analysis and isolated one fraction enriched in this cytoprotective factors. *L. obliqua* venomous secretions induced significant increase in hESCs viability, proliferation and migration mainly by the protection against oxidative damage and ERK-dependent pathway activation.

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6. Conflict of interest statement

The authors declare that there is no conflict of interest involved.

7. References

[1] V.A. Prianishnikov, On the concept of stem cell and a model of functional-morphological structure of the endometrium, Contraception. 18 (1978) 213–223. https://doi.org/10.1016/S0010-7824(78)80015-8.

[2] R.W.S. Chan, K.E. Schwab, C.E. Gargett, Clonogenicity of Human Endometrial Epithelial and Stromal Cells1, Biol. Reprod. 70 (2004) 1738–1750. https://doi.org/10.1095/biolreprod.103.024109.

[3] E.S. Lucas, N.P. Dyer, K. Fishwick, S. Ott, J.J. Brosens, Success after failure: The role of endometrial stem cells in recurrent miscarriage, Reproduction. 152 (2016) R159–R166. https://doi.org/10.1530/REP-16-0306.

[4] M. Dominici, K. Le Blanc, I. Mueller, I. Slaper-Cortenbach, F.C. Marini, D.S. Krause, R.J. Deans, A. Keating, D.J. Prockop, E.M. Horwitz, Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement, Cytotherapy. 8 (2006) 315–317. https://doi.org/10.1080/14653240600855905.

[5] E.S. Lucas, N.P. Dyer, K. Murakami, Y. Hou Lee, Y.W. Chan, G. Grimaldi, J. Muter, P.J. Brighton, J.D. Moore, G. Patel, J.K.Y. Chan, S. Takeda, E.W.F. Lam, S. Quenby, S. Ott, J.J. Brosens, Loss of Endometrial Plasticity in Recurrent Pregnancy Loss, Stem Cells. 34 (2016) 346–356. https://doi.org/10.1002/stem.2222.

[6] A.J. Wilcox, D.D. Baird, C.R. Weinberg, Time of Implantation of the Conceptus and Loss of Pregnancy, N. Engl. J. Med. 340 (1999) 1796–1799. https://doi.org/10.1056/nejm199906103402304.

[7] J.J. Brosens, M.S. Salker, G. Teklenburg, J. Nautiyal, S. Salter, E.S. Lucas, J.H. Steel, M. Christian, Y.W. Chan, C.M. Boomsma, J.D. Moore, G.M. Hartshorne, S. Šućurović, B. Mulac-Jericevic, C.J. Heijnen, S. Quenby, M.J. Groot Koerkamp, F.C.P. Holstege, A. Shmygol, N.S. Macklon, Uterine selection of human embryos at implantation, Sci. Rep. 4 (2014). https://doi.org/10.1038/srep03894.

[8] M. Salker, G. Teklenburg, M. Molokhia, S. Lavery, G. Trew, T. Aojanepong, H.J. Mardon, A.U. Lokugamage, R. Rai, C. Landles, B.A.J. Roelen, S. Quenby, E.W. Kuijk, A. Kavelaars, C.J. Heijnen, L. Regan, N.S. Macklon, J.J. Brosens, Natural selection of human embryos: Impaired decidualization of endometrium disables embryo-maternal interactions and causes recurrent pregnancy loss, PLoS One. 5 (2010). https://doi.org/10.1371/journal.pone.0010287.

[9] H. El Hachem, V. Crepaux, P. May-Panloup, P. Descamps, G. Legendre, P.E. Bouet, Recurrent pregnancy loss: Current perspectives, Int. J. Womens. Health. 9 (2017) 331–345. https://doi.org/10.2147/IJWH.S100817.

[10] C. Bon, The natural toxins, Biochimie. 82 (2000) 791–792. https://doi.org/10.1016/S0300-9084(00)01186-X.

[11] A.B.G. Veiga, J.M.C. Ribeiro, J. a Guimarães, I.M.B. Francischetti, A catalog for the transcripts from the venomous structures of the caterpillar Lonomia obliqua: identification of the proteins potentially involved in the coagulation disorder and hemorrhagic syndrome., Gene. 355 (2005) 11–27. https://doi.org/10.1016/j.gene.2005.05.002.

[12] A.F.M. Pinto, M. Berger, J. Reck, R.M.S. Terra, J. a Guimarães, Lonomia obliqua venom: In vivo effects and molecular aspects associated with the hemorrhagic syndrome., Toxicon. 56 (2010) 1103–12. https://doi.org/10.1016/j.toxicon.2010.01.013.

[13] A.C.M. Rocha-Campos, L.R.C. Gonçalves, H.G. Higashi, I.K. Yamagushi, I. Fernandes, J.E. Oliveira, M.T.C.P. Ribela, M.C.C. Sousa-E-Silva, W. Dias Da Silva, Specific heterologous F(ab′) 2 antibodies revert blood incoagulability resulting from envenoming by Lonomia obliqua caterpillars, Am. J. Trop. Med. Hyg. 64 (2001) 283–289. https://doi.org/10.4269/ajtmh.2001.64.283.

[14] A.F.M. Pinto, K.R.L.M. Silva, J. a Guimarães, Proteases from Lonomia obliqua venomous secretions: comparison of procoagulant, fibrin(ogen)olytic and amidolytic activities., Toxicon. 47 (2006) 113–21. https://doi.org/10.1016/j.toxicon.2005.10.004.
[15] M. Berger, A.F.M. Pinto, J.A. Guimarães, Purification and functional characterization of bothrojaractivase, a prothrombin-activating metalloproteinase isolated from Bothrops jararaca snake venom, Toxicon. 51 (2008) 488–501.
[16] M.I. Klein, J. Xiao, B. Lu, C.M. Delahunty, J.R. Yates, H. Koo, Streptococcus mutans Protein Synthesis during Mixed-Species Biofilm Development by High-Throughput Quantitative Proteomics, PLoS One. 7 (2012). https://doi.org/10.1371/journal.pone.0045795.
[17] M.P. Washburn, D. Wolters, J.R. Yates, Large-scale analysis of the yeast proteome by multidimensional protein identification technology, Nat. Biotechnol. 19 (2001) 242–247. https://doi.org/10.1038/85686.
[18] W.H. McDonald, D.L. Tabb, R.G. Sadygov, M.J. MacCoss, J. Venable, J. Graumann, J.R. Johnson, D. Cociorva, J.R. Yates, MS1, MS2, and SQT - Three unified, compact, and easily parsed file formats for the storage of shotgun proteomic spectra and identifications, Rapid Commun. Mass Spectrom. 18 (2004) 2162–2168. https://doi.org/10.1002/rcm.1603.
[19] T. Xu, S.K. Park, J.D. Venable, J.A. Wohlschlegel, J.K. Diedrich, D. Cociorva, B. Lu, L. Liao, J. Hewel, X. Han, C.C.L. Wong, B. Fonslow, C. Delahunty, Y. Gao, H. Shah, J.R. Yates, ProLuCID: An improved SEQUEST-like algorithm with enhanced sensitivity and specificity, J. Proteomics. 129 (2015) 16–24. https://doi.org/10.1016/j.jprot.2015.07.001.
[20] J. Peng, J.E. Elias, C.C. Thoreen, L.J. Licklider, S.P. Gygi, Evaluation of multidimensional chromatography coupled with tandem mass spectrometry (LC/LC-MS/MS) for large-scale protein analysis: The yeast proteome, J. Proteome Res. 2 (2003) 43–50. https://doi.org/10.1021/pr025556v.
[21] D.L. Tabb, W.H. McDonald, J.R. Yates, DTASelect and contrast: Tools for assembling and comparing protein identifications from shotgun proteomics, J. Proteome Res. 1 (2002) 21–26. https://doi.org/10.1021/pr015504q.
[22] P. Terraciano, T. Garcez, L. Ayres, I. Durli, M. Baggio, C.P. Kuhl, C. Laurino, E. Passos, A.H. Paz, E. Cirne-Lima, Cell therapy for chemically induced ovarian failure in mice, Stem Cells Int. 2014 (2014). https://doi.org/10.1155/2014/720753.
[23] E. Orellana, A. Kasinski, Sulforhodamine B (SRB) Assay in Cell Culture to Investigate Cell Proliferation, BIO-PROTOCOL. 6 (2016). https://doi.org/10.21769/bioprotoc.1984.
[24] M. Berger, J.A. de Moraes, W.O. Beys-da-Silva, L. Santi, P.B. Terraciano, D. Driemeier, E.O. Cirne-Lima, E.P. Passos, M.A.R. Vieira, T.C. Barja-Fidalgo, J.A. Guimarães, Renal and vascular effects of kallikrein inhibition in a model of Lonomia obliqua venom-induced acute kidney injury, PLoS Negl. Trop. Dis. 13 (2019). https://doi.org/10.1371/journal.pntd.0007197.
[25] K.M. Miranda, M.G. Espey, D. a Wink, A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite., Nitric Oxide. 5 (2001) 62–71. https://doi.org/10.1006/niox.2000.0319.
[26] M.S.N. Hohmann, R.D.R. Cardoso, F.A. Pinho-Ribeiro, J. Crespigio, T.M. Cunha, J.C. Alves-Filho, R. V. Da Silva, P. Pinge-Filho, S.H. Ferreira, F.Q. Cunha, R. Casagrande, W.A. Verri, 5-Lipoxygenase Deficiency Reduces Acetaminophen-Induced Hepatotoxicity and Lethality, Biomed Res. Int. 2013 (2013). https://doi.org/10.1155/2013/627046.

[27] M.H. Pinar, K. Gibbins, M. He, S. Kostadinov, R. Silver, Early Pregnancy Losses: Review of Nomenclature, Histopathology, and Possible Etiologies, Fetal Pediatr. Pathol. 37 (2018) 191–209. https://doi.org/10.1080/15513815.2018.1455775.
[28] L.J. Ewington, S. Tewary, J.J. Brosens, New insights into the mechanisms underlying recurrent pregnancy loss, J. Obstet. Gynaecol. Res. 45 (2019) 258–265. https://doi.org/10.1111/jog.13837.
[29] M. Berger, J. Reck, R.M.S. Terra, A.F.M. Pinto, C. Termignoni, J. a Guimarães, Lonomia obliqua caterpillar envenomation causes platelet hypoaggregation and blood incoagulability in rats., Toxicon. 55 (2010) 33–44. https://doi.org/10.1016/j.toxicon.2009.06.033.
[30] A.B.G. Veiga, B. Blochtein, J.A. Guimarães, Structures involved in production, secretion and injection of the venom produced by the caterpillar Lonomia obliqua (Lepidoptera, Saturniidae), Toxicon. 39 (2001) 1343–1351. https://doi.org/10.1016/S0041-0101(01)00086-1.
[31] S. Takeda, H. Takeya, S. Iwanaga, Snake venom metalloproteinases: Structure, function and relevance to the mammalian ADAM/ADAMTS family proteins, Biochim. Biophys. Acta - Proteins Proteomics. 1824 (2012) 164–176. https://doi.org/10.1016/j.bbapap.2011.04.009.
[32] D. Trevisan-Silva, L.H. Gremski, O.M. Chaim, R.B. da Silveira, G.O. Meissner, O.C. Mangili, K.C. Barbaro, W. Gremski, S.S. Veiga, A. Senff-Ribeiro, Astacin-like metalloproteases are a gene family of toxins present in the venom of different species of the brown spider (genus Loxosceles)., Biochimie. 92 (2010) 21–32. https://doi.org/10.1016/j.biochi.2009.10.003.
[33] C. V. Reis, F.C.V. Portaro, S.A. Andrade, M. Fritzen, B.L. Fernandes, C.A.M. Sampaio, A.C.M. Camargo, A.M. Chudzinski-Tavassi, A prothrombin activator serine protease from the Lonomia obliqua caterpillar venom (Lopap): Biochemical characterization, Thromb. Res. 102 (2001) 427–436. https://doi.org/10.1016/S0049-3848(01)00265-1.
[34] M.P. Alvarez Flores, M. Fritzen, C. V Reis, A.M. Chudzinski-Tavassi, Losac, a factor X activator from Lonomia obliqua bristle extract: its role in the pathophysiological mechanisms and cell survival., Biochem. Biophys. Res. Commun. 343 (2006) 1216–23. https://doi.org/10.1016/j.bbrc.2006.03.068.
[35] R.Z. Mendonça, K.N. Greco, A.P.B. Sousa, R.H.P. Moraes, R.M. Astray, C.A. Pereira, Enhancing effect of a protein from Lonomia obliqua hemolymph on recombinant protein production, Cytotechnology. 57 (2008) 83–91. https://doi.org/10.1007/s10616-008-9141-4.
[36] H.L.A. Vieira, A.C.P. Pereira, C.C. Peixoto, R.H.P. Moraes, P.M. Alves, R.Z. Mendonça, Improvement of recombinant protein production by an anti-apoptotic protein from hemolymph of Lonomia obliqua., Cytotechnology. 62 (2010) 547–55. https://doi.org/10.1007/s10616-010-9305-x.
[37] T.E. Heinen, C.B. De Farias, A.L. Abujamra, R.Z. Mendonça, R. Roesler, A.B.G. Da Veiga, Effects of Lonomia obliqua caterpillar venom upon the proliferation and viability of cell lines, Cytotechnology. 66 (2014) 63–74. https://doi.org/10.1007/s10616-013-9537-7.
[38] C.V. Reis, S.A. Andrade, O.H.P. Ramos, C.R.R. Ramos, P.L. Ho, I.D.F.C. Batista, A.M. Chudzinski-Tavassi, Lopap, a prothrombin activator from Lonomia obliqua belonging to the lipocalin family: Recombinant production, biochemical characterization and structure-function insights, Biochem. J. 398 (2006) 295–302. https://doi.org/10.1042/BJ20060325.
[39] M. Fritzen, M.P.A. Flores, C.V. Reis, A.M. Chudzinski-Tavassi, A prothrombin activator (Lopap) modulating inflammation, coagulation and cell survival mechanisms.,

Biochem. Biophys. Res. Commun. 333 (2005) 517–23. https://doi.org/10.1016/j.bbrc.2005.05.140.
[40] A.C.V. Carmo, D.N.S. Giovanni, T.P. Corrêa, L.M. Martins, R.C. Stocco, C.A.T. Suazo, R.H.P. Moraes, A.B.G. Veiga, R.Z. Mendonça, Expression of an antiviral protein from Lonomia obliqua hemolymph in baculovirus/insect cell system, Antiviral Res. 94 (2012) 126–130. https://doi.org/10.1016/j.antiviral.2011.12.010.
[41] A.C.V. Carmo, L.H.T. Yamasaki, C.A. Figueiredo, D.N. da Silva Giovanni, M.I. de Oliveira, F.C.P. dos Santos, S.P. Curti, P. Rahal, R.Z. Mendonça, Discovery of a new antiviral protein isolated Lonomia obliqua analysed by bioinformatics and real-time approaches, Cytotechnology. 67 (2014) 1011–1022. https://doi.org/10.1007/s10616-014-9740-1.
[42] A.C. Sato, R.V. Bosch, S.E.A. Will, M.P. Alvarez-Flores, M.B. Goldfeder, K.F.M. Pasqualoto, B.A.V.G. da Silva, S.A. de Andrade, A.M. Chudzinski-Tavassi, Exploring the in vivo wound healing effects of a recombinant hemolin from the caterpillar Lonomia obliqua, J. Venom. Anim. Toxins Incl. Trop. Dis. 22 (2016). https://doi.org/10.1186/s40409-016-0093-4.
[43] R.V. Bosch, M.P. Alvarez-Flores, D.A. Maria, A.M. Chudzinski-Tavassi, Hemolin triggers cell survival on fibroblasts in response to serum deprivation by inhibition of apoptosis, Biomed. Pharmacother. 82 (2016) 537–546. https://doi.org/10.1016/j.biopha.2016.05.043.
[44] J.A. Moraes, G. Rodrigues, V. Nascimento-Silva, M. Renovato-Martins, M. Berger, J.A. Guimarães, C. Barja-Fidalgo, Effects of Lonomia obliqua Venom on Vascular Smooth Muscle Cells: Contribution of NADPH Oxidase-Derived Reactive Oxygen Species., Toxins (Basel). 9 (2017). https://doi.org/10.3390/toxins9110360.

Figures

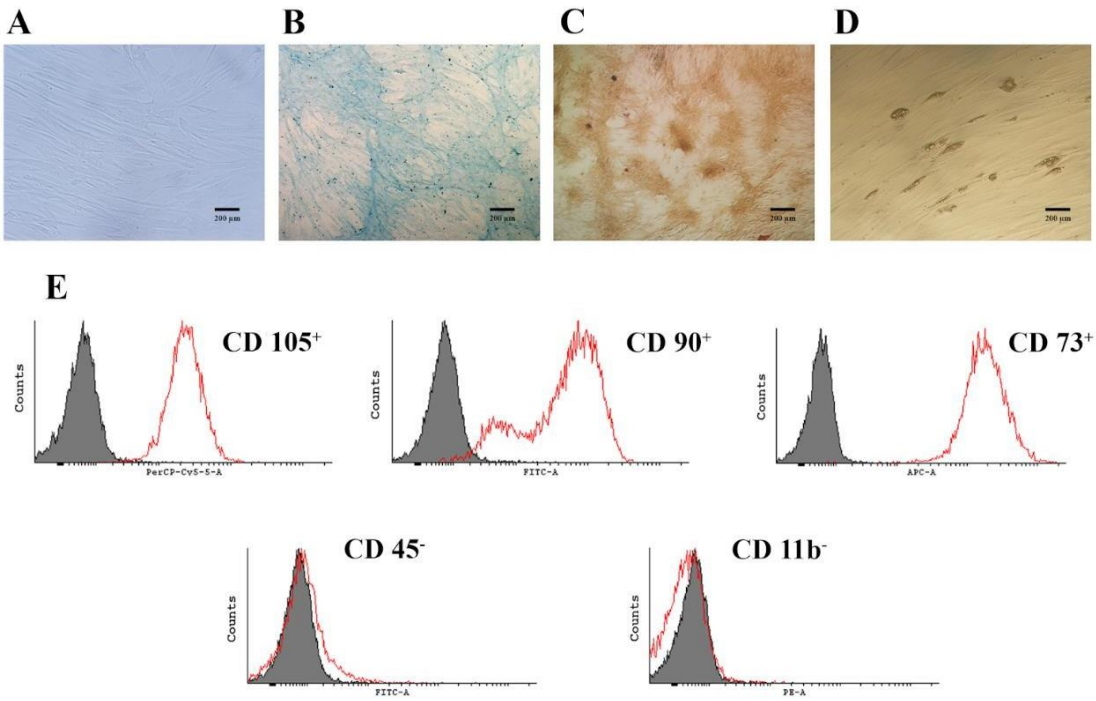

Figure 1. Human endometrial stromal cell (hESC) isolation and characterization. hESCs were isolated from seven endometrial tissue biopsies of healthy donors undergoing *in vitro* fertilization procedures. The images presented here are representative of isolation and characterization steps performed for all seven samples. **(A).** Morphological aspects of isolated hESCs under optical microscopy. **(B).** Morphological aspects of hESCs differentiated in chondrocytes. **(C).** Morphological aspects of hESCs differentiated in osteocytes. **(D).** Morphological aspects of hESCs differentiated in adipocytes. **(E).** Immunophenotypic characterization was performed by flow cytometry. It was analyzed mesenchymal (CD105, CD90 and CD73) and hematopoietic markers (CD45 and CD11).

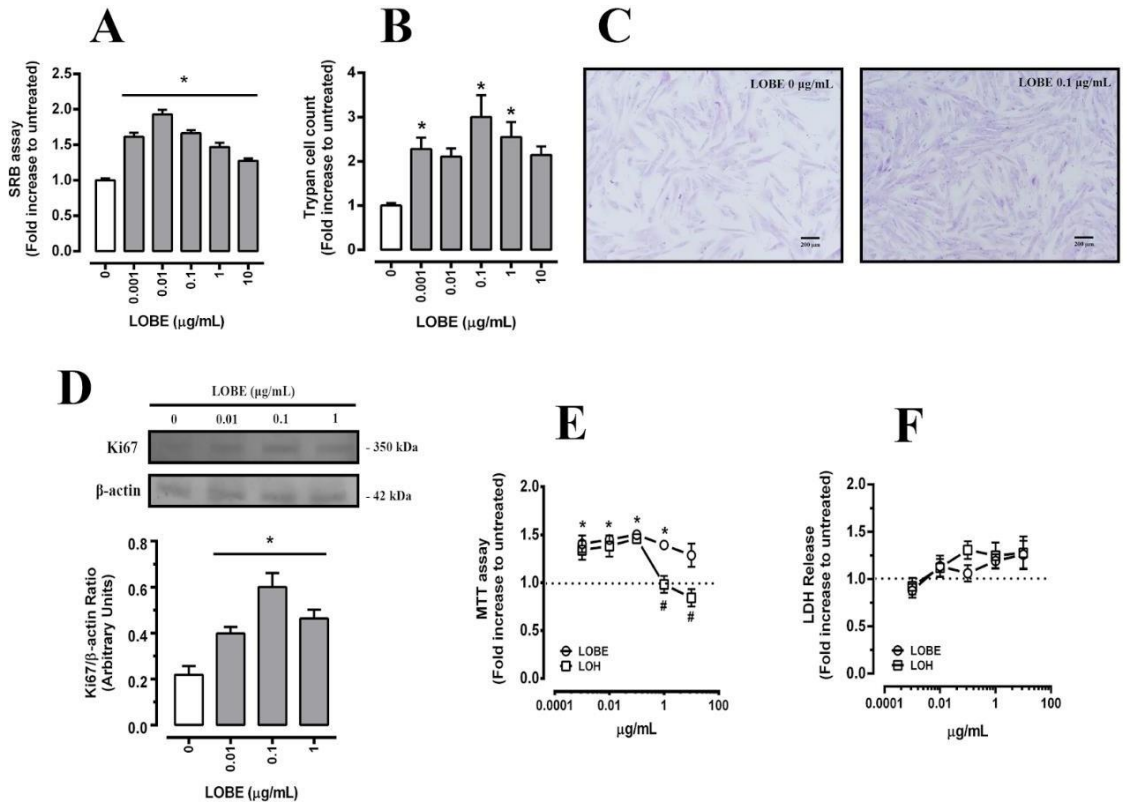

Figure 2. Effects of *L. obliqua* venomous secretions on hESC viability and proliferation. (A). hESCs in culture were treated with different concentrations of *L. obliqua* bristle extract (LOBE) and cell proliferation was evaluated by the sulforhodamine B (SRB) assay. **(B).** hESCs in culture were treated with different LOBE concentrations and cell proliferation was alternatively analyzed by the trypan-blue exclusion cell counts. **(C).** Differences in LOBE-induced cell proliferation were also verified by optical microscopy in Giemsa-stained hESCs. **(D).** hESCs were treated with LOBE and the immunocontent of the main cell proliferation marker (Ki67) was determined by western-blot. **(E).** Comparative effects of LOBE and *L. obliqua* hemolymph (LOH) on hESCs viability was studied by MTT assay. **(F).** Cell toxicity induced by LOBE and LOH was evaluated by LDH release. Results represents mean values ± SE. Were considered statistically different values of *$p < 0.05$ vs untreated cells (0 µg/mL LOBE) or #$p < 0.05$ vs LOBE treated cells (1 and 10 µg/mL).

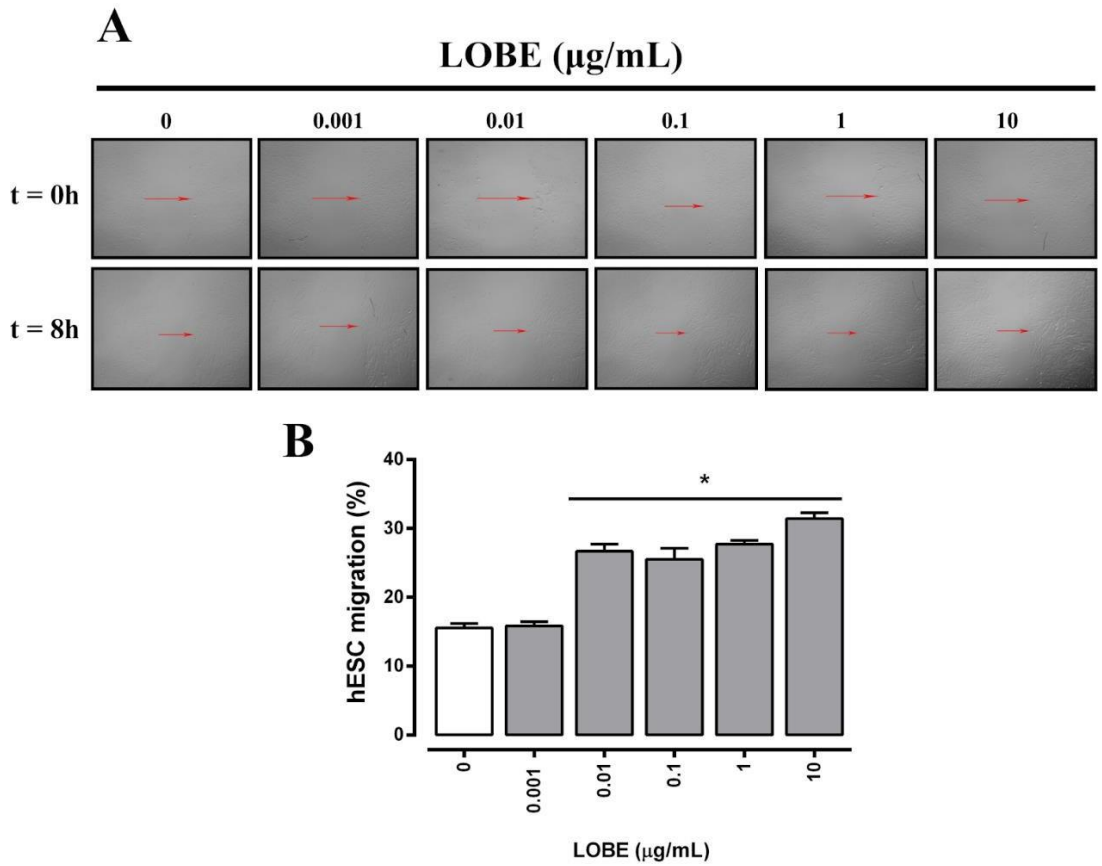

Figure 3. Effects of *L. obliqua* venomous secretions on hESC migration. Cell migration was evaluated by wound healing assay. **(A).** hESCs were cultured until reach confluence, a scratch with yellow tip was performed and cells were treated with different LOBE concentrations. Microscopic photographs were captured at the beginning (0 h) and after 8 h to follow cell migration through the scratched area. Red arrows represent the distance between scratch edges. **(B).** Clear areas were measured and compared between treatment groups using Image J software and the results were expressed as percent values of wound repaired area (% migration). Data are presented as mean ± SE and values of *p < 0.05 vs untreated cells (0 µg/mL LOBE) were considered statistically significant.

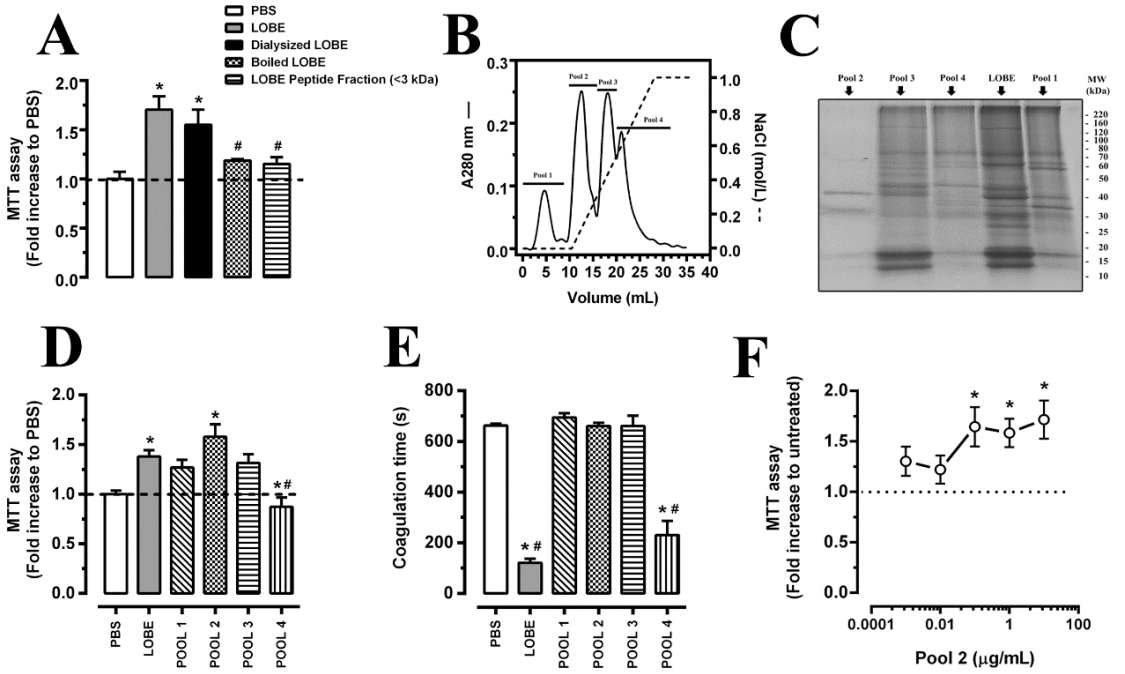

Figure 4. Characterization of *L. obliqua* **venom components involved in hESC cytoprotection. (A).** hESCs were treated with different LOBE preparations (crude LOBE, dialyzed LOBE, boiled LOBE and LOBE peptide fraction) at concentration of 0.1 µg/mL. Cell viability was measured by MTT assay. **(B).** LOBE components were fractionated by Q-sepharose anion-exchange chromatography. Fractions corresponding to each protein peak were pooled and used in subsequent analysis. **(C).** Pooled fractions from Q-sepharose were analyzed by gradient SDS-PAGE (5-20%) electrophoresis. **(D).** Effects of pooled fraction on hESC viability was tested in MTT assay. **(E)**. Procoagulant effects of pooled fractions was tested in plasma recalcification assay. **(F).** Dose-response action of Q-sepharose-derived pool 2 on hESC viability was verified by MTT assay. Results represents mean values ± SE. Were considered statistically different values of *p < 0.05 vs PBS-treated cells or PBS treatment (0 µg/mL LOBE) or #p < 0.05 vs LOBE treated cells or pool 2 treated cells.

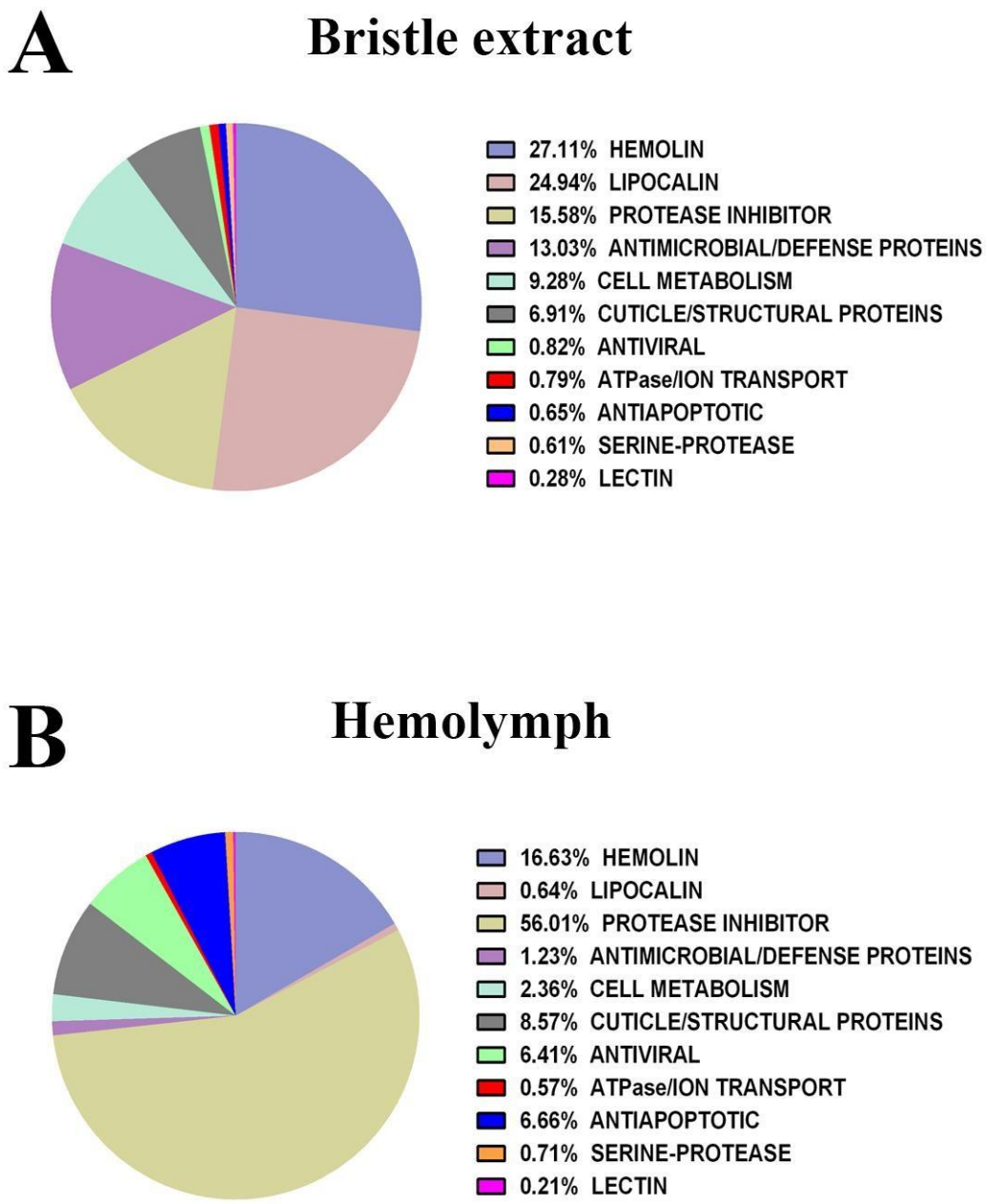

Figure 5. Proteomic analysis of *L. obliqua* **venomous secretions.** Proteins from *L. obliqua* bristle extract (LOBE) and hemolymph (LOH) were identified by mass spectrometry-based proteomic analysis. It was identified a total number of 430 proteins in LOBE and 312 in LOH (a complete list with all identified protein can be found in supplementary tables S1 and S2). **(A).** Proteins identified in bristle extracts were categorized accordingly to its classes and/or biological function. **(B).** Proteins identified in hemolymph were categorized accordingly to its classes and/or biological function.

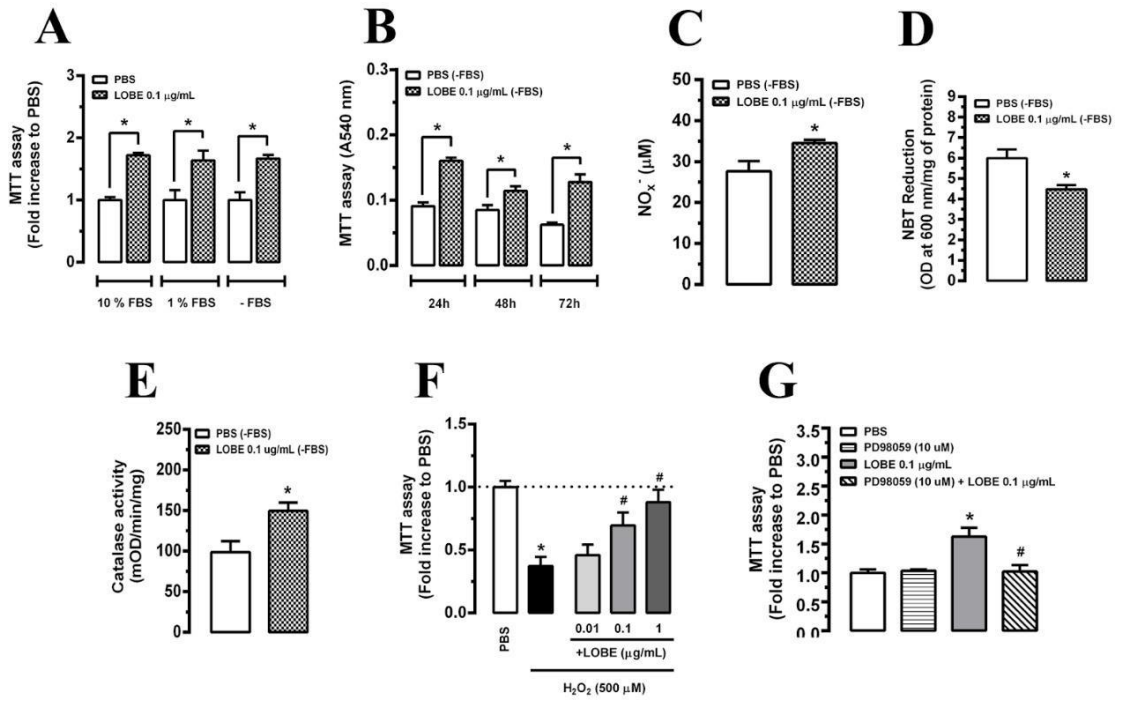

Figure 6. Mechanisms involved in *L. obliqua* **venom-induced cytoprotective effects. (A).** hESCs were treated with LOBE in the presence of 1 and 10 % fetal bovine serum (FBS) and in complete serum deprivation conditions. After 24 h cell viability was evaluated by MTT assay. **(B).** hESCs were treated with LOBE in serum deprivation conditions (-FBS) and cell viability was analyzed after 24, 48 and 72 h by MTT assay. **(C).** LOBE-treated hESCs cultivated in absence of FBS were collected after 24 h and nitrate/nitrite (NO_x^-) concentrations were evaluated by the Griess method. **(D).** LOBE-treated hESCs cultivated in absence of FBS were collected after 24 h and generated superoxide anion was estimated by nitroblue tetrazolium (NBT) reduction. **(E).** LOBE-treated hESCs cultivated in absence of FBS were collected after 24 h and catalase activity was determined by hydrogen peroxide decomposition. **(F).** An oxidative insult was triggered by hydrogen peroxide and hESCs were treated by different LOBE concentrations. Cell viability was determined by MTT assay. **(G).** The participation of ERK-dependent pathway was estimated in hESCs pretreated with PD98059, an ERK inhibitor. Cell viability was determined by MTT assay. Results represents mean values ± SE. Were considered statistically different values of *$p < 0.05$ vs PBS-treated cells (0 µg/mL LOBE) or #$p < 0.05$ vs LOBE treated cells.

Tables

Table 1. Effects of *L. obliqua* bristle extract (LOBE) on hESC viability obtained from seven healthy donors.

Patient ID[a]	PBS	LOBE (µg/mL)				
		0.001	**0.01**	**0.1**	**1**	**10**
1	1 ± 0.010	1.43 ± 0.010[b]	1.33 ± 0.005[b]	1.46 ± 0.005[b]	1.49 ± 0.014[b]	1.32 ± 0.009[b]
2	1 ± 0.003	1.42 ± 0.003[b]	1.30 ± 0.003[b]	1.13 ± 0.004[b]	1.04 ± 0.010[b]	1.17 ± 0.006[b]
3	1 ± 0.010	1.60 ± 0.006[b]	2.00 ± 0.020[b]	2.03 ± 0.023[b]	1.60 ± 0.010[b]	1.31 ± 0.006[b]
4	1 ± 0.050	1.28 ± 0.096	1.44 ± 0.140[b]	1.20 ± 0.020	1.18 ± 0.010	1.22 ± 0.010
5	1 ± 0.030	1.32 ± 0.040[b]	1.21 ± 0.027[b]	1.01 ± 0.014	1.21 ± 0.030[b]	1.36 ± 0.032[b]
6	1 ± 0.030	1.31 ± 0.008[b]	1.39 ± 0.030[b]	1.31 ± 0.024[b]	1.41 ± 0.030[b]	1.40 ± 0.030[b]
7	1 ± 0.010	1.56 ± 0.010[b]	1.47 ± 0.005[b]	1.60 ± 0.005[b]	1.48 ± 0.007[b]	1.43 ± 0.009[b]

[a] Human endometrial stem cells (hESC) were isolated from seven different patients and then treated with *L. obliqua* bristle extract (LOBE) for 24 h. Cell proliferation was estimated by MTT assay.

[b] Values of $p < 0.05$ were considered statistically significant when compared to cells treated with PBS (One-way ANOVA, followed by bonferroni's post-hoc test). Data (mean ± SE) were expressed as fold-increase to control (PBS)-value.

Table 2. Proteomic data and functional classification of the main potential cytoprotective molecules identified in *L. obliqua*.

Accession Number[1]	Identified Proteins[2]	Proteomic Data[3]					Protein MW[4] (Da)	Protein Class/Function[5]
		Detection		Spectral Counts				
		B	H	B	H	Fold change		
ABF21070.1	hemolin	✔	✔	658	677	-1.03	45,140	immunoglobulin-like superfamily hemolin family immunoglobulin cell adhesion molecule
ABF21073.1	hemolin	✔	✔	606	474	1.27	45,114	
ABF 21072.1	hemolin	✔	✔	603	380	1.59	45,154	
ABF 21071.1	hemolin	✔	✔	534	435	1.23	45,590	
AAV91447.1	lipocalin 1	✔	✔	589	29	20.3	22,432	lipocalin family cytosolic fatty-acid binding protein family biliverdin binding protein serine peptidase
Q5ECE3.2	lipocalin 1/4; Lopap	✔	✔	583	29	20.1	22,432	
AAV91423.1	lipocalin 4	✔	✔	526	15	35.1	22,432	
AAW88441.1	prothrombin activator	✔	✔	499	3	166	22,432	
AAV91470.1	lipocalin 3	✔	ND	9	-	-	15,423	lipocalin family odorant binding-protein
P85195.2	lipocalin 2	✔	ND	3	-	-	17,937	lipocalin family cuticle protein

AAV91437.1	hypothetical protein 22, partial	✔	✔	21	653	-31.1	19,121	hemocyanin/hexamerin superfamily hemocyanin C
AEZ02311.1	putative antiviral protein	✔	✔	73	758	-10.4	19,792	undefined protein class antiviral protein
Q5MGQ0.1	Putative defense protein 1	✔	✔	574	62	9.26	17,801	insect defense protein superfamily defensin antibiotic, antimicrobial peptide
AAV91350.1	defense protein 1	✔	✔	574	62	9.26	17,801	
Q5MGD8.1	defense protein 4	✔	✔	2	4	-2.0	7,215	antibacterial peptide cecropin-like protein cecropin C
AAV91462.1	defense protein 4	✔	✔	2	4	-2.0	7,215	
AAV91454.1	defense protein 3	✔	✔	1	7	-7.0	25,053	antibacterial peptide attacin/sarcotoxin-2 family attacin C
Q5MGE6.1	defense protein 3	✔	✔	1	7	-7.0	25,053	
AAV91431.1	serine protease inhibitor 4, partial	✔	✔	448	2191	-4.89	43,194	hydrolase inhibitor serpin superfamily antitrypsin
AAV91430.1	serpin 3, partial	✔	✔	433	2181	-5.04	43,194	
Q5MGH0.1	Serine protease inhibitor 3/4	✔	✔	433	2181	-5.04	43,194	
AAV91429.1	serpin 2	✔	✔	27	7	3.86	43,764	hydrolase inhibitor serpin superfamily

AAV91428.1	serpin 1	✔	✔	21	6	3.50	43,740	hydrolase inhibitor serpin superfamily antichymotrypsin
AAV91424.1	protease inhibitor 1	✔	✔	13	2	6.50	43,740	
AAV91426.1	putative protease inhibitor 4	✔	✔	3	26	-8.60	10,971	hydrolase inhibitor TIL superfamily trypsin inhibitor-like
AAV91461.1	serpin 6, partial	✔	ND	2	-	-	28,562	hydrolase inhibitor serpin superfamily
AAV91449.1	protease inhibitor 7	ND	✔	-	25	-	10,225	hydrolase inhibitor Kazal-type protease inhibitor
AAV91427.1	protease inhibitor 5, partial	ND	✔	-	3	-	9,658	hydrolase inhibitor TIL superfamily trypsin inhibitor-like

1. Acession number in UniProtKB/Swiss-Prot database.

2. All proteins were identified based on sequences deposited in *L. obliqua* data bases.

3. Semi-quantitative proteomic data was obtained based on spectral counts for each identified protein in bristles (B) and hemolymph (H) and represent its abundance. Spectral count ratio (B/H) was calculated to obtain the fold change for those proteins present in both B and H. Positive values indicate abundant proteins in B while negative values indicate abundant proteins in H. ND denoted not detected proteins.

4. Predicted molecular weight (MW) of identified proteins.

5. Proteins were manually searched in UniProtKB/Swiss-Prot database and classified according to its class/function.

Table 3. Potential cytoprotective function described for proteins identified in *L. obliqua* proteome.

Protein/Protein Class[1]	Potential Cytoprotective Function[2]	References
Hemolins	Stimulates the activation of fibroblasts, proliferation of epithelial cells, healing and decrease of inflammation in a rat model of full-thickness wounds; Blocks apoptosis, increases NO production and proliferation of HUVECs; induces protection of FN-1 cells in conditions of stress by serum deprivation preventing ROS-mediated damage and loss of mitochondrial function by inhibiting apoptosis. Stimulates HDF cell migration and wound repair.	Alvarez-Flores et al., 2006; Sato *et al*., 2016; Bosch *et al*., 2016
Lipocalins	Increases cell viability, NO production and inhibits apoptosis induced by serum deprivation in HUVECs; Induces suppression of caspase 3 activity and upregulates Bcl-2 and Ki-67 in primary human fibroblasts; Increases extracellular matrix proteins and matrix remodelling in mice skin.	Reis et al., 2001; Reis et al., 2006; Fritzen et al., 2005;
Hemocyanins	Prevents apoptosis; increases viability and proliferation of Sf-9 and V-79 cells via cAMP signalling pathway activation; protection of Sf-9 cells against t-BHP- and H_2O_2 oxidative damage; improves recombinant protein production.	Mendonça *et al.*, 2008; Vieira *et al.*, 2010; Heinen *et al.*, 2014
Antiviral proteins	Antiviral action against rubella, herpes virus and picornavirus tested in VERO, SIRC and L929 cells.	Carmo *et al.*, 2012; Carmo *et al.*, 2014
Antimicrobial proteins	The present study identified several sequences with homology to antimicrobial peptides from attacin and cecropin family. Similar findings were obtained by a cDNA library study. Even though these peptides may have an immunity function in caterpillar physiology and a potential cytoprotective function its mechanism of action has not been explored so far.	Veiga *et al.*, 2005
Protease Inhibitors	Mainly hemolymph is enriched in serpins, cystatins, TIL and Kazal-type inhibitors, which participate in coagulation and immunity process in the caterpillar and also may have cytoprotective functions. However, the specificity and mechanisms remain to be determined.	Veiga *et al.*, 2005

1 Protein class identified in *L. obliqua* proteome. For details see Table 2 and Table S1 and S2.
2 Potential function for each class already reported in literature

PERSPECTIVAS

Além do trauma físico, a perda recorrente da gravidez, e até mesmo a falha de implantação recorrente em procedimentos de reprodução assistda, também está associada a considerável morbidade psicológica. Infelizmente, excluindo causas genéticas e anatômicas, não há opções de tratamento. Uma vez que a deficiência de hESC, senescência e uma capacidade de resposta aberrante aos sinais deciduogênicos estão fortemente envolvidos na perda gestacional, decidimos estudar o papel das moléculas citoprotetoras obtidas das secreções venenosas de *Lonomia obliqua* na proliferação de hESC e nos mecanismos de sobrevivência. Neste trabalho relatamos a identificação de pelo menos seis classes de proteínas com propriedades citoprotetoras por meio de análise proteômica e isolamos uma fração enriquecida nestes fatores citoprotetores. As secreções venenosas de L. *obliqua* induziram aumento significativo na viabilidade, proliferação e migração de hESCs, principalmente pela proteção contra danos oxidativos e ativação da via dependente de ERK. Além disso, a ação proliferativa do veneno da L. obliqua poderá ser usado em outras desordens endometriais, como a falha implantacional recorrente, situação que acomete até 75% dos casais que passam por ciclos de reprodução assistida e que pode ter como causa o endométrio fino.