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VALDETE KALIANE DA SILVA CALISTO

EFEITO DO ÓLEO ESSENCIAL DA CASCA DE LARANJA (*Citrus sinensis* L.) NO TRATAMENTO DA ASMA INDUZIDA POR OVALBUMINA EM CAMUNDONGOS

ARACAJU

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Tese apresentada ao Programa de Pós-Graduação em Ciências da Saúde da Universidade Federal de Sergipe como requisito à obtenção do grau de Doutora em Ciências da Saúde.

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RESUMO

A asma é uma doença inflamatória crônica das vias aéreas caracterizada por episódios recorrentes de sibilos, falta de ar e tosse incessante. Estima-se que afete cerca de 300 milhões de pessoas em todo o mundo. Ervas medicinais e metabólitos secundários estão ganhando considerável atenção devido ao seu potencial papel terapêutico e mecanismos farmacológicos como ferramentas adjuntas aos broncodilatadores sintéticos. Buscando estudar o efeito anti-inflamatório da casca de laranja (*Citrus sinensis* L.) como possível medicamento para a melhora do tratamento asmático e levando em consideração que os óleos essenciais possuem boa aceitabilidade no mercado e conservam características primordiais das suas fontes, este trabalho visa avaliar o efeito do óleo essencial da casca de laranja (OECL) na inflamação das vias aéreas desencadeada por alérgeno na asma experimental em camundongos Swiss e BALB/c. O estudo foi realizado em modelos experimentais nessas duas espécies, através da análise cromatográfica dos compostos bioativos do OECL, presença de neutrófilos, eosinófilos e macrófagos nos pulmões, presença de eosinófilos no lavado broncoalveolar (LBA), perfil das citocinas interleucina (IL)-5, 10, 17, -1β e fator de necrose tumoral alfa (TNF-α) nos pulmões. Através destes foi possível relatar que: o OECL é rico no terpeno D- Limoneno, a análise do LBA das duas espécies de animais induzidas à asma demonstrou que o tratamento com o OECL nas duas maiores doses (30 e 90 mg/kg) reduziu o número de células inflamatórias para valores próximos ao encontrado nos animais do grupo controle (sem estímulo alérgico), o resultado da peroxidase eosinofílica (EPO) no tecido pulmonar do grupo dos animais sensibilizados e desafiados com ovalbumina (OVA) foi significativamente maior quando comparada aos demais grupos, com exceção dos grupos de animais tratados com o OECL que não apresentou nenhuma diferença significativa. Além disso, o tratamento com o OECL nas doses de 10 mg/kg e 30 mg/kg, foi capaz de reduzir significativamente a atividade da mieloperoxidase (MPO), quando comparado ao grupo OVA. Neste estudo demonstrou-se também que as citocinas IL-10 e TNF-α comportaram-se de maneira semelhante no OECL e na dexametasona (DEXA) e nas outras citocinas estudadas (IL-5,17 e -1β) não foi encontrada uma correlação no OECL. Sendo assim pode-se concluir que o OECL é capaz de reduzir a resposta inflamatória relacionada ao processo asmático.

Palavra- chave: Asma, citocinas, óleos de plantas, *Citrus sinensis* L.

ABSTRACT

Asthma is a chronic inflammatory disease of the airways characterized by recurrent episodes of wheezing, shortness of breath and incessant coughing. It is estimated to affect around 300 million people worldwide. Seeking to study the anti-inflammatory effect of orange peel (*Citrus sinensis L.)* as a possible medicine to improve asthmatic treatment and taking into account that essential oils have good acceptability in the market and retain key characteristics of their sources, this work aims to to evaluate the effect of orange peel essential oil (OECL) on allergen-triggered airway inflammation in experimental asthma in Swiss and BALB/c mice. The study was carried out in experimental models in these two species, through the chromatographic analysis of the bioactive compounds of the OECL, presence of neutrophils, eosinophils and macrophages in the lungs, presence of eosinophils in the bronchoalveolar lavage (LBA), profile of cytokines interleukin (IL)-5, 10, 17, -1β and tumor necrosis factor alpha (TNF-α) in the lungs. Through these it was possible to report that: the OECL is rich in the terpene D-Limonene, the analysis of the LBA of the two species of animals induced to asthma demonstrated that the treatment with the OECL in the two highest doses (30 and 90 mg/kg) reduced the number of inflammatory cells to values close to those found in animals in the control group (without allergic stimulus), the result of Eosinophil peroxidase (EPO) in the lung tissue of the group of animals sensitized and challenged with ovalbumin (OVA) was significantly higher when compared to the other groups , with the exception of groups of animals treated with OECL, which did not show any significant difference. Furthermore, treatment with OECL at doses of 10 mg/kg and 30 mg/kg was able to significantly reduce myeloperoxidase (MPO) activity when compared to the OVA group. In this study, it was also shown that the cytokines IL-10 and TNF-α behaved similarly in OECL and in dexamethasone and in the other cytokines studied (IL-5,17 and -1β) it was not found a correlation in OECL. Therefore, it can be concluded that OECL is able to reduce the inflammatory response related to the asthmatic process.

Keywords: Asthma, cytokines, plant oils, *Citrus sinensis L*.

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LISTA DE ABREVIATURAS E SIGLAS

OECL	óleo essencial da casca de laranja
LBA	lavado broncoalveolar
IL	interleucina
EPO	Peroxidase Eosinofílica
MPO	mieloperoxidase
OVA	ovalbumina
TNF-α	fator de necrose tumoral alfa
AAIO	asma alérgica induzida por ovalbumina
IgE	Imunoglobulina E
SUS	Sistema Único de Saúde
Th1	células T helper 1
Th2	células T helper 2
IFN	interferon
TGF-β	fator de crescimento transformador **beta**
GM-CSF	fator estimulador de colônias de granulócitos
Tregs	células T reguladoras
DEXA	dexametasona
i.n.	intranasal
i.p.	intraperitoneal
$AlOH_3$	hidróxido de alumínio
NaCl 0,9%	salina estéril

SUMÁRIO

1. INTRODUÇÃO

A asma é uma doença inflamatória crônica das vias aéreas caracterizada por episódios recorrentes de sibilos, falta de ar e tosse incessante (Mims, 2015). A prevalência da asma aumentou nas últimas décadas e é atualmente uma das causas mais comuns de morbidade respiratória no mundo (Nunes et al., 2017), atingindo indivíduos de todas as idades (Tang et al., 2018), estima-se que afete cerca de 339 milhões de pessoas em todo o mundo, e sua incidência continua a aumentar (The Global Asthma Report 2018). Embora a etiologia da asma ainda não seja totalmente esclarecida, ela está associada a fatores como exposição a alérgenos, fumaça de tabaco e arranjo do microbioma, o que pode levar ao desenvolvimento da doença em indivíduos geneticamente suscetíveis (Mims, 2015).

Os principais sintomas dessa patologia incluem a produção excessiva de muco, levando a obstrução das vias aéreas (Doeing e Solway, 2013), que é diversas vezes acompanhada por infiltração dessas por eosinófilos, mastócitos e linfócitos além do espessamento das paredes brônquicas e hipertrofia/hiperplasia do músculo liso das vias aéreas (Cheng et al., 2018). Durante esse processo a resposta inflamatória da asma brônquica inclui a estimulação de reações do sistema imunológico, bem como a produção e aumento da expressão de citocinas e quimiocinas (Xin, et al., 2018).

A patologia da condição asmática é caracterizada principalmente por um desequilíbrio entre citocinas do tipo Th1 e Th2 (Lu, et al., 2019), visto que a inflamação das vias aéreas está associada à estimulação de respostas imunes derivadas de células T helper (Th) 2 e produção de interleucinas (IL)-4, 5 e 13 (Ku e Lin, 2016) e outras citocinas, como IL-1 e 33, fator de necrose tumoral alfa (TNF-α) e fator de crescimento transformador beta (TGF-β), que desempenham um papel fundamental na fisiopatologia das reações alérgicas (Verheijden et al., 2016; Tettamanti et al., 2018).

Os tratamentos mais utilizados para esta patologia são corticosteróides, β-agonistas e antagonistas dos receptores de leucotrienos. Embora muito eficazes no controle dos sintomas da doença, esses agentes sintéticos podem causar efeitos adversos significativos aos pacientes. Além dos corticosteróides, que visam reduzir o processo inflamatório, esses outros medicamentos atuam apenas para aliviar os sintomas (McCracken, et al., 2017).

Nesse contexto, a fitoterapia tem sido usada para tratar a asma há vários anos devido à sua fácil acessibilidade, baixa toxicidade e maior biodisponibilidade em comparação com agentes sintéticos (Yu, et al., 2017). Recentemente plantas medicinais e seus metabólitos secundários como terpenóides, alcalóides, flavonóides, saponinas e compostos fenólicos, têm sido amplamente pesquisados para o tratamento e manejo da asma, devido às suas características promissoras e eficazes (De Almeida, et al., 2017; Ye et al. , 2019). Há evidências crescentes da aplicabilidade de extratos vegetais na modulação de interleucinas (IL) como IL-1β, 4, 5, 6 e 13 para gerenciar e tratar a síndrome asmática usando sistemas experimentais *in vivo* e *in vitro* (Fouladi et al., 2019), o que poderia levar ao desenvolvimento de novas substâncias terapêuticas para reduzir a dependência dos tratamentos convencionais.

Vale ressaltar que os remédios fitoterápicos são uma forma popular de medicina complementar ou alternativa para a asma e quase 40% dos asmáticos já usaram remédios à base de ervas (Ernst, 1995). É sabido que frutas cítricas, tal como a laranja (*Citrus sinensis*), possuem inúmeros compostos bioativos, que são capazes de auxiliar no bom funcionamento do corpo humano, porém suas cascas são habitualmente descartadas, sendo estas uma considerável fonte de substâncias anti-inflamatórias e antioxidantes (Abeysinghe et al., 2007) com grande potencial fitoterápico. Também é sabido que óleos essenciais extraídos de plantas são misturas de compostos voláteis, contendo centenas de constituintes químicos bioativos. Graças à sua volatilidade, esses óleos podem facilmente atingir o trato respiratório superior e inferior (Levy et al., 2018), onde eles poderiam reduzir os níveis de células inflamatórias, Imunoglobulina E (IgE) e citocinas que atuam diretamente na cascata inflamatória asmática, como IL-4, 5 e 13 (Horváth e Kamilla, 2015).

Buscando elucidar o efeito anti-inflamatório da casca de laranja como possível medicamento para a melhora do tratamento asmático e levando em consideração que os óleos essenciais possuem boa aceitabilidade no mercado e conservam características primordiais das suas fontes, este trabalho objetiva investigar os efeitos do óleo essencial da casca da laranja (OECL) no processo inflamatório na asma.

2. REVISÃO BIBLIOGRÁFICA

2.1 Fisiopatologia da Asma

A asma vem se tornando um problema de saúde em nível mundial, acometendo cerca de 300 milhões de pessoas (WHO, 2007). No Brasil, estima-se uma prevalência de aproximadamente 20 milhões de asmáticos. Segundo o DATASUS, o banco de dados do Sistema Único de Saúde (SUS), em média, cerca de 350.000 internações são devidas à essa doença, representando a terceira ou quarta causa de hospitalizações pelo SUS (SBPT, 2013).

Nas últimas 2 décadas, tem havido uma maior compreensão de que a asma é uma condição crônica mediada imunologicamente para reparar as vias aéreas, resultando em alterações inflamatórias e remodelação das vias aéreas. E essas manifestações juntas, caracterizam e explicam as manifestações clínicas da asma. Os mecanismos pelos quais fatores ambientais externos, juntamente com as complexas ações genéticas, propagam o processo inflamatório que caracteriza a asma ainda estão sob investigação.

A resposta aumentada da IgE à fatores ambientais, como ácaros da poeira doméstica, alérgenos de animais, mofo e animais de fazenda, contribui para sensibilização à asma, exacerbando seus sintomas e atribuindo-a ao aumento da reatividade das vias aéreas. A razão é que há um aumento da exposição a esses alérgenos, mas há menos dados disponíveis sobre a causalidade. A poluição do ar e a causa da asma também são menos claras. No entanto, fatores comportamentais como tabagismo e obesidade podem aumentar o risco ao desenvolvimento da doença. (Camargo et al., 1999; Gilliland et al., 2006).

Neste processo, há a participação ativa de elementos estruturais, como o epitélio e músculo liso das vias aéreas, além do endotélio. Na fisiologia respiratória normal, a complacência pulmonar é a vontade de os pulmões se distenderem, enquanto a elastância é a capacidade dos pulmões de retornar à sua posição de repouso. Em pacientes com asma, o mecanismo fisiológico muda devido à inflamação, diminuindo o raio da via aérea (Grinnan and Truwit, 2005). Todos esses mecanismos juntos alteram ligeiramente a complacência dos pulmões para aumentar o trabalho respiratório (Sinyor and Perez, 2022).

Assim, como um componente primordial para entendimento dos mecanismos envolvidos no desenvolvimento da asma. O processo inflamatório das vias aéreas abrange a estimulação de células imunes como neutrófilos, células dendríticas, basófilos e eosinófilos, cada um presente em diferentes estágios de progressão da asma, produzindo e secretando citocinas e outros fatores (Figura 1), respondendo de maneira coordenada, embora disfuncional, através de uma série de vias de sinalização complexas que facilitam a comunicação entre elas. (Xin et al., 2018). A característica imunológica da asma é uma mudança de equilíbrio das citocinas de perfil T helper (Th)1 para Th2 (Jalali et al., 2013). O que ocorre é que as cascatas inflamatórias durante as crises de asma estimulam o aumento da expressão de linfócitos do tipo T helper (Th) 2 (Xin et al., 2018) que liberam citocinas como IL-4, IL-5 e IL-13, causando inflamação das vias aéreas decorrente de uma maior secreção de IgE (Lambrecht, 2015). O aumento da produção dessas citocinas foi demonstrado em Lavado Broncoalveolar (LBA) e biópsias de vias aéreas de pacientes com asma leve ou assintomática (Walker et al., 1992). Vale ressaltar que além da asma alérgica eosinofílica, norteada pela liberação de citocinas de perfil Th2 (Ku et al., 2016), foi relatado que as respostas imunes Th1 e Th17 (Xin et al., 2018), com predominância de neutrófilos, ou combinada, com neutrófilos e eosinófilos (Gungl et al., 2018), também podem atuar nessa patologia,com uma resposta imune heterogênea (Russell et al., 2018; Xin et al., 2018).

Essencialmente, as citocinas orquestram e perpetuam a inflamação crônica das vias aéreas (Barnes, 2018). Algumas citocinas como a IL-17 (Choy et al., 2015), IL-6 (Jevnikar et al., 2019), e interferon (IFN) –γ (Gauthier et al., 2017) são importantes reguladoras do processo imunológico na asma. Concomitante a isso, a IL-10 é um regulador primordial do equilíbrio entre as respostas imunes (NG et al., 2017).

Outra citocina reguladora do processo inflamatório na asma é a IL-4, promovendo a diferenciação de linfócitos T em células Th2 com atração e ativação de mastócitos e liberação de broncoconstritores, aumentando o nível de IgE, estimulando a produção de imunoglobulinas, e liberação de citocinas de perfil Th2, como a IL-5 e IL-13, enquanto reduz a atividade das citocinas derivadas de linfócitos Th1 (Wynn et al., 2015).

As células Th1 inibem a resposta Th2 através da secreção de IFN-γ, IL-2 e fator de crescimento transformador beta (TGF-β) e, portanto, o objetivo da terapia da asma deve ser o equilíbrio Th1/Th2 (Randolph et al., 1999). Estudos experimentais recentes mostraram que a manutenção do equilíbrio entre as respostas imunes Th1/Th2 poderia

proteger contra exacerbações de asma (Rao et al., 2017) e vários ensaios objetivam inibir citocinas derivadas de células Th2, como IL-4 e IL-5 (Rivera et al., 2011).

Nesse sentido, a utilização de substâncias funcionais, oriundas de plantas naturais, são sugeridas para uso na prevenção ou tratamento da asma, a fim de melhorar tal alteração na proporção das respostas Th1 e Th2 (Ku et al.,2017), visto que tratamentos convencionais utilizados por um grande período estão relacionados a predisposições de implicações não desejadas, como hiperglicemia, crise adrenal, taquicardia, tremores musculares, convulsões, etc (Sharma et al.,2017).

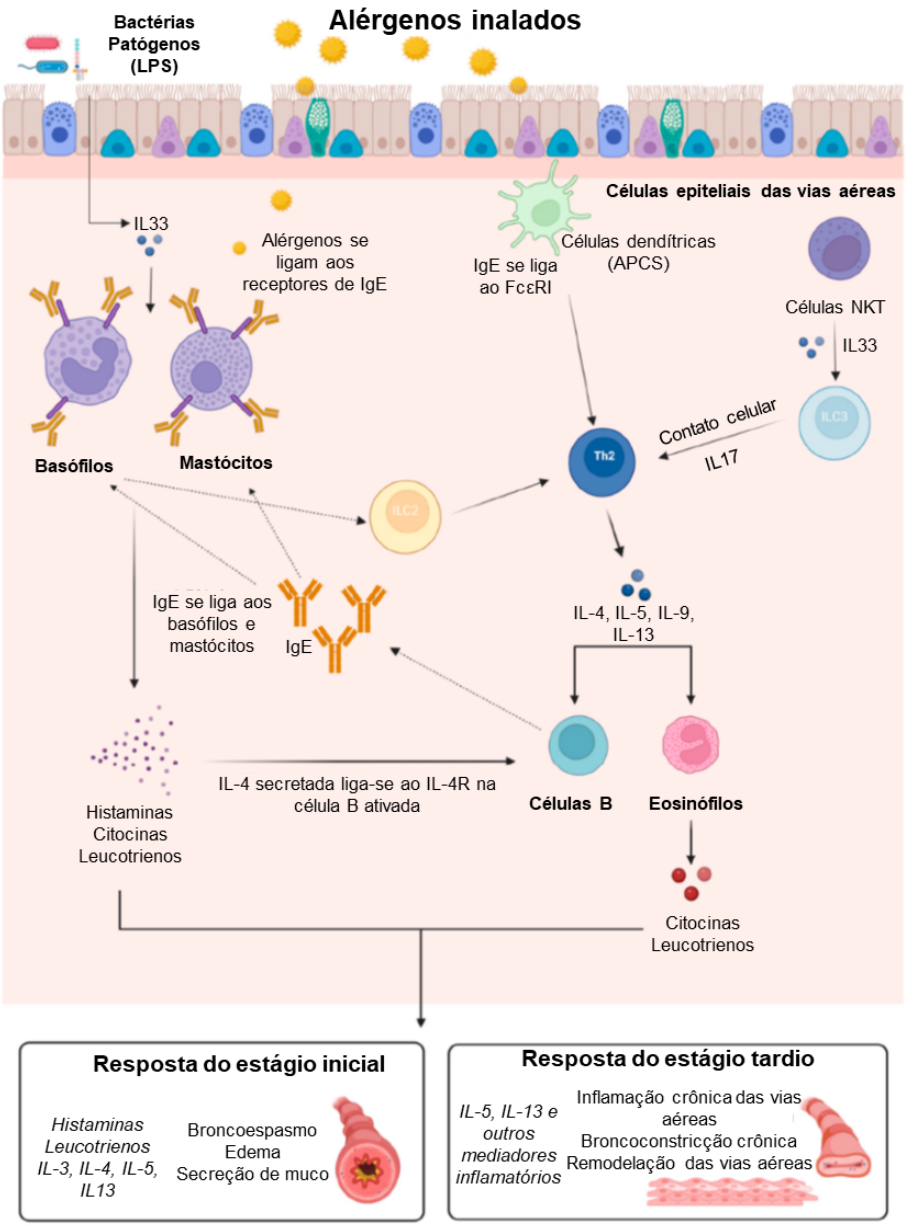

Figura 1. IgE e seus mediadores desempenham um papel nas respostas do estágio inicial e tardio da asma após a exposição a alérgenos. Adaptada de Hachim et al., 2022.

Existem duas fases de uma exacerbação da asma, que incluem a fase inicial e a fase tardia (Figura 1). Na fase inicial, também chamada de fase de sensibilização, os anticorpos IgE específico para o alérgeno, produzidos pelas células B, se ligam aos receptores de alta afinidade FcεR1 na superfície dos mastócitos e basófilos (Han et al., 2017; Aun et al., 2017). Quando um poluente ou fator de risco é inalado, os mastócitos liberam citocinas, histamina, prostaglandinas e leucotrienos e eventualmente degranulam. Essas células, por sua vez, contraem o músculo liso e causam o estreitamento das vias aéreas (Liu et al., 1991). Concomitante a isso, linfócitos Th2 desempenham um papel integral onde produzem, além das citocinas de perfil Th2 mencionadas, fatores de crescimento como fator estimulador de colônias de granulócitos (GM-CSF), que auxiliam na comunicação com outras células e sustentam a inflamação. As citocinas Th2 são necessárias para o desenvolvimento de eosinofilia das vias aéreas e para estimular uma resposta inflamatória que resulta em asma (Ray e Cohn, 1999). Dito isso, a IL-5, além de estimular o aumento da produção de IL-6, possui diversas funções relacionadas eosinófilos (SO-HYEON et al., 2019), como maturação, ativação, proliferação e persistência no tecido pulmonar, determinantes na patologia da asma eosinofílica ou seja, é responsável pelo desenvolvimento e maturação de eosinófilos na medula óssea e seus recrutamento para o pulmão e mucosa intersticial durante a inflamação alérgica (Bok, et al., 2019). A IL-13 foi atribuída à remodelação, fibrose, hiperplasia, produção de IgE por linfócitos B e eosinófilos e a produção de muco no tecido pulmonar (Zhu et al., 1999; Zhang et al., 2019; Lin et al., 2015; Bok, et al., 2019).

Nas horas seguintes do processo fisiopatológico da asma, ocorre a fase tardia, na qual eosinófilos, basófilos, neutrófilos e células T auxiliares e de memória localizam-se também nos pulmões, que realizam broncoconstrição e causam inflamação. Os mastócitos também desempenham um papel essencial em trazer os reagentes de fase tardia para os locais inflamados (Stewart et al., 1995). É fundamental reconhecer esses dois mecanismos para direcionar a terapia e aliviar a broncoconstrição e a inflamação, dependendo da gravidade da doença. Curiosamente, aqueles com vias aéreas mais espessas ao longo do tempo têm uma duração mais longa da doença, devido a uma via aérea mais estreita (Doeing et al., 2013).

Como resultado da inflamação e broncoconstrição, há uma obstrução do fluxo aéreo, resultando em aumento do trabalho respiratório. A hiperresponsividade das vias aéreas é uma característica crucial da asma, pois trata-se de uma resposta

broncoconstritora exagerada. Há uma variedade de mecanismos que levam à hiperresponsividade das vias aéreas, dos quais, o aumento da histamina dos mastócitos ou ao aumento da massa muscular lisa das vias aéreas e aumento do cálcio livre intracelular que promove a contratilidade das células musculares lisas das vias aéreas (Doeing e Solway, 2013).

A remodelação ocorre pela transição das células epiteliais para mesenquimais, aumentando o conteúdo de músculo liso (Kudo et al., 2013). Além disso, os eosinófilos podem exacerbar ainda mais a remodelação das vias aéreas devido à liberação de TGF-β e citocinas por interações com mastócitos. Esses mecanismos de remodelação das vias aéreas são cruciais para a piora da inflamação e agravamento da asma ao longo do tempo se não forem tratados e manejados corretamente (Doeing et al., 2013).

2.2 Tratamentos da Asma

A asma causa alterações nas vias aéreas (Panettieri et al., 2018) tais como hiper-responsividade, obstrução reversível (Boyman et al,2015; Palomares et al.,2017) recrutamento de células inflamatórias e produção excessiva de muco (Wang et al., 2018). Tal patologia é tratada de diversas formas, e dentre os tratamentos destaca-se a classe dos glicocorticoides, porém seu uso prolongado pode facilitar algumas infecções que causam candidíase, rouquidão, osteopenia e lesões cutâneas (Vanfleteren et al., 2018). Os β-agonistas e os antagonistas dos receptores de leucotrienos apresentam eventos adversos menos frequentes, sendo os mais comuns, exacerbações de asma e sintomas neuropsiquiátricos, mas eles precisam ser usados em conjunto com outros medicamentos para aumentar sua eficácia (Leung et al., 2017).

A classe dos medicamentos β-agonistas inclui os de ação curta ou prolongada, O mecanismo por trás dos beta-agonistas é que eles são receptores de proteína G que ativam o AMPc. O cAMP então ativa o relaxamento do músculo liso por um mecanismo não totalmente compreendido. A ideia por trás de um β-agonista é tentar broncodilatar os pulmões do paciente quando eles se contraem durante um ataque de asma (Moore et al., 2001).

Os glicocorticoides inalatórios e sistêmicos (por exemplo, budesonida) são usados para diminuir a inflamação e a remodelação dos pulmões. O principal mecanismo de um glicocorticóide é aumentar a produção de IL-10. Essa citocina tem o papel inibitório para outras citocinas inflamatórias, além de ativação de células T e diferentes

leucócitos, como mastócitos e eosinófilos (Moore et al., 2001). Esses efeitos diminuem a inflamação e ajudam o paciente a respirar melhor a curto e longo prazo.

Os antagonistas muscarínicos bloqueiam o efeito inflamatório diminuindo a atração e a vida das células inflamatórias, diminuindo a liberação de citocinas (D'Amato et al., 2017). Os antagonistas muscarínicos podem ser combinados com um β-agonista ou glicocorticóide para um efeito sinérgico (Sinyor and Perez, 2022).

Embora tenha sido demonstrado que os medicamentos para controle dos sintomas podem melhorar fortemente qualidade de vida dos pacientes (controlando a inflamação das vias aéreas, diminuindo a hiperresponsividade e gravidade das exacerbações), é amplamente conhecido que eles não curam, portanto devem ser tomados continuamente (Han et al., 2013). Todas essas terapias citadas destinam-se a tratar a inflamação e a broncoconstrição, porém, considerando que a remodelação brônquica ainda permanece insensível aos tratamentos atuais para asma, (Keramidas et al., 2013) novas terapias são bem vindas para aumentar o leque de opções.

2.3 Modelos experimentais em Asma

Apesar dos estudos clínicos serem o objetivo das pesquisas científicas, na asma não são capazes de esclarecer todos os aspectos da fisiopatologia da doença. Assim, modelos animais foram desenvolvidos com o objetivo de investigar os mecanismos e avaliar a segurança e eficácia das terapias antes de iniciar os ensaios clínicos. Várias espécies de animais têm utilizado em modelos experimentais de asma, como *Drosophila melanogaster*, no qual há um foco para o processo de remodelação das vias aéreas, por se tratar de uma espécie que possui a arquitetura das vias aéreas simples e são ausentes de imunidade inata e adaptativa (Ehrhardt et al., 2022). Outros animais como ratos (Thakur et al., 2019), cobaias (Lui et al., 2022), gatos, cachorros, suínos, primatas e equinos (Aun, et al., 2017). O principal achado dentre diversos estudos in vivo é que linhagens de camundongos, como BALB/c, são adequadas para protocolos experimentais de reações inflamatórias asmáticas. Modelos animais de camundongos BALB/c têm sido usados extensivamente em doenças inflamatórias relacionadas à asma com responsividade idêntica das vias aéreas e inflamação brônquica com hiperprodução de citocinas Th2 (Gueders et al., 2009).

O alérgeno mais utilizado para indução da hiperresponsividade é a proteína ovalbumina (OVA), por induzir intensa inflamação pulmonar alérgica (Aun, et al.,

2017). Estudo em ratos, que adicionou o lipopolysaccharide (LPS) ao protocolo de indução da asma com OVA obteve resultados obtiveram fenótipo da asma severa com aumentada produção de citocinas IL-4, IL-5 e IL-13, bem como infiltração exacerbada de eosinófilos, neutrófilos e linfócitos, quando comparado ao grupo OVA somente (Thakur et al., 2019). No entanto, as espécies mais comuns estudadas nas últimas duas décadas são camundongos, particularmente BALB/c. Modelos animais de asma tentam imitar a fisiopatologia da doença humana, embora apresentem algumas limitações, são ainda uma alternativa importante para estudo para elucidar os mecanismos imunológicos e não imunológicos envolvidos na patogênese da asma e identificar possíveis alvos para controlar inflamação alérgica (Nials and Uddin, 2008; Aun et al., 2017).

2.4 Produtos naturais

Óleos essenciais são encontrados originalmente na natureza e podem ser descritos como mistura de substâncias voláteis de hidrocarbonetos com ou sem insaturações, álcool, aldeídos, ésteres, éteres, cetonas, óxidos, fenóis e terpenos (Köteles et al.,2018), possuindo eficácia contra ácaros, vírus, bactérias e fungos. O seu consumo vem aumentando gradativamente pela população, inclusive portadores de asma (Levy et al., 2018), objetivando a melhora do sistema imunológico e, consequentemente da alergia (Pina et al.,2018). Ressaltando que há muito tempo são empregados pela população como tratamento alternativo para o sistema respiratório e infecções como faringite, bronquite e sinusite (Köteles et al.,2018). Os óleos nos trazem diversas possibilidades bioquímicas e farmacológicas que podem ter atividade nos mecanismos fisiológicos e fisiopatológicos da asma.

Observa-se que metabólitos secundários de plantas, principalmente pertencentes à classe dos flavonóides, podem ser considerados reguladores de alguns alvos moleculares do sistema imunológico e exercer forte ação sobre a população de células inflamatórias nas vias aéreas. Os flavonóides são a classe mais estudada de metabólitos vegetais, podendo diminuir a expressão e produção de moléculas inflamatórias, incluindo histamina e leucotrienos (Lago et al., 2014) e inibem a degranulação de mastócitos e promovem a produção de citocinas Th1/Th2. Eles também regulam para baixo a produção e atividade de mensageiros secundários ligados às vias aéreas de inflamação e controlam o dano tecidual (Fortunato et al., 2012).

Os polifenóis da estrutura dos flavonóides são especialmente eficazes nas vias aéreas inflamatórias, pois há evidências substanciais de que estes desempenham um papel importante na regulação das citocinas Th2, IL-4, IL-5 e supressão de níveis de IgE e IgG1 em LBA (Magrone e Jirillo, 2012). De fato, os compostos flavonoides e polifenóis são potentes moduladores de citocinas presentes na cascata de início da asma e outras reações inflamatórias (Leyva-López et al., 2016).

3. OBJETIVOS

3.1 Geral

Avaliar o efeito do óleo essencial da casca de laranja (*Citrus sinensis* L.) (OECL) na inflamação das vias aéreas desencadeada por alérgeno em modelo de asma experimental com camundongos.

3.2 Específicos

Investigar o efeito do OECL no tratamento da asma em camundongos sobre:

- Caracterização por análise cromatográfica os compostos bioativos do OECL
- Presença de neutrófilos, eosinófilos e macrófagos nos pulmões;
- Presença de eosinófilos no lavado broncoalveolar (LBA);
- O perfil de citocinas IL-5, 10, 17, 1β e fator de necrose tumoral alfa (TNF-α) nos pulmões.

4. MATERIAS E MÉTODOS

4.1 Material vegetal

Frutos de *Citrus sinensis* (L.) foram colhidos da fazenda agrícola de Cristinápolis, Sergipe, Brasil e foram devidamente autenticados pelo Prof. Dr. Marcelo Duarte Cavalcante, botânico da UFS. As cascas foram cortadas em pedaços, secas em estufa (30°C) por 24 horas e pulverizadas em moinho elétrico.

4.2 Óleo essencial da casca de laranja (*Citrus sinensis* L.)

4.2.1 Extração do óleo essencial da casca de laranja (*Citrus sinensis* L.)

A extração do OECL foi realizada por meio da técnica de hidrodestilação com aparelho de Clevenger modificado, onde 60 g de casca, devidamente seca e triturada, junto com 450 ml de água destilada foram colocados em um balão de 1 L e aquecidos em manta de aquecimento até a 100 °C e contadas três horas a partir do decaimento da primeira gota até o fim da extração (BERISTAIN, 1996). O OECL foi armazenado em um recipiente de vidro hermeticamente fechado a 4-5 °C até à sua utilização.

4.2.2 Compostos voláteis encontrados no óleo essencial da casca de laranja por Cromatografia Gasosa acoplado ao detector por Espectrometria de Massas (GC-MS)

A análise dos compostos voláteis dos óleos essenciais foi realizada através da injeção líquida na quantidade de 0,5 μL de amostra de OECL, diluída em hexano na proporção de 1:1. Em um cromatógrafo a gás acoplado a um espectrômetro de massas da marca Varian (modelo 3900), equipado com coluna apolar (HP-5MS - 30 m x 25 mm x 0,25 μm) com temperatura do injetor de 250 °C e programação de forno iniciando a 60 °C e subindo até 240 ºC a uma taxa de 3ºC/min. Os compostos foram identificados por comparação dos espectros dos compostos, com os espectros disponíveis na biblioteca NIST e também através do cálculo do índice de Kovats dos compostos calculado através da injeção de um série homóloga de padrões de alcanos (C_7-C_{30}).

4.3 Animais

4.3.1 Camundongos Swiss

Foram utilizados camundongos Swiss fêmeas (25-30 g), provenientes do Biotério Central da Universidade Federal de Alagoas (UFAL). Os animais foram mantidos em condições controladas de temperatura (22 ± 2°C) e luminosidade (ciclo claro/escuro de 12 horas) com livre acesso a ração e água antes dos experimentos. Os animais permaneceram no laboratório para sua adaptação por um período de pelo menos 1 hora (h) antes da realização dos experimentos. O experimento foi aprovado pelo Comitê de Ética em Pesquisa com Animal da Universidade Federal de Alagoas (Processo nº 18/2019).

4.3.2 Camundongos BALB/c

Foram utilizados camundongos BALB/c, fêmeas, não prenhes, saudáveis e pesando entre 20-30 g. Os animais foram obtidos do Biotério Central da Universidade Federal de Sergipe (UFS), após a aprovação pelo Comitê de Ética em Pesquisa Animal da Universidade Federal de Sergipe, e mantidos no Laboratório de Farmacologia do Processo Inflamatório da UFS. Os camundongos foram mantidos em gaiolas de polipropileno, temperatura entre 21 ± 2 °C, 60 ± 5% de umidade e ciclo claro/escuro de 12/12 horas, além de suprimento de ração e água *ad libitum*.

4.4 Modelos de Asma

4.4.1 Modelo de Asma Alérgica em camundongos Swiss

Os animais foram sensibilizados nos dias 0, 7 e 14 através da administração subcutânea de 200 µl de uma mistura contendo, 50 µg/animal de ovalbumina (OVA) e 5 mg/animal de hidróxido de alumínio (Al(OH)3) diluído em solução salina (NaCl 0,9%). Nos dias 21, 22 e 23 após a primeira sensibilização, os animais foram anestesiados com isoflurano inalatório e submetidos, por via intranasal, ao tratamento com salina (NaCl,

0,9%), OECL, nas doses de 10, 30 ou 90 mg/kg ou budesonida (7,5 mg/kg) como fármaco referência. Após 1 h cada tratamento, os animais foram provocados também por via intranasal com salina ou OVA (50 µg/25 µl). No dia 24 foi realizada a eutanásia e análises. A distribuição dos grupos tratados e o desenho experimental estão demonstrados no quadro 1 e figura 2 respectivamente.

Quadro 1: Grupos experimentais utilizados no modelo experimental de asma alérgica em camundongos Swiss

Grupo	Desafio intranasal com OVA	Tratamento intranasal
SAL	-	Salina
OVA	+	Salina
BUD	+	Budesonida (7,5 mg/Kg)
OECL 10	+	OECL (10 mg/Kg)
OECL 30	+	OECL (30 mg/Kg)
OECL 90	+	OECL (90 mg/Kg)

Os grupos experimentais foram compostos de camundongos Swiss fêmeas. Grupo SAL = solução salina (NaCl, 0,9%); Grupo OVA = solução de ovalbumina (50 µg/25 µl). BUD = solução de budesonida. OECL = óleo essencial da casca de laranja nas doses de 10, 30 ou 90 mg/kg.

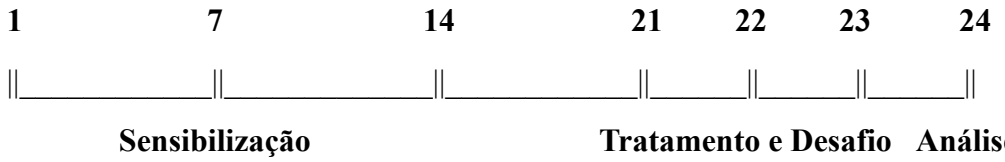

Figura 2. Desenho experimental demonstrando a sensibilização nos dias 1, 7 e 14, o tratamento e desafio nos dias 21, 22, 23 e as análises no dia 24 em camundongos Swiss.

Após 48 h do último estímulo intranasal com OVA, os animais foram eutanasiados através de aprofundamento da anestesia com injeção intraperitoneal de tiopental (200 mg/Kg) (Thiopentax, Cristália) e tiveram a traqueia dissecada e canulada para obtenção do LBA. O LBA foi obtido por três lavagens consecutivas com 0,5 mL de

PBS com EDTA (10 mM). Em seguida, o lavado obtido foi centrifugado a 1500 rpm (g), 4 °C por 10 minutos. No pellet foi adicionado 1 mL de PBS contendo EDTA (10 mM) para contagem de células. A contagem de leucócitos totais foi realizada em câmara de Neubauer sob microscopia de luz, utilizando uma alíquota de células diluída em solução de Turk (1:100). A contagem diferencial foi realizada em citoesfregaços corados pelo método do Panótico e avaliados por microscopia de luz na objetiva de ×100.

4.4.2 Modelo de Asma Alérgica em camundongos BALB/c

O protocolo de indução teve duração de 24 dias. Os camundongos foram sensibilizados nos dias 0, 7 e 14 através de injeção intraperitoneal (i.p.) com 100 µg de ovalbumina (OVA *Grade V*; *Sigma – Aldrich*) e 20 mg de hidróxido de alumínio ($AlOH_3$), solubilizados em 100 µl de salina estéril (NaCl 0,9%). Posteriormente, nos dias 21, 22 e 23, os camundongos foram anestesiados com solução de cetamina (2 mg/mL) e xilazina (1 mg/mL) e tratados com OECL nas doses de 30 ou 90 mg/kg por via intranasal ou dexametasona (DEXA), 1h depois, foi realizado o desafio por via intranasal (i.n.) com solução contendo apenas 100 µg de OVA em 25 µL de solução salina estéril. O grupo controle foi submetido aos mesmos procedimentos, porém a sensibilização e os desafios foram realizados somente com solução salina estéril (Quadro 2). As coletas foram realizados no 24º dia, quando houve a eutanásia desses animais para a coleta de sangue, do LBA e dos pulmões para as análises.

Quadro 2: Grupos experimentais utilizados no modelo experimental de asma alérgica em camundongos BALB/c

Grupos	Sensibilização/Desafio/Tratamento	Abreviações	N
1	Camundongos sensibilizados e desafiados com salina. Receberam solução salina durante o tratamento.	SAL	10
2	Camundongos sensibilizados e desafiados com OVA. Receberam solução salina durante o tratamento.	OVA	10
3	Camundongos sensibilizados e desafiados com OVA. Receberam dose de 30mg/kg de peso de OECL.	OVA + OECL 30	10
4	Camundongos sensibilizados e desafiados com OVA. Receberam dose de 90mg/kg de peso de OECL.	OVA + OECL 90	10
5	Camundongos sensibilizados e desafiados com OVA. Receberam dexametasona durante o tratamento	OVA + DEXA	10

Os grupos experimentais foram compostos de camundongos BALB/c fêmeas. Grupo SA = solução salina (NaCl, 0,9%); Grupo OVA = solução de ovalbumina (100 µg/25 µl). DEXA= solução de dexametasona. OECL = óleo essencial da casca de laranja nas doses de 30 ou 90 mg/kg.

4.5 Análises

4.5.1 Obtenção do Lavado Broncoalveolar e contagem de leucócitos

Após 24 horas do último desafio, os camundongos foram anestesiados e o sangue coletado através do plexo retro orbital para a obtenção do soro e quantificação citocinas. Posteriormente, foi realizada traqueostomia para a coleta do LBA. Para tanto, uma cânula foi inserida na traquéia através de um orifício formado e acoplada a uma seringa de 1 mL. Logo em seguida foram realizadas cinco instilações intratraqueais de 300 µL de PBS e BSA 3%, perfazendo um volume total de 1500 µL lentamente injetado. O fluido do lavado recuperado (em média 1200 µL) foi mantido em gelo e centrifugado por 10 min a 1500 rpm e 4°C. O sobrenadante do fluido centrifugado foi coletado e armazenado a -80°C, para dosagens posteriores, e o *pellet* de células foi ressuspenso em 200 µL de PBS com BSA 3%. Para contagem total de

leucócitos no LBA, um volume de 10 µL da solução contendo o *pellet* ressuspenso foi adicionado ao volume de 190 µL de líquido de Turk, cuja solução final foi utilizada para contagem do número total de células. A contagem foi realizada em câmara de Neubauer com o auxílio de microscópio óptico (aumento de 100x) e contador manual.

4.5.2 Contagem total e diferencial de leucócitos no sangue

Foi coletado 10 ul de sangue e adicionado em 190 ul de líquido de turk para contagem total em câmera de Neubauer. Além disso, cerca de 5 ul foi usado para realização do esfregaço sanguíneo. As lâminas foram coradas com H&E para contagem diferencial de leucócitos.

4.5.3 Ensaio de atividade da Mieloperoxidase pulmonar

A quantificação do acúmulo de neutrófilos no pulmão foi determinada pelo ensaio da mieloperoxidase (MPO), de acordo com Camargo et al. (2014). Após 24h do último desafio, os animais foram anestesiados e perfundidos com 50 ml de tampão PBS-HEPARINA (0,1%). Em seguida amostras do pulmão foram coletadas, pesadas, cortadas em pequenos pedaços e homogeneizadas em tampão fosfato (50 mM, pH 6,0) com 0,5% de brometo de hexadeciltrimetilamônio (HTAB), na proporção de 1 mL de HTAB para cada 50 mg de tecido, com auxílio de um homogeneizador de tecidos Polytron® (13000 rpm). O homogenato foi centrifugado (2 min a 14000 rpm, 4°C) para a obtenção do sobrenadantes e, em uma placa contendo 96 poços, 10 µL do sobrenadante foi adicionado a 200 µL de uma solução contendo dihidrocloreto de o-dianisidina (0,167 mg/mL, preparada em PBS 50 mM contendo 0,005% de H_2O_2). As alterações nos valores das absorbâncias detectadas a 460 nm foram registradas com leitor de microplaca (Synergy MX®, Biotek, USA), com intervalos de 30 segundos por 10 min. Uma UMPO foi considerada como a quantidade de enzima que degrada 1 µmol de H2O2/min.

4.5.4 Ensaio de atividade da Peroxidase Eosinofílica no Lavado Broncoalveolar

A Peroxidase Eosinofílica (EPO) foi estimada no LBA, de acordo com método inicialmente descrito por Strath et al. (1985). Em uma microplaca contendo 96 poços foi adicionado 100 µL de uma solução de substrato contendo o-fenilenodiamina dihidrocloreto - OPD (0.1 mM) - e Triton X-100 (0.1%), na presença de H2O2 (1 mM) solubilizados em Tris– HCl (0,05 M, pH 8.0), a 50 µL de amostra do sobrenadante do LBA. A reação foi incubada por 30 min a 37°C e interrompida com a adição de 50 µL de ácido sulfúrico (4 M), sendo a densidade óptica da reação analisada a 492 nm (BOŠNJAK et al., 2009; STRATH et al., 1985).

4.5.5 Ensaio da Determinação da Peroxidase Eosinofílica pulmonar

A atividade de EPO foi estimada no LBA e no tecido pulmonar de acordo com o método descrito por Strath et al. (1985). Após coletado tecido pulmonar e conservação no gelo, para a obtenção do homogenato, 100 mg do lobo esquerdo do pulmão foi homogeneizado utilizando 1 mL de PBS e 0,05% de Tween 20. As amostras foram centrifugadas a 440 g por 15 minutos a 4°C e o sobrenadante armazenado para quantificação de citocinas e quimiocinas. O pellet formado foi ressuspendido novamente em 1 mL de PBS e 0,05% de Tween 20, homogeneizado e centrifugado a 440 g por 15 minutos a 4°C. O sobrenadante foi descartado e o pellet ressuspendido em 1,9 mL/100 mg de tecido de HTAB 0,5 % em PBS e homogeneizado. Após a homogeneização, as amostras foram congeladas em nitrogênio líquido e descongeladas em banho-maria a 37°C.

4.5.6 Quantificação de citocinas

As citocinas IL-5, 10, 17, TNF-α e IL-1β foram avaliadas no sobrenadante do homogenato do pulmão. Para o ensaio de citocinas, 50µL do sobrenadante foi adicionado à placa de 96 poços de alta ligação, com o anticorpo de captura previamente aderido, e a placa bloqueada. Após isso, as amostras foram incubadas por 3 a 24 horas, lavadas e adicionadas ao anticorpo de detecção. Em seguida, o conjugado foi adicionado à placa e por último, o substrato, para revelação da cor. As placas foram

analisadas no espectrofotômetro, em O.D, de acordo com o protocolo do fabricante (Peprotech®).

5. RESULTADOS

5.1 Identificação dos compostos presentes no Óleo Essencial da casca de Laranja

Na Tabela 1 estão listados os compostos identificados no óleo essencial da casca de laranja (OECL) de acordo com os padrões utilizados. Como pode ser verificado, dentre os compostos, observa-se uma predominante presença do terpeno D- Limoneno (Área % = 99,15).

Tabela 1: Compostos voláteis encontrados no óleo essencial da casca de laranja (OECL) por GC-MS.

#	Composto	LRI_{Lit}	$LRI_{Exp.}$	Área (%)
1	α-Pineno	937	929	0,490
2	Sabineno	976	968	0,148
3	(-)-β-Pineno	981	985	0,146
4	β-Mirceno	990	1002	0,009
5	3-Careno	1009	1007	0,054
6	D-Limoneno	1025	1026	99,153

Índice de retenção linear experimental ($LRI_{Exp.}$, do inglês *Linear Retention Index Experimental)***; Índice de retenção linear da literatura ($LRI_{Lit.}$,** do inglês *Linear Retention Index Literatura*).

5.2 Efeito do Óleo Essencial da Casca de Laranja sobre o infiltrado inflamatório do lavado broncoalveolar de camundongos Swiss submetidos ao modelo de asma alérgica induzida por ovalbumina

Em um primeiro momento, foi investigado a influência do OECL sobre o infiltrado inflamatório do LBA de camundongos Swiss submetidos ao modelo de asma alérgica induzida por ovalbumina (AAIO) (Figura 3). Nesse sentido, a análise de variância de uma via (ANOVA one way) demonstrou que existe diferença significativa na quantidade total de leucócitos e diferencialmente nas contagens de células mononucleares, eosinófilos e neutrófilos no LBA entre os grupos. O teste de comparações múltiplas de Tukey entre os pares de média dos grupos demonstrou que o LBA de animais induzidos à AAIO e tratados com veículo (Grupo OVA) exibiu um acúmulo no número de células inflamatórias representado por aumento na contagem

total de leucócitos e, diferencialmente, na quantidade de células mononucleares, eosinófilos e neutrófilos, quando comparados ao grupo controle ($p < 0,05$).

O tratamento com o OECL nas duas maiores doses (30 e 90 mg/kg) reduziu significativamente, para valores próximos ao encontrado nos animais do grupo controle (sem estímulo alérgico), o acúmulo de células inflamatórias no LBA. Como esperado, o tratamento com budesonida (7,5 mg/Kg), fármaco de referência, reduziu significativamente o infiltrado inflamatório no LBA.

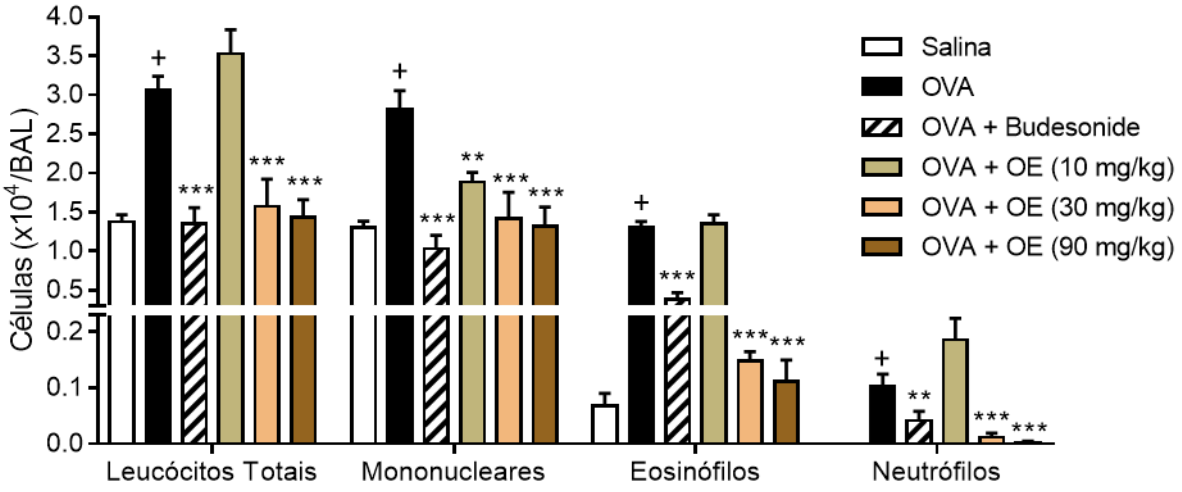

Figura 3: Efeito do Óleo Essencial da Casca de Laranja (OECL) sobre o número total de leucócitos e sobre a contagem de células mononucleares, eosinófilos e neutrófilos no lavado broncoalveolar (LBA) de camundongos Swiss submetidos ao modelo de asma induzida por Ovalbumina (AAIO). Os animais foram tratados com o OECL (OECL, 10, 30 e 90 mg/Kg), budesonida (7,5 mg/Kg) ou salina por via intranasal 1 h antes de cada desafio intranasal com OVA. As barras representam a média ± E.P.M de no mínimo 4 animais. As diferenças estatísticas foram detectadas com ANOVA seguida com o teste de Tukey. (+) representa $p<0,001$ quando comparado ao grupo desafiado com salina. (**) representa $p<0,01$, (***) representa $p<0,001$ quando comparados ao grupo desafiado com OVA e tratado com salina.

5.3 Efeito do Óleo Essencial da Casca de Laranja sobre o número total de leucócitos no lavado broncoalveolar de camundongos BALB/c submetidos ao modelo de asma alérgica induzida por ovalbumina

Buscou-se avaliar a influência do OECL sobre o número total de leucócitos no LBA de camundongos BALB/c submetidos ao modelo de AAIO (Figura 4). Para tanto, a análise de variância de uma via (ANOVA *one way*) demonstrou a presença de diferença

significativa na quantidade total de leucócitos no LBA entre os grupos [$F_{4,42} = 7,185$, $p = 0,002$]. O pós-teste para comparações múltiplas de Tukey entre os pares de média dos grupos, como esperado, evidenciou que o infiltrado de células inflamatórias no LBA dos animais sensibilizados e desafiados com OVA foi significativamente maior quando comparado ao grupo de animais sensibilizados e desafiados com veículo (Salina) (0,6756 ± 0,1269 *versus* 0,1730 ± 0,03340 x 10^6 leucócitos por LBA, respectivamente, $p < 0,001$). Como esperado, o infiltrado de células inflamatórias no LBA dos animais sensibilizados e desafiados com OVA, mas tratados com dexametasona (OVA + Dexa), foi reduzido significativamente quando comparado ao grupo de animais sensibilizados e desafiados com OVA (0,2520 ± 0,03514 *versus* 0,6756 ± 0,1269 x 10^6 leucócitos por LBA, respectivamente, $p < 0,01$). Ainda, o tratamento com o OECL nas doses de 10 mg/kg (OVA + OECL 10) e 30 mg/kg (OVA + OECL 30), 24 horas após o último desafio, foi capaz de reduzir significativamente o quantitativo de leucócitos do LBA, quando comparado ao grupo OVA (OVA versus OVA + OECL 10: 0,6756 ± 0,1269 *versus* 0,3025 ± 0,04720 x 10^6 leucócitos por LBA, respectivamente, $p < 0,01$; OVA versus OVA + OECL 30: 0,6756 ± 0,1269 *versus* 0,3830 ± 0,0773 x 10^6 leucócitos por LBA, respectivamente, $p < 0,05$).

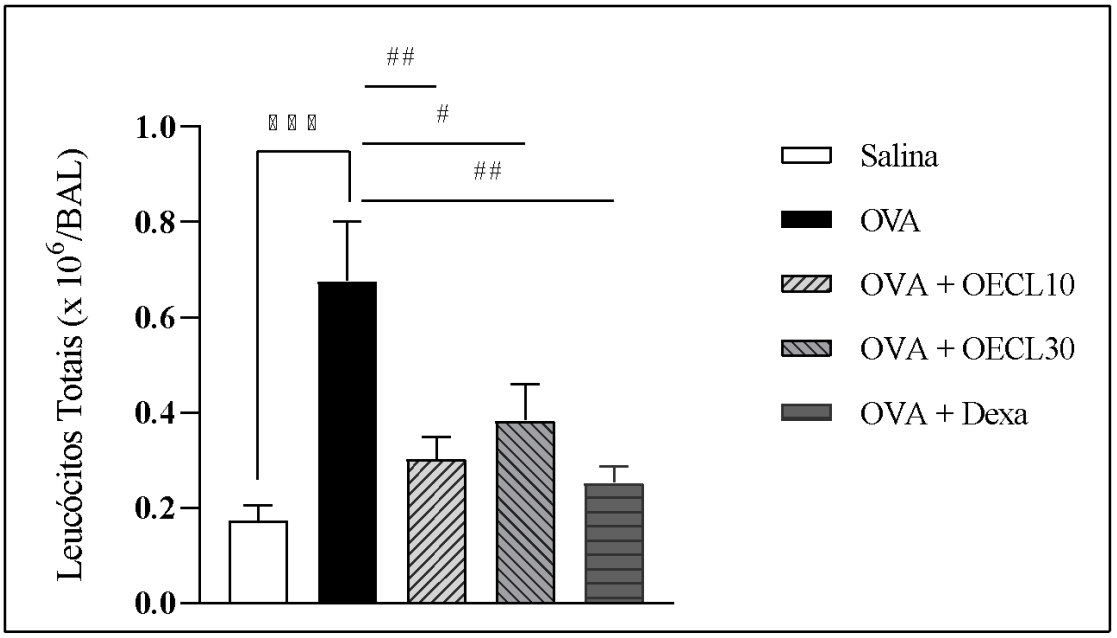

Figura 4: Influência do Óleo Essencial da Casca de Laranja (OECL) sobre o número total de leucócitos no lavado broncoalveolar (LBA) de camundongos BALB/c submetidos ao modelo de asma alérgica induzida por ovalbumina (AAIO). Os dados, expressos como média ± E.P.M, representam a quantidade de leucócitos no LBA mensuradas 24h após o último dia de desafio. Análise estatística: ANOVA *one way* seguida pelo pós-teste de Tukey. (***) $p < 0,001$ *versus* Salina; ($^{\#\#}$) $p < 0,01$ *versus* OVA; ($^{\#}$) $p < 0,05$ *versus* OVA; ($^{\#\#}$) $p < 0,01$ *versus* OVA. Salina (n=10); OVA (n=9); OVA + OECL 10 (n=8); OVA + OECL 30 (n=10); e, OVA + Dexa (n=10). Salina: animais sensibilizados nos dias 0, 7 e 14 com 20 mg de $Al(OH_3)$ solubilizado em 100 µl de salina estéril, administrado por via intraperitoneal (i.p.) e desafiados nos dias 21, 22 e 23 com 25 µl de salina estéril (NaCl 0,9%) por via intranasal (i.n.); OVA: animais sensibilizados nos dias 0, 7 e 14 com 100 µg de OVA adsorvida a 20 mg de $Al(OH_3)$ em 100 µl de salina estéril (NaCl 0,9%) administrado por via intraperitoneal (i.p.) e desafiados nos dias 21, 22 e 23 com 100 µg de OVA em 25 µl de salina estéril (NaCl 0,9%) por via intranasal (i.n.); OVA + OECL 10: animais sensibilizados nos dias 0, 7 e 14 com 100 µg de OVA adsorvida a 20 mg de $Al(OH_3)$ em 100 µl de salina estéril (NaCl 0,9%) administrado por via intraperitoneal (i.p.) e desafiados nos dias 21, 22 e 23 com 100 µg de OVA em 25 µl de salina estéril (NaCl 0,9%) por via intranasal (i.n.) e tratados com OECL na dose de 10 mg/kg de massa corpórea, por via intranasal, 1 hora antes do desafio; OVA + OECL 30: animais sensibilizados nos dias 0, 7 e 14 com 100 µg de OVA adsorvida a 20 mg de $Al(OH_3)$ em 100 µl de salina estéril (NaCl 0,9%) administrado por via intraperitoneal (i.p.) e desafiados nos dias 21, 22 e 23 com 100 µg de OVA em 25 µl de salina estéril (NaCl 0,9%) por via intranasal (i.n.) e tratados com OECL na dose de 30 mg/kg de massa corpórea, por via intranasal, 1 hora antes do desafio; OVA + Dexa: animais sensibilizados nos dias 0, 7 e 14 com 100 µg de OVA adsorvida a 20 mg de $Al(OH_3)$ em 100 µl de salina estéril (NaCl 0,9%) administrado por via intraperitoneal (i.p.) e desafiados nos dias 21, 22 e 23 com 100 µg de OVA em 25 µl de salina estéril (NaCl 0,9%) por via intranasal (i.n.) e tratados com dexa 2 mg/kg de massa corpórea, por via intranasal, 1 hora antes do desafio;

5.4 Efeito do Óleo Essencial da Casca de Laranja sobre a atividade da mieloperoxidase no tecido pulmonar de camundongos BALB/c submetidos ao modelo de asma alérgica induzida por ovalbumina

Ao investigar o efeito do OECL sobre a atividade da mieloperoxidase (MPO) no pulmão de camundongos BALB/c submetidos ao modelo de AAIO após 24h do último dia de desafio (Figura 5), a análise de variância de uma via (ANOVA *one way*) apresentou o seguinte resultado: [$F_{4,15} = 17,12$, $p < 0,0001$]. A análise do pós-teste demonstrou que a atividade da MPO do grupo dos animais sensibilizados e desafiados com OVA foi significativamente maior quando comparado ao grupo de animais sensibilizados e desafiados com veículo (Salina) (7,600 ± 0,6853 *versus* 1,388 ± 0,1276, respectivamente, $p < 0,0001$). Como esperado, a atividade da MPO dos animais sensibilizados e desafiados com OVA, mas tratados com dexametasona (OVA + Dexa), foi reduzida significativamente quando comparado ao grupo de animais sensibilizados e desafiados com OVA (3,455 ± 0,4305 *versus* 7,600 ± 0,6853, respectivamente, $p < 0,001$). Ademais, o tratamento com o OECL nas doses de 10 mg/kg (OVA + OECL 10) e 30 mg/kg (OVA + OECL 30), 24 horas após o último desafio, foi capaz de reduzir significativamente a atividade da MPO, quando comparado ao grupo OVA (OVA versus OVA + OECL 10: 7,600 ± 0,6853 *versus* 3,843 ± 0,8342, respectivamente, $p < 0,01$; OVA versus OVA + OECL 30: 7,600 ± 0,6853 *versus* 4,428 ± 0,3273, respectivamente, $p < 0,01$).

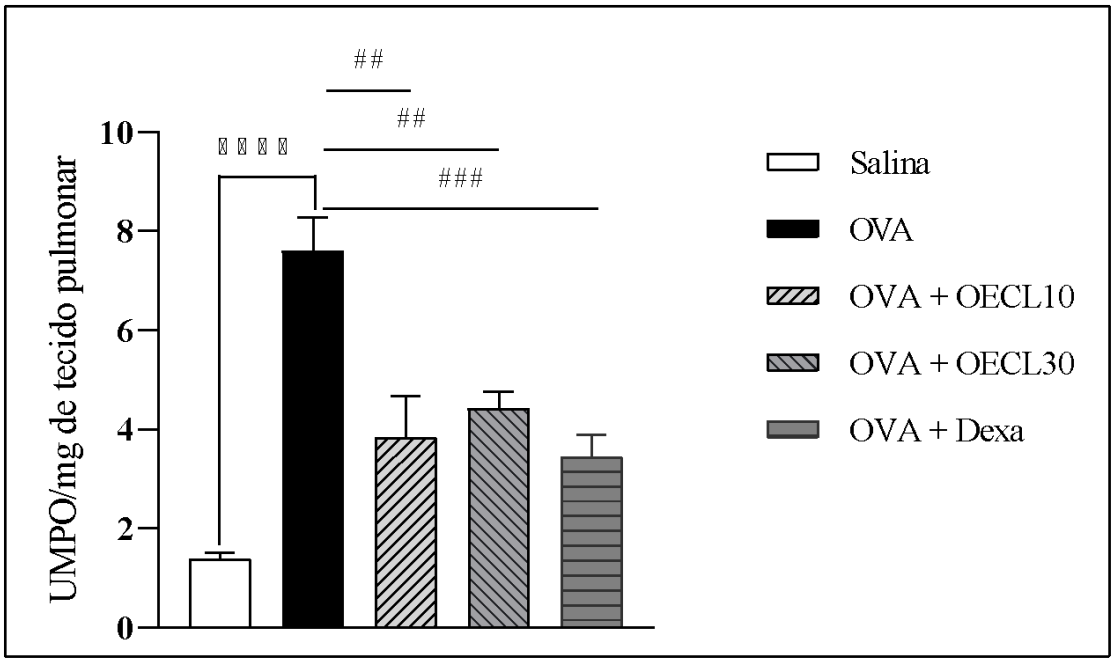

Figura 5: Efeito do Óleo Essencial da Casca de Laranja (OECL) sobre a atividade da mieloperoxidase (MPO) no tecido pulmonar de camundongos BALB/c submetidos ao modelo de asma alérgica induzida por ovalbumina (AAIO). Os dados, expressos como média ± E.P.M, representam a atividade de MPO pulmonar mensurada 24h após o último dia de desafio. Análise estatística: ANOVA *one way* seguida pelo pós-teste de Tukey. (****) $p < 0,0001$ *versus* Salina; ($^{###}$) $p < 0,001$ *versus* OVA; ($^{##}$) $p < 0,01$ *versus* OVA. Salina (n=4); OVA (n=4); OVA + OECL 10 (n=4); OVA + OECL 30 (n=4); e, OVA + Dexa (n=4). Salina: animais sensibilizados nos dias 0, 7 e 14 com 20 mg de $Al(OH_3)$ solubilizado em 100 µl de salina estéril, administrado por via intraperitoneal (i.p.) e desafiados nos dias 21, 22 e 23 com 25 µl de salina estéril (NaCl 0,9%) por via intranasal (i.n.); OVA: animais sensibilizados nos dias 0, 7 e 14 com 100 µg de OVA adsorvida a 20 mg de $Al(OH_3)$ em 100 µl de salina estéril (NaCl 0,9%) administrado por via intraperitoneal (i.p.) e desafiados nos dias 21, 22 e 23 com 100 µg de OVA em 25 µl de salina estéril (NaCl 0,9%) por via intranasal (i.n.); OVA + OECL 10: animais sensibilizados nos dias 0, 7 e 14 com 100 µg de OVA adsorvida a 20 mg de $Al(OH_3)$ em 100 µl de salina estéril (NaCl 0,9%) administrado por via intraperitoneal (i.p.) e desafiados nos dias 21, 22 e 23 com 100 µg de OVA em 25 µl de salina estéril (NaCl 0,9%) por via intranasal (i.n.) e tratados com OECL na dose de 10 mg/kg de massa corpórea, por via intranasal, 1 hora antes do desafio; OVA + OECL 30: animais sensibilizados nos dias 0, 7 e 14 com 100 µg de OVA adsorvida a 20 mg de $Al(OH_3)$ em 100 µl de salina estéril (NaCl 0,9%) administrado por via intraperitoneal (i.p.) e desafiados nos dias 21, 22 e 23 com 100 µg de OVA em 25 µl de salina estéril (NaCl 0,9%) por via intranasal (i.n.) e tratados com OECL na dose de 30 mg/kg de massa corpórea, por via intranasal, 1 hora antes do desafio; OVA + Dexa: animais sensibilizados nos dias 0, 7 e 14 com 100 µg de OVA adsorvida a 20 mg de $Al(OH_3)$ em 100 µl de salina estéril (NaCl 0,9%) administrado por via intraperitoneal (i.p.) e desafiados nos dias 21, 22 e 23 com 100 µg de OVA em 25 µl de salina estéril (NaCl 0,9%) por via intranasal (i.n.) e tratados com dexa 2 mg/kg de massa corpórea, por via intranasal, 1 hora antes do desafio;

5.5 Efeito do Óleo Essencial da Casca de Laranja sobre a atividade da peroxidase eosinofílica no tecido pulmonar de camundongos BALB/c submetidos ao modelo de asma alérgica induzida por ovalbumina

Com o objetivo de mensurar o efeito do OECL sobre a quantidade de eosinófilos presentes no tecido pulmonar, o conteúdo de EPO foi determinado em amostras pulmonares coletadas 24h após o último dia de desafio. Para tanto, através do teste estatístico ANOVA *one-way*, obteve-se o seguinte resultado: [$F_{4,79} = 18,55$, $p < 0,0001$]. Como pode ser observado na Figura 6, a análise do pós-teste mostrou que o conteúdo de EPO no tecido pulmonar do grupo dos animais sensibilizados e desafiados com OVA foi significativamente maior quando comparado ao grupo de animais sensibilizados e desafiados com veículo (Salina) (0,6956 ± 0,06711 *versus* 0,1227 ± 0,01132, respectivamente, $p < 0,0001$). Como esperado, o conteúdo de EPO no pulmão dos animais sensibilizados e desafiados com OVA, mas tratados com dexametasona (OVA + Dexa), foi reduzido significativamente quando comparado ao grupo de animais sensibilizados e desafiados com OVA (0,4879 ± 0,04103 *versus* 0,6956 ± 0,06711, respectivamente, $p < 0,05$). Enquanto nenhuma diferença estatisticamente significativa foi observada entre os conteúdos de EPO dos grupos de animais tratados com o óleo essencial da casca de laranja nas doses de 10 mg/kg (OVA + OECL 10) e 30 mg/kg (OVA + OECL 30), 24 horas após o último desafio, quando comparados ao grupo OVA (OVA versus OVA + OECL 10: 0,6956 ± 0,0671 *versus* 0,5919 ± 0,06346, respectivamente, p = n.s.; OVA versus OVA + OECL 30: 0,6956 ± 0,0671 *versus* 0,5684 ± 0,06278, respectivamente, p = n.s.).

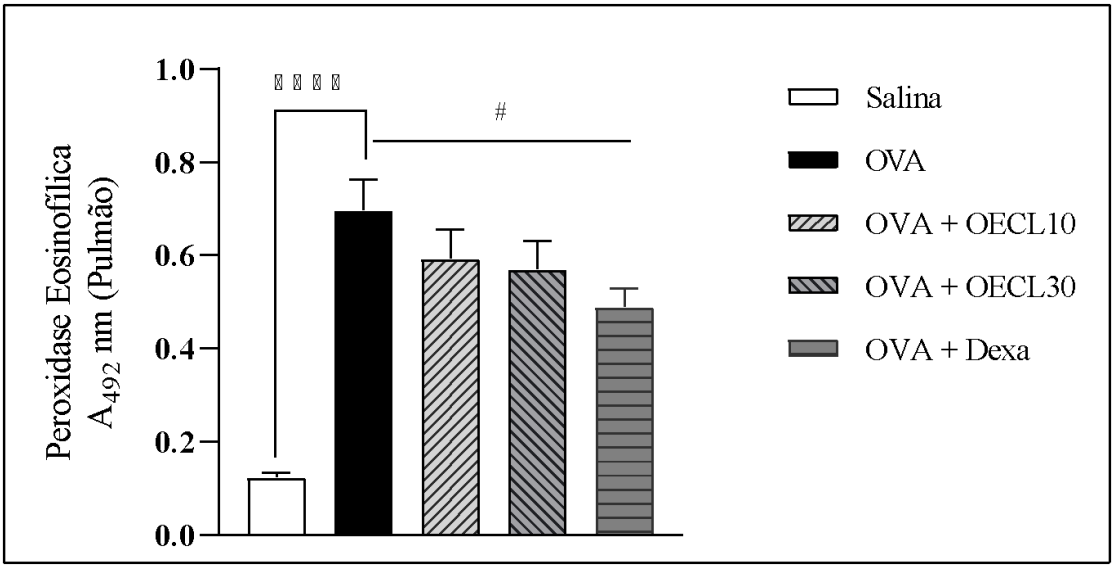

Figura 6: Efeito do Óleo Essencial da Casca de Laranja (OECL) sobre a atividade da peroxidase eosinofílica (EPO) no tecido pulmonar de camundongos BALB/c submetidos ao modelo de asma alérgica induzida por ovalbumina (AAIO). Os dados, expressos como média ± E.P.M, representam a atividade de EPO no tecido pulmonar mensurada 24h após o último dia de desafio. Análise estatística: ANOVA *one way* seguida pelo pós-teste de Tukey. (****) $p < 0,0001$ *versus* Salina; ($^{\#}$) $p < 0,05$ *versus* OVA. Salina (n=19); OVA (n=20); OVA + OECL 10 (n=9); OVA + OECL 30 (n=19); e, OVA + Dexa (n=17). Salina: animais sensibilizados nos dias 0, 7 e 14 com 20 mg de $Al(OH_3)$ solubilizado em 100 µl de salina estéril, administrado por via intraperitoneal (i.p.) e desafiados nos dias 21, 22 e 23 com 25 µl de salina estéril (NaCl 0,9%) por via intranasal (i.n.); OVA: animais sensibilizados nos dias 0, 7 e 14 com 100 µg de OVA adsorvida a 20 mg de $Al(OH_3)$ em 100 µl de salina estéril (NaCl 0,9%) administrado por via intraperitoneal (i.p.) e desafiados nos dias 21, 22 e 23 com 100 µg de OVA em 25 µl de salina estéril (NaCl 0,9%) por via intranasal (i.n.); OVA + OECL 10: animais sensibilizados nos dias 0, 7 e 14 com 100 µg de OVA adsorvida a 20 mg de $Al(OH_3)$ em 100 µl de salina estéril (NaCl 0,9%) administrado por via intraperitoneal (i.p.) e desafiados nos dias 21, 22 e 23 com 100 µg de OVA em 25 µl de salina estéril (NaCl 0,9%) por via intranasal (i.n.) e tratados com OECL na dose de 10 mg/kg de massa corpórea, por via intranasal, 1 hora antes do desafio; OVA + OECL 30: animais sensibilizados nos dias 0, 7 e 14 com 100 µg de OVA adsorvida a 20 mg de $Al(OH_3)$ em 100 µl de salina estéril (NaCl 0,9%) administrado por via intraperitoneal (i.p.) e desafiados nos dias 21, 22 e 23 com 100 µg de OVA em 25 µl de salina estéril (NaCl 0,9%) por via intranasal (i.n.) e tratados com OECL na dose de 30 mg/kg de massa corpórea, por via intranasal, 1 hora antes do desafio; OVA + Dexa: animais sensibilizados nos dias 0, 7 e 14 com 100 µg de OVA adsorvida a 20 mg de $Al(OH_3)$ em 100 µl de salina estéril (NaCl 0,9%) administrado por via intraperitoneal (i.p.) e desafiados nos dias 21, 22 e 23 com 100 µg de OVA em 25 µl de salina estéril (NaCl 0,9%) por via intranasal (i.n.) e tratados com dexa 2 mg/kg de massa corpórea, por via intranasal, 1 hora antes do desafio;

5.6 Efeito do Óleo Essencial da Casca de Laranja sobre as citocinas IL-5, 10, 17, TNF-α e IL-1β no tecido pulmonar de camundongos BALB/c submetidos ao modelo de asma alérgica induzida por ovalbumina

Com o objetivo de verificar a existência de correlação de citocinas presentes no tecido pulmonar dos animais dos grupos experimentais 24 horas após o último desafio, a Correlação de Pearson foi realizada e os resultados estão representados na Figura 7, nos painéis de A-F. Verificou-se a presença de correlação estatisticamente significativa nos seguintes cenários: entre IL-10 e TNF-α no grupo de animais submetidos à AAIO e tratados com OECL na dose de 30 mg/kg de massa corpórea (OECL 30) ($p = 0,0242$; $r = 0,7741$; IC 95% = 0,15 a 0,95) (Figura 7, Painel A); entre IL-5 e IL-17 ($p = 0,0019$; $r = 0,9062$; IC 95% = 0,55 a 0,98), IL-10 e TNF-α ($p = 0,0013$; $r = 0,8630$; IC 95% = 0,51 a 0,96), IL-17 e TNF-α ($p = 0,0136$; $r = 0,8157$; IC 95% = 0,26 a 0,96), IL-17 e IL-10 ($p < 0,0001$; $r = 0,9799$; IC 95% = 0,89 a 0,99), e, por fim, entre IL-1β e IL-17 ($p = 0,0144$; $r = 0,8119$; IC 95% = 0,25 a 0,94) (Figura 7, Painéis de B a F, respectivamente), no grupo de animais submetidos à AAIO e tratados com dexametasona (DEXA).

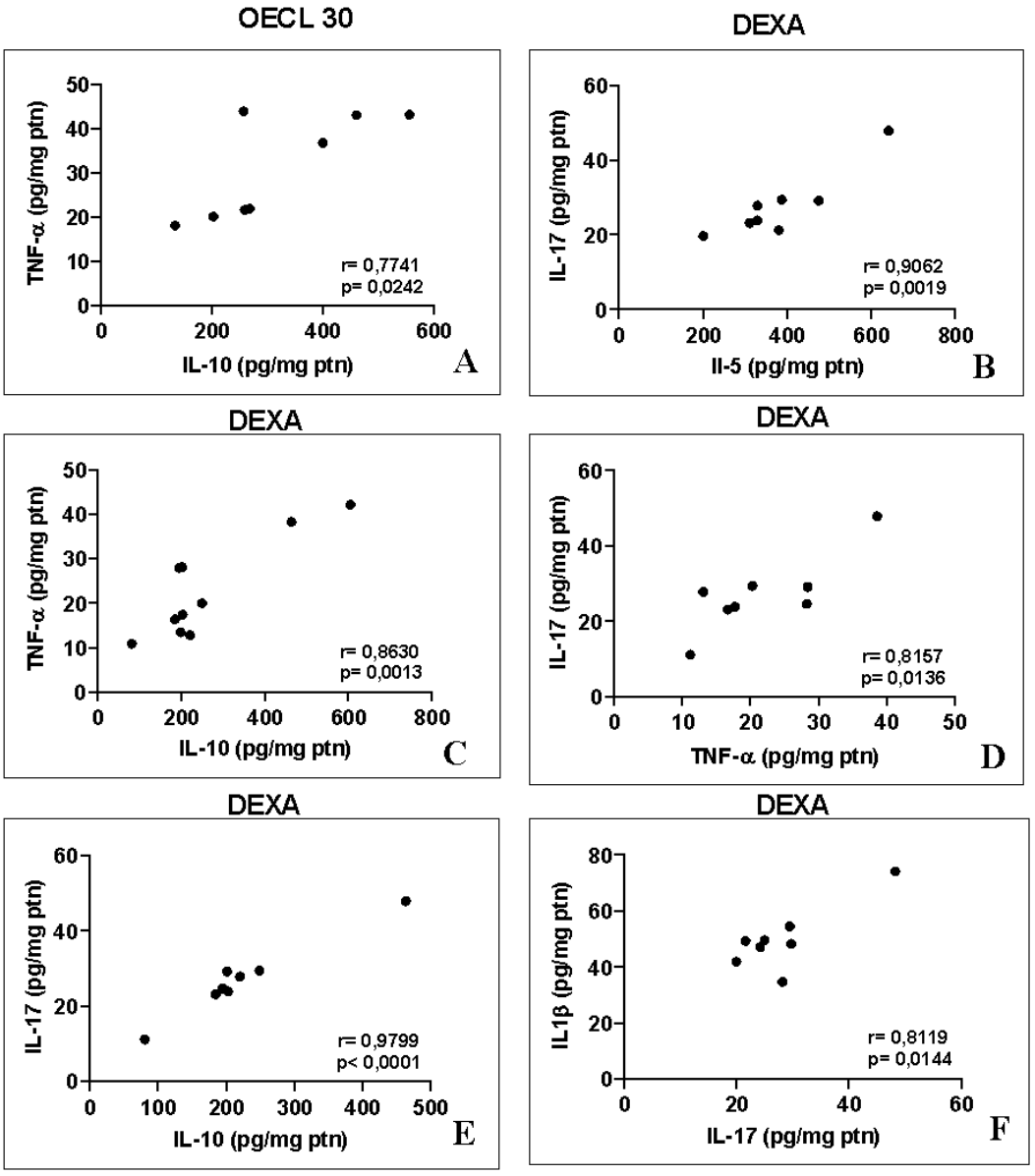

Figura 7. Efeito do óleo essencial da casca de laranja (OECL) sobre a concentração de citocinas no tecido pulmonar de animais submetidos ao modelo de asma induzida por Ovalbumina (AAIO) 24 horas após o último desafio. Análise estatística: Correlação de Pearson. Entre IL-10 e TNF-α no grupo OECL 30 mg/kg, i.n, (Painel A) (n = 10); Entre IL-17 e IL-5 no grupo DEXA (Painel B) (n = 10); Entre TNF-α e IL-10 no grupo DEXA (Painel C) (n = 10); Entre IL-17 e TNF-α no grupo DEXA (Painel D) (n = 10); Entre IL-17 e IL-10 no grupo DEXA (Painel E) (n = 10); Entre IL-1β e IL-17 no grupo DEXA (Painel E) (n = 10). OECL: OECL; AAIO: asma alérgica induzida por ovalbumina; OECL 30: animais sensibilizados nos dias 0, 7 e 14 com 100 µg de OVA adsorvida a 20 mg de $Al(OH_3)$ em 100 µl de salina estéril (NaCl 0,9%) administrado por via intraperitoneal (i.p.) e desafiados nos dias 21, 22 e 23 com 100 µg de OVA em 25 µl de salina estéril (NaCl 0,9%) por via intranasal (i.n.) e tratados com OECL na dose de 30 mg/kg de massa corpórea, por via intranasal, 1 hora antes do desafio; Dexa: animais sensibilizados nos dias 0, 7 e 14 com 100 µg de OVA adsorvida a 20 mg de $Al(OH_3)$ em 100 µl de salina estéril (NaCl 0,9%) administrado por via intraperitoneal (i.p.) e desafiados nos dias 21, 22 e 23 com 100 µg de OVA em 25 µl de salina estéril (NaCl 0,9%) por via intranasal (i.n.) e tratados com dexametasona 2 mg/kg de massa corpórea, por via intranasal, 1 hora antes do desafio;

6. DISCUSSÃO

O D- Limoneno foi o terpeno mais prevalente no óleo da casca laranja, este achado encontra-se em concordância com diversos estudos. Simas et al., 2017, determinou o teor e composição química dos óleos essenciais de 11 espécies, dentre 15 variedades de frutas cítricas e, foi verificado a principal substância dos óleos cítricos estudados por eles, o limoneno. Conforme Mehl et al., 2014 o limoneno compõe de 30% a 97% dos óleos essenciais de espécies cítricas, sendo na maioria das vezes a principal substância química dos óleos essenciais desse tipo de espécie. Vale ressaltar que a Food and Drug Administration (FDA, 2015) considerou o limoneno um composto seguro para administração, sendo seu uso autorizado como inseticida natural e como repelente, pela U.S. Environmental Protection Agency (EPA, 1993)

A análise do LBA das duas espécies de animais induzidas à asma e tratadas com veículo (Grupo OVA) exibiu um acúmulo no número de células inflamatórias maior quando comparada aos demais grupos, já o tratamento com o OECL nas duas maiores doses (30 e 90 mg/kg) reduziu o acúmulo de células inflamatórias no LBA para valores próximos ao encontrado nos animais do grupo controle (sem estímulo alérgico). Como esperado, o tratamento com o fármaco de referência em ambas espécies, reduziu significativamente o infiltrado inflamatório no LBA. É importante ressaltar que os flavonoides, presente significativamente no OECL através do Limoneno, podem ter atividade sobre a regulação do sistema imunológico em alguns pontos e atuar de forma expressiva sobre as células inflamatórias nas vias aéreas.

Os flavonoides são a classe mais estudada de metabólitos vegetais, que podem diminuir a expressão e produção de moléculas inflamatórias, incluindo histamina e leucotrienos (Lago et al., 2014). O tratamento da asma alérgica com *Gleditsia sinensis* L., planta rica em flavonoides, induziu a redução significativa no número de células inflamatórias das vias aéreas no LBA em um modelo animal asmático (Lee et al., 2011). Pré e pós-tratamentos com safranal, um componente ativo do *Crocus sativus* Linn. (Iridaceae), reduziu a infiltração celular nas vias aéreas (Bukhari et al., 2015). O extrato hidroetanólico de *Mandevilla longiflora* (Desf.) Pichon (Apocynaceae), rico em componentes flavonoides como hesperidina, naringina, rutina, naringenina e luteolina,

neutralizaram significativamente o processo inflamatório no pulmão induzido por OVA e a resposta do tipo Th2 (De Almeida et al., 2017).

A inflamação causada pela asma alérgica tem como principais colaboradores os eosinófilos (YANG et al., 2013) e sua atividade foi mensurada através da EPO. Demonstrou-se que o resultado da EPO no tecido pulmonar do grupo dos animais sensibilizados e desafiados com OVA foi significativamente maior quando comparado aos demais grupos, com exceção dos grupos de animais tratados com o OECL que não apresentaram nenhuma diferença significativa. A maior parte dos estudos encontrados apresentaram uma redução de eosinofilia no pulmão dos camundongos, tais estudos analisaram extratos de diversas plantas (Jantan et al., 2015, Heo et al., 2008, Rogerio et al., 2008, Huang et al., 2008) e óleos essenciais, como o óleo essencial de *C. verbenace*a (Rogerio et al., 2009), e o óleo essencial de lavanda, cuja inalação diminuiu significativamente os eosinófilos no LBA e tecido pulmonar em camundongos asmáticos (Ueno-Iio et al., 2014).

As citocinas orquestram e perpetuam a inflamação crônica das vias aéreas (Barnes, 2018), através de cascatas inflamatórias durante as crises de asma que incluem a estimulação de reações do sistema imunológico com aumento da produção e expressão de linfócitos T auxiliares (Th) 2 (Xin et al., 2018), que é caracterizada por uma liberação de citocinas Th1 e Th2 e desequilíbrio funcional, com maior secreção de citocinas Th2, causando inflamação das vias aéreas, resultando em infiltração, ativação e diferenciação de eosinófilos, IgE e secreção de muco (Lu et al., 2019).

O óleo da casca da laranja foi analisado com ênfase em sua ação sobre a regulação das citocinas IL-5, 10, 17, TNF-α e IL-1β. Neste estudo demonstrou-se que as citocinas IL-10 e TNF-α comportaram-se de maneira semelhante no óleo da casca de laranja e na dexametasona, (figura 7), ou seja o OECL comportou-se de maneira semelhante ao fármaco de referência utilizado, demonstrando assim atividade anti-inflamatória satisfatória relacionada a estas citocinas. Já em relação a outras citocinas estudadas (IL-5,17 e -1β) não foram encontradas correlações significativas em relação ao OECL, por fatores ainda desconhecidos pelos autores. O objetivo de estudar tais citocinas seria avaliar a ação do OECL sobre elas visto que a IL-5 desempenha um papel fundamental no desenvolvimento e migração de eosinófilos e se correlaciona com a gravidade da inflamação eosinofílica (Sanderson, 1992), a IL-10 é produzida

principalmente por células Th2, embora a secreção de IL-10 possa inibir a produção de IL-1β e TNF-α (Iyer e Chen, 2012), o TNF-α é considerado um mediador importante em asmáticos, sendo produzido por células do tipo TH1, sendo capaz de induzir a inflamação das vias aéreas, aumentar a secreção de muco e ativar macrófagos (Chung et al., 2015).

Por estarem altamente envolvidas no processo inflamatório asmático, as citocinas são amplamente discutidas em grande parte de estudos que envolvem essa patologia. Nos últimos anos os estudos associando a melhora do processo inflamatório asmático a produtos naturais vem crescendo, sendo cada vez mais discutido a função destes compostos na cascata inflamatória que envolve as citocinas. O óleo essencial de *Angelicae sinensis* (Oliv.) foi capaz de desempenhar um papel benéfico na patologia nos ratos sendo utilizado como tratamento em ratos sensibilizados com OVA, e foram observadas melhoras nas características clássicas que são alteradas na asma, tais como melhora na função pulmonar, elevação dos níveis de IL-10 no LBA e no pulmão dos animais asmáticos (Wang et al., 2015). Outros óleos essenciais estudados demonstraram sua capacidade anti-inflamatória em estudos *in vivo.* O óleo de perila diminuiu a inflamação broncoalveolar, diminuindo a secreção de citocinas pró-inflamatórias (Chang et al., 2008). A timoquinona também demonstrou o aumento da IL-10 em camundongos sensibilizados e desafiados com OVA (EL Gazzar et al., 2006). O farnesol, outro composto natural, um álcool sesquiterpênico amplamente presente em frutas, vegetais e óleos essenciais (Duncan e Archer, 2008) restaurou os níveis de diversas citocinas, entre elas IL-10 e TNF-α em LBA em camundongos sensibilizados e desafiados com OVA, sugerindo que este pode ter potencial para modular o equilíbrio Th1/Th2 nos pulmões (Ku e Lin, 2015). Camundongos tratados com limoneno (maior composto encontrado no OECL) reduziram os níveis de IFN-γ, IL-5, IL-13 e TGF-β e de eosinófilos nos pulmões em um modelo de asma (Hirota et al., 2012).

Vale destacar os monoterpenos alcoólicos citronelol, α-terpineol e carvacrol, amplamente presente em plantas dos gêneros Cymbopogon, Eucalyptus e Origanum, que são utilizados para o tratamento de doenças inflamatórias (Guimarães et al., 2013). Citronelol, α-terpineol e carvacrol modulam a migração de eosinófilos e diminuem os níveis de TNF-α na cavidade pleural de camundongos desafiados com OVA. Esses efeitos podem estar associados à capacidade dos monoterpenos de inibir alvos importantes de mediadores inflamatórios e podem ser potenciais candidatos a drogas

que estejam associadas a terapia de inflamação alérgica e asma. Tais estudos corroboram com o fato de que compostos naturais, assim como o OECL, são capazes de melhorar o quadro inflamatório relacionado ao equilíbrio de citocinas, especialmente IL-10 e TNF-α.

Tais estudos demonstram a necessidade de aprofundar pesquisas em torno dos óleos essenciais, especialmente o OECL, para elucidarmos melhor seu mecanismo de ação e suas funções anti-inflamatórias para que possamos explorar da melhor forma possível suas características anti-asmáticas em benefício da população e indústria. A fitoterapia tem sido utilizada para tratar a asma devido à sua acessibilidade a populações de baixa renda e baixa toxicidade percebida (Yu et al., 2017). Os óleos essenciais se encaixam perfeitamente nesse perfil.

7. CONCLUSÃO

O óleo essencial da casca da laranja mostrou-se capaz de reduzir grande parte dos eventos inflamatórios investigados, causados pela asma alérgica, tanto em camundongos swiss quanto em camundongos BALB/c. Foi possível visualizar através dos resultados acima citados o possível potencial efeito anti-inflamatório do óleo essencial da casca da laranja em seres humanos.

Estudos vêm demonstrando que extratos vegetais, de forma geral, mostram-se capazes de regular a reatividade respiratória, reduzindo a cascata de eventos inflamatórios relacionados ao processo asmático ou potencializando as condições pulmonares. Este estudo demonstrou que a pesquisa científica, em torno dos óleos essenciais de plantas medicinais ricas em compostos polifenólicos, como os flavonóides, deve ser aprofundada pois estes têm demonstrado uma promissora ação anti-inflamatória capaz de reduzir células inflamatórias e possivelmente modular as citocinas Th1/Th2 além de oferecer novas opções terapêuticas, se seus mecanismos farmacológicos e de segurança farmacológica para uso clínico forem melhor demonstrados. A tradução desses resultados para humanos por meio da realização de ensaios clínicos é necessária para estimular o desenvolvimento de pesquisas relacionadas às plantas medicinais visto que há uma lacuna científica com a escassez de estudos para corroborar essa abordagem para os fabricantes de medicamentos e pacientes.

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APÊNDICE I

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Review

Modulation of interleukin expression by medicinal plants and their secondary metabolites: A systematic review on anti-asthmatic and immunopharmacological mechanisms

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ABSTRACT

Background: Asthma is one of the most common chronic inflammatory conditions of the lungs in modern society. Asthma is associated with airway hyperresponsiveness and remodeling of the airways, with typical symptoms of cough, wheezing, shortness of breath and chest tightness. Interleukins (IL) play an integral role in its inflammatory pathogenesis. Medicinal herbs and secondary metabolites are gaining considerable attention due to their potential therapeutic role and pharmacological mechanisms as adjunct tools to synthetic bronchodilator drugs.

Purpose: To systematically review the literature on the use of single or mixed plants extracts therapy *in vivo* experimental systems for asthma, emphasizing their regulations on IL production to improve lung.

Methods: Literature searches were performed on PubMed, EMBASE, Scopus and Web of Science databases. All articles in English were extracted from 1999 up to September 2019, assessed critically for data extraction. Studies investigating the effectiveness and safety of plant extracts administered; inflammatory cell count, immunoglobulin E (IgE) production and regulation of pro-inflammatory cytokine and T helper (Th) 1 and Th2-driven cytokine expression in bronchoalveolar lavage fluid (BALF) and lung of asthmatic animals were included.

Results: Four hundred and eighteen publications were identified and 51 met the inclusion criteria. Twenty-six studies described bioactive compounds from plant extracts. The most frequent immunopharmacological mechanisms described included reduction in IgE and eosinophilic recruitment, decreased mucus hypersecretion and airway hyperreactivity, enhancement of the balance of Th1/Th2 cytokine ratio, suppression of matrix metallopeptidase 9 (MMP-9) and reversal of structural alterations.

Conclusion: Plant extract therapies have potential control activities on asthma symptoms by modulating the secretion of pro-inflammatory (IL-1β, IL-8), Th17 (IL-17), anti-inflammatory (IL-10, IL-23, IL-31, IL-33), Th1 (IL-2, IL-12) and Th2 (IL-4, IL-5, IL-6, IL-13) cytokines, reducing the level of biomarkers of airway inflammation.

Abbreviations: AHR, airway hyperresponsiveness; BALF, Bronchoalveolar Lavage Fluid; ELISA, Enzyme-Linked Immunosorbent Assay; HO, Heme Oxygenase; H&E, Hematoxylin and Eosin; IL, Interleukin; IgE, Immunoglobulin E; ICAM-1, Intercellular Adhesion Molecule-1; LPS, Lipopolysaccharide; MCP-1, Monocyte Chemoattractant Protein 1; MMP-9, Matrix Metallopeptidase 9; NFκB, Nuclear Factor Kappa B; OVA, Ovalbumin; PAS, Periodic acid-Schiff; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; PCR, Polymerase Chain Reaction; TGF-β, Transforming Growth Factor Beta; Th, T helper; VCAM-1, Vascular Cell Adhesion Molecule

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Introduction

Asthma is a chronic inflammatory condition of the airways characterized by recurrent episodes of wheeze, mucus overproduction, narrowing of the airways leading to bronchial hyperreactivity and hyperresponsiveness (Mims, 2015). This condition affects around 339 million people worldwide, and its incidence is increasing (The Global Asthma Report 2018). Although the etiology of asthma is not fully understood, the exposure to allergens or tobacco smoke and multiple genetic and environmental factors are implicated (Beasley et al., 2015).

The most widely used pharmacological treatments for asthma are corticosteroids, β-agonists and leukotriene receptor antagonists (McCracken et al., 2017). Although very effective for symptomatic treatment, these agents have well established side effects. The prolonged use of glucocorticoid can facilitate opportunistic infections such as candidiasis, hoarseness, osteopenia and skin lesions (Vanfleteren et al., 2018). β-agonists and leukotriene receptor antagonists have infrequent adverse events such as asthma exacerbations and neuropsychiatric symptoms, but they need to be used in tandem with other medicines to increase their effectiveness (Leung et al., 2017). Besides corticosteroids, other medicaments relieve symptomatic shortness of breath, chest tightness and coughing without reducing inflammation and mucus secretion (McCracken et al., 2017).

The inflammatory cascades during asthma crises include the stimulation of immune system reactions with increased production and expression of T helper (Th) 2-type lymphocytes (Xin et al., 2018), which is characterized by a Th1 and Th2 cytokines release and function imbalance, with higher secretion of Th2 cytokines such as interleukin (IL)-4, IL-5 and IL-13, causing airway inflammation resulting in airway infiltration, eosinophil activation and differentiation, immunoglobulin E (IgE) production and mucus secretion (Lu et al., 2019). Cytokines orchestrate and perpetuate chronic airway inflammation (Barnes, 2018). Thus; IL-4 promotes the differentiation of T lymphocytes into Th2-cells with attraction and activation of mastocytes and the release of bronchoconstrictors (Wynn, 2015). IL-5 is responsible for the development and maturation of eosinophils in the bone marrow and their recruitment into the lung and interstitial mucosa during allergic inflammation; whereas IL-13 plays an important role in production of mucus in the lung tissue and promotes optimal induction of eosinophils and stimulates B lymphocytes to synthesize IgE (Bok et al., 2019).

Herbal medicine has been used to treat asthma due to their accessibility to low income populations, perceived low toxicity (Yu et al., 2017). Plants and derived metabolites such as terpenoids, alkaloids, flavonoids, saponins and phenolic compounds, have been extensively researched for treatment and management of asthma (De Almeida et al., 2017; Ye et al., 2019) with an increasing number of reports on their use modulating interleukins to control and reverse asthma exacerbations *in vivo* and *in vitro* experiments (Fouladi et al., 2019). Thus, we aimed to review the use of medicinal plants extracts and mixed formulations for the prevention of asthma, through emphasis on their action on IL regulations and immunopharmacological mechanisms.

Materials and methods

This review follows the guidelines for Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) report (Moher et al., 2009).

Search strategy

The PubMed, EMBASE, Scopus and Web of Science databases were systematically searched for relevant publications in English, classified to Medical Subjects Headings Index (MeSH/DeCS) from January 1999 to September 2019. We used the keywords: "Medicinal Plants", "Cytokines", "Interleukins", "Anti-asthmatic Effect", "Natural Products", "Inflammation", "Immunoregulatory" and "Immune Modulation". In addition, we reviewed the references quoted by the articles selected to identify further studies not encompassed in the database searches.

Study selection

An initial selection based on titles and abstracts of the publications was conducted by three authors (GRG, GCSL and VKSC). In case of dissimilarities, a fourth author (RQG) screened whether the study encountered the inclusion and exclusion criteria. In order to remove partiality in the data assortment and selection strategy, the information was independently recovered by the four authors (GRG, GCSL, VKSC, and RQG) and analyzed. Only experimental studies investigating the anti-asthmatic action were included. Reviews, conference proceedings, and editorial notes, as well as unpublished studies, were excluded. The studies were chosen if they investigated the effect of plant extracts/formulations for the treatment of asthma and describing mechanisms of action through specific IL-mediated signaling pathways, including murine models. Studies were excluded if they investigated isolated, synthetic, essential oils and fractions from plant extracts; *in vitro* studies of inflammatory airway diseases or only reporting the *in vitro* anti-asthmatic effect; or reporting the use of crude extracts for the treatment of bronchial asthma without highlighting immunopharmacological mechanisms.

Data extraction

Data were extracted and presented in tables to summarize publication characteristics; substances (plant species, families or polyherbal formulation); experimental models (animal, strain, sex and treatment description) and experimental procedures, methods of induction, biochemical variations; and the putative mechanisms of biochemical and treatment response; plant species, plant-derived substances and phytochemicals and their immunopharmacological mechanisms and therapeutic reaction.

Methodological quality and risk of bias

The methodological quality was assessed using standard criteria and checklists (Hooijmans et al., 2014) to describe essential characteristics of the studies using animal models. The quality analysis was represented by using colors as recommended by Roskosk-Jr (2017).

Data analysis

The data is presented as a narrative because experimental procedures and the parameters describing mediators of inflammation were heterogeneous, and studies followed different protocols for assessment and biochemical examinations. Pooling statistics and meta-analysis were not feasible.

Results and discussion

Included studies

The PRISMA diagram describes the method to select studies (Fig. 1). A total of 418 papers (PubMed: 325, EMBASE: 22, Scopus: 41 and Web of Science: 30) were identified, of which 15 were duplicate. Of these, 175 studies were excluded after evaluating the titles and abstract. The remaining 51 publications were selected and the references listed in these papers were checked to identify further reports missed by the database searches, but no further articles were identified.

Qualitative analysis

The studies selected originated from twelve countries, including The Republic of Korea (n = 21), China (n = 8), Taiwan (n = 5), Brazil

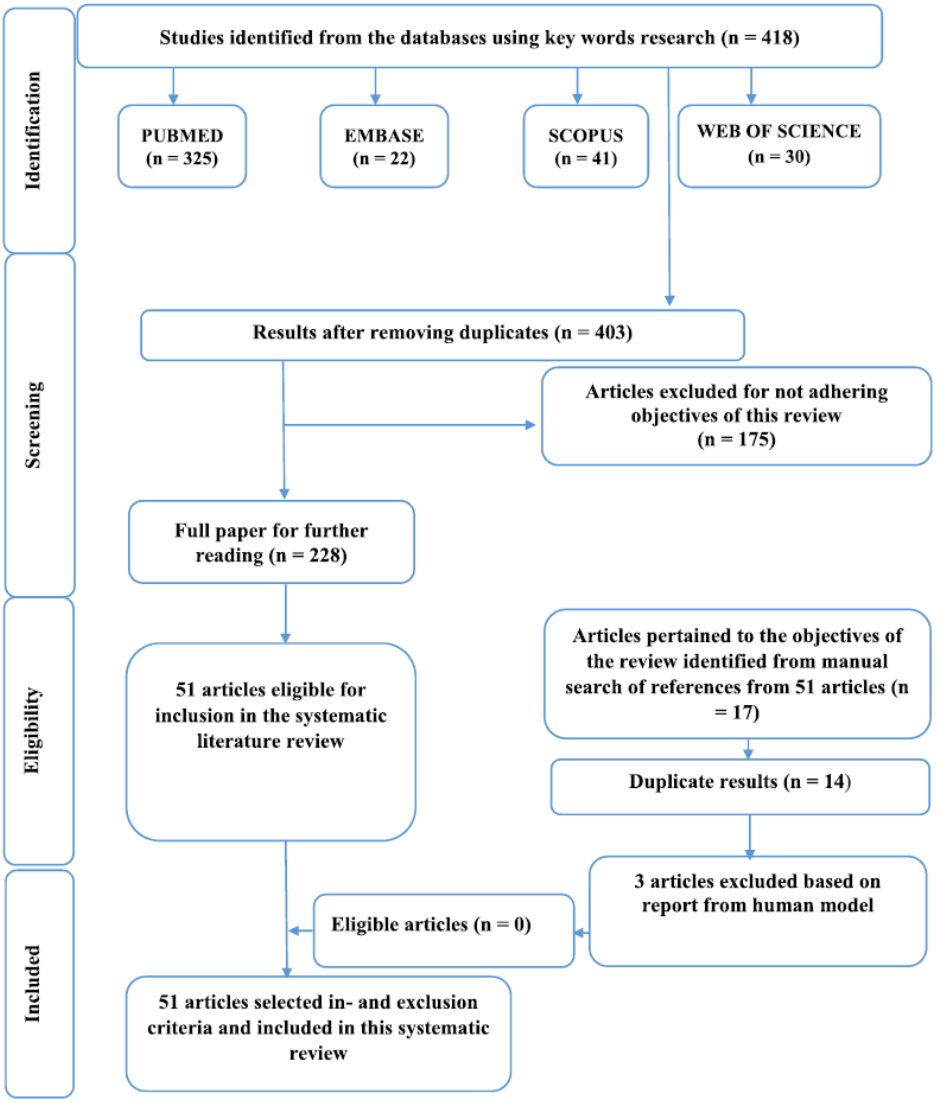

Fig. 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram of number of articles selected for review.

(n = 5) and Japan (n = 4). Two studies were from India and one each from Cuba, Iran, Mexico, USA, Pakistan and Switzerland. Seventy-eight plant species were investigated in 51 studies, either as single-plant (n = 31) or mixed-plant extracts or formulations (n = 20). The following plant families were included: Acanthaceae, Alismataceae, Amaryllidaceae, Anacardiaceae, Apiaceae, Apocynaceae, Araceae, Araliaceae, Aristolochiaceae, Asteraceae, Berberidaceae, Campanulaceae, Caprifoliaceae, Chenopodiaceae, Convolvulaceae, Cruciferae, Cucurbitaceae, Cupressaceae, Ebenaceae, Ephedraceae, Fabaceae, Ganodermataceae, Guttiferae, Iridaceae, Labiatae, Lamiaceae, Lauraceae, Lythraceae, Mimosaceae, Moraceae, Oleaceae, Ophioglossaceae, Paeoniaceae, Phytolaccaceae, Pinaceae, Piperaceae, Ranunculaceae, Rhamnaceae, Rosaceae, Rutaceae, Schisandraceae, Simaroubaceae, Solanaceae, Theaceae, Verbenaceae, Violaceae, Vitaceae and Zingiberaceae.

The most frequently used plant parts were roots (n = 41), rhizomes (n = 24), and whole plants (n = 12), followed by fruits (n = 11), leaves (n = 11), seeds (n = 10) and stems (n = 6). Few studies used tubers (n = 4), pericarps (n = 3), cortexes (n = 3), barks (n = 2), thorns (n = 1), stigmas (n = 1), pells (n = 1), galls (n = 1), flowers (n = 1), branches (n = 1) or aerial parts (n = 1), while eleven studies did not provide this information. Toxicity tests were assessed in 37 of

Fig. 2. Summary of the studies outlining the plant species, families, list of countries reported, used parts of plants and toxicity investigations.

the 51 publications, as shown in Fig. 2. *In vivo* studies used BALB/c mice (n = 38), Sprague-Dawley rats (n = 4), ICR mice (n = 3), Dunkin-Hartley guinea pigs (n = 2), AJ mice (n = 2), Wistar rats (n = 2), SPF mice (n = 1), Swiss-Webster mice (n = 1), NMARI mice (n = 1), By-JNarl mice (n = 1), SPF guinea pigs (n = 1) and BDF1 mice (n = 1). Asthmatic murine models were achieved by immunization of ovalbumin (OVA)-sensitized and challenged airway inflammation (n = 45), *Blomia tropicalis* (n = 2) and *Dermatophagoides pteronyssinus* (n = 1) induced allergic asthma, Phorbol 12-yristate 13-acetate (n = 2) and ragweed (n = 1) administered asthmatic respiratory allergy.

Table. 1
Description of the main characteristics of studies using plant extracts for the management of asthma in animal models.

Authors, Year, Country	Plant species/ Formulation	Animal/ Strains	Dose/ Route	Objectives	Interleukins	Assays Biochemical	Molecular	Improved Characteristics
Suzuki et al., 1999;Japan	Bu-Zhong-Yi-Qi-Tang (Hochu-ekki-to) (BZYQT)	BDF1 Mice and Wistar Rats	0.085 and 0.85 mg/g (*p.o.*)	To demonstrate that water-extracted BZYQT can suppress immunoglobulin (Ig) E antibody production and histamine release in mice immunized with ovalbumin (OVA)	Interleukin (IL) −4 and IL-2	Enzyme-Linked Immunosorbent Assay (ELISA)	Reverse Transcription Polymerase Chain Reaction (RT-PCR)	BZYQT revealed potential efficacy in treating asthma due to reduced cell proliferation, histamine release, IgE production and IL-4 secretion and expression.
Ishimitsu et al., 2001; Japan	Bu-Zhong-Yi-Qi-Tang (Hochu-ekki-to) (BZYQT)	BALB/c Mice	1000 mg/kg (*p.o.*)	To determine thera-peutic potential of BZYQT on an OVA-induced murine model of asthma.	IL-4 and IL-5	ELISA		BZYQT ameliorated airway inflammation through T helper (Th) 2 cytokines release reduction only during induction phase of the experiment, which indicates its possible prophylactic use in asthma.
Ikeda et al., 2002; Japan	Xiao-Qing-Long-Tang (Sho-seiryu-to) (XQLT)	BALB/c Mice	0.5 and 1 g/kg (*p.o.*)	To investigate the effect of aqueous extract of XQLT on an OVA-induced allergic mice model.	IL-4	ELISA		XQLT reduced sneezing, IgE production, IL-4 secretion in spleen cells, possibly acting in Th1/Th2 differentiation and indicating possible curative and prophylactic uses in asthma.
Fang et al., 2005; Japan	Gyokuheifusan (GHS)	BALB/c Mice	25 mL/kg (*p.o.*)	To study the immunological effect of GHS on the Th1/Th2 balance in an OVA-induced asthma model mice.	IL-4	ELISA		GHS reduced the production of IgE and IL-4 through increment of interferon gamma (IFN-γ). This reestablishes Th1/Th2 balance, inordinate in asthma.
Do et al., 2006; Republic of Korea	Semen Armeniacae Amarum mature seeds (SAA) (Rosaceae)	BALB/c Mice	1 or 10 mg/mL (*p.o.*)	To assess therapeutic potential of water extract of SAA on prevention or control of asthma in an OVA-induced mouse model through suppression of Th2 response.	IL −4 IL-5, and IL-13	ELISA		SAA attenuates airway hyperresponsiveness (AHR) and airway inflammation, which possibly result from inhibition of IL-4 and IL-5 production.
Lee et al., 2006; Republic of Korea	*Mosla dianthera* (Buch.-Ham. ex Roxb.) Maxim. (Labiatae)	ICR Mice and Sprague-Dawley Rats	1, 10, 100 and 1000 mg/kg (*i.p.*)	To assess anti-allergic effect of aqueous extract of *M. dianthera* on a phorbol 12-myristate 13-acetate sensitized mast cell-mediated allergy model.	IL-6 and IL-8	ELISA		*M. dianthera* inhibited systemic allergic reactions in mice and reduced IL-6 and IL-8 production in human mast cells, showing potential to treat allergic diseases.
Jin et al., 2006; Republic of Korea	*Ailanthus altissima* (Mill.) Swingle (Simaroubaceae)	BALB/c Mice	100, 200 and 400 mg/kg (*p.o.*)	To determine the role of ethanol extract of *A. altissima* in the arachidonic cascade enzymes metabolism and its potential use in asthma.	IL-4, IL-5 and IL-13		RT-PCR	*A. altissima* inhibited formation of arachidonic-derived enzymes and reduced eosinophilic infiltration in the airway through down regulation of Th2 response and inhibition of inflammatory mediators.
Bae et al., 2007; Republic of Korea	*Artemisia princeps* Pamp. (Asteraceae) and fermented *A. princeps* (FAP)	BALB/c Mice	50 mg/kg (*p.o.*)	To examine anti- asthamatic activity of *A. princeps* ethanolic extract and its fermentation with *Bifidobacterium infantis* K-525 in OVA-immunized mice.	IL-4 and IL-6	ELISA	RT-PCR	*A. princeps* and FAP reduced IgE and IL levels. Antiasthamatic effects of FAP may be been due to the inhibition of cytokine biosynthesis and degranulation.
Rogerio et al., 2008; Brazil	*Lafoensia pacari* A. St. Hil (Lythraceae)	BALB/c Mice	*L. pacari* - 200 mg/kg (*p.o.*) Ellagic acid - 10 mg/kg (*p.o.*)	To determine whether *L. pacari* ethanolic extract or ellagic acid could improve airway allergic inflammation in an OVA-induced murine model.	IL-4, IL-5 and IL-13	ELISA		*L. pacari* and ellagic acid reduced eosinophilic recruitment and production of Th2 cytokines. In addition, ellagic acid showed a tendency to reduce AHR.

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Table. 1 (*continued*)

Authors, Year, Country	Plant species/ Formulation	Animal/ Strains	Dose/ Route	Objectives	Interleukins	Assays Biochemical	Molecular	Improved Characteristics
Heo et al., 2008; Republic of Korea	*Camellia sinensis* (L.) Kuntze (Theaceae)	BALB/c Mice	25 μg/mL (*p.o.*)	To evaluate the anti-asthmatic activity of the methanol extract of *C. sinensis* in an OVA-induced asthmatic model.	IL −4,IL −10, IL- 13, IL- 17 and IL- 31	ELISA	RT- PCR	*C. sinensis* exhibits anti-asthmatic activity by increasing expression of TNF-α and IFN-γ and by reducing IL-4 and IL-13 in lung tissue of mice.
Huang et al., 2008; Taiwan	*Gynostemma pentaphyllum* (Thunb.) Makino (Cucurbitaceae)	BALB/c Mice	5 g/kg (*p.o.*)	To assess whether the aqueous extract of *G. pentaphyllum* is able to influence airway inflammatory responses in OVA sensitive mice.	IL-4, IL-5 and IL-13	ELISA		*G. pentaphyllum* might be beneficial for the airway inflammation of asthma through suppression of Th2 activity and increment of IFN-γ response.
Ok et al., 2009; Republic of Korea	*Pinellia ternate* (Thunb.) Makino (Araceae) and *Citrus reticulate* Blanco (Rutaceae)	BALB/c Mice	200 and 400 mg/ kg (*p.o.*)	To analyze the effect of aqueous extracts of *P. ternate* and *C. reticulate* on physiological features of asthma in OVA sensitized murine model.	IL-4, IL-5 and IL-13	ELISA	RT-PCR	*P. ternate, C. reticulate* and their combined prescription ameliorated AHR and eosinophilic infiltration through suppression of Th2 cytokines.
Brattström et al., 2010; Switzerland	*Petasites hybridus* (Linn.) G. Gaertn., B. Mey. and Scherb. (Asteraceae)	BALBc/Mice	10, 30 and 100 μg dissolved in 40 μL of saline (*i.n.*)	To test the ability of *P. hybridus* extract (using liquid carbon dioxide) of inhibiting Th2 response on and OVA-induced murine model.	IL-4, IL-5, IL-10, IL-12 and IL-13	ELISA		*P. hybridus* reduced allergic airway inflammation and AHR by inhibiting the production of the Th2 cytokines IL-4 and IL-5.
Lee et al., 2010; Republic of Korea	*Viola mandshurica W. Becker* (Violaceae)	BALB/c Mice	20, 30 and 50 mg/ kg (*p.o.*)	To investigate the efficacy of *V. mandshurica* ethanolic extract in the treatment of asthma in an OVA-induced mouse model.	IL-5 and IL-13	ELISA		*V. mandshurica* inhibited AHR, eosinophilia, and mucus hyper secretion by suppression of IgE, IL-5 and IL-13 levels.
Brugiolo et al., 2011; Brazil	*Echinodorus grandiflorus* (Cham. & Schltr.) Mitcheli. (Alismataceae)	BALB/c Mice	23 mg/kg (*p.o.*)	To verify whether the aqueous extract of *E. grandiflorus* was able to decrease OVA-induced inflammation in a murine model of pulmonary allergy.	IL-4 and IL-13		RT-PCR	*E. grandiflorus* reduced inflammatory cell migration to the airways, IgE levels in the serum and IL-4 and IL-13 expression, due to inhibition of Th2 lymphocytes.
Chang et al., 2011; Taiwan	Xiao-Qing-Long-Tang (Sho-seiryu-to) (XQLT)	Dunkin-Hartley guinea Pigs	0.06 g/mL (nebulization)	To evaluate the effects of the water extract of XQLT on pulmonary resistance and airway inflammation in OVA-sensitized guinea pigs.	IL-5	ELISA		XQLT reduced respiratory resistance (possibly from a β_2-agonist effect on bronchial smooth muscles), IL-5 levels and eosinophilic infiltration in Bronchoalveolar Lavage Fluid (BALF).
Ghafourian Boroujerdnia et al., 2011; Iran	*Astragalus gypsicola* Maassoumi & Podlech (Fabaceae)	NMARI Mice	250 and 500 mg/ kg (*i.p.*)	To investigate the effects of the *A. gypsicola* hydroalcoholic extract in OVA-induced allergic mice.	IL-4	ELISA		*A. gypsicola* decreased IL-4 levels and appears to have the potential of reestablishing the balance of Th1/Th2 cytokines.
Gu et al., 2011; China	San'ao Decotion (SAD)	BALB/c Mice	2.2, 4.4 and 8.8 g/ kg (*i.n.*)	To evaluate the effect of the water extract of SAD on airway inflammation and AHR on an OVA-challenged and lipopolysaccharide (LPS) enhanced asthma model.	IL-4 and IL-5	ELISA		SAD presented therapeutic effect on AHR by reducing asthma outbreaks. It also promoted Th1/ Th2 balance through elevation of IFN-γ and reduction of IL-4 and IL-5.
Lee at al., 2011a; Republic of Korea	*Gleditsia sinensis* Lam. (Fabaceae)	BALB/c Mice	25, 50 and 100 mg/kg (*p.o.*)	To evaluate anti-asthmatic effects of *G. sinensis* ethanolic extract and its mechanisms in a murine model of OVA-induced asthma.	IL-4 and IL-5	ELISA		*G. sinensis* reduced airway inflammation, IgE, IL-4 and IL-5 levels in BALF and plasma, eosinophilia and mucus secretion, which indicates protective effect of the herb on allergic asthma.

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Table. 1 (*continued*)

Authors, Year, Country	Plant species/ Formulation	Animal/ Strains	Dose/ Route	Objectives	Interleukins	Assays Biochemical	Molecular	Improved Characteristics
Lee et al., 2011b; Republic of Korea	*Angelica dahurica* (Hoffm.) Benth. & Hook.f. ex Franch. & Sav. (Apiaceae)	BALB/c Mice	25, 50 and 100 mg/kg (*p.o.*)	To investigate anti-asthmatic effects of *A. dahurica* ethanolic extract in a murine model of OVA-induced asthma, and determine involvement of heme-oxygenase-1 (HO-1) in its mechanism of action.	IL-4 and IL-5	ELISA		*A. dahurica* reduced airway eosinophilia, IL-4, IL-5, tumor necrosis factor (TNF)-α and IgE levels, mucus production and suppressed oxidative stress. These effects are partially mediated by HO-1.
Lee et al., 2011c; Republic of Korea	*Kochia scoparia* (Linn.) Schrad (Chenopodiaceae)	BALB/c Mice	100 and 200 mg/ kg (*p.o.*)	To investigate the role of *K. scoparia* ethanolic extract in airway inflammation in an OVA-induced mouse asthma model.	IL-4 and IL-5	ELISA		*K. scoparia* attenuated levels of Th2 cytokines and IgE in BALF and plasma. It also suppressed leukocyte infiltration and mucus secretion in the lungs.
Lee et al., 2011d; Republic of Korea	*Mentha haplocalyx* Briq. (Lamiaceae)	BALB/c Mice	100 mg/kg (*p.o.*)	To investigate whether *M. haplocalyx* ethanolic extract was effective against allergic asthma in an OVA-induced mouse model.	IL-4 and IL-5	ELISA	RT- PCR	*M. haplocalyx* reduced mucus secretion and inflammatory cells infiltration, besides Th2 cytokines mRNA expression and their levels in BALF.
Rivera et al., 2011; Cuba	*Mangifera indica* L. (Anacardiaceae)	BALB/c Mice	*M. indica* −50, 100 or 250 mg/kg (*p.o.*) Mangiferin -50 mg/kg (*p.o.*)	To study the effects of *M. indica* water extract and its component mangiferin on inflammatory response and Th2 cytokine production on a murine model of OVA-induced asthma.	IL-4 and IL-5	ELISA		*M. indica* and mangiferin reduced production of both IL-4 and IL-5, levels of IgE, airway inflammation and lymphocyte proliferation, which indicates possible future uses in asthma.
Suh et al., 2011,Republic of Korea	*Lindera obtusiloba* Blume (Lauraceae)	ICR Mice and Sprague–Dawley Rats	1, 10 and 100 mg/ kg (*i.p.*)	To evaluate antiallergic effects of *L. obtusiloba* water extract and to understand the mechanism of action.	IL-6		Real-Time Polymerase Chain Reaction (Real-Time PCR)	*L. obtusiloba* inhibited inflammation in human mast cells, by reducing intracellular calcium and proinflammatory cytokines expression.
Yang et al., 2011; Republic of Korea	*Mimosa pudica* L. (Mimosaceae)	BALB/c Mice	50, 125 and 250 mg/kg (*p.o.*)	To investigate the effects of *M. pudica* ethanolic extract on asthmatic responses using OVA-challenged mice.	IL-5, IL-6 and IL-8	ELISA		*M. pudica* suppressed eosinophilia, mucus hypersecretion and IgE levels in asthmatic lungs of mice, probably through reduction of IL-5 and IgE expression.
Wang et al., 2012; Taiwan	Xiao-Qing-Long-Tang (Sho-seiryu-to) (XQLT)	BALB/cMice	1000 mg/kg (*p.o.*)	To investigate whether water extract of XQLT attenuates asthma symptoms in a *Dermatogpides pteronyssinus* challenged asthmatic mice model.	IL-5, IL-6, IL-10 IL-12 and IL-13	ELISA	RT-PCR	XQLT attenuates allergic airway inflammation, remodeling and antigen-induced AHR through modulating Th1/Th2 responses and inhibiting NF-κB activation.
Costa et al., 2012; Brazil	*Ocimum gratissimum* Linn. (Lamiaceae)	AJ Mice	25, 50 and 100 mg/kg (*p.o.*)	To evaluate immuno-modulatory effects of *O. gratissimum.* methanolic extract and rosmarinic acid in a murine model of respiratory allergy induced by *Blomia tropicalis* mite.	IL-4	ELISA		*O. gratissimum* and rosmarinic acid reduced levels of Th2 cytokine, numbers of leukocytes/eosinophil and eosinophil peroxidase activity, presence of mucus in respiratory tract and histopathological changes.
Lee et al., 2012; Republic of Korea	*Diospyros blancoi* A. DC. (Ebenaceae)	BALB/c Mice	20 and 40 mg/kg (*p.o.*)	To investigate the anti-inflammatory and anti-asthmatic effects of *D. blancoi* in an OVA-induced mouse airway inflammation model.	IL-4 and IL-5	ELISA		*D. blancoi* inhibited allergen induced inflammation by reducing the Th2 immune response, suppressing Matrix Metalloproteinase-9 (MMP-9) activity and inducing HO expression.

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Table. 1 (*continued*)

Authors, Year, Country	Plant species/ Formulation	Animal/ Strains	Dose/ Route	Objectives	Interleukins	Assays Biochemical	Molecular	Improved Characteristics
Chang et al., 2013;Taiwan	Xiao-Qing-Long-Tang (Sho-seiryu-to) (XQLT)	Dunkin-Hartley Guinea Pigs	60 g/ml (nebulization)	To investigate the effect of the water extract of XQLT in an OVA-induced asthma model.	IL- 4	ELISA		XQLT inhibited histamine and IL-4 by stabilizing mast cells; has bronchodilation effects and exerts an anti-inflammatory effect.
Chen-Xue et al., 2013;China	Qi`ao Decoction (QAD)	BALB/cMice	6.7, 13.4 and 26.8 g/kg (*i.n.*)	To evaluate the effect of QAD (water extracted) on inflammation and AHR on OVA-induced and LPS-enhanced asthma mice model.	IL-4, IL-12	ELISA		QAD can inhibit airway inflammation and AHR and lipopolysaccharide, adjust the balance of cytokines, and improve lung histopathological condition.
Hong et al., 2013; Republic of Korea	*Broussonetia papyrifera* (Linn.) L'Hér'Hér. ex Vent. (Moraceae) and *Lonicera japonica* Thunb. (Caprifoliacea)	BALB/c Mice	100, 200 and 400 mg/kg (*p.o.*)	To investigate the therapeutic efficacy of *B. papyrifera* and *L. japonica* ethanolic extracts in a murine model of OVA-induced asthma.	IL-4	ELISA		The extracts could be used in asthma because of their potent antioxidant, anti-inflammatory, and antiasthmatic effects.
Xu et al., 2013; China	*Astragali radix* Antiasthmatic Decotion (AAD)	BALB/cMice	7.5 and 15 g/kg (*i.g.*)	To determine whether water extract of AAD could suppress allergen induced AHR and remodeling in OVA-induced mice.	IL-10 and IL-13	ELISA		AAD decreased AHR, eosinophilic airway inflammation and expression of IL-13 and TGF-β1.
Shin et al., 2014; Republic of Korea	*Picrasma quassioides* (D.Don) Benn. (Simaroubaceae)	BALB/c Mice	15 and 30 mg/ kg (*p.o.*)	To evaluate the inhibitory effects of *P. quassioides* on the inflammatory responses in mice with allergic asthma induced by OVA and in LPS-stimulated RAW264.7 cells	IL-4, IL-5 and IL-13	ELISA		*P. quassioides* decreased inflammatory cell count and reduced IL-4, IL-5, IL-13, IgE and AHR. It also attenuated mucus production in the airways.
Srivastava et al., 2014; USA	Antiasthma Simplified Herbal Medicine Intervention (ASHMI) and refined (ASHMI^{R})	BALB/c Mice	9 mg/ml (*i.g.*)	To determine the effects of standard ASHMI and ASHMI^{R} in a murine model of ragweed asthma and explore it mechanisms.	IL-5, IL-8, IL-10, IL-13 and IL-17	ELISA		ASHMI and ASHMI^{R} reduced hyper reactivity, mucus production, neutrophil and eosinophilic inflammation, and Th2 responses.
Zhou et al., 2014; China	Qu Feng Xuan Bi Formula (QFXBF)	Sprague Dawley Rats	20.7 and 10.35 g/ kg (*i.g.*)	To investigate the effect of the aqueous extract of QFXBF in airway inflammation in rat OVA-induced asthma models	IL-4 and IL-13	ELISA		QFXBF is potent for alleviating lung damage of asthma and efficient for diseased nasal mucous recovery. It also reduces eosinophil's count.
Bukhari et al., 2015; India	*Crocus sativus* Linn. (Iridaceae)	BALB/cMice	1 and10 mg/kg (*p.o.*)	To evaluate the antioxidant potential of *C. sativus* hydro-alcoholic extract and its constituents, safranal and crocin, in normal human bronchial epithelial cells and its antiasthmatic potential in an OVA-induced murine model of asthma.	IL-5 and IL-13	ELISA		*C. sativus* and safranal reduced oxidative stress, prevented epithelial cell damage during allergic airway inflammation and decrease in airway cellular infiltration and AHR.
Jeon et al., 2015; Republic of Korea	Soshiho-tang	BALB/cMice	100 and 200 mg/ kg (*p.o.*)	To investigate the anti-allergic effect related to inflammation and oxidative stress of Soshiho-tang water extract on bronchial asthma using an OVA-induced mouse model.	IL-4, IL-5, IL-13, IL-17 and IL-33	ELISA		Soshiho-tang had a suppressive effect on eosinophil influx, decreased Th2-type cytokines, mucus hypersecretion, IgE levels and significantly induced HO-1 protein expression.
Liu et al., 2015; China	Pingchuan Formula (PCF)	BALB/cMice	20 mL/kg (p.o.)	To investigate the effect of PCF (water and 95% ethanol) on lungs damage of asthmatic mice in OVA-induced mice.	IL-6, IL-17 and IL-23	ELISA		PCF restored Th17/Treg balance, improving airway inflammation and reducing asthma symptoms.

(*continued on next page*)

Table. 1 (*continued*)

Authors, Year, Country	Plant species/ Formulation	Animal/ Strains	Dose/ Route	Objectives	Interleukins	Assays Biochemical	Molecular	Improved Characteristics
Oliveira et al., 2015; Brazil	*Allium cepa* Linn. (Amaryllidaceae)	A/J Mice	100 and 1000 mg/ kg (*p.o.*)	Investigate the effect of *A. cepa* methanolic extract and quercetin on cytokine release and muscle contraction in a murine model of asthma.	IL-4, IL-5 and IL-13	ELISA		*A. cepa* or quercetin reduced the production of inflammatory cytokines, a relaxation of tracheal rings, and a reduction in total number of inflammatory cells.
Shin et al., 2015a; Republic of Korea	*Thuja orientalis* Linn. (Cupressaceae)	BALB/c Mice	30 mg/ kg (*p.o.*)	To examine the effects of *T. orientalis* on airway inflammation, in LPS-stimulated RAW264.7 cells and in an OVA-induced allergic asthma murine model.	IL-4, IL-5, IL-6, and IL-13	ELISA	RT-PCR	*T. orientalis* decreased the inflammatory cell, IL-4, IL-5, IL-13, eotaxin, IgE and AHR and attenuated airway inflammation and mucus hypersecretion.
Shin et al., 2015b; Republic of Korea	*Zingiber mioga* (Thunb.) Roscoe (Zingiberaceae)	BALB/cMice	30 mg/ kg (*p.o.*)	To investigate the inhibitory potential of *Z. mioga* on airway inflammation in asthma, using a murine model of OVA-induced asthma	IL-4, IL-5 and IL-13	ELISA		*Z. mioga* decreased the inflammatory cell, AHR and attenuated the infiltration of inflammatory cells and mucus production in the airways.
Yang et al., 2015; Taiwan	Bu-Zhong-Yi-Qi-Tang (Hochu-ekki-to) (BZYQT)	BALB/c and ByJNarl Mices	500 and 1000 mg/ kg (*i.g.*)	To determine whether water extract of BZYQT alleviates AHR and inflammation of the airways in an OVA-sensitized asthmatic murine model	IL-4, IL-5 and IL-13	ELISA		BZYQT ameliorated AHR and eosinophilia and the cytokines presented significantly lower levels.
Arora et al., 2016; India	Kanakasava	Wistar Rats	1.23 and 2.46 mL/ kg (*p.o.*)	To assess the safety and therapeutic effi-cacy of Kanakasava (8% ethanolic water) against OVA-induced asthma and airway inflammation in rats.	IL-4, IL-5 and IL-1β	ELISA		Kanakasava decresed IgE, cytokines, nitrites and influx of eosinophils and neutrophils; improved lung functions and suppression of degranulation of mast cells.
Rana et al., 2016; Pakistan	*Pistacia integerrima* J.L. Stewart ex Brandis (Anacardiaceae)	BALB/cMice	200 mg/ kg (*i.n.*)	To investigate the immunomodulatory and anti-inflammatory activities of a methanolic extract *P. integerrima* in mouse model of OVA-induced allergic asthma.	IL-4 and IL-5		RT-PCR	*P. integerrima* possesses significant anti-asthmatic activity which may be attributed to reduction in TNF-α, IL-4 and IL-5 expression levels
Song et al., 2016; Republic of Korea	*Rosa multiflora* Thunb. (Rosaceae)	BALB/cMice	200 mg/ kg (*p.o.*)	To evaluate the therapeutic efficacy of *R. multiflora* water extract in allergic asthma via the suppression of Th2 cytokine production in an OVA-induced murine asthma model; and histamine release from mast cells.	IL-4 and IL-6	ELISA	–	*R. multiflora* suppressed the infiltration of inflammatory cells, the hyperplasia of goblet cells and the deposit of collagen fiber and reduced Th2-type cytokines.
Bui et al., 2017; Republic of Korea	*Citrus tachibana* (Makino) Yu.Tanaka (Rutaceae)	SPF, BALB/c and ICR Mice	200 mg/kg (*p.o.*)	To evaluate the therapeutic efficacy of *C. tachibana* ethanolic extract in allergic asthma via the suppression of Th2 cytokine production from Th2 cells on an OVA-induced murine asthma model.	IL-4, IL-5, IL-10 and IL-12	ELISA		*C. tachibana* inhibited OVA-induced asthmatic response by reducing airway inflammation, restoring Th1/Th2 balance
De Almeida et al., 2017; Brazil	*Mandevilla longiflora* (Desf.) Pichon (Apocynaceae)	Swiss-Webster Mice	20, 50 and 200 mg/kg (*p.o.*)	To evaluate the anti-inflammatory potential of *M. longiflora* hydro ethanolic extract 70% in a model of hypersensitivity induced by OVA.	IL-4, IL-5, and IL-13	ELISA		*M. longiflora* attenuated leukocyte migration into the airways, which was evidenced by a decrease in eosinophils, neutrophils and mononuclear cells.

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Table 1 (*continued*)

Authors, Year, Country	Plant species/ Formulation	Animal/ Strains	Dose/ Route	Objectives	Interleukins	Assays Biochemical	Molecular	Improved Characteristics
Huang et al., 2017; China	*Scoptidium ternatum* (Thunb.) Lyon (Ophioglossaceae)	BALB/c Mice	10, 12, 30 and 40 mg/kg (*p.o.*)	To screen different solvent extracts of *S. ternatum for* anti-tussive and anti-asthmatic effects and study their associated mechanisms.	IL - 4	ELISA		*S. ternatum* alleviated cough, airway inflammation, Th2 cytokine release possibly due to reduced expression of cysteinyl leukotriene receptors.
Tang et al., 2017; China	*Epimedium brevicornu* Maxim (Berberidaceae) and *Ligustrum lucidum* W.T.Aiton (Oleaceae)	Sprague-Dawley Rats	100 mg/ kg (*p.o.*)	Investigate the effects of the co administration of combined extracts of *E. brevicornu* and *L. lucidum* with inhaled budesonide on airway remodeling in the rat asthmatic model induced by OVA.	IL-4 and IL-5	ELISA		Co-administration of extracts and budesonide reduced allergen-induced increased IL-4, IL-5, IgE, eosinophils and the mRNA expression of TGF-β2.
Gutiérrez and Flores, 2018; Mexico	*Petiveria alliacea* Linn. (Phytolaccaceae)	BALB/c Mice	100, 200 and 400 mg/ kg (*p.o.*)	To examine the effect of methanol extract of *P. alliacea* on airway inflammation in a murine model of chronic asthma induced by OVA.	IL-4, IL-5 and IL-13	ELISA		*P. alliacea* inhibited airway inflammation, regulate cytokines, chemokines and enhance pulmonary conditions.
Ye et al., 2019; China	*Castanea Mollissima* Blume (Fagaceae)	SPF Guinea Pigs	250, 500 and 1000 mg/kg (*p. o.*)	To verify the effect of ethanol extract of *C. Mollissima* on airway inflammation in OVA-induced asthmatic guinea pigs.	IL-5	ELISA	RT-PCR	*C. mollissima* alleviated airway inflammation and smooth muscle thickening by reducing the levels and expression of IL-5.

There was a large variation in the amount of extracts administered and these were administered orally in 38 studies. Doses ranged from 1 to 5 g/kg body weight. Intranasal instillation was executed in 4 studies, with doses ranging from 10 μg to 26.8 g/kg. Four studies reported intragastric administration, with doses ranging from 0.5 to 15 g/kg. Intraperitoneal injection was reported in 3 studies, with doses ranging from 0.01 to 1 g/kg and two 2 studies reported nebulization, with doses of 0.06 to 60 g/mL, respectively, as shown in Table 1.

Study characteristics and description

The main parameter used to analyze plant-extract effect on the management of asthma was the reduction of IgE (n = 37) and eosinophil count (n = 39). Estimation of leukocytes count was described in 35 studies. Twenty-four studies measured the balance of Th1/Th2 cytokines, 49 measured regulations of proinflammatory cytokines and 20 the biochemical parameters examining intensity of inflammation associated with airway hyperreactivity. Lung histology was described in 36 studies and 23 measured the effectiveness of the extracts on airway goblet-cell hyperplasia and mucus production. Immunohistochemistry was described in 9 reports.

The therapeutic approaches of plant extracts on asthma allergic immunopathogenesis were explored through parameters of the adaptive and innate immune responses. Plant-extract action containing secondary metabolites predominantly ellagic acid, petasins, caffeic acid, (-)-epicatechin, eriodictyol, ethyl gallate, quercetin, nodakenin, oxypeucedanin hydrate, byakangelicin, oxypeucedanin, imperatorin, isoimperatorin, mangiferin, mimosine, rosmarinic acid, safranal, crocin, procyanidin B-3, entguibourtinidol -(4β 6)-catechin, hesperidin, naringin, rutin, (±)-naringenin, and luteolin regulated the levels of the following markers: neutrophils, eosinophils, and mononuclear cells count, leukocyte infiltration, eosinophil peroxidase activity, production of IL-4, IL-5, IL-13 and IFN-γ, IgE levels, epidermal growth factor (EGF) and transforming growth factor beta (TGF-β), heme oxygenase (HO) enzyme activity, pathologic changes of asthmatic lung tissues and macrophages activity. The levels of the following markers were modulated by plant-extract treatment rich in phytochemicals like alkaloids, terpenes, phenolics, tannins, gypenosides, cucurbitacins, flavonoids, iridoid glycosides, polyphenols and saponins: number of eosinophils and lymphocytes, levels of IgE, IL-5, and IFN-γ, hematoxylin and eosin (H&E) staining of lung tissue sections, lung histomorphometry, biochemical and molecular markers of airway remodeling, activity of the TGF-β signaling, secretion of Th2-related cytokines, expression of matrix metallopeptidase 9 (MMP-9), intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule (VCAM-1) (Table 2). *In vivo* studies of antibodies that block IL-4, IL-5 and IL-13 reported reduced acute exacerbations in nominated asthmatic patients (Barnes, 2018). Tables 1 and 2 summarize the products or extract that act on these cytokines.

The measurement on IL and IgE production, regulation of Th1/Th2 cytokine ratio, determination of airway hyperresponsiveness (AHR) indicated by lung resistance and stimulated by acetylcholine and the histopathological examination of lung and cytokines assays showed significant change after plant-formulations treatment, which contained alkaloids, flavonoids, organic acids, anthraquinones, polyphenols, and saponins (Table 2). Significant suppression in IL-4 productions in splenocytes induced by OVA treatment related to plant-formulation usage were reported in 2 studies, whose predominant compounds were calycosin, atratylenolide III, 4′-O-β-D-glucosyl-5-O-methylvisamminol and alkaloids. One study reported fewer bronchial inflammatory cells, inconspicuous bronchial epithelial degeneration and little significant exudation of bronchial cavity in lung histology of plant formulation extract containing ephedrine and pseudoephedrine as active components (Table 2). The impact of anti-asthma simplified herbal formulation extracts in neutrophil-predominant murine model of ragweed asthma was analysed (n = 1), with a significant reduction of airway

Table. 2
Phytochemicals, mechanistic role and therapeutic effects for asthmatic disease of the studies using plant extracts.

Authors, Year, Country	Plant species/Formulation	Phytochemicals	Mechanistic role	Therapeutic effects
Ikeda et al., 2002; Japan	Xiao-Qing-Long-Tang (Sho-seiryu-to)	Alkaloids from *Ephedra sinica* Stapf. a herb present in Sho-seiryu-to formulation	Alkaloids could possibly participate in the modulation of Th1/Th2 response through adrenergic effect on CD4+ T cells	Immunomodulatory and antiallergic
Fang et al., 2005; Japan	Gyokuheifusan (GHS)	Calycosin, atratylenolide III, 4′-O-β-D-Glucosyl-5-O-methylvisamminol	Components from GHS could be responsible for the decrease of IgE and IL-4 levels, due to persistent increase of IFN-α.	Immunomodulatory and antiasthmatic
Rogerio et al., 2008, Brazil	*Lafoensia pacari*	Ellagic acid	Ellagic acid suppressed eosinophilic inflammation via the modulation of Th2 cytokines (IL-4, 5 and 13)	Antiinflammatory, antiallergic and antiasthmatic
Heo et al., 2008; Republic of Korea	*Camellia sinensis*	Polyphenolic compounds	Polyphenols rich *C. sinensis* increased the expression level of TNF-β and IFN-γ and by decreasing the expression level of IL-4 and IL-13 in the lungs.	Antiasthmatic
Huang et al., 2008; Taiwan	*Gynostemma pentaphyllum*	Gypenosides	*G. pentaphyllum* containing gypenosides suppressed airway inflammation, eosinophilia and Th2 cytokines	Antiinflammatory and antiasthmatic
Brattström et al., 2010; Switzerland	*Petasites hybridus*	Petasins	Petasins from *P. hybridus* reduced airway inflammation and hyperreactivity by reducing the Th2 cytokines mediated immune response	Antiasthmatic
Brugiolo et al., 2011, Brazil	*Echinodorus grandiflorus*	Flavonoids, phenolic compounds, saponins, triterpenes, and tannins	The mechanism of antiasthmatic action of extract containing phytochemicals may be related to inhibition of migration and function of eosinophils with reduced production of IL-4 and IL-13.	Antiallergic and antiasthmatic
Ghafourian Boroujerdnia et al., 2011, Iran	*Astragalus gypsicolus*	Alkaloids, terpenoids, flavonoids, saponins, cucurbitacins and phenolic compounds	Phytochemicals from *A. gypsicolus* are believed to be responsible for the immunomodulatory effects (decrease in IL-4 and increase in IFN-γ levels)	Immunomodulatory and antiasthmatic
Gu et al., 2011; China	San'ao Decotion	Ephedrine and pseudoephedrine from *E. sinica* and amygdalin from Semen Armeniacae Amarum	Components exerted bronchial smooth muscle relaxation and respiratory center inhibition, which could reduce airway hyperresponsiveness	Antiasthmatic
Lee at al., 2011a; Republic of Korea	*Gleditsia sinensis*	Caffeic acid, (-)-epicatechin, eriodictyol, ethyl gallate, and quercetin	*G. sinensis* containing phytoconstituents played a key role in the inhibition of recruitment of eosinophils in the lung via regulation of IL-4 and 5 cytokines	Antiasthmatic
Lee et al., 2011b; Republic of Korea	*Angelica dahurica*	Nodakenin, oxypeucedanin hydrate, byakangelicin, oxypeucedanin, imperatorin and isoimperatorin	*A. dahurica* comprising coumarins has protective effect on airway inflammation and significantly attenuated the increase in total cells, eosinophils and other inflammatory cells in the BALF of asthmatic mice	Antiinflammatory and antiasthmatic
Lee et al., 2011c; Republic of Korea	*Kochia scoparia*	Saponins	Saponins from *K. scoparia* significantly reduced the Th2-type cytokines and supressed the infiltration of inflammatory cell and mucus secretion in lung tissue	Antiinflammatory and antiasthmatic
Rivera et al., 2011, Cuba	*Mangifera indica*	Mangiferin	Mangiferin suppressed IgE levels and modulated eosinophilic function by reducing the levels of IL-4 and 5	Antiinflammatory and antiasthmatic
Yang et al.,2011; Republic of Korea	*Mimosa pudica*	Mimosine, tannins, sitosterol and flavonoid glycosides	Natural compounds from *M. pudica* might be responsible for the decrease in the mucus hypersecretion and associated with the regulation of IL-5 and IgE expression	Antiinflammatory and antiasthmatic
Costa et al., 2012; Brazil	*Ocimum gratissimum*	Rosmarinic acid	Rosmarinic acid reduced eosinophilia, mucus production and levels of IL-4 in the BALF and exert antiasthmatic effect	Immunomodulatory and antiasthmatic
Chen-Xue et al., 2013; China	Qi`ao Decoction (QAD)	Alkaloids and organic acids	Alkaloids and organic acids from decoction significantly inhibited hyperresponsiveness and influenced on airway inflammation via adjusting the balance of Th1/Th2 cytokine	Antiinflammatory and antiasthmatic
Srivastava et al.,2014; USA	Antiasthma Simplified Herbal Medicine Intervention (ASHMI)	Ganoderic acid C1 (GAC1)	ASHMI and its active constituent GAC1 might markedly reduce airway inflammation, mucous production, neutrophilic inflammation and IL-8 and 17 levels	Antiinflammatory and antiasthmatic
Bukhari et al., 2015; India	*Crocus sativus*	Safranal and crocin	Safranal and crocin reduced airway hyper-responsiveness and airway cellular infiltration via the modulation of IL-5 and 13 levels	Antiinflammatory and antiasthmatic
Jeon et al., 2015; Republic of Korea	Soshiho-tang	Liquritin, glycyrrhizin and baicalin	Soshiho-tang containing active components has antiallergic effects through modulation of Th2-type cytokines	Antiinflammatory, anti allergic
Liu et al., 2015; China	Pingchuan Formula	Scopoletin, ephedrine, hydro-chloride, pseudoephedrine, hydro-chloride, baicalin, rosmarinic acid, sinapine, laetrile, liquiritin and glycyrrhizic acid and ephedrine hydrochloride	This active ingredient might reduce asthma symptoms, improve Th1/Th2 balance, decreased IL-5, IL-4 and IL-13 and increased INF -γ	Antiinflammatory and antiasthmatic

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Table. 2 (*continued*)

Authors, Year, Country	Plant species/Formulation	Phytochemicals	Mechanistic role	Therapeutic effects
Oliveira et al., 2015	*Allium cepa*	Quercetin	Quercetin as an active constituent from *A. cepa* reduced inflammatory cells, regulated IL-1, IL- 4 and IL- 5 levels and relaxed tracheal rings	Antiasthamatic and antiallergic
Arora et al., 2016; India	Kanakasava	Ethyl gallate and gallic acid	Ethyl gallate and gallic acid reduced the secretion of Th2 cytokines mainly, IL-4 and 5. The phenolic compounds also suppressed IL-1β, TNF-α, COX-2 and production of NO2 and PGE2	Antiinflammatory and antiasthmatic
Song et al., 2016; Republic of Korea	*Rosa multiflora*	Tannins, procyanidin B-3, enguilbourtinidol -(4β 6) -catechin	The hot water extract of *R. multiflora* contain bioactive components reduced eosinophils, IgE and decreased the production of Th2 cytokines IL-4 and 6	Antiinflammatory, antiasthmatic and antiallergic
De Almeida et al., 2017; Brazil	*Mandevilla longiflora*	Ellagic acid, hesperidin, naringin, rutin, (±)-naringenin, and luteolin	The active constituents in *M. longiflora* reduced the concentration of Th2 cytokines, decreased the eosinophilia in the airways and mucus secretion in the lung	Antiasthmatic
Tang et al., 2017; China	*Epimedium brevicornu* and *Ligustrum lucidum*	Flavonoids, iridoid glycosides, alkaloids and oleanolic acids	Airway remodeling effects of the extracts might be a result of synergistic actions among bioactive compounds and reduced the IL-4, 5 and IgE levels	Antiasthmatic and immunomodulatory
Ye et al., 2019; China	*Castanea mollissima*	Flavonoids, terpenoids and phenolic compounds	The ethanol extract of *I. castaneae* containing bioactive metabolites significantly reduced the levels of inflammation-related factors IgE and IL-5 and inhibited asthmatic airway inflammation	Antiasthmatic

reactivity to acetylcholine provocation, mucus production, neutrophilic inflammation, IL-8 and IL-17 levels and decreased eosinophilic inflammation after treatment with refined plant formulation containing ganoderic acid C1 (a triterpenoid compound). They also investigated the lung sections stained with Periodic acid-Schiff (PAS) that stains mucus to measure treatment effectiveness on airway tissue inflammation and mucus hyper production.

Bias analyses

All 51 reports were randomized and followed established experimental designs; participants included both sexes in the murine models; random sequencing and allocation concealment was used by the investigators and there was a low risk of bias because of the absence of incomplete outcome data. None of the studies reported a blinded assessor or informed blinded investigation. The source of funding bias was clear in majority of the studies. Fig. 3 describes the assessments of methodological quality in different classifications. Fig. 4 provides a graphical demonstration of the overall risk of bias (high and low).

Studies of interleukin responses to polyherbal formulations and mixed-plant extracts in asthmatic condition

The *in vivo* asthmatic disease model was chosen instead of *in vitro* to mimic mechanisms and pathophysiology of human conditions, usually preferred to assess both safety and efficacy of asthma therapies before commencing clinical trials (Aun et al., 2017). To identify experimental models interrelated to human studies; we focused on murine models, with asthmatic induction protocols suited for the murine lineage as they were considered most relevant for the study of new therapies (Mullane and Williams, 2014). Fifty-one studies analyzed animal models of asthma, induced by sensitization (intraperitoneal and subcutaneous routes), and challenge (aerosol, intranasal or intratracheal instillation) with allergens. In addition, intranasal instillation of allergens was administered in four studies. OVA studies were replaced by dust mites' allergens in two studies to cause physiological processes similar to human-like airway hyperresponsiveness, inflammation and remodeling.

The balance of Th1/Th2 was mainly assessed by the biochemical and molecular examinations in bronchoalveolar lavage fluid (BALF), in which higher percentages of Th1/Th2-mediated cytokines particularly IL-2, IL-4, IL-6 and IL-13 in the asthmatic late-phase were reported. The publications mostly analyzed IL-4, IL-5 and IL-13 (so called Th2 cytokines) derived from Th2 cells, since these shares and play major roles in the development of airway eosinophilia and IgE (Barnes, 2001; Goh et al., 2012). The studies described the complexity of the pathogenetic mechanisms underlying the development of allergic bronchial asthma and discuss the predominant role of ILs within these processes. A positive involvement of Th1-mediated IL-2 and IL-12, release of pro-inflammatory (IL-1β) and regulation of anti-inflammatory cytokines (IL-10, IL-23, IL-33) in asthmatic mice followed by the administration of natural substances was reported. Fig. 5 describes the action of phytochemicals on Th1/Th2 cytokines orchestrated inflammatory network.

Arora et al. (2016) demonstrated that OVA in antigen sensitized control animals showed significant increases in IgE production, IL-4, IL-5, and IL-1β compared to the non-sensitized group. Treatment with kanakasava, a standardized polyherbal formulation for gallic acid and ethyl gallate contents decreased IgE-mediated hypersensitivity reactions and reversed the over expression of Th2-mediated cytokines. Two studies reported that Sho-seiryu-to and Qi`ao Decoction rich in alkaloids inhibited airway hyperresponsiveness and influenced airway inflammation by adjusting the balance of Th1/Th2 cells producing IL-4 and IL-12 (Ikeda et al., 2002; Chen-Xue et al., 2013). Data corroborates previous literature reporting; plant alkaloids derived from Datura stramonium Linn. (Solanaceae) has antiasthmatic effect through blockade of abnormal immune cell accumulation in the airways of

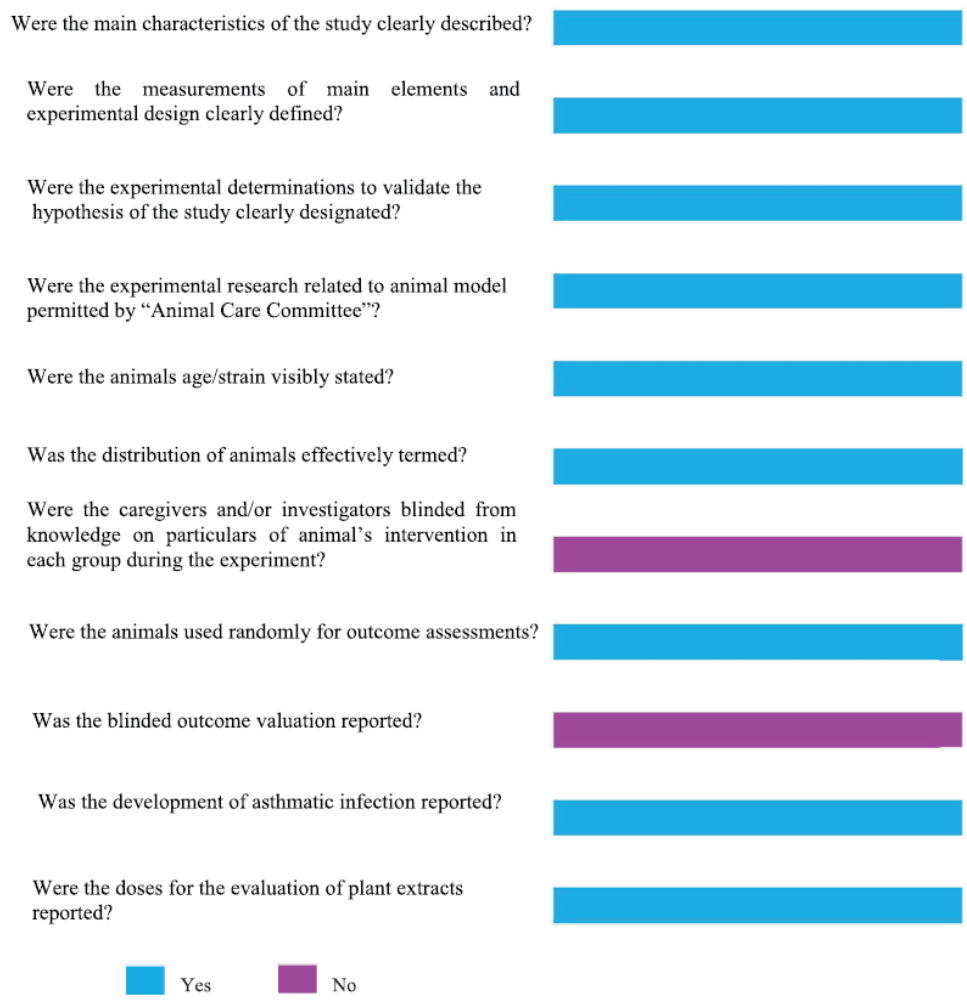

Fig. 3. Evaluation on methodological quality and results of studies. Blue and magenta bars represent the proportion of studies for which the element was or was not appropriate.

prenatal pregnancy women in Southern Africa (Pretorius and Marx, 2006). Atropine, the major active anticholinergic alkaloid derived from D. stramonium, possesses antiparasitic property and blocks the increase in mucus secretion as well as inhibit the action of bronchoconstriction induced by exercise, allergens and parasitic infections (Zdanowicz, 2007).

The presence of major active components *viz.* calycosin, atratylenolide III, 4′-O-β-D-glucosyl-5-O-methylvisamminol, ephedrine, pseudoephedrine, amygdalin, ganoderic acid C, liquritin, glycyrrhizin, baicalin, scopoletin, ephedrine hydrochloride, pseudoephedrine hydrochloride, rosmarinic acid, sinapine, laetrile, liquiritin, glycyrrhizic acid and ephedrine hydrochloride in different combined-herb formulas like Gyokuheifusan, San'ao decotion, Qi`ao Decoction, Antiasthma Simplified Herbal Medicine Intervention, Soshiho-tang and Pingchuan consequently regulate IL-4, IL-6, IL-8, IL-13, IL-17, IL-23, IL-33 productions (Fang et al., 2005; Gu et al., 2011; Srivastava et al., 2014; Jeon et al., 2015; Liu et al., 2015). The anti-Th2 cytokine action of these metabolites in herbs had shown strong anti-asthmatic activity *in vivo* and that it has reduced IgE, eosinophilic lung inflammation and mucus hypersecretion, exercising these activities through inhibition of Th2 cytokines secretion by regulating the shift of Th1 to Th2 accordingly. The outcomes from the studies suggest it can modulate Th1/Th2 function and enhance the immune-function of asthma individuals by improving Th1-deficiency.

It is also observed that these plant secondary metabolites mostly belong to the class of flavonoids, might consider regulating some molecular targets of immune system and exert strong action on inflammatory cell population in the airway. Flavonoids are the most studied class of plant metabolites that can decrease the expression and production of inflammatory molecules including histamine and leukotrienes (Lago et al., 2014) and inhibits mast cell degranulation and promote the Th1/Th2 cytokine production. They also down-regulate

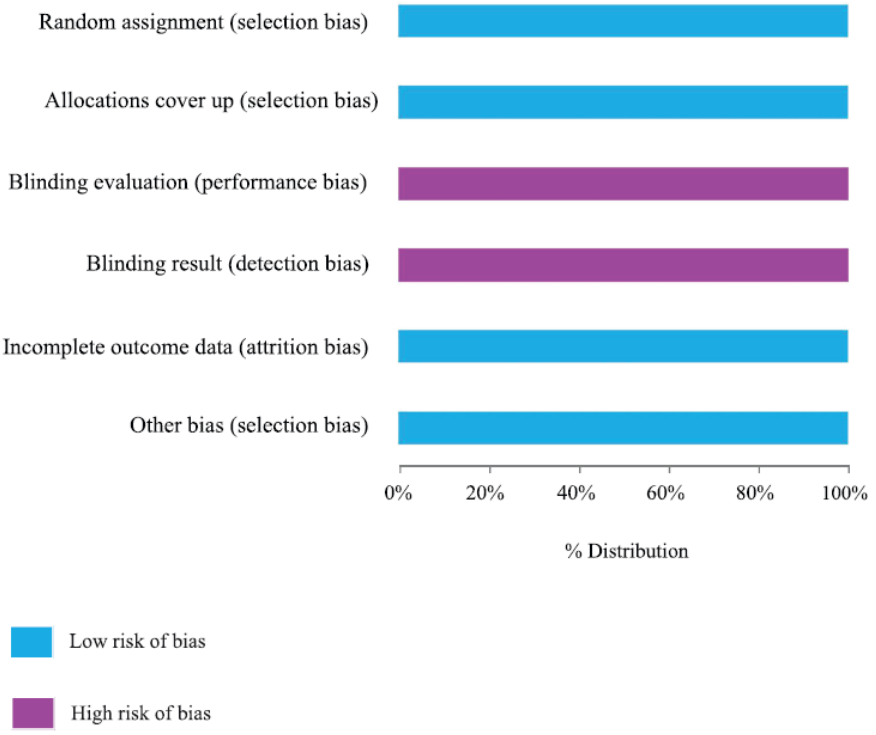

Fig. 4. The Risk of bias graph. Blue: Low; Magenta: High.

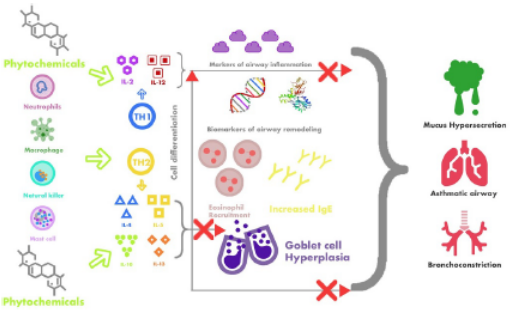

Fig. 5. Immunopharmacologic mechanisms through modulation of cytokine (interleukin) expression for the antiasthmatic effect of phytochemicals derived from medicinal plants.

the production and activity of secondary messengers linked to airway inflammation and control tissue damage (Fortunato et al., 2012). Treatment with combined extracts of *Epimedium brevicornu* and *Ligustrum lucidum* in combination with administration of budesonide, a glucocorticoid significantly reduced allergen-induced increased productions of IL-4, IL-5 and IgE, the amount of eosinophils in BALF, goblet cell hyperplasia, and expressions of signaling markers related to airway remodeling (Tang et al., 2017). These findings suggest that the combination of budesonide and the herbal extracts rich in flavonoids, iridoid glycosides and alkaloids have a better synergistic effect on asthma airway remodeling than the use of budesonide alone.

The levels of ILs were modulated by polyherbal preparations such as Bu-Zhong-Yi-Qi-Tang, Semen Armeniacae Amarum, Xiao-Qing-Long-Tang, *Astragali radix* Antiasthmatic Decotion, Qu Feng Xuan Bi Formula and combined selected plant extracts like *Pinellia ternate* (Thunb.) Makino plus *Citrus reticulate* Blanco and *Broussonetia papyrifera* (Linn.) L'Hér. ex Vent. plus *Lonicera japonica* Thunb treatment: IL-2, IL-4, IL-5, IL-6, IL-10, IL-12 and IL-13. On the other hand, the following immunological markers had their levels regulated after treatment with herbal formulations and extracts: IgE, leucocytes and neutrophils count, pulmonary eosinophilic accumulation, histamine production, activation of NFκB, MCP-1 mRNA expression, MMP-2 and MMP-9 activities, inflammatory infiltration in lung tissue and mediators of T cell function (Suzuki et al., 1999; Ishimitsu et al., 2001; Do et al., 2006; Ok et al., 2009; Chang et al., 2011; Wang et al., 2012; Chang et al., 2013; Hong et al., 2013; Xu et al., 2013; Zhou et al., 2014; Yang et al., 2015). Joint analysis of the data demonstrates that preparations and extracts from plants are effective in decreasing Th2 cell-driven inflammation and maintaining pulmonary homeostasis, contributing to a subsequent immune regulatory function in the airways to limit the inflammatory consequences of asthmatic infection and to maintain tolerance to aeroallergen sensitization.

Studies also indicate that the use of combined plant products/extracts show promising immunomodulatory potential via synergistic interactions between their multiple phytochemicals on inflammatory profiles that are largely contributed by the involvement of Th2 cell. The findings reveal that the benefits of the mixed plant formulations were greater than the single plant extract, which indicated a combination of active constituents synergize or enhance the therapeutic application for

the management of complex diseases like asthma and the efforts to isolate the individual components might be justified for clinical medicine. Our analysis also displays that using these combinations resulted in a reduction in the dose required for effective anti-asthmatic effects, which is interesting because it may decrease both the risk of side effects and the costs of treatment. However, these formulations, if confirmed by human studies, could have synergism with anti-asthmatics to potentiate their effect and help individuals to overcome the syndrome.

Studies of interleukin responses to single-plant extracts in asthmatic condition

Some plant-derived phytochemicals such as alkaloids, flavonoids, phenolic compounds, glycosides, terpenes, polysaccharides, lactones, terpenoids, saponins and glycosides, present in several plants, have been reported to be accountable for the plants immunomodulating properties (Jantan et al., 2015). The extract of *Camellia sinensis* (L.) Kuntze (Theaceae) rich in polyphenols, has immune-modulatory and anti-asthmatic characteristics and might be utilized as good, natural, immune-modulatory and anti-asthmatic agents (Heo et al., 2008). Ellagic acid, a natural phenolic compound from *Lafoensia pacari* A. St. Hil (Lythraceae) has been found to exert anti-inflammatory and anti-asthmatic activities (Rogerio et al., 2008). The triterpenoid saponins gypenosides, from *Gynostemma pentaphyllum* (Thunb.) Makino (Cucurbitaceae) enhances the production of Th1 cytokine (IFN-γ) and suppress the activity of IL-4, IL-5 and IL-13 (Huang et al., 2008). These findings show the curative anti-inflammatory potential of plant extracts on allergic lung inflammation, since the results revealed that the extracts reduced eosinophil infiltration and production of Th2 cytokines, by increasing the expression level of TNF-β and IFN-γ. An active component of *Petasites hybridus* (Linn.) G. Gaertn., B. Mey. and Scherb. (Asteraceae), petasins, a sesquiterpene, inhibits leukotriene activity, reduced allergic airway inflammation and airway hyperresponsiveness by inhibiting the production of the IL-4 and IL-5 (Brattström et al., 2010).

Polyphenols of flavonoids structure are especially effective in the airway inflammatory sites, as there is substantial evidence that these play an important role in the regulation of Th2 cytokines IL-4, IL-5 and suppression of IgE and IgG1 levels in BALF (Magrone and Jirillo, 2012). In fact, the flavonoids and polyphenols compounds are potent modulators of key cytokines in the cascade of asthma onset and other reported inflammatory conditions (Leyva-López et al., 2016). In addition, Singh et al. (2011) postulated that interference of dietary polyphenols from fruits and vegetables with Th2 cells activation, proliferation and function seems to be the main mechanism of their inhibitory effects on asthma development. Polyphenols also maintain the immune homoeostasis at mucosal levels via activation of regulatory T cells (Tregs) function which seem to play a pathogenic role in allergic disease and asthma. These elements may represent a major target of polyphenol activity. Mangiferin, a kind of natural polyphenol of C-glycosylxanthone structure found in *Mangifera indica* L. (Anacardiaceae) inhibit IL-4 and IL-5 in BALF and lymphocyte culture supernatant, IgE levels and lymphocyte proliferation by suppressing vascular and bronchial positive inflammatory reaction in OVA-sensitized/challenged mouse lung (Rivera et al., 2011). Rosmarinic acid, a polyphenol flavonoid and *Ocimum gratissimum* Linn. (Lamiaceae) exerted its immunomodulatory effect in a murine model of respiratory allergy induced by the *Blomia tropicalis* mite by targeting the level of Th2 cytokine (IL-4) in BALF and hindering the inflammatory process of the factors involved in airway and lung inflammation induced by a clinically-relevant aeroallergen (Costa et al., 2012).

Echinodorus grandiflorus (Cham. & Schltr.) Mitcheli. (Alismataceae) extract rich in flavonoids, phenolic compounds, saponins, triterpenes, and tannins modulate allergic pulmonary inflammation through significant reduction in the expression of IL-4 and IL-13 in lung tissue homogenate (Brugiolo et al., 2011). The concentration of IL-4 was decreased after *Astragalus gypsicola* Maassoumi & Podlech (Fabaceae) extract treatment with a high concentration of terpenoids and flavonoids and enhanced INF-γ in OVA-sensitized mice groups (Ghafourian Boroujerdnia et al., 2011). These findings show that extracts restored the balance of Th1/Th2 cytokines. The treatment of allergic asthma with *Gleditsia sinensis* Lam, Fabaceae, a plant rich in flavonoids, resulted in the inhibition of the IL-4 and IL-5 dependent eosinophil recruitment to the lungs and induced the significant reduction in the numbers of airway inflammatory cells in BALF and plasma in the asthmatic animal model (Lee et al., 2011a). Lee et al., 2011b reported the anti-asthmatic action of *Angelica dahurica* (Hoffm.) Benth. & Hook.f. ex Franch. & Sav. (Apiaceae) extract comprising coumarins on airway inflammation lowered eosinophilia and cytokine levels including IL-4, IL-5 and TNFα and suppressed oxidative stress via induction of HO-1 activity.

Kochia scoparia (Linn.) Schrad (Chenopodiaceae) extracts containing saponins had anti-asthmatic activity *in vivo* and reduced airway eosinophilia, mucus hypersecretion and elevations in IL-4 and IL-5 levels in a dose-dependent manner, exercising these activities through attenuating the increase in protein expression of ICAM-1, VCAM-1 and MMP-9 in lung tissue (Lee et al., 2011c). ICAM-1 and VCAM-1 found on lung airway epithelium is accompanied with activation and migration of inflammatory cell, critical for pathogenesis of airway inflammatory diseases (Lee et al., 2003). *Mimosa pudica* L. (Mimosaceae), consisted of substantial quantity of mimosine (an alkaloid), inhibited leukocytosis, eosinophilia and mucus hypersecretion into the airways of mouse model of asthma. The extract displayed anti-asthmatic activity diminishing IL-4, IL-5 and IL-13 secretions including modulation of EGF and TGF-β (Yang et al., 2011).

Pre- and post-treatments with safranal, an active constituent from *Crocus sativus* Linn. (Iridaceae), attenuated asthmatic features by reducing iNOS levels in lungs of allergically inflamed mice associated with decreased airway cellular infiltration and Th2 type of cytokine production IL-5 and IL-13 (Bukhari et al., 2015). Oliveira et al., 2015 investigated the effect of *Allium cepa* Linn. (Amaryllidaceae) extract and its flavonoid quercetin on IL-4, IL-5, IL-13 concentrations, relaxation on tracheal rings and its therapeutic potential in a murine model of allergic airway disease induced by *Blomia tropicalis* mite. The extract and quercetin displayed a greater efficacy to regulate bronchoconstriction, maintenance of the IL response and suppressed mucus production. This study corroborated previous studies on the anti-inflammatory and antiallergic properties of quercetin an important constituent from *A. cepa*, inhibited airway inflammatory processes, regulated Th1/Th2 cytokines balance and restored bronchial epithelial cell damage (Park et al., 2009; Joskova et al., 2011).

Rosa multiflora Thunb. (Rosaceae) rich in quercetin glycosides and condensed tannins, decreased production of Th2 cytokines TNF-α, IL-4, and IL-6, and suppressed the infiltration of inflammatory cells, the hyperplasia of goblet cells and the deposit of collagen fiber in the lungs of mice with OVA-induced asthma (Song et al., 2016). These findings show the therapeutic potential of plant extracts on Th2-mediated or mast cell derived-allergic asthma, since the results of the study also revealed that the extracts had lowered histamine secreted from mast cells (Song et al., 2016). The hydroethanolic extract of *Mandevilla longiflora* (Desf.) Pichon (Apocynaceae), rich in flavonoid components like hesperidin, naringin, rutin, naringenin and luteolin, significantly neutralized the OVA-induced lung inflammation and Th2-type response. The extract attenuated leukocyte migration into the airways, which was evidenced by a decrease in concentrations of eosinophils, neutrophils and mononuclear cells, both in BALF quantification and by histopathological analysis, as well as decreasing the levels of IL-4, IL-5, IL-13 and IgE (De Almeida et al., 2017).

Ye et al. (2019) hypothesized that ethanol extract of *Involucrum castaneae* Blume (Fagaceae) comprising steroids, polyphenols and flavonoids protected guinea pigs from asthmatic airway inflammation. The extract reduced the number of inflammatory cells in BALF of OVA-induced guinea pig model. Meanwhile, it also significantly reduced the thickening of the bronchial smooth muscle and repaired the infiltration damage of lung tissues and reduced the levels of inflammation-related factors like IgE and IL-5. Numerous reports found in this review display that extracts from different parts of plants, such as, *Mosla dianthera* (Buch. -Ham. ex Roxb.) Maxim. (Labiatae), *Ailanthus altissima* (Mill.)

Swingle (Simaroubaceae), *Viola mandshurica W. Becker* (Violaceae), *Mentha haplocalyx* Briq. (Lamiaceae), *Lindera obtusiloba* Blume (Lauraceae), *Diospyros blancoi* A. DC. (Ebenaceae), *Picrasma quassioides* (D.Don) Benn. (Simaroubaceae), *Thuja orientalis* Linn. (Cupressaceae), *Zingiber mioga* (Thunb.) Roscoe (Zingiberaceae), *Pistacia integerrima* J.L. Stewart ex Brandis (Anacardiaceae), *Citrus tachibana* (Makino) Yu.Tanaka (Rutaceae), *Sceptridium ternatum* (Thunb.) Lyon (Ophioglossaceae), *Petiveria alliacea* Linn. (Phytolaccaceae) (Lee et al., 2006; Jin et al., 2006, 2010; Lee et al., 2011d; Suh et al., 2011, 2012; Shin et al., 2014, 2015a, 2015b; Rana et al., 2016; Bui et al., 2017; Huang et al., 2017; Gutierrez and Flores, 2018) were independently found to inhibit mast cell-mediated allergic reactions with no apparent toxicity, preventing eosinophil infiltration into the airway, attenuating allergen induced-lung inflammation and vastly regulating IL-4, IL-5, IL-6, IL-8, IL-12 and IL-13 secretions in murine model of allergic asthma. The active constituents were not identified and isolated from the above plants. Therefore, further phytochemical standardization is recommended in order to discover the active constituents from the plants in larger quantities and seemly to exhibit superior inhibitory activity against airway inflammatory diseases than the crude extract.

One distinctive study was designed to find *Artemisia princeps* Pampanini (Asteraceae) fermented with *Bifidobacterium infantis* K-525 exerting anti-asthmic property, the effect was evaluated *in vivo* system. Fermented *A. princeps* decreased IgE and reduced IL-6 and IL-4 levels in the trachea, as well as in the lung of the OVA-sensitized mice. The *in vivo* anti-asthmic effect of fermented *A. princeps* was more potent than the non-fermented ethanol extract. Therefore, these findings suggest that the improved anti-asthmic effect of extract after *B. infantis* K-525 fermentation was possibly due to the enhancement in the antioxidant status and raising phytochemicals-linked functionality (Bae et al., 2007).

Conclusion

Plant extracts regulate respiratory reactivity, reducing the cascade of inflammatory events related to the asthmatic process or enhancing pulmonary conditions. Airway hyperreactivity disorderly increases IgE production and enhance mucus hypersecretion and selective recruitment of eosinophils into inflamed airway site. Medicinal plants rich in polyphenolic compounds such as flavonoids have shown promise in the modulation of Th1/Th2 cytokine balance and may offer new therapeutic options, if their pharmacological mechanisms and pharmacological safety for clinical use are better demonstrated. The translation of these results to humans via the conduction of clinical trials is needed to stimulate the development of medicinal plants. There is a scientific gap with the scarcity of systematic reviews to corroborate this approach for drug makers.

Declaration of Competing Interest

The authors declare that there is no conflict of interest on the publication of this paper.

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APÊNDICE II

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Review

Essential oils and its bioactive compounds modulating cytokines: A systematic review on anti-asthmatic and immunomodulatory properties

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ABSTRACT

Background: Asthma, the main inflammatory chronic condition affecting the respiratory system, is characterized by hyperresponsiveness and reversible airway obstruction, recruitment of inflammatory cells and excessive production of mucus. Cytokines as biochemical messengers of immune cells, play an important role in the regulation of allergic inflammatory and infectious airway processes. Essential oils of plant origin are complex mixtures of volatile and semi volatile organic compounds that determine the specific aroma of plants and are categorized by their biological activities.

Purpose: We reviewed whether essential oils and their bioactive compounds of plant origin could modulate cytokines' immune responses and improve asthma therapy in experimental systems *in vitro* and *in vivo*.

Methods: Electronic and manual search of articles in English available from inception up to November 2018 reporting the immunomodulatory activity of essential oils and their bioactive compounds for the management of asthma. We used PubMed, EMBASE, Scopus and Web of Science. Publications reporting preclinical experiments where cytokines were examined to evaluate the consequence of anti-asthmatic therapy were included.

Results: 914 publications were identified and 13 were included in the systematic review. Four articles described the role of essential oils and their bioactive compounds on bronchial asthma using cell lines; nine *in vivo* studies evaluated the anti-inflammatory efficacy and immunomodulating effects of essential oil and their secondary metabolites on cytokines production and inflammatory responses. The most important immunopharmacological mechanisms reported were the regulation of cytokine production, inhibition of reactive oxygen species accumulation, inactivation of eosinophil migration and remodeling of the airways and lung tissue, modulation of FOXP3 gene expression, regulation of inflammatory cells in the airways and decreasing inflammatory mediator expression levels.

Conclusion: Plant derived essential oils and related active compounds have potential therapeutic activity for the treatment of asthma by modulating the release of pro-inflammatory (TNF-α, IL-1β, IL-8), Th17 (IL-17), anti-inflammatory (IL-10), Th1 (IFN-γ, IL-2, IL-12) and Th2 (IL-4, IL-5, IL-6, IL-13) cytokines and the suppression of inflammatory cell accumulation.

Abbreviations: BALF, bronchoalveolar lavage fluid; COPD, chronic obstructive pulmonary disease; DEP, diesel exhaust particles; ELISA, enzyme-linked immunosorbent assay; OVA, ovalbumin; PRISMA, preferred reporting items for systematic reviews and meta-analyses; qPCR, quantitative real-time polymerase chain reaction; ROS, reactive oxygen species

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Introduction

Asthma is a chronic inflammation of the airways characterized by the infiltration of inflammatory cells, inflammation, hyperresponsiveness and remodeling (Murdoch and Lloyd, 2010) that can be induced or exacerbated by exposure to environmental triggers (Hardy et al., 2015). Its symptoms include excessive production of mucus leading to airway obstruction (Doeing and Solway, 2013) and is often accompanied by infiltration of the airways by eosinophils, mast cells and lymphocytes, thickening of the bronchial walls, and hypertrophy/hyperplasia of airway smooth muscle (Cheng et al., 2018).

The prevalence of asthma has increased in recent decades and is currently one of the most common causes of respiratory morbidity in the world (Nunes et al., 2017), affecting individuals across the age spectrum (Tang et al., 2018). It is estimated that more than 300 million people worldwide have asthma (Nunes et al., 2017). Inflammation of the airways in asthma is associated with stimulation of T helper (Th) 2 cell-derived immune responses and production of Interleukins (IL) – 4, 5, and 13 (Ku and Lin, 2016) and other cytokines, such as IL-1 and 33, tumor necrosis factor alpha (TNF-α) and transforming growth factor beta (TGF-β), play a pivotal role in the pathophysiology of allergic reactions (Verheijden et al., 2016; Tettamanti et al., 2018). Recent experimental studies have shown that maintaining the Th1/Th2 immune balance could protect against asthma exacerbations (Rao et al., 2017) and several trials have aimed to enhance the inhibition of Th2 cell-derived cytokines such as IL-4 and 5 (Rivera et al., 2011).

Herbal remedies are a popular form of complementary or alternative medicine for asthma and nearly 40% of asthmatics have used herbal remedies (Ernst, 1998). Essential oils extracted from plants are mixtures of volatile compounds, mainly mono- and sesquiterpenoids, phenylpropanoids containing hundreds of bioactive chemical constituents. These oils have antifungal, acaricidal, antiviral and bactericidal properties and could have potential roles for the management asthma (Pina et al., 2018). Thanks to their volatility these oils can easily reach the upper and lower respiratory tract (Levy et al., 2018), where they could reduce IgE, IL-4, 5 and 13 levels and inflammatory cells (Horváth and Kamilla, 2015). This review examines *in vitro* and *in vivo* studies reporting the effect of essential oils and their bioactive compounds in anti-asthmatic activity, highlighting the specific cytokines immunomodulated.

Materials and methods

The review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher et al., 2009).

Search strategy

Peer reviewed publications in English were extracted from the PubMed, EMBASE, Scopus and Web of Science databases, restricted to Medical Subjects Headings Index (MeSH/DeCS) up to November 2018. The search used the keywords: "Essential oil", "Medicinal Plants", "Bioactive Compounds", "Cytokines", "Asthma", "Anti-asthmatic Effect", "Natural Products", "Inflammation", "Immunomodulatory" and "Immune Response". In addition, we reviewed the references listed in the articles selected to identify additional reports not included in the databases.

Study selection

Two authors (GRG and ABSV) extracted and checked the titles and abstracts of the articles independently. Studies investigating the anti-asthmatic action of essential oils and bioactive compounds in pre-clinical models and discussing possible mechanisms of action through specific cytokine-mediated signaling pathways were included. We excluded human studies, review articles, meta-analyses, book chapters,

Table. 1
In vitro studies of cytokine responses to essential oil and bioactive constituents in asthmatic condition.

Authors, year, country	Substance	Concentration	Cell lines	Objectives	Cytokines studied	Assays	Improved characteristics
Hirota et al. (2010); Japan	Limonene from *Citrus junos*	7.34 mMol/l	HL-60 clone 15 cells (Human leukemia cell line)	To investigate anti-inflammatory effect of limonene on human eosinophilic leukemia	Tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β), interleukin (IL)-8, IL-10	ELISA	Limonene may have potential anti-inflammatory efficacy against bronchial asthma by inhibiting cytokines production
Podlogar and Verspohl (2011); Germany	Ginger volatile oil (*Zingiber officinale*)	0.1–1 μg/ml	BEAS-2B (Human bronchial epithelial cell line)	Ginger volatile oil and its compounds were tested against human bronchial epithelial cells	IL-8 and TNF-α	ELISA	Ginger oil and its terpenoid compounds could be used as anti-inflammatory drugs in respiratory infections
Kianmehr et al. (2016); Iran	Carvacrol	75, 150 and 300 μg/ml	Splenocytes	Effects of carvacrol on cytokines genes expression in splenocytes of asthmatic mice	IL-4, IFN-γ, transforming growth factor beta (TGF-β) and IL-17	qPCR	Immunomodulatory effect of carvacrol indicating increased IFN-γ and FOXP3 but decreased IL-4, TGF-β, and IL-17 expression
Khosravi and Erle (2016); USA	Carvacrol and Thymol	200 μm (equal to 30 μg/ml)/ 80 μg/ml	Beas-2B, H292 (Mucoepidermoid carcinoma cells) and A549 (Adenocarcinomic human alveolar basal epithelial cells)	To hypothesize that chitin directly stimulates airway epithelial cells to release cytokines that promotes type 2 immune responses	IL-25 and IL-33	ELISA	Direct effects of chitin on airway epithelial cells are likely to contribute to allergic airway diseases like asthma, and that carvacrol/thymol directly inhibits epithelial cell pro-inflammatory responses to chitin

Table. 2
In vivo studies of cytokine responses to essential oil and bioactive constituents in asthmatic condition.

Authors, year, country	Substance	Strains/ animal	Dose/ route	Objectives	Cytokines studied	Assays Biochemical	Molecular	Improved characteristics
EI Mezayen et al. (2006); Japan	Thymoquinone	BALB/c Mice	3 mg/kg (*i.p.*)	To investigate the potential anti-inflammatory role of thymoquinone by examining its effect on mouse challenged through the airways with ovalbumin (OVA)	IL-4, IL-5 and IL-13	ELISA	Western blot	Thymoquinone has an anti-inflammatory effect during the allergic response in the lung through the inhibition of Th2 cytokine mediated immune response
EI Gazzar et al. (2006); USA	Thymoquinone	BALB/c Mice	3 mg/kg (*i.p.*)	To examine the anti-inflammatory effect of thymoquinone in a mice sensitized and challenged with OVA	IL-4, IL-5, IL-13 and IL-10	ELISA		Anti-inflammatory effect of thymoquinone on allergic lung is mediated by a decrease in Th2 cytokine production
Chang et al. (2008); Taiwan	5% Perilla oil	BALB/c Mice	Experimental food-oral	To evaluate the anti-inflammatory effect of perilla oil on OVA- sensitized and challenged asthmatic mice	IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, Interferon gamma (IFN-γ) and TNF-α	ELISA		Dietary perilla oil alleviates inflammation by decreasing the secretion of pro-inflammatory cytokines in BALF, but failed to regulate the Th1/Th2 balance during allergic airway inflammation.
Hirota et al. (2012); Japan	Limonene isolated from *Citrus junos*	BALB/c Mice	4 μg (*i.t.*)	To evaluate the inhibitory effect of limonene on *Dermatophagoides farinae*-induced airway hyper responsiveness and airway modeling in a mice model of asthma	IFN-γ, IL-5, IL-13, and TGF-β	ELISA		The goblet cell metaplasia, thickness of airway smooth muscle, and fibrosis were markedly decreased in limonene treated mice. It has a potential to reduce airway remodeling and hyperresponsiveness
Ueno-Iio et al. (2014); Japan	Lavender essential oil isolated from *Lavandula angustifolia*	BALB/c Mice	Aerosolized 5–20 μl of Lvn	To evaluate the anti-inflammatory effect of lavender essential oil on OVA-induced bronchial asthma	IL-4, IL-5 and IL-13	ELISA		Essential oil inhibits allergic inflammation and mucous cell hyperplasia with suppression of Th2 cytokines
Ku and Lin (2015); Taiwan	Farnesol	BALB/c Mice	5, 25 and 100 mg/kg (mixed in feed)	To investigate the effect of farnesol on OVA-sensitized and challenged asthmatic mice	IL-4, IL-5, IL-2, IFN-γ, IL-1β, TNF-α and IL-10	ELISA		Farnesol supplementation may be beneficial to improve the Th2-cytokine mediated allergic asthmatic inflammation
Ku and Lin (2016); Taiwan	Farnesol	BALB/c Mice	5, 25 and 100 mg/kg (mixed in feed)	To investigate the effect of farnesol on allergic asthma by OVA-sensitized and challenged mice	IL-2, IL-4, IL-5 and IFN-γ	ELISA		Farnesol improved allergic antibody levels and regulated Th2 responses towards Th1 immune balance and ameliorate inflammation
Kianmehr et al. (2017); Iran	Thymol from *Zataria multiflora*	BALB/c Mice	200, 400 and 800 μg/ml (*p.o.*)	To investigate the effect of *Z. multiflora* containing thymol on cytokine genes expression on OVA-injected experimental mouse model of asthma	IFN-γ, IL-4, TGF-β and IL-17		Real time-PCR	*Z. multiflora* containing thymol decreased pro inflammatory cytokines but increased anti-inflammatory cytokines gene expression and the number of Treg in splenocytes of asthmatic mice
Pina et al. (2018); Brazil	Citronellol, α-terpineol and carvacrol	Swiss Mice	25, 50 or 100 mg/kg (*i.p.*)	Effects of citronellol, α-terpineol and carvacrol on allergic airway inflammation established by OVA	TNF-α	ELISA		Monoterpenes decreased leucocyte migration and TNF-α levels, it can be an alternative for treatment of allergic airway inflammation

conference abstracts, editorials/letters, patents and case reports. Disagreements among the two authors were resolved by a third author (RQG) through discussion.

Data extraction

Data were extracted and summarized in tables. Table 1 summarizes data of *in vitro* studies, including (a) author's name and publication year (b) substances and their concentrations (c) cytokines assessed (d) components identified (e) cell lines or strains used (f) proposed mechanisms of biochemical results. Table 2 provides data on experimental studies, including (a) author's name and year (b) experiment design (c) substances and concentrations (d) dose/route of administration/animal model (e) outcomes (f) biochemical mechanisms and results.

Methodological quality and risk of bias

The methodological quality of the studies was assessed using standard checklists and mandatory statements of random allocation and concealment of treatment, compliance with welfare regulations, blinding of drug administration, evaluation of outcomes, depiction of animal losses and comprehensiveness of outcome data (Hooijmans et al., 2014). The quality analysis was depicted using colors as suggested by Roskosk-Jr (2017).

Data analysis

The data is presented as a narrative. Pooling statistics and meta-analysis were not used due to the heterogeneity of the studies.

Results and discussion

Search results

Fig. 1 presents a flowchart of the search. Nine hundred and fourteen articles were identified (PubMed: 381, EMBASE: 99, Scopus: 228 and Web of Science: 206), including 251 duplicates. Of these, 640 were excluded after screening the abstracts because they did not report cytokines, were case reports or review articles. Twenty-three publications were selected for full-text review and of these, thirteen, four *in vitro* and nine *in vivo,* met the eligibility criteria.

Study characteristics and description

Four *in vitro* studies investigated the effect of plant-derived essential oils and their components against inflammatory airway disease in cell lines of bronchial asthma. A further nine *in vivo* studies assessed the essential oils and their components effect in mouse models of asthma characterized by eosinophil-dominant inflammation in actively sensitized mice. The chemical structures of the essential oil components are shown in Figs. 2 and 3. Most of the studies used monoterpenes, sesquiterpene and triterpene alcohols ($n = 6$) and quinones ($n = 2$). Two studies used cyclic monoterpene and three used crude essential oils. Four studies originated from Japan, three from Taiwan, two each from USA and Iran and one each from Brazil and Germany.

In vitro experiments were performed in human bronchial epithelial (BEAS-2B), lung mucoepidermoid carcinoma (H292), lung carcinoma (A549), eosinophilic leukemia (HL-60 clone 15) and mouse splenocytes cell lines. Numerous experimental approaches were used in the *in vitro* studies, including the inhibition of eosinophil migration, cytokine assays, level of reactive oxygen species and determination of levels and expression of molecules in innate immune responses. The main finding of the *in vivo* studies is that mice strains, such as BALB/c, are suited for experimental protocols of asthmatic inflammatory reactions. BALB/c mice models have been used extensively in asthma-related inflammatory disease with identical airway responsiveness and bronchial inflammation with hyperproduction of Th2 cytokines to humans (Gueders et al., 2009). This model was recurrently reported in the *in vivo* studies included.

Two experimental mouse models of allergic inflammation and asthmatic disorders have been used to describe the immunomodulation effect of essential oils and their components. These include the ovalbumin-sensitized airway inflammation and the *Dermatophagoides farinae*-induced airway hyperresponsiveness and eosinophilic infiltration. Several methods have been used to study changes in mouse models of asthma, including the assessment of airway responsiveness, leukocyte counts, proinflammatory and inflammatory mediators and markers, cytokine levels, antibody titres, lung resistance, histopathology and histomorphometric analyses, levels of immunostimulatory dependent signalling molecules and transcription factors.

In vitro studies of cytokine responses to essential oil and constituents in asthmatic condition

In vitro studies investigating the systemic treatment of asthma with essential oils of ginger and bioactive compounds, such as limonene from *Citrus junos*, terpenoids from *Zingiber officinale*, carvacrol and thymol have used established cell lines models of asthmatic syndromes via regulating proinflammatory, Th1 and Th2 cytokines to enhance immune responses in experimental bronchial asthma (Table 1). Limonene from *Citrus junos* inhibits the diesel exhaust particles (DEP)-stimulated p38 mitogen activated protein kinase (MAPK) signaling pathway and modulates eotaxin-induced chemotaxis by inhibiting TNF-α, IL-1β, IL-8 and IL-10 (Hirota et al., 2010). In addition, monocyte chemoattractant protein (MCP)-1 production decreases, suggesting that limonene might decrease monocyte and eosinophil infiltration in the lungs. The bioactive constituents of essential oils of various plants may have potential therapeutic roles for the management of bronchial asthma through their role in controlling reactive oxygen species (ROS) production, inactivating eosinophil migration and ameliorating oxidative damage to the lung (Beck-Speier et al., 2005).

Regarding the attenuation of airway inflammation, the increased number of inflammatory cells in the bronchioalveolar lavage fluid (BALF) of rats after lipopolysaccharide treatment is significantly reduced by a ginger extract (Aimbire et al., 2007). Ginger volatile oils and their terpenoid compounds have anti-inflammatory and regulatory effects on lipopolysaccharide-stimulated BEAS-2B-induced IL-8 and TNF-α secretion and might be promising against inflammatory airway diseases (Podlogar and Verspohl, 2011).

Carvacrol or cymophenol is considered the foremost constituent of several plants including *Zataria multiflora,* that demonstrate beneficial properties on respiratory diseases, including asthma (Alavinezhad et al., 2017). It has been hypothesized that carvacrol from *Carum copticum* essential oil has relaxant effects on smooth muscles of guinea pig tracheal chains and bronchodilator effects (Boskabady et al., 2003). The immunologic feature of asthma is a balance shift from Th1 to Th2 (Jalali et al., 2013). The increased cytokine production of IL-4, -5 and -13 has been shown in BALF and airway biopsies of patients with mild or asymptomatic asthma (Walker et al., 1992). Th2 cytokines are required for the development of airway eosinophilia and to stimulate an inflammatory response that results in asthma (Ray and Cohn, 1999). Th1 inhibits Th2 responses through the secretion of INF-γ, IL-2 and TGF-β and thus the goal of asthma therapy should be the balance of Th1/Th2 (Randolph et al., 1999). Carvacrol potentiates anti-inflammatory reactions through the improved balance of Th1 (IFN-γ) and Th2 (IL-4) cytokine gene expression in splenocytes of sensitized mice. The immune modulatory effect of carvacrol is more effective than dexamethasone in sensitized mice splenocytes, indicating it has potential therapeutic value in allergy, autoimmunity and infection (Kianmehr et al., 2016).

The major phenolic monoterpenoids found in genera *Origanum* and *Thymus* are carvacrol and thymol, the most abundant essential oil

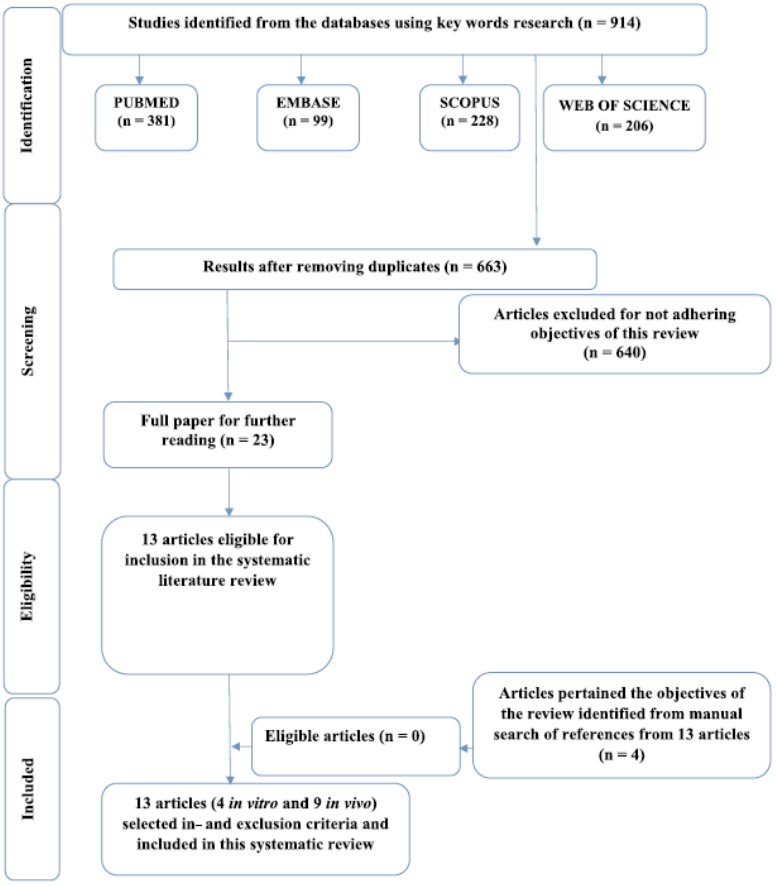

Fig. 1. Preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow diagram of article search and selection included in this review.

constituents of various plants (Alavinezhad et al., 2018). In *in vitro* T-cell models, carvacrol and thymol can affect transcriptional factors that regulate inflammation by reducing IL-2 and IFN-γ production (Gholijani et al., 2015, 2016). Carvacrol and thymol inhibit the effects of chitin on type-2 promoting cytokines IL-25 and IL-33 released in asthmatic conditions. Chitin is a component of the cell walls of fungi, crab, shrimp, insects and house dust mites which correlates with asthma (Koch et al., 2015). Previous reports have found increases in IL-25 and IL-33 or the receptors in mice lungs inflamed after chitin administration (Yasuda et al., 2012; Van Dyken et al., 2014). Moreover, IL-33 is involved in other pathological conditions, such as cardiovascular diseases, arthritis, infection, sepsis, atherosclerosis, neurological disorders, cancer and allergy (Pan et al., 2018). The direct administration of chitin to airway epithelial cells is likely to contribute to allergic airway reactions and carvacrol and thymol may inhibit epithelial pro-inflammatory cell responses to chitin by inhibiting type-2 promoting cytokine release and immune responses (Khosravi and Erle, 2016).

In vivo studies of cytokine responses to essential oil and constituents in asthmatic condition

Thymoquinone reduces elevated levels of IL-4, 5 and 13 secretion and increases IL-10 in ovalbumin (OVA)-sensitized and challenged mice (El Gazzar et al., 2006). IL-4 is required for Th2 cell differentiation and isotype switching in B cells from IgM to IgE production (Kopf et al., 1993; Holgate, 2004), whereas, IL-5 and IL-13 regulate growth, differentiation, and survival of eosinophils (Domae et al., 2003). IL-13 promotes IgE isotype switching in B cells and mucus production by goblet cells in the airway mucosa (Zhu et al., 1999). Thymoquinone causes obstruction of lung tissue eosinophilia and goblet cells hyperplasia which could be due to its intense action on Th2 cytokines and inflammatory cells in the airways.

El Mezayen et al. (2006) reported that thymoquinone attenuates OVA-induced airway inflammation by inhibiting cyclooxygenase-2 (COX-2) expression and prostaglandin D2 production and IL-4, 5 and 13 levels. The inhibition of prostaglandin D2 synthesis plays an important role in modulating Th2 cytokine levels, lung eosinophilia, goblet cell hyperplasia and mucus hyperproduction in allergic airway inflammation (Larche et al., 2002; Herrick and Bottomly, 2003). Thymoquinone suppressed the immunologic and inflammatory responses induced by Th2 cytokines in a mouse model of airway inflammation and had anti-inflammatory effects in allergic lung responses.

Perilla oil diminished bronchoalveolar inflammation by decreasing the secretion of pro-inflammatory and Th1 cytokines into the local lung

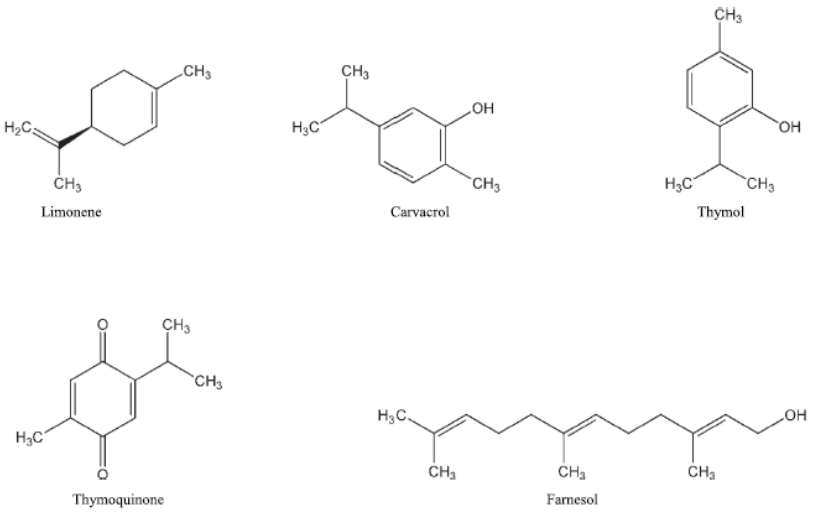

Fig. 2. Chemical structures of essential oil constituents (I).

and airway tissues but failed to regulate the Th1/Th2 balance toward the Th1 pole in Th2-skewed allergic airway inflammation (Chang et al., 2008). Limonene treated mice had reduced IFN-γ, IL-5, IL-13 and TGF-β levels and lower numbers of eosinophils in the lungs of *Dermatophagoides farina*-induced airway hyperresponsiveness asthma model (Hirota et al., 2012). IL-13 is linked to mucus hypersecretion by hyperplastic goblet cells that create airway mucus plugs, especially in peripheral airways of asthmatics (Li et al., 2003). TGF-β is associated with the development of airway remodeling in asthma and correlates with thickening of the basement membrane (Matsukuara et al., 2010; Tian et al., 2011).

Lavender essential oil inhalation extinguishes eosinophils in BALF and lung tissue concomitantly with a decrease in IL-5 and IL-13 levels in bronchial tissue of experimental asthmatic mice (Ueno-Iio et al., 2014). Eosinophils play a key role in the pathogenesis of bronchial asthma, while IL-5 plays a key role in the development and migration of eosinophils and correlates with the severity of eosinophilic inflammation (Sanderson, 1992). IL-13 remodels the infiltration of inflammatory cells into BALF and peri-bronchial and perivascular tissues (Zhou et al., 2012).

Farnesol, a sesquiterpene alcohol widely present in fruits, vegetables and essential oils (Duncan and Archer, 2008) restores IL-2. IL-4, IL-5, IL-10, IL-1β and TNF-α levels in distorted BALF due to OVA sensitization and challenge in asthmatic mice, suggesting it may have potential to modulate the Th1/Th2 balance in lungs (Ku and Lin, 2015). Th2 cells are the major source of IL-10 production, although excessive IL-10 secretion may inhibit the production of IL-1β and TNF-α (Iyer and Cheng, 2012). Farnesol supplementation ameliorates serum lipid profiles in OVA-sensitised and challenged mice and reduces increased non-specific IgE, IgA, IgM, and IgG levels after OVA sensitisation and challenge, suggesting that its supplementation regulates Th2 cytokine ratios in BALF and reduces inflammation.

Zataria multiflora has a relaxant effect on tracheal responsiveness, bronchodilator and regulation of inflammatory mediators in OVA-sensitized guinea pigs (Boskabady et al., 2014) and a preventive effect on emphysema and pathological changes of the lung and systematic inflammation in guineas pigs' models of chronic obstructive pulmonary disease (COPD) (Gholami Mahtaj et al., 2015). The extract of *Zataria multiflora* thymol as main constituent decreases pro-inflammatory cytokines (IL-4 and IL-17 and TGF-β) but increases anti-inflammatory (IFN-γ) cytokine and Forkhead box P3 (FOXP3) gene expression in splenocytes of asthmatic mice, suggesting a specific therapeutic effect in allergy, autoimmunity and infection potentiating Th1 and suppressing Th2 and Th17 cells. (Kianmehr et al., 2017)

The alcoholic monoterpenes citronellol, α-terpineol and carvacrol largely present in plants of the genus *Cymbopogon, Eucalyptus* and *Origanum* are used for the treatment of inflammatory diseases (Guimaraes et al., 2013). Citronellol, α-terpineol and carvacrol modulates eosinophil migration and decreases TNF-α levels in the mice pleural cavity after induction by OVA. These effects can be associated

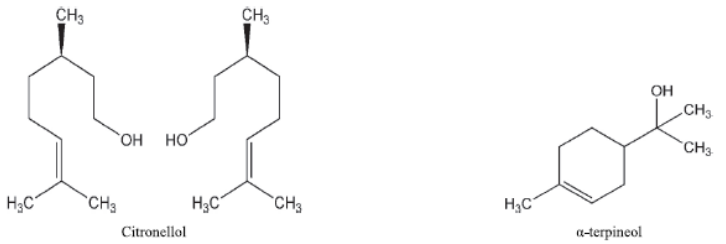

Fig. 3. Chemical structures of essential oil constituents (II).

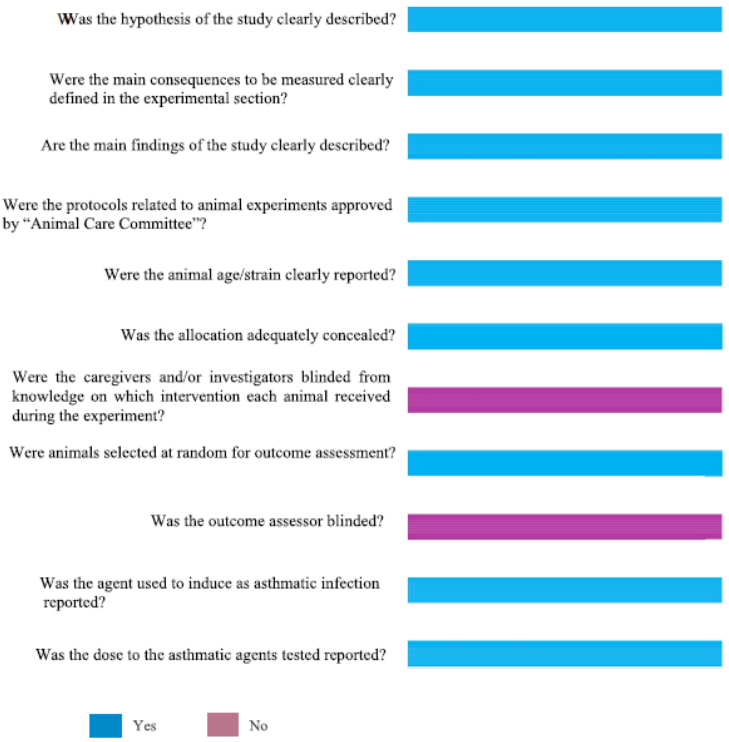

Fig. 4. Analysis on methodological quality and results of studies. Blue and magenta bars represent the proportion of studies for which the item was or was not applicable.

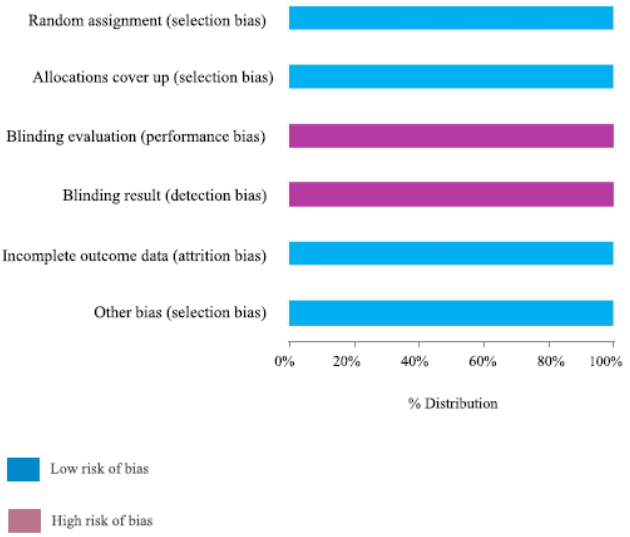

Fig. 5. Risk of bias level. Blue: Low; Magenta: High.

with the monoterpenes' ability to inhibit important targets of inflammatory mediators and could be prospective candidates as drugs in the therapy of allergic inflammation and asthma.

Methodological quality/risk of bias

The methodological features of the *in vivo* studies are detailed in Fig. 4. All papers selected reported the allocation sequence generation with random procedures. However; none of them reported blinding procedures. The animal experiments had a lack of blinding of the interventions allocated (performance bias) and outcome assessment (detection bias) (Fig. 5).

Conclusion

This review assessed the anti-inflammatory and immune-modulating activities of essential oils and their bioactive compounds linked to cytokine expression and secretion in airway pathologic reactions, highlighting the immunoregulatory mechanisms enhancing cytokine responses, triggering of immune cells, orientate immune regulation and decreased inflammation associated with asthma. The pharmacology and pharmacokinetics of several plant-derived essential oils remain to be clearly established using cellular and animal models of asthma.

Conflict of interest

The authors declare they have no conflicts of interest regarding the publication of this paper.

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Review article

Cytokines in the management of rotavirus infection: A systematic review of *in vivo* studies

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ABSTRACT

Objective: Rotavirus is a leading cause of childhood diarrhoea. Rotavirus vaccines are effective against severe rotavirus gastroenteritis, but have lower efficacy in low income countries in Africa. Anti-rotavirus treatment is not available. This study reviews the literature of animal studies evaluating whether cytokine mediated pathways of immune activation could improve rotavirus therapy.

Methods: We performed a systematic review of articles in English published from 2010 to 2016 reporting agents with *in vivo* antirotavirus activity for the management of rotavirus infection. The search was carried in PubMed, EMBASE, Scopus and Web of Science. Animal experiments where cytokines were investigated to assess the outcome of rotavirus therapy were included.

Results: A total of 869 publications were identified. Of these, 19 pertained the objectives of the review, and 11 articles described the effect of probiotics/commensals on rotavirus infection and immune responses in animals. Eight further *in vivo* studies evaluated the immunomodulating effects of herbs, secondary metabolites and food-derived products on cytokine responses of rotavirus-infected animals. Studies extensively reported the regulatory roles for T-helper (Th)1 (interferon gamma (IFN-γ), interleukin (IL)-2, IL-12) and Th2 (IL-4, IL-6, IL-10) cytokines responses to rotavirus pathogenesis and immunity, inhibiting rotavirus infection through suppression of inflammation by viral inhibition.

Conclusion: Th1 and Th2 cytokines stimulate the immune system, inhibiting rotavirus binding and/or replication in animal models. Th1/Th2 cytokine responses have optimal immunomodulating effects to reduce rotavirus diarrhoea and enhance immune responses in experimental rotavirus infection.

1. Introduction

Rotavirus is a segmented, double-stranded RNA virus [1] that, causes severe dehydrating diarrhoea in children worldwide [2]. Two oral rotavirus vaccines, RotaTeq (Merck), a pentavalent human-bovine vaccine containing five rotavirus strains, and Rotarix (GlaxoSmithKline), a monovalent human rotavirus vaccine containing a G1P8 strain, are universally recommended to prevent severe diarrhoea in children [3]. Although the vaccines have significantly reduced diarrhoea incidence and diarrhoea related hospitalizations [4], they are less effective in preventing rotavirus infections and their efficacy is lower in some countries in Southern Africa [5].

Although synthetic compounds, such as, ribavirin, cimetidine, famotidine, dipyridamole, nifedipine and isoprinosine and the effect of plants and their natural molecules have been studied using *in vitro* and *in vivo* experimental systems for the treatment of rotavirus

Abbreviations: ELISA, Enzyme-Linked Immunosorbent Assay; ELISPOT, Enzyme-Linked Immunospot Assay; PCR, Polymerase Chain Reaction; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; RT-PCR, Reverse transcription-Polymerase Chain Reaction

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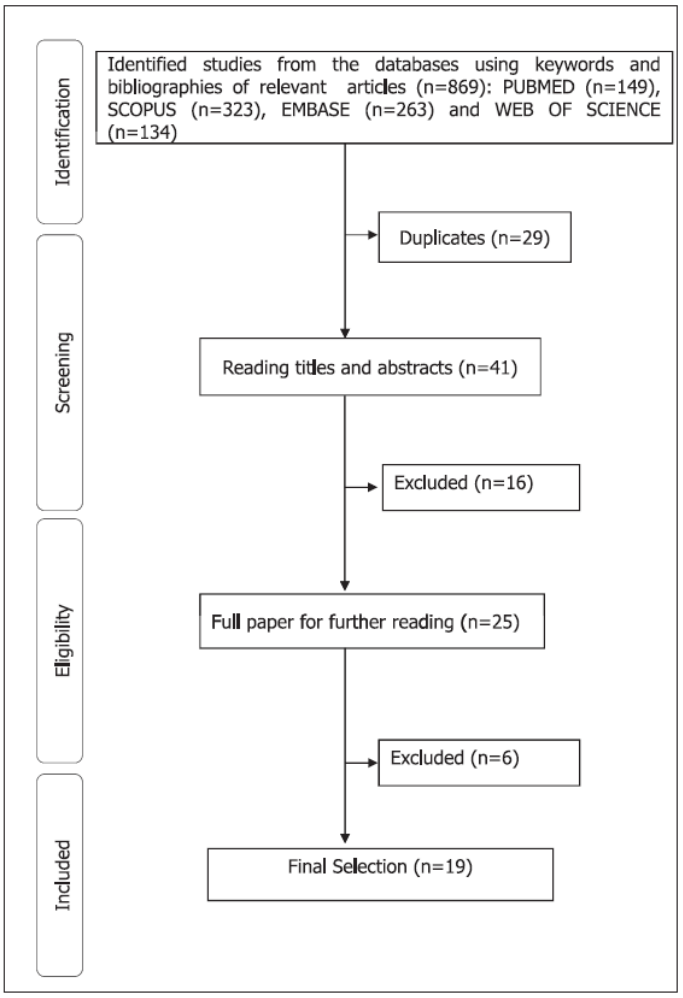

Fig. 1. Flow chart of study selection process.

gastroenteritis [6–9], but, there are no promising anti-rotavirus drugs to date [9]. The prevention of dehydration, fluid replacement and zinc supplementation continue to be the key interventions to manage severe diarrhoea in childhood [10].

Rotavirus infection triggers an innate immune response of the intestinal epithelial cells, activating proinflammatory signalling pathways with the release of type I and type III interferons (INFs) and other cytokines [11]. These responses are critical to reduce rotavirus replication and to build up protective immunity in later stages of the infection [12,13]. An increasing number of studies have reported the effect of biotherapeutic substances on antiviral immune responses and the underlying cytokine mediated pro- and anti-inflammatory responses for the inhibition of rotavirus replication. This review examines studies reporting the effect of substances with *in vivo* antirotavirus activity for the management of rotavirus infection, highlighting specific cytokines that regulate the immunomodulation on rotavirus infection.

2. Materials and methods

This report followed the guidelines for Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [14].

2.1. Search strategy

We conducted a comprehensive search for papers in PubMed, EMBASE, Scopus and Web of Science limited to Medical Subjects Headings Index (MeSH/DeCS) using the key words “Rotavirus”, “Rotavirus infection”, “Cytokine” and “Rotavirus vaccine” and related terms. The databases were searched for publication years 2010–2016 inclusive. In addition, we reviewed the references lists of the articles

selected to identify further reports not included in the database search.

2.2. Study selection

Two independent reviewers (GRG and VKSC) screened the titles and abstracts for relevance. Articles considered to have original material were obtained and assessed in detail. Anti-rotavirus studies that examined immune responses by promoting cytokine dependent signalling events in animal models were included. However, *in vivo* studies in humans, *in vitro* studies, review articles, meta-analyses, abstracts, conference proceedings, editorials/letters, patents and case reports were excluded. In case of disagreement between the two reviewers, a third reviewer (JSSQ) was consulted regarding the non-consensus features.

2.3. Data extraction

Data were extracted into a pre-defined standardized form containing, article information (first author, year, study location), methods (substances studied, animal model, strain, randomization, blinding procedures, cytokines assessed, study design, outcomes, biochemical assays, molecular mechanisms) and results.

2.4. Methodological quality and assessment of the risk of bias

The risk of bias in the experimental design of studies included was explored using checklists and standard scales [15]. The methodological quality was assessed by whether there was adequate randomization and distribution of animal numbers, blinding of drug administration and, assessment of outcomes, description of animal losses and completeness of outcome data.

3. Results and discussion

3.1. Search results

The selection steps of the studies are summarized in Fig. 1. We identified 869 citations (PubMed: 149, Scopus: 323, EMBASE: 263 and Web of Science: 134). After elimination of duplicates and screening for eligible titles and abstracts, 25 publications were selected for full-text review. Of these, 19 fulfilled the inclusion criteria and were characterized into (a) studies of probiotics for cytokine responses, (b) studies of plant-derived products and secondary metabolites for cytokine responses and (c) studies of fungal secondary metabolites and food-derived products for cytokine responses.

3.2. Study description

The characteristics of the studies selected are described in Tables 1–3. Eleven articles investigated the immunoregulatory and immunostimulatory properties of probiotics/commensals supplementation against oral inoculation of live mammalian viral strains such as human (Wa), porcine (RVA K85 and OSU) and murine (EW) rotavirus strains individually infected in neonatal gnotobiotic pig or mice models. Three studies evaluated the immunomodulatory and protective effect of natural phytochemicals and five assessed the effects of dietary supplements and fungal secondary metabolites on rotavirus infectivity. Neonatal gnotobiotic pigs have been used widely as a model of human rotavirus diarrhoea and exhibits a similar intestinal epithelial structure to humans [16]. This model is preferred in experimental studies, since it furnishes significant details of the intestinal immunological homeostasis after rotavirus infection [17].

Several methods have been described to study changes in rotavirus infectivity, including the faecal consistency score, faecal virus shedding, intestinal lesions score, histomorphology, analysis of non-structural protein-4 (NSP4) mRNA expression, analysis of intestinal epithelial barrier function, levels of innate, T-helper (Th)1, Th2, Th17, T regulatory, pro- and anti-inflammatory cytokine concentrations, levels of immunoregulatory and immunostimulatory dependent signalling molecules and transcription factors. Among the methods examined for rotavirus inhibition, faecal consistency, faecal virus shedding, histology of small intestine and levels of Th1/Th2 cytokines were used in a larger number of studies.

3.3. Cytokine responses to probiotics in rotavirus infection

Five studies reported that probiotic strains, such as; *Lactobacillus acidophilus* NCFM™ plus *Lactobacillus reuteri* ATCC 23272, *Lactobacillus rhamnosus* GG ATCC 53103 plus *Bifidobacterium lactis* Bb12, *Escherichia coli* Nissle plus *L. rhamnosus* GG and *L. rhamnosus* GG plus *E. coli* Nissle 1917 synergistically colonize the gut and reduce the duration and severity of rotavirus diarrhoea, enhancing virus-specific immune responses and preventing viral replication in the gnotobiotic pig [18–22] (Table 1). *L. rhamnosus* GG, *Lactobacillus ruminis* SPM 0211, *Bifidobacterium longum* SPM 1205 and SPM 1206 individually had antiviral activity against rotavirus infection, alleviating diarrhoea, protecting ileal epithelium, improving intestinal microbiota, promoting the intestinal immunoregulatory properties and reducing the levels of virus shedding in the gnotobiotic pig and neonatal mice models [23–27] (Table 1). Innate (interferon alpha (IFN-α), interferon beta (IFN-β)), Th1 (interferon gamma (IFN-γ), interleukin (IL)-2, IL-12), Th2 (IL-4, IL-6, IL-10), pro-inflammatory (tumor necrosis factor alpha (TNF-α), IL-1, IL-8), Th17 (IL-17), anti-inflammatory (IFN-γ) and T regulatory (transforming growth factor beta (TGF-β)) cytokines were measured to describe immune response patterns and its modulations by commensal colonizations.

The most defined therapeutic immunomodulating mechanisms for probiotics strains was to enhance the induction of Th cells including Th1, Th2 and Th17 to promote responses against rotavirus and maintain immunological homeostasis by controlling the production of T regulatory (TGF-β) cytokines. The initial release of Th1/Th2 cytokines such as IFN-γ, IL-4 and IL-12 inhibits significant viral replication by promoting cell-mediated immunity [28]. In addition, IFN-γ production is a major contributing factor against infections caused by a diverse groups of cytopathic viruses [29], and it plays a vital role in the defence against viral infections. Furthermore, IL-6 and IL-10 play potential roles in the pathogenicity and immunity against rotavirus infection [30], with the latter influencing the modulation of immune responses, ion transport in the small intestine and anti-inflammatory effects against infectious diseases [31].

Probiotic strains belonging to *Lactobacilli* (*Lactobacillaceae*) individually or jointly with *Bifidobacteria* (*Bifidobacteriaceae*) reduced the levels of rotavirus infectivity by blocking or inhibiting the viral replication and modulation of the immune response through promoting Th1/Th2 cytokines, such as IFN-γ, IL-2, IL-4, IL-10, IL-12 which act as key regulators to enhance immune response against human rotavirus infection [32]. The review also included a comprehensive investigation of other commensal species from *Lactobacilli* and *Bifidobacteria* which could enable studies of novel anti-rotavirus agents to enhance Th1/Th2 cytokines modulators and immunomodulating effects to control human rotavirus.

The early colonization of probiotic species can play a role enhancing the human rotavirus vaccine efficacy [33]. Among the bacteriotherapies, a considerable number of studies using probiotics such as *L. rhamnosus* GG and *B. lactis* Bb12 reported to regulate innate immune responses, with enhanced innate and Th1 cytokines (IFN-α, IFN-β and IL-12) responses to the vaccines [19,20,27] and reduced the severity of rotavirus diarrhoea in gnotobiotic pigs vaccinated with the human rotavirus vaccines. These probiotics are described as vaccine adjuvants, with significant impact on vaccine immunogenicity and efficacy upregulating Th1 cytokine production. Many of these probiotic species claim functional properties and health benefits and may enhance

Table 1
Studies of cytokine responses to probiotics in rotavirus infection.

Authors, year, country	Cytokines	Substances	Animals	Methods	Cytokine assays	Improved characteristics	R	B
Azevedo et al. [18], USA	Interferon alpha (IFN-α), interferongamma (IFN-γ), interleukin (IL)-4, IL-12 and IL-10	*Lactobacillus acidophilus* NCFM™ and *Lactobacillus reuteri* ATCC 23272	Neonatal gnotobiotic pigs	Combination of probiotic strains *L. acidophilus* and *L. reuteri* on the development of cytokine responses in neonatal gnotobiotic pigs infected with human rotavirus (Wa) strain was investigated	The cytokine levels were determined in serum and small intestinal contents by enzyme-linked immunospot assay (ELISPOT) and enzyme-linked immunosorbent assay (ELISA)	Probiotic strains significantly enhanced T-helper (Th)1/Th2 cytokine levels and reduced diarrhoea in rotavirus-infected pigs	Y	N
Liu et al. [23], USA	IL-6, IL-8, IFN-γ, transforming growth factor beta (TGF-β) and tumor necrosis factor alpha (TNF-α)	*Lactobacillus rhamnosus* GG ATCC 53103	Gnotobiotic pigs	The study was carried to assess the impact on continued *L. rhamnosus* GG supplementation on rotavirus gastroenteritis in the gnotobiotic pig model of virulent human rotavirus (Wa) strain infection	Porcine cytokine levels in serum was measured by ELISA	*L. rhamnosus* GG ameliorated rotavirus diarrhoea by preventing injuries to epithelium and maintained the levels of anti-inflammatory cytokines in serum	Y	N
Zhang et al. [24], China	IFN-γ and TNF-α	*Lactobacillus rhamnosus* GG ATCC 53103	Neonatal mice	The efficacy of different timing and dosage of *L. rhamnosus* GG against human rotavirus (Wa) strain-infected neonatal mouse model was evaluated	Levels of cytokine in serum was measured by ELISA	*L. rhamnosus* GG extensively protected mice against rotavirus-induced diarrhoea and preserved intestinal mucosa by increasing the function of IFN-γ as well as suppressing the secretions and activities of TNF-α	Y	N
Vlasova et al. [19], USA	TGF-β, TNF-α, IL-10, IL-12 and IFN-α	*Lactobacillus rhamnosus* GG ATCC 53103 and *Bifidobacterium lactis* Bb12	Neonatal Gnotobiotic Pigs	Effects of co-colonization with *L. rhamnosus* GG and *B. lactis* Bb12 on rotavirus vaccine challenged with human rotavirus (Wa) strain in gnotobiotic pigs was assessed	Cytokine levels in spleen was measured by ELISA	Probiotic strains contributed to immunomaturation, regulated immune homeostasis and enhanced vaccine efficacy in pig model of rotavirus vaccination and infection, thereby moderated rotavirus diarrhoea	Y	N
Chattha et al. [20], USA	TGF-β, IL-4, IL-12, IFN-γ, IL-6, TNF-α, IL-10, IL-8, IL-17 and IFN-α	*Lactobacillus rhamnosus* GG ATCC 53103 and *Bifidobacterium lactis Bb12*	Neonatal Gnotobiotic Piglets	T cell and cytokine responses to rotavirus vaccine and virulent human rotavirus (Wa) strain in gnotobiotic pigs colonized with *L. rhamnosus* GG and *B. lactis Bb12* was investigated	Cytokines in serum, intestinal contents and bile were measured by ELISA	Probiotic strains moderated rotavirus infection and enhanced vaccine efficacy by increasing systemic Th1 dependent immune responses	Y	N
Kang et al. [25], Republic of Korea	IFN-α and interferonbeta (IFN-β)	*Lactobacillus ruminis* SPM 0211, *Bifidobacterium* longum SPM 1205 and SPM 1206	BALB/c mice	Antiviral effects of probiotic strains in human rotavirus (Wa) strain-infected neonatal mouse model and their mechanism of rotavirus inhibition was studied	Real-time quantitative-*Polymerase Chain Reaction* (PCR) analysis was conducted with primers specific to cytokine in intestinal tissues	*L. ruminis* SPM 0211, *B. longum* SPM 1205 and 1206 efficiently inhibited rotavirus replication. Anti-rotaviral effects of probiotics are likely due to their modulation of immune responses through promoting type I interferons (IFNs)	Y	N
Yang et al. [34], USA	IFN-γ	Rice bran plus *Lactobacillus rhamnosus GG* ATCC 53103 and *Escherichia coli* Nissle	Gnotobiotic pigs	Effect of rice bran in combination with *L. rhamnosus* GG and *E. coli* Nissle on diarrhoea, gut epithelial health and innate immune responses during virulent human rotavirus (Wa) strain infection was investigated	IFN-γ concentrations in small and large intestines was measured using ELISA	Rice bran in combination with probiotic strains signifcantly protected against rotavirus diarrhoea and enhanced the innate immune response during rotavirus infection. Rice bran strengthened the probiotic growth in gut	Y	Y
Mao et al. [26], China	IL-2 and IL-4	*Lactobacillus rhamnosus* GG ATCC 53103	Piglets	The efficacy of *L. rhamnosus* GG against rotavirus diarrhoea via evaluating jejunal mucosal barrier function in the weaned piglets challenged by porcine rotavirus (OSU) strain was investigated	Cytokine concentrations in jejunal mucosa was determined using ELISA	*L. rhamnosus* GG supplementation alleviated diarrhoea via improving jejunal mucosal barrier function, including non-specific barrier mechanisms and specific immunological responses	Y	N
Wang et al. [27], USA	IL-6, IL-8, IL-10 and TNF-α	*Lactobacillus rhamnosus* GG ATCC 53103	Gnotobiotic pigs	The study evaluated the modulatory effects of *L. rhamnosus* GG on transplanted human gut microbiota and intestinal immune cell signalling molecules in gnotobiotic pigs vaccinated with rotavirus vaccine	Reverse Transcription-Polymerase Chain Reaction (RT-PCR) was done using specific primers to cytokine	*L. rhamnosus* GG exerted significant modulatory effects on the intestinal immune cell signalling molecules in pigs vaccinated with rotavirus vaccine	Y	N
Kandasamy et al. [21], USA	IL-6 and IL-10	*Escherichia coli* Nissle and *Lactobacillus rhamnosus* GG ATCC 53103	Gnotobiotic piglets	The comparative effects of *L. rhamnosus* GG and *E. coli* Nissle strains were determined on virulent human rotavirus (Wa) strain	IL-6 and IL-10 cytokine levels in spleen and ileal mononuclear cells were determined by ELISA	*E. coli* Nissle when compared with *L. rhamnosus* GG had greater beneficial effects in ameliorating rotavirus infection and	Y	N

(continued on next page)

Table 1 *(continued)*

Authors, year, country	Cytokines	Substances	Animals	Methods	Cytokine assays	Improved characteristics	R	B
				infection in piglet model		promoted the development of intestinal immune responses		
Vlasova et al. [22], USA	IFN-α, IL-6, TNF-α, IL-12 and IL-10	*Lactobacillus rhamnosus* GG ATCC 53103 and *Escherichia coli* Nissle 1917	Gnotobiotic pigs	Anti-rotavirus effects of *L. rhamnosus* GG *and E. coli* Nissle 1917 was evaluated on human rotavirus (Wa) strain infected pigs and their influence on immunoregulatory and immunostimulatory cytokines were studied	The levels of cytokine in the spleen, blood, ileum and duodenum were examined by ELISA	*E. coli* Nissle 1917 mediated greater protection than *L. rhamnosus* GG against rotavirus infection. It enhanced innate immune response and modulated mucosal immunity	Y	N

R, Reporting of randomization; B, Reporting of Blinding; Y, Yes; N, No.

Table 2
Studies of cytokine responses to plant-derived products and secondary metabolites in rotavirus infection.

Authors, year, country	Cytokines	Substances	Animals	Methods	Cytokine assays	Improved characteristics	R	B
Wu et al. [35], China	IL-2, IFN-γ, IL-4 and IL-10	Qiwei Baizhu powder (QWBZP), a herbal product	Suckling mice	Suckling mice with human rotavirus (Wa) strain induced diarrhoea was used to study the densities of T cell subsets and expressions of their cytokines in small intestinal mucosa epithelial cells	Determination of cytokine expressions in small intestine by semi-quantitative RT-PCR was done	QWBZP exhibited antiviral effect through modulating the densities of T cell subsets and expressions of Th1/Th2 cytokines in small intestinal epithelial cells	Y	N
Alfajaro et al.[36], Republic of Korea	IL-8, IL-10, IFN-β, IFN-γ and TNF-α	*Glycyrrhiza uralensis* Fisch (Fabaceae)	Piglets	Anti-rotavirus activity of *G. uralensis* in colostrums-deprived piglets inoculated with porcine rotavirus (RVA K85) strain was investigated. Determination of mRNA expression levels of inflammation-related cytokines, signalling molecules and transcription factor in small intestine and spleen	RT-PCR assays using specific primer pairs to cytokines in the small intestine and spleen were performed	*G. uralensis* lessened rotavirus-induced diarrhoea in colostrum-deprived piglets. It markedly improved the mRNA expression levels of inflammation-related cytokines, signalling molecules and transcription factors	Y	N
Hendricks et al. [37], USA	IFN-γ and IL-10	18β-Glycyrrhetinic acid	C57BL/6 mice	Effect of 18β-Glycyrrhetinic acid to modulate immune responses in mice inoculated with murine rotavirus (EW) strain was determined	PCR analysis was carried to measure the mRNA expression levels of cytokine in duodenal and ileal tissues	18β-Glycyrrhetinic acid shortened the duration of faecal rotavirus antigen shedding, modulated immune cell responses in the gut and attenuated viral replication	N	N

R, Reporting of randomization; B, Reporting of Blinding; Y, Yes; N, No.

Table 3
Studies of cytokine responses to fungal secondary metabolite and food-derived products in rotavirus infection.

Authors, year, country	Cytokines	Substances	Animals	Methods	Cytokine assays	Improved characteristics	R	B
Hester et al. [42], USA	TNF-α, IL-1 and IL-8	Human milk oligosaccharides	Piglets	Anti-rotavirus activity of human milk oligosaccharides was tested against porcine (OSU) and human (Wa) rotavirus strains infected piglet model	RT-PCR using porcine-specific primers for cytokine were analyzed in ileal mucosa	Human milk oligosaccharides reduced rotavirus infection by inhibiting the binding of virus to the epithelial cells and lowered rotavirus replication	Y	N
Shen et al. [43], China	IL-4, IL-2 and IFN-γ	Cyclosporin A	BALB/c mice	The mechanism of action of cyclosporin A on human rotavirus (Wa) strain in mice model was explored	IL-4, IL-2, and IFN-γ concentrations in small intestine were determined by ELISA	Cyclosporin A enhanced the elimination of rotavirus antigen from mouse. It inhibited rotavirus replication through promoting type 1 IFNs-based intracellular innate immunity in host cells	Y	N
Zhao et al. [45], China	IL-2, IL-6 and IFN-β	Dietary Vitamin D	Pigs	The study was conducted to analyze the vitamin D supplementation against a pig model of porcine rotavirus (OSU) strain infection	Levels of cytokine in serum was determined using ELISA kits	Vitamin D supplementation improved the faecal consistency of rotavirus challenged pigs and reversed the damage caused to intestinal epithelium. It mediated innate antiviral immune response by stimulating the expressions of IFNs-stimulated genes	Y	N
Li et al. [46], USA	IL-4, IL-6, IL-8, IL-10, IL-12 and IFN-γ	Human milk oligosaccharides	Piglets	Effectiveness of human milk oligosaccharides on mucosal immunity, gut microbiota in porcine rotavirus (OSU) strain inoculated piglets was investigated	RT-PCR was performed to assay the cytokines gene expression in ileal tissue	Human milk oligosaccharides reduced the duration of rotavirus-induced diarrhoea. It modulated colonic microbiota and enhanced Th1/Th2 cytokine mediated immune responses to rotavirus infection	Y	N
Shen et al. [44], China	IFN-α, IFN-β, IL-8, IL-10, IFN-γ and TNF-α	Cyclosporin A	BALB/c mice	Anti-rotavirus efficacy of cyclosporin A in neonatal mice inoculated with murine (EW) rotavirus strain was studied	RT-PCR with primers specific to cytokines were studied in spleen and small intestine	Cyclosporin A inhibited rotavirus diarrhoea and improved faecal virus shedding and intestinal lesion changes. It modulated the inflammatory reaction alleviating intestinal lesions produced by host inflammation	Y	N

R, Reporting of randomization; B, Reporting of Blinding; Y, Yes; N, No.

Fig. 2. Methodological quality of studies. Green bars represent the proportion of studies for which the item was applicable; red bars represent the proportion of studies for which the item was not applicable; yellow bars represent the proportion of studies for which the item was unclear. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

vaccine efficacy due to their enhanced induction of innate immune responses.

In the gnotobiotic pig model, the use of dietary rice bran in combination with probiotic *L. rhamnosus GG* and *E. coli* Nissle species reduced human rotavirus induced diarrhoea severity and the interval of episodes and strengthened probiotic growth, gut epithelial health and innate immune responses [34]. In addition, the combination enhanced intestinal IFN-γ concentrations, resulting in Th1 dependent immune responses. These have led to investigators hypothesizing this combination could lead to anti-inflammatory and intestinal epithelial cure for rotavirus infections due to its ability to promote intestinal innate immune system responses.

3.4. Cytokine responses to plant-derived products and secondary metabolites in rotavirus infection

Three studies reported the use of phytotherapeutics in the regulation of immune responses in rotavirus-inoculated mice and piglets including Qiwei Baizhu powder (QWBZP), a herbal product from China, *Glycyrrhiza uralensis* Fisch (Fabaceae) and 18β-Glycyrrhetinic acid. These substances are reported to improve small intestinal pathological changes, shorten the duration of diarrhoea and reduce the levels of virus shedding [35–37] (Table 2). Phytochemicals effects on mRNA concentrations of pro-inflammatory, Th1 and Th2 cytokines were investigated to describe their regulatory roles in rotavirus-induced reactive oxygen species formation [35,36]. IFN-γ, IL-5, INF-α and IL-12 secreted from intra-epithelial lymphocytes exhibited strong anti-infection properties and immune tolerance protecting the small intestinal mucosa epithelial cells [38–40]. Plant extracts and natural molecules improved the mRNA expression of IL-2, IL-4, IL-8, and IL-10, IFN-γ, IFN-β and TNF-α and related signalling molecules in small intestinal mucosa epithelial cells. These cytokines have special characteristics, interfering rotavirus-induced reactive oxygen species formation and protecting intestinal epithelial cells [41]. These studies

Fig. 3. Risk level and type of bias in the studies. Green: Low risk; Yellow: Unclear risk; Red: High risk. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

highlight the potential of Th1/Th2 cytokine components as anti-rotavirus drug targets as novel antivirals for rotavirus gastroenteritis.

3.5. Cytokine responses to fungal secondary metabolite and food-derived products in rotavirus infection

Table 3 describes the role of fungal secondary metabolites and food-derived products for the management of rotavirus gastroenteritis and their immunomodulatory effect on virulent rotavirus infection [42–46]. Human milk oligosaccharides and vitamin D significantly inhibits rotavirus infectivity and enhances Th1/Th2 cytokine-mediated antiviral responses against experimental rotavirus diarrhoea [42,45,46]. Dietary supplements that contains medicinal value are useful as functional foods with physiological benefits for immunocompromised children [47]. Human milk is likely to contain anti-rotavirus functional components and breast-fed infants have a lower incidence of diarrhoeal disease than formula-fed children [48,49].

Cyclosporin A, a secondary metabolite of *Tolypocladium inflatum* has been used to treat autoimmune disease [50]. Its role on immunoregulatory, anti-infective and immune responses to rotavirus infection include the enhancement of mucosal Th1/Th2 cytokine responses, modulating mucosal immunity and inhibiting rotavirus binding and/or replication [43,44]. Intestinal mucosa Th1/Th2 cytokine concentrations replicate the immunomodulatory action of therapeutic agents on rotavirus-induced acute gastroenteritis [46] and may reflect the curative effects of the agents in animals challenged with rotavirus and the development of intestinal immune responses through customized Th1/Th2 cytokines quantities. A balanced Th1/Th2 cytokine expression is essential in the initiation and coordination of the immune activities against viral infections [28]. These dietary supplements and secondary metabolites may constitute a novel nutritional approach to protect against rotavirus infection in infants and animals.

3.6. Methodological quality/risk of bias

Studies lacking well designed randomization and inadequate outcome blinding of outcome measurements have a high risk of bias [51]. We found that most experimental studies carried a high risk of bias in relation to measurements to assess precision, directness and applicability of the interventions or exposures. Eighteen of the 19 studies allocated the experimental animals randomly, but only one reported the blinding procedures. The protocol details for the blind assessment of the experimental drugs on animal groups or the investigator awareness of the group allocation and results was not clearly described. The methodological quality of the studies is displayed in Figs. 2 and 3.

4. Conclusion

This systematic review combined scattered information on the impact of therapeutic substances in the immune function and cytokine responses against rotavirus infection. The review may further our understanding of the antivirals mechanisms enhancing Th1/Th2 cytokine responses, the stimulation of the immune system and inhibition of rotavirus binding and/or replication in animal models. This report highlights the immunomodulating effects that reduce rotavirus diarrhoea and enhance immune responses during rotavirus infection.

Conflicts of interest

The authors declare they have no conflicts of interest for this publication.

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