VAGNER DIAS RAIMUNDO

EFEITOS DOS TERPENOS NO TRATAMENTO DA LEISHMANIOSE VISCERAL: UMA REVISÃO SISTEMÁTICA DA EVIDÊNCIA PRÉ-CLÍNICA

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Biologia Celular e Estrutural, para obtenção do título de *Magister Scientiae*.

Orientador: Eduardo de Almeida Marques da Silva

Coorientadora: Mariana Machado Neves

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Assentimento:

Vagner Dias Raimundo
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Orientador

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De Deus é a vingança, o início e o final.
(Moisés Cleyton)

Não há vitória sem barreiras no caminho.
(Anderson Freire)

RESUMO

RAIMUNDO, Vagner Dias, M.Sc., Universidade Federal de Viçosa, fevereiro de 2022. **Efeitos Dos Terpenos No Tratamento Da Leishmaniose Visceral: Uma Revisão Sistemática Da Evidência Pré-Clínica**. Orientador: Eduardo de Almeida Marques da Silva. Coorientadora: Mariana Machado Neves.

As leishmanioses são antropozoonoses de transmissão vetorial e constituem um problema de saúde pública atualmente, sobretudo por serem doenças negligenciadas. A leishmaniose visceral (LV) é a forma mais grave e potencialmente fatal. Ainda não existem vacinas contra a leishmaniose humana, e a quimioterapia é tóxica e potencialmente teratogênica. As plantas, usadas terapeuticamente há milhares de anos, contribuem na busca por novas drogas contra LV. Terpenos, um dos grupos de metabólitos secundários das plantas, já tiveram várias atividades descritas e foram escolhidos para terem os seus potenciais efeitos leishmanicidas em modelos murinos revisados sistematicamente. Os artigos foram pesquisados nas bases Web of Science, Scopus e PubMed. Trinta e quatro artigos foram incluídos na síntese e posteriormente, realizou-se a análise de viés dos trabalhos com a ferramenta SYRCLE. Em geral, o tratamento com os terpenos não apresentou toxicidade ou a toxicidade era desprezível, resultando em aumento da resposta imune do tipo Th1, redução de citocinas Th2, aumento na produção de óxido nítrico (NO) e redução da carga parasitária (> 90% até > 99%). Os terpenos também atuaram na membrana plasmática e na DNA topoisomerase do parasita, induzindo a apoptose. Os estudos também revelaram o uso de carreadores de terpenos. Isso evita a rápida excreção dos fármacos e os libera no sítio da infecção. O uso de alguns derivados de terpeno destacou-se por reduzir a toxicidade. Essas ações positivas do tratamento de LV com terpenos abrem caminho para outros testes pré-clínicos e dão um direcionamento para testes em humanos.

Palavras-chave: Leishmaniose visceral. Terpenos. Tratamento. Terapia. Fitoterápicos.

ABSTRACT

RAIMUNDO, Vagner Dias, M.Sc., Universidade Federal de Viçosa, February, 2022. **Effects of terpenes in the treatment of visceral leishmaniasis: a systematic review of preclinical evidence.** Advisor: Eduardo de Almeida Marques da Silva. Co-advisor: Mariana Machado Neves.

Leishmaniasis is a vector-borne anthropozoonosis and is currently a public health problem, mainly because it is a neglected disease. Visceral leishmaniasis (VL) is the most serious and potentially fatal form. There are still no vaccines against human leishmaniasis, and chemotherapy is toxic and potentially teratogenic. Plants, used therapeutically for thousands of years, contribute to the search for new drugs against VL. Terpenes, one of the groups of secondary plant metabolites, have already had several activities described and were chosen to have their potential leishmanicidal effects in murine models systematically reviewed. Articles were searched in the Web of Science, Scopus and PubMed databases. Thirty-four articles were included in the synthesis and later, a bias analysis was performed with the SYRCLE tool. In general, the treatment with terpenes showed no toxicity or the toxicity was negligible, resulting in an increase in the Th1-type immune response, a reduction in Th2 cytokines, an increase in the production of nitric oxide (NO) and a reduction in the parasite load (> 90% up to >99%). Terpenes also acted on the plasma membrane and DNA topoisomerase of the parasite, inducing apoptosis. Studies have also revealed the use of terpene carriers. This prevents the rapid excretion of drugs and releases them at the site of infection. The use of some terpene derivatives stood out for reducing toxicity. These positive actions of the treatment of VL with terpenes pave the way for other preclinical trials and provide a direction for human trials.

Keywords: Visceral leishmaniasis. Terpenes. Treatment. Therapy. Herbal Medicines.

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INTRODUÇÃO GERAL

Leishmanioses constituem um grupo de doenças tropicais negligenciadas e permanecem como um grande problema de saúde pública (KARAMYSHEVA *et al*., 2020). As leishmanioses são transmitidas pela picada de insetos flebotomíneos fêmeas que estejam infectadas com protozoários do gênero *Leishmania*. Considerando as doenças tropicais negligenciadas, as leishmanioses são a terceira maior causa de mortalidade, ficando atrás apenas da doença do sono e da doença de Chagas (TABBABI, 2019).

De acordo com informações da Organização Mundial da Saúde (OMS), em 2020, Brasil, Sudão, Etiópia, Quênia, Índia e Eritréia identificaram mais de mil novos casos de LV, enquanto outros países da América, África e Ásia reportaram até 999 novos casos, conforme mostra a Figura 1. A OMS estima, ainda, que anualmente ocorram entre 50.000 a 90.000 novos casos de LV no mundo (WHO, 2021).

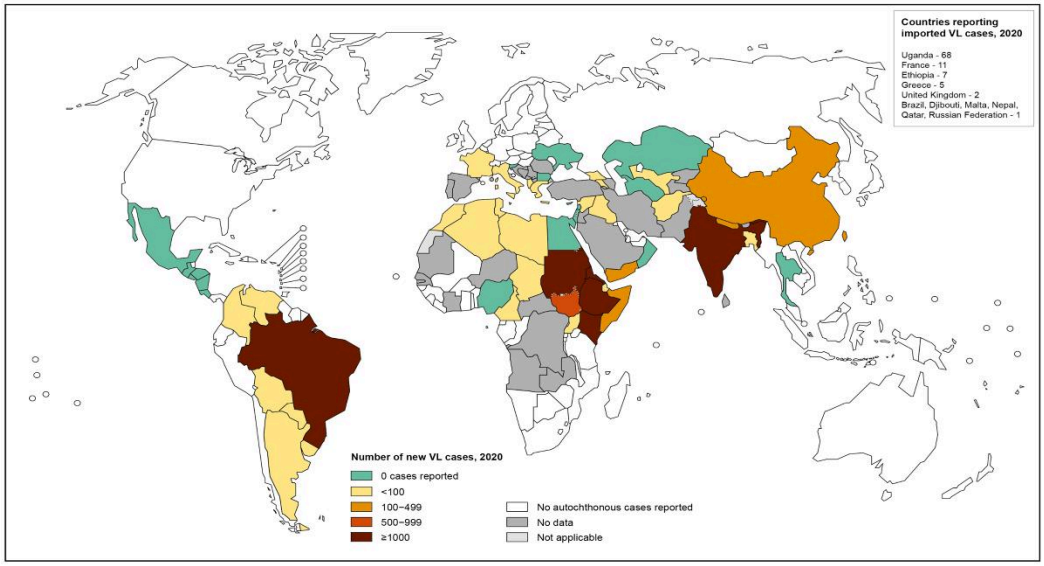

Figura 1. Mapa mostrando o status da endemicidade da LV no ano de 2020. O padrão de cores da figura indica que as regiões do mapa coloridas mais fortemente demonstram maior número de novos casos da doença relatados no ano da análise.

Diferentes espécies de *Leishmania* causam diferentes formas clínicas da enfermidade. Dentre as manifestações de leishmaniose pode-se destacar a existência da leishmaniose tegumentar (LT) e da LV. A LT, também referida como leishmaniose cutânea, pode ser causada pelas espécies *Leishmania major*, *Leishmania tropica*, *Leishmania aethiopica*. A leishmaniose mucocutânea pode ser causada por *Leishmania braziliensis*, enquanto a LV pode ser causada por *Leishmania donovani* ou *Leishmania infantum chagasi* (AMARASINGHE & WICKRAMASINGHE, 2020). Existe, ainda, a chamada leishmaniose dérmica pós-calazar

(PKDL), que se caracteriza por manifestações na pele algum tempo depois da cura da LV em um indivíduo (GEDDA *et al*., 2020).

O ciclo de vida de *Leishmania* acontece em dois estágios: um deles é caracterizado por transformações do parasito no hospedeiro invertebrado, ou seja, o inseto vetor. No outro estágio acontecem transformações do parasito quando ele se localiza no hospedeiro vertebrado (Figura 2). Durante o repasto sanguíneo em hospedeiros vertebrados infectados, os flebotomíneos fêmeas adquirem o protozoário, por ingerirem junto com o sangue os macrófagos infectados com formas amastigotas do patógeno. Estas formas são liberadas no intestino do vetor após a ruptura dos fagócitos, iniciando o ciclo de vida do parasito neste hospedeiro (FREITAS-MESQUITA *et al*., 2021). Durante esta etapa, as amastigotas transformam-se em promastigotas procíclicas; tal mudança é induzida pelas condições do ambiente no interior do intestino do vetor em relação às condições do ambiente do hospedeiro vertebrado, como o aumento do pH e diminuição da temperatura (DOSTÁLOVÁ & VOLF, 2012). As promastigotas procíclicas começam a se multiplicar rapidamente, e diferenciam-se em promastigotas metacíclicas, responsáveis pela infecciosidade do parasito. As formas metacíclicas migram para a faringe do inseto vetor para que, ao realizar uma nova hematofagia, ele inocule os parasitas em outro hospedeiro vertebrado (BEATTIE & KAYE, 2011). Ao serem inoculadas, as promastigotas metacíclicas são fagocitadas por células do sistema mononuclear fagocitário e por neutrófilos, os quais são recrutados para o sítio de infecção. Dentro dos macrófagos, as promastigotas metacíclicas transformam-se em amastigotas e se multiplicam bastante ao ponto de causar a lise dessas células, momento em que há a liberação das amastigotas multiplicadas. Essas amastigotas podem ser fagocitadas por outros macrófagos ou podem ser ingeridas por outro inseto vetor durante o repasto sanguíneo, possibilitando a manutenção do ciclo da doença (BEATTIE & KAYE, 2011; DOSTÁLOVÁ & VOLF, 2012).

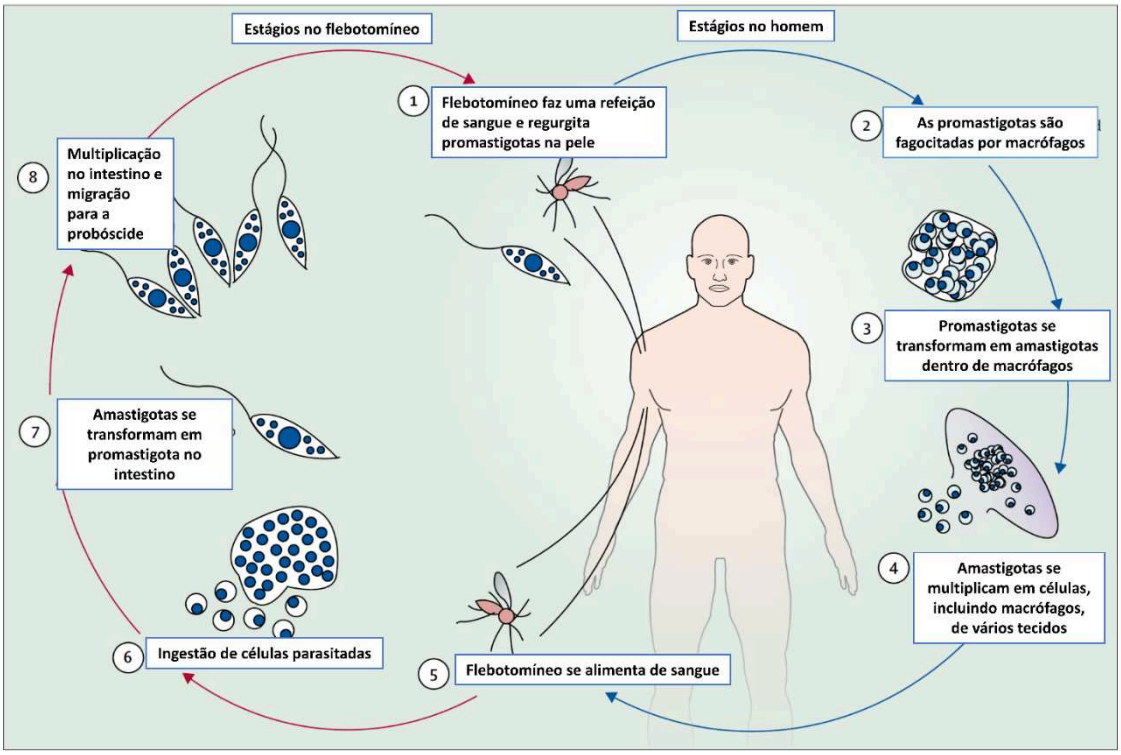

Figura 2. Ciclo de vida de *Leishmania* spp. Conforme evidenciado pela imagem acima, o ciclo compreende dois estágios, um no hospedeiro invertebrado (o inseto vetor) e outro no hospedeiro vertebrado (o ser humano, por exemplo), com mudanças nas formas protozoárias em cada estágio. Adaptado de Burza *et al*., 2018.

A LV, considerada a forma mais grave das leishmanioses, é potencialmente fatal se não for tratada. Ela é caracterizada por febre irregular e persistente, hepatoesplenomegalia, pancitopenia, hipergamaglobulinemia e perda de peso (BURZA *et al*., 2018).

Não existe nenhuma vacina que previna a leishmaniose humana, e a quimioterapia atualmente utilizada provoca efeitos colaterais (TABBABI, 2019). Além disso, os parasitos têm apresentado resistência às drogas, o que acaba obstruindo os progressos obtidos durante o tratamento (KARAMYSHEVA *et al*., 2020). Enquanto uma vacina não é descoberta, o caminho a ser seguido é o tratamento dos doentes. Porém, em virtude da toxicidade das drogas administradas (ARONSON & JOYA, 2019; CAPELLI-PEIXOTO *et al*., 2019) e por elas apresentarem um potencial teratogênico (SUNDAR & SINGH, 2018), revela-se a necessidade da busca por terapias alternativas.

Considerando a potencial letalidade da LV (BUENO *et al*., 2019; SUNDAR & RAI, 2005), foi realizada esta revisão sistemática sobre os efeitos de alguns compostos contra esta doença. Em suma, revisão sistemática é a reunião, em um documento único, de resultados dos estudos originais. Este tipo de revisão se caracteriza por objetivos claramente articulados, critérios de inclusão e exclusão previamente definidos, pesquisa abrangente de estudos

originais, avaliação da qualidade dos estudos, análise de dados, síntese e apresentação dos resultados (AROMATARIS & PEARSON, 2014; SIDDAWAY *et al*., 2019).

As plantas fornecem muitos compostos que são utilizados para a base de muitas drogas elaboradas pela indústria farmacêutica (LI & WENG, 2017). Esses compostos são provenientes dos chamados metabólitos secundários. Existem três grupos principais de metabólitos secundários: compostos contendo nitrogênio, compostos fenólicos e terpenos (LI *et al*., 2020).

Dentre esses três grupos, os terpenos possuem representantes que já tiveram um amplo espectro de atividades descrito, como atividades antimicrobiana, antitumoral (DA CRUZ NIZER *et al*., 2021), antioxidante e anti-inflamatória (DIAMOND & BAILEY, 2013). Além disso, os terpenos são os principais constituintes dos óleos essenciais (SOMMANO *et al*., 2020), muito utilizados na indústria de perfumaria, farmacêutica, cosmética e alimentos (DOSOKY & SETZER, 2018).

Terpenos são formados a partir da "regra do isopreno", sendo construídos em blocos de cinco carbonos. Com base na quantidade de resíduos de isoprenos que os constituem, os terpenos podem ser classificados em hemiterpenos, monoterpenos, sesquiterpenos, diterpenos, sesterterpenos, triterpenos e tetraterpenos, cujas constituições variam entre 1 e 8 unidades de resíduos de isopreno. Existem, ainda, os politerpenos, com mais de 8 unidades de resíduos de isopreno, e os denominados terpenos irregulares, conforme ilustrado pela Figura 3 (ASHOUR *et al*., 2010; FELIPE *et al*., 2017).

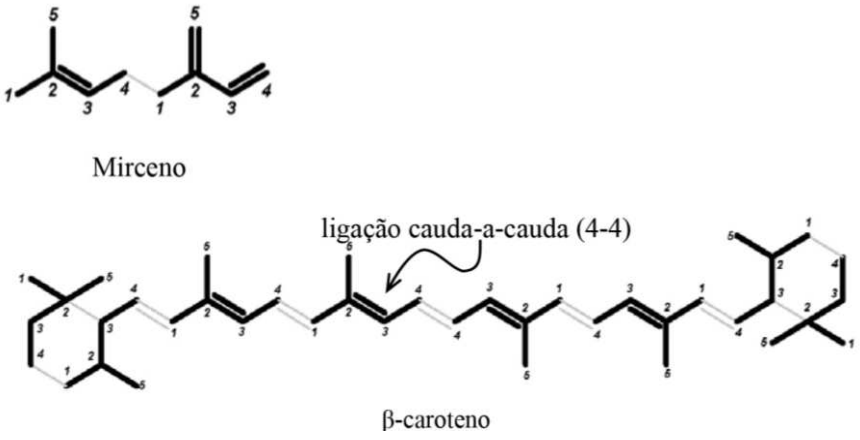

Figura 3. A: Resíduos de isopreno, unidos pela ligação do carbono 1 ao carbono 4, que dá origem aos terpenos regulares. **B:** Ligação do carbono 4 ao carbono 4 entre resíduos de isopreno, que origina os terpenos irregulares. Essas duas formas de ligação caracterizam a chamada "regra do isopreno". Adaptado de FELIPE *et al*., 2018.

Nesse contexto de busca por terapias alternativas, a expectativa sobre o tratamento de uma doença parasitária é de que ele seja capaz de aniquilar diretamente o patógeno que causa determinada enfermidade, ou então que ele possa agir sobre o sistema imunológico para que ele seja capaz de debelar o agente estranho. É conhecido que uma resposta imune eficaz contra a LV deve apresentar um perfil de citocinas Th1 que prevaleça sobre as citocinas Th2 (RAJA *et al*., 2021; GANGNEUX *et al*., 2013). Além disso, elicitar a produção de óxido nítrico por macrófagos, que é um outro mecanismo de defesa do organismo contra a infecção por *Leishmania* (PANDEY *et al*., 2016), é desejável. Dessa forma, realizou-se a presente revisão sistemática com o objetivo de avaliar os efeitos dos terpenos sobre a LV, para verificar se eles são capazes de agir diretamente sobre os parasitos ou se são capazes de modular a resposta imune, ou se eles agem das duas maneiras.

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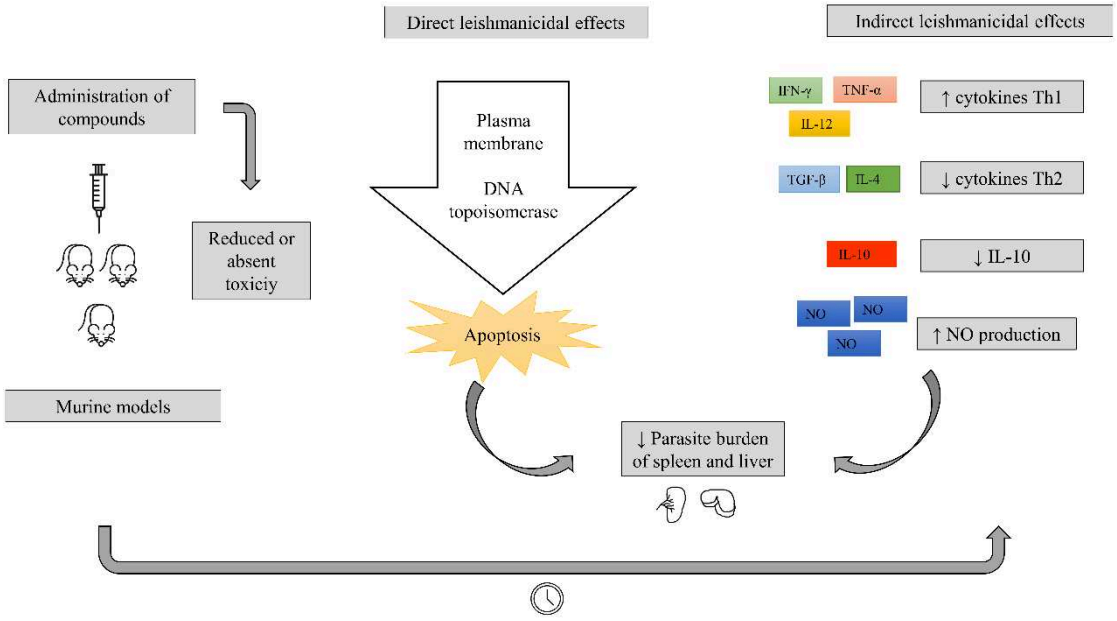
ARTIGO

Effects of terpenes in the treatment of visceral leishmaniasis: a systematic review of preclinical evidence

*Publicado na revista Pharmacological Research.

Graphical Abstract



Abstract

Visceral leishmaniasis (VL) is a serious and potentially fatal neglected disease that is a public health problem in many countries around the world. There are still no vaccines against human VL, and existing chemotherapy is usually toxic. In this sense, viable alternatives to treatments are sought, and plants make this possible through their secondary metabolites. Among these, terpenes have already had a broad spectrum of activities described. This systematic review aimed to evaluate the effects of terpenes in the treatment of VL in rodents using three databases: Pubmed, Scopus, and Web of Science, from what thirty-four articles were included in the synthesis. Bias analysis was done using the SYRCLE. Results showed that triterpenes were the most used terpenes among the selected articles. In general, the treatment with the compounds presents non or negligible toxicity, resulting in an increased Th1 type of immune response profile, reduction of Th2 cytokines, production of nitric oxide (NO), and reduction in the parasite load (> 90% and even > 99%). Terpenes also acted on the plasma membrane and on the DNA topoisomerase of the parasite, inducing apoptosis. The studies also revealed the use of carriers for terpenes, which increase their bioavailability in the body, preventing its rapid excretion, and delivering the drug at the site of infection. The use of some terpene derivatives was highlighted by the improvement in their pharmacokinetics. These positive actions of the VL treatment with terpenes pave the way for other pre-clinical tests and give a direction for tests in humans.

Keywords: Visceral leishmaniasis; Terpenes; Treatment; Therapy; Herbal Medicines.

Some chemical compounds studied in this article:

1) Artemisinin - PubChem CID: 68827

2) Asiaticoside - PubChem CID: 11954171

3) Bassic acid - PubChem CID: 160465

4) Cedrol - PubChem CID: 65575

4) Glycyrrizic acid - PubChem CID: 14982

6) Lupeol - PubChem CID: 259846

7) Oleanolic acid - PubChem CID: 10494

8) Thymol - PubChem CID: 6989

9) Ursolic acid - PubChem CID: 64945

10) Amarogentin - PubChem CID: 115149

1. Introduction

Leishmaniasis remains a current public health problem. It is part of the group of neglected tropical diseases, that do not receive the necessary government attention. There are no sufficient investments in research areas focused on vaccines and treatments development [1,2]. Its transmission is vectorial, that is, it occurs through the bite of infected female phlebotomine sandflies with protozoan parasites of the genus *Leishmania* [3,4]. An individual affected by this disease may present a cutaneous manifestation mainly [5], besides mucocutaneous and visceral forms. VL, also named kala-azar, is the most severe and potentially fatal form of this disease [6].

Leishmaniasis is endemic in North Africa, Asia, the Middle East, the Mediterranean region and Central and South America, with about 1.5-2 million new records per year [7]. In 2019, more than 87% of skin cases occurred in Afghanistan, Algeria, Brazil, Colombia, Iran, Iraq, Libya, Pakistan, Syrian Arab Republic and Tunisia. More than 90% of mucocutaneous leishmaniasis cases occurred in Bolivia, Brazil, Ethiopia and Peru, while Brazil, Ethiopia, Eritrea, India, Iraq, Kenya, Nepal, Somalia, South Sudan and Sudan concentrated more than 90% of VL cases [8].

There are still no vaccines against human leishmaniasis. Currently, the first-line treatments of this disease comprise the use of pentavalent antimonials, sodium stibogluconate, and meglumine antimoniate [9]. Amphotericin B (AmB), pentamidine, and paromomicin are second-line drugs used to replace these treatments in case of unsuccess [10]. Overall, the administration of these substances uses the parenteral route, which may cause toxic side

effects to kidneys and liver [11]. The toxicity of these drugs and the resistance developed by the parasites [12-14] highlight the need to seek new alternatives to these treatments.

In this context, plants represent viable options for current treatments through their secondary metabolites. Natural compounds from plant sources have shown potential pharmacological activity against *Leishmania* spp., including alkaloids, phenolic compounds, terpenes and flavonoids [15]. Among them, terpenes found in essential plant oils (e. g., lavender, peppermint, and lemongrass) have received attention for their antimicrobial, antioxidant, anti-inflammatory, and antitumor activities [16].

Thus, we classify terpenes regarding the number of units into hemiterpenes, monoterpenes, iridoids, sesquiterpenes, diterpenes, sesterterpenes, triterpenes, tetraterpenes, polyterpenes, and irregular terpenes. There are also polyterpenes, with more than eight isoprene units, and irregular terpenes [20].

Interestingly, no reviews were found that addressed the *in vivo* effects of terpenes in the VL treatment. Studies have reported their activity against trypanosomatids [21,22] with low *in vitro* and *in vivo* toxicity, confirming their promising potential for pharmacotherapy [23,24]. In this light, we carried out this systematic review focused on gathering and evaluating the effects of terpenes in the VL treatment in murine models.

2. Methods

2.1 Elaboration of the main question

The main question we sought to answer in this systematic review was: Do the effects of terpenes from plant sources in the VL treatment in murine models demonstrate potential for therapeutic use in humans?

2.2 Protocol, registration and PRISMA

This review was registered at PROSPERO (International Prospective Register of Systematic Review, http://www.crd.york.ac.uk/PROSPERO) with registration number CRD 42020221969. It was also conducted according to the Preferred Reporting Items for Systematic and Meta Reviews-Analyses (PRISMA) statement [25].

2.3 Search strategy

The search was performed using three databases, PubMed/MEDLINE, Scopus, and Web of Science [26], and the following terms: "visceral leishmaniasis", "kala-azar", "*Leishmania donovani", "Leishmania infantum", "Leishmania chagasi", "Leishmania infantum chagasi"* in combination with "terpenes", "cannabinoids", "carotenoids", "diterpenes", "dolichols", "gefarnate", "hemiterpenes", "monoterpenes", "polyisoprenyl phosphates", "polyprenols", "sesquiterpenes", "sesterterpenes", and "triterpenes". There was no restriction regarding the year of publication of the articles. The reference lists of all studies were read carefully to find additional studies potentially relevant to be included in the systematic review.

2.4 Selection of studies

Studies were included in the review once they met the following criteria: 1) used naturally occurring terpenes in plants for VL treatment in rodents; 2) published in indexed journals and peer-reviewed. Articles were excluded if 1) experiments were conducted using *in vitro* assays; 2) were secondary studies (reviews and conference proceedings). After the literature search, duplicates were removed using the Mendeley® software, and titles, abstracts, and keywords were then read for the initial screening. Two researchers (VDR and RPRC) independently recovered the studies in their full text and analyzed their eligibility

based on the defined criteria using the StArt® software. Doubts and disagreements between researchers were discussed with a third researcher (EAMS) and resolved by consensus [26].

2.5 Data extraction

Data extraction was also performed independently by two researchers (VDR and RPRC). The information extracted from the studies was as follows: *i)* publication characteristics: authors, year and journal of publication, title, and country; *ii)* characteristics of the experimental model: animal model, sex, age, species of *Leishmania*, inoculation route of parasites, type of terpene, source, dosage of terpene, administration route, frequency, treatment duration, control groups, iii) toxicity, animal death occurred during the treatment weight, suggested mechanisms of action, increase of NO and cytokines production and reduction of parasite load [27]. These data were inserted into a Microsoft Excel software version 2016 spreadsheet.

2.6 Bias Analysis

The risk of bias was analyzed based on the guidelines provided in the Risk of Bias Tool for Animal Studies SYRCLE (Systematic Review Center for Laboratory Animal Experimentation) [28]. The use of this tool requires reading the methodology and results sections of the primary studies included in the systematic review. Analyzes of these sections were based on reports of random sequence generation for group allocation, group similarity at baseline, allocation concealment (selection bias), random housing during the experiment, blinding of researchers (performance bias), random selection of animals to evaluate the results, blinding of the evaluator (detection bias), adequate treatment of incomplete data (attrition bias), reporting free of selective results (reporting bias) and other biases, considered here as reports of approval of the Ethics Committee and proper treatment of the animals used,

such as supply of feed and water *ad libitum*. For each type of bias, there are signaling questions: if the answers to these questions are "yes", it indicates "low risk of bias"; if they are "no", it means "high risk of bias"; if there is uncertainty about the risk of bias, the "unclear" option is considered. Review Manager software version 5.4 was also used to conduct this analysis and to construct the risk of bias figures [29].

3. Results

3.1 Studies included in the qualitative synthesis and in the qualitative analysis

The flowchart summarizing the literature search process is shown in Fig. 1. The initial search resulted in 692 records identified through database searches in PubMed/MEDLINE, Scopus, and Web of Science. Following the removal of duplicates and exclusions, 32 studies were considered eligible for this review. In addition, two studies were selected through the reference lists, totalizing 34 studies. From those eligible studies, most of them were from India (n = 22), followed by Belgium (n = 3), Brazil, China, Spain (n = 2, each), Saudi Arabia, Iran, and Kenya (n = 1, each) (Supplementary Table 1).

Regarding the animal model, most studies used BALB/c mice (n = 19) and golden hamsters (n = 13), and two studies used both species in their experiments. Male animals were mainly used in the studies (n = 7), whereas females were the animal sex present in five studies. Six articles used both male and female animals, 14 did not mention this information, and two used the unclear term "same sex". The age of the animals ranged between three and eight weeks (n = 15 studies). Sixteen studies did not report the animal age, and three articles used the term same age to refer to the animal age. *L. donovani* was the main species used for inducing leishmaniasis (n = 28 articles), followed by *L. infantum* (n = 5 articles) and *L. infantum chagasi* (n = 1 article). These studies inoculated promastigote (n = 20) and amastigote forms (n = 13). One study did not report the form used, mentioning only the

success of the induced infection. Intravenous (n = 13 studies), intracardiac (n = 13 studies) and intraperitoneal (n = 6 studies) vias were used as routes of parasite inoculation. Only two studies did not report the route of inoculation used (Supplementary Table 2).

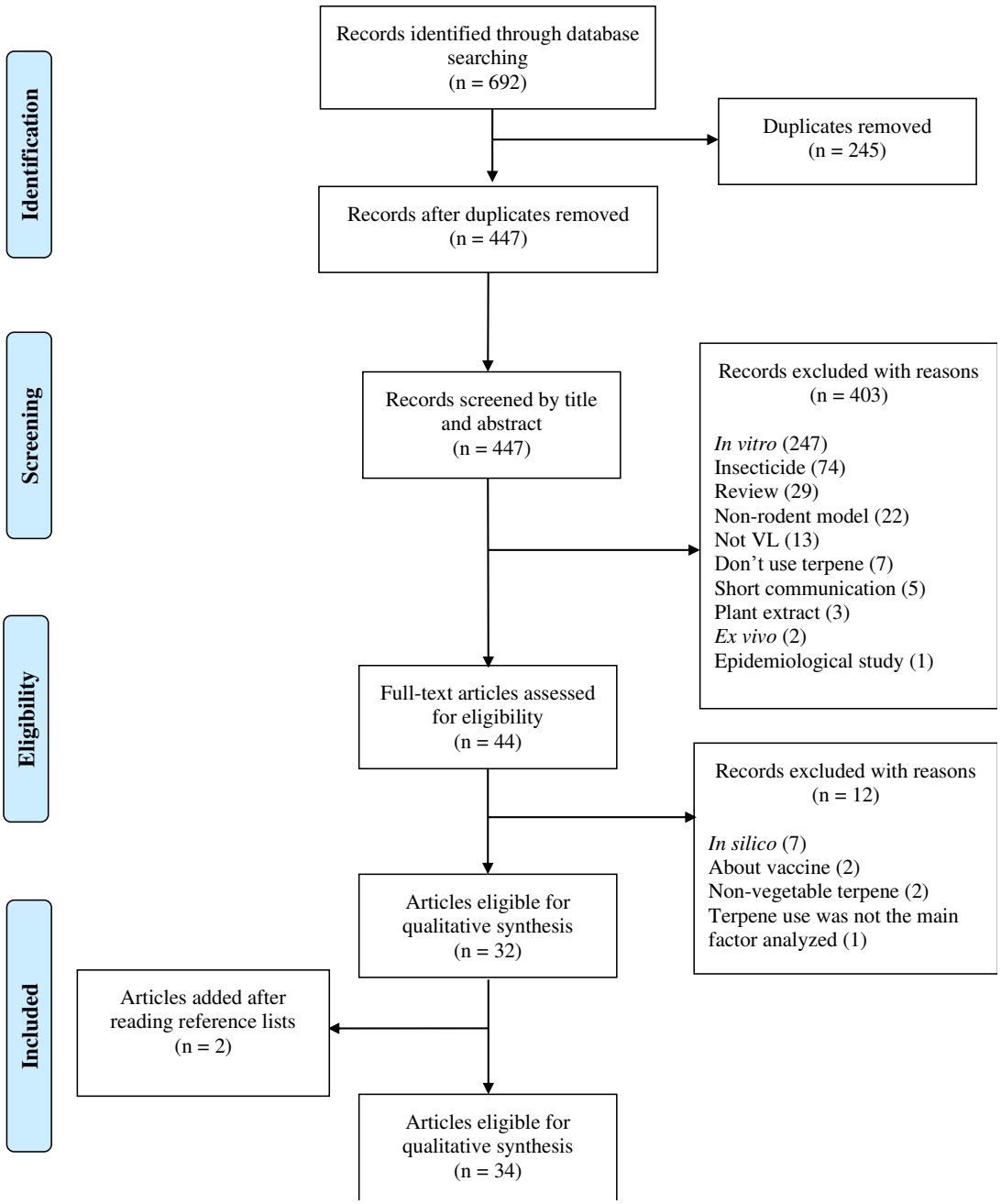


Figure 1 – Flowchart highlighting the steps of article selection based on PRISMA recommendations. Adapted from [103].

3.2 Types of terpenes, sources, routes of administration, treatment time and controls used

Triterpenes were the main compounds studied (n = 19), followed by sesquiterpenes (n = 7), diterpenes (n = 5), and monoterpenes (n = 3; Table 1). Fifteen studies used only free forms of terpenes, including maesabalides (n = 3), glycyrrhizic acid (n = 2), ursolic acid (n = 2), lupeol (n = 2), dihydrobetulinic acid, asiaticoside, clerodane, artemisinin, α-bisabolol and "thymol, carvacrol and linalool" (n = 1, each). One article evaluated associations of vitamin D3 with retinoic acid and retinoic acid with chenodeoxycholic acid in the VL treatment, while another study used the combination of glycyrrhizic acid and sodium stibogluconate (n = 1; Table 2). Other studies used terpene-derived compounds, such as those derived from glycyrrhizic acid (18β-glycyrrhetinic acid; n = 2) as treatment, thymol derivatives (acetyl thymol and benzoyl thymol), "heteroretinoid amide derivatives", and artemisinin derivative (dihydroartemisinin) (n = 1, each). Only one article used a mixture of artesunate (derived from artemisinin) with diminazene (Table 3).

Ten studies used terpene in carriers (Table 4), such as nanostructured lipid carrier alone, nanostructured lipid carrier plus chitosan, nanoliposome alone, poly-lactic co-glycolic acid nanoparticles, D-alpha- tocopherol polyethylene glycol 1000 succinate nanoparticles, oil-in- water microemulsion, and poly-DL-lactide (PLA) nanoparticles, nanogel and PLA nanoparticle, liposome alone, liposome, niosome, microsphere and nanocapsule, liposome and niosome (n = 1, each). One study used a terpene derivative loaded in a carrier 14-deoxy-1-oxoandrographolide in liposome, noisome, and microsphere (Table 4). Thirteen studies obtained it from plants after using methanolic extraction (n = 7) and ethanolic extraction (n = 2), whereas four studies only mentioned the plants used. Moreover, 18 used synthesized terpenes purchased from companies, and three studies did not report the source of the compound used.

The dosage of compounds ranged between 200 µg and 1000 mg/ kg^{-1} body weight (Table 1). The compounds were mainly administered through the intraperitoneal route (n = 14), followed by oral (n = 9) and subcutaneous vias (n = 9). Two articles did not describe the route of administration used. The duration of treatment varied between 1 day and 30 days, as shown in Table 1. Likewise, the control group also varied. The control group was composed of infected and untreated mice in nine studies. Other studies used phosphate-buffered saline (PBS; n = 5), dimethylsulfoxide (DMSO; n = 1), AmB (n = 1), sodium stibogluconate (n = 1), and unspecified placebo (n = 1) in control animals. Moreover, eligible articles used more than one control group, such as uninfected mice, untreated infected mice, and AmB (n = 1); uninfected control and infected control receiving water (n = 2); PBS + DMSO and PBS groups (n = 1); no treatment group and miltefosine treated (n = 1); uninfected untreated mice, infected mice treated with PBS, and infected mice receiving AmB (n = 1); empty nanoparticles and AmB (n = 1); Glucantime and water (n = 1); no treatment group and AmB treatment (n = 1); untreated control and miltefosine (n = 1); uninfected mice, untreated infected mice, infected mice receiving antimony sodium gluconate, and infected mice receiving DMSO (n = 1); Pentostam and AmB (n = 1); placebo of PLA nanogels and nanoparticles (n = 1), unspecified placebo and AmB (n = 1). Some studies used olive oil (n = 1) and vegetable oil (n = 1) as control groups (Table 1).

Table 1. Description of the terpenes used, ways of obtaining and information regarding dosages, frequency, treatment time, routes of administration of the compounds and controls used

Reference	Tested compound	Type of terpene	Source	Dosage	Frequency and time of treatment	Rout of administration	Control
Bilbao-Ramos *et al*., 2020	Ursolic acid	Triterpenoid	Sigma-Aldrich	5 mg/kg	Daily for 7 days	Intraperitoneal	Untreated infected animals
Gogulamudi *et al*., 2019	Vitamin D3 + retinoic acid / Retinoic acid + chenodeoxycholic acid	Diterpene	Not reported	384 ng+30 ng/kg / 78 ng+30 ng/kg	Daily for 4 weeks	Oral	Phosphate-buffered saline
Kaur *et al*., 2019	Lupeol	Triterpenoid	Not reported	25 and 50 mg/kg^{-1}	14 days	Oral	Uninfected animals; infected and untreated; amphotericin B
Youssefi *et al*., 2019	Thymol / Carvacrol	Monoterpenes	Sigma-Aldrich	100 mg/kg / 100 mg/kg	Single dose	Intraperitoneal	Dimethylsulfoxide
Das *et al*., 2017a	Ursolic acid in nanostructured lipid carrier	Triterpene	Sigma-Aldrich	0,2 mL	Four times for 20 days	Oral	Uninfected animals; infected receiving sterile water
Das *et al*., 2017b	Lupeol	Triterpene	Methanol extract of *Sterculia villosa* bark	5, 25, 50, 75, and 100 mg/kg	Five times for 30 days	Intraperitoneal	Untreated infected animals

Table 1. Description of the terpenes used, ways of obtaining and information regarding dosages, frequency, treatment time, routes of administration of the compounds and controls used (continuation)

Reference	Tested compound	Type of terpene	Source	Dosage	Frequency and time of treatment	Rout of administration	Control
Jesus *et al*., 2017	Ursolic acid	Triterpene	Ethanol extract of *Baccharis uncinella* leaves	1 e 2 mg/kg	Daily for 15 days	Intraperitoneal	Phosphate-buffered saline+dimethylsulfoxide and phosphate-buffered saline alone
Kar *et al*., 2017	Cedrol in nanostructured lipid carrier system	Sesquiterpene	Sigma-Aldrich	28 mg/kg	Once a week for 1 or 2 weeks	Oral	Untreated animals; treated with miltefosine
Want *et al*., 2017	Nanoliposome-associated artemisinin	Sesquiterpene	Baoji Herbest Bio-Tech Ltd	10 or 20 mg/kg	Alternate days for 10 days	Intraperitoneal	Uninfected untreated animals; infected treated with phosphate buffered saline; infected receiving amphotericin B
Ghosh *et al*., 2016	Oleanolic acid in D-alpha-tocopherol polyethylene glycol 1000 succinate nanoparticles	Triterpene	Sigma-Aldrich	2 mg/kg	One dose every five days	Intraperitoneal	Uninfected animals; infected receiving sterile water
Bandyopadhyay *et al*., 2015	Glycyrrhizic acid	Triterpenoid	Licorice root	75 mg/kg of body weight	Five times in alternate days	Intraperitoneal	Phosphate-buffered saline

Table 1. Description of the terpenes used, ways of obtaining and information regarding dosages, frequency, treatment time, routes of administration of the compounds and controls used (continuation)

Reference	Tested compound	Type of terpene	Source	Dosage	Frequency and time of treatment	Rout of administration	Control
Bhattacharjee *et al*., 2015	Glycyrrhizic acid + sodium gluconate antimony	Triterpenoid	Licorice root (glycyrrhizic acid)	Glycyrrhizic acid (50 mg/kg) and sodium gluconate antimony (250 mg/kg)	Five times in em alternate days	Intraperitoneal	Phosphate-buffered saline
Corpas-López *et al*., 2015	α-bisabolol	Sesquiterpene	Sigma-Aldrich	50, 200, and 1000 mg/kg	Daily for 14 days	Oral	Olive oil
Want *et al*., 2015	Artemisinin loaded on poly-lactic co-glycolic acid nanoparticles	Sesquiterpene	Baoji Herbest Bio-Tech Ltd	10 and 20 mg/kg	Alternate days for 10 days	Intraperitoneal	Empty nanoparticles and amphotericin B
Morais *et al*., 2014	Thymol derivatives	Monoterpene	VETEC, Fortaleza, Brazil	100 mg/kg	Daily for 30 days	Intraperitoneal	Glucantime and water
Bhattacharjee *et al*., 2012	Glycyrrhizic acid	Triterpenoid	Licorice root	75 mg/kg	Five times in alternate days	Intraperitoneal	Phosphate-buffered saline
Bhaumik *et al*., 2012	Asiaticoside	Triterpenoid saponin	Sigma Chemicals	1–10 mg/kg	Daily for 10 days	Oral	Untreated infected animals

Table 1. Description of the terpenes used, ways of obtaining and information regarding dosages, frequency, treatment time, routes of administration of the compounds and controls used (continuation)

Reference	Tested compound	Type of terpene	Source	Dosage	Frequency and time of treatment	Rout of administration	Control
Suryawanshi *et al*., 2012	Isoxazole containing heteroretinoid and its amide derivatives	Diterpene	Not reported	50 mg/kg^{-1}	Daily for five consecutive days	Intraperitoneal	Sodium stibogluconate
Mutiso *et al*., 2011	Artesunate + diminazene	Sesquiterpene	Dr. Alain Bourdichon (TropMed, Alemanha)	12.5 mg/kg of body weight	Consecutively for 28 days	Not reported	Untreated and amphotericin B treated animals
Ukil *et al*., 2011	18β-glycyrrhetinic acid	Triterpenoid	Sigma-Aldrich	10-100 mg/kg	Three times, five days apart	Intraperitoneal	Not reported
Misra *et al*., 2010	Clerodane (16α - hydroxycleroda – 3,13 (14) Z - dien – 15,16 - olide)	Diterpene	Ethanol extract of *Polyalthia longifolia* leaves	25, 50, 100 and 250 mg/kg^{-1}	Daily for five days	Not reported	Untreated control and miltefosine

Table 1. Description of the terpenes used, ways of obtaining and information regarding dosages, frequency, treatment time, routes of administration of the compounds and controls used (continuation)

Reference	Tested compound	Type of terpene	Source	Dosage	Frequency and time of treatment	Rout of administration	Control
Sen *et al*., 2010	Artemisinin	Sesquiterpene	Sigma-Aldrich	10 and 25 mg/kg	Five alternate days	Oral	Uninfected animals, infected untreated, infected receiving sodium gluconate antimony and infected receiving dimethylsulfoxide
Lala *et al*., 2006	Bassic acid in oil-in-water microemulsion and poly-DL-lactide nanoparticles	Triterpene	Methanol extract of *Mimusops elangii* seeds	200 µg in 0.2 mL microemulsion and 9.33 mg poly-DL-lactide or diluted in 0.5 mL of PBS	Every three days for 15 days	Subcutaneous	Untreated infected animals
Germonprez *et al*., 2005	Maesabalides	Triterpenoid saponin	Methanolic extract of *Maesa balansae* leaves	0.2 mg/kg	Single dose	Subcutaneous	Pentostam and amphotericin B
Tyagi *et al*., 2005	Arjunglucoside in nanogel and poly-DL-lactide nanoparticle	Triterpene	Methanol extract of *Terminalia bellerica* Roxb stem bark	400 µg of free drug and 400 µg of drug associated with nanogel or poly-DL-lactide nanoparticles	Every three days, totaling six doses	Subcutaneous	Placebo of nanogels and poly-DL-lactide nanoparticles
Ukil *et al*., 2005	18β-glycyrrhetinic acid	Triterpene	Sigma-Aldrich	1-100 mg/kg	Three times for 10 days	Intraperitoneal	Unspecified placebo

Table 1. Description of the terpenes used, ways of obtaining and information regarding dosages, frequency, treatment time, routes of administration of the compounds and controls used (continuation)

Reference	Tested compound	Type of terpene	Source	Dosage	Frequency and time of treatment	Rout of administration	Control
Maes *et al*., 2004a	Maesabalide III (PX-6518)	Triterpenoid saponin	Methanolic extract of *Maesa balansae* leaves	0.2, 0.4 and 0.8 mg/kg	Single dose	Subcutaneous	Amphotericin B
Maes *et al*., 2004b	PX-6518	Triterpenoid saponin	Methanol extract *of Maesa balansae*, *M. sinensis*, *M. tomentella* and *M. crassifolia*	0.4, 0.8, 1.6, and 2.5 mg/kg	Single dose	Subcutaneous	Unspecified placebo and amphotericin B
Ma *et al*., 2004	Dihydroartemisinin	Sesquiterpene	Beijing Nº 6 Pharmaceutical Factory	25 mg/kg^{-1} and 50 mg/kg^{-1}	Daily for 14 days	Oral	Vegetable oil (thinner)
Chowdhury *et al*., 2003	Dihydrobetulinic acid (DHBA)	Triterpene	Betulinic acid isolated from *Bacopa monnieri* leaves and subjected to hydrogenation to create DHBA	10 mg/kg of body weight	6 weeks	Oral and intramuscular	Phosphate-buffered saline

Table 1. Description of the terpenes used, ways of obtaining and information regarding dosages, frequency, treatment time, routes of administration of the compounds and controls used (continuation)

Reference	Tested compound	Type of terpene	Source	Dosage	Frequency and time of treatment	Rout of administration	Control
Lala *et al*., 2003	14-deoxy-1-oxoandrographolide in free form, liposomal, niosomal and associated with microsphere	Diterpene	Authors' Department of Medicinal Chemistry	200 µg in liposomes, niosomes and microspheres or diluted in 0.5 mL of PBS	Every three days for 15 days	Subcutaneous	Untreated infected animals
Sinha *et al*., 2002	Free bacosaponin-c, or in liposome, niosome, microsphere, or nanocapsule	Triterpene	Authors' Department of Medicinal Chemistry	0.175 mg in 0.5 ml of liposomal, niosomal, microsphere and nanocapsule suspension or in 0.5 ml of PBS	Every three days for 15 days	Subcutaneous	Untreated infected animals
Sinha *et al*., 2000	Liposomal andrographolide	Diterpene	Department of Medicinal Chemistry at the Authors' Institute	250 µg intercalated with 0.5 ml liposomal suspension or 0.5 mL of PBS	Every three days for 15 days	Subcutaneous	Untreated infected animals
Medda *et al*., 1999	Free, liposomal and niosomal amarogentin	Monoterpene	Methanol extract of *S. chirata*	2.5 mg/kg of body weight	Every three days for 15 days	Subcutaneous	Untreated infected animals

Table 2. Main findings after treating leishmaniasis with terpenes administered in their free form or in combination with other substances

Compounds	Main findings	Reference
α -bisabolol	Reduced parasite load by more than 70% in the spleen and more than 80% in the liver without showing toxicity.	Corpas-López *et al*., 2015
Dihydrobetulinic acid (DHBA)	Oral treatment reduced more than 95% of the parasite load in the spleen and more than 90% in the liver; Intramuscular treatment reduced more than 95% in the spleen and liver, with no toxicity in both treatments.	Chowdhury *et al*., 2003
Glycyrrhizic acid	Increased NO production and iNOS gene induction; increase in Th1 cytokines and decrease in Th2 cytokines.	Bandyopadhyay *et al*., 2015
	Induction of the iNOS gene and increase in NO production; increase in Th1 cytokines and decrease in Th2 cytokines; more than 90% reduction in the parasite load in the spleen and more than 95% in the liver with reduced toxicity.	Bhattacharjee *et al*., 2012
Glycyrrhizic acid + sodium stibogluconate (SAG)	Significant increase in NO production; increase in Th1 cytokines and reduction in Th2 cytokines; reduction of more than 90% of the parasite load in the spleen and liver.	Bhattacharjee *et al*., 2015
Ursolic acid	Increased Th1 cytokines. Reduction of more than 95% of the parasite load in the spleen and liver without presenting toxicity.	Bilbao-Ramos *et al*., 2020
	NOS2 positive areas were detected; increase in Th1 cytokine mRNA and decrease in Th2 cytokine mRNA. 90% reduction in the parasite load in the spleen was observed with treatment with 1 and 2mg/kg. The same dosages reduced more than 95% of the parasite load in the liver, without presenting toxicity.	Jesus *et al*., 2017
Artemisinin	Increased NO and iNOS mRNA production; increase in Th1 cytokines; reduction of more than 80% of the parasitic load in the spleen.	Sen *et al*., 2010

Table 2. Main findings after treating leishmaniasis with terpenes administered in their free form or in combination with other substances (continuation)

Compounds	Main findings	Reference
Asiaticoside	Increased iNOS and NO expression; increase in Th1 cytokines and decrease in Th2 cytokines; reduction of more than 95% in the spleen and liver, with no toxicity.	Bhaumik *et al*., 2012
Clerodane (16α - hydroxycleroda – 3,13 (14) Z - dien – 15,16 - olide)	Reduction of more than 90% of the parasite load in the spleen and more than 80% in the liver and bone marrow, without any toxicity.	Misra *et al*., 2010
Lupeol	Increased iNOS expression and NO production; increase in Th1 cytokines and decrease in Th2 cytokines; reduction of more than 80% and 95% of the parasite load in the spleen at dosages of 25 and 50mg/kg, respectively, without presenting toxicity.	Kaur *et al*., 2019
	Increased NO production; increase in Th1 cytokines and decrease in Th2 cytokines; reduction of more than 80% of the parasite load in the spleen and more than 70% in the liver, without presenting toxicity.	Das *et al*., 2017b
Maesabalides	Maesabalide 3 reduced more than 90% of the parasite load in the liver.	Germonprez *et al*., 2005
Maesabalide III	Complete reduction of the parasite load in the liver, but it did not prevent infection in the spleen and bone marrow, without presenting toxicity.	Maes *et al*., 2004a
	Dose-dependent reduction in parasite load, and 2.5mg/kg reduced more than 95% of parasites in the liver, with minimal toxicity.	Maes *et al*., 2004b
Thymol	Highly reduced parasite load in the spleen and liver.	Youssefi *et al*., 2019
Carvacrol	Moderate reduction in spleen and low in liver.	
Vitamin D3 + retinoic acid	Increase in Th1 cytokines and decrease in Th2 cytokines; reduction of more than 70% of the parasite load in the spleen and more than 80% in the liver.	Gogulamudi *et al*., 2019
Retinoic acid + chenodeoxycholic acid	Increase in Th1 cytokines and decrease in Th2 cytokines; reduction of more than 45% of the parasite load in the spleen and liver.	

Table 3. Main findings after treating leishmaniasis with terpene derivatives

Derivatives	Original compounds	Main findings	Reference
18β-glycyrrhetinic acid	Glycyrrhizic acid	NO production greater than observed *in vitro*; increase in Th1 cytokines and decrease in Th2 cytokines; reduction of more than 70% of the parasite load in the spleen, without presenting toxicity.	Ukil *et al*., 2011
		Dose-dependent increase in NO, with an increase after 24h; increase in Th1 cytokines and decrease in Th2 cytokines; complete reduction of the parasite load in the spleen and liver; toxicity only at concentrations above 20 μM.	Ukil *et al*., 2005
Acetil-thymol	Thymol	Reduction of the parasite load in the liver.	Morais *et al*., 2014
Benzoil-thymol		Reduced parasite load in the spleen and liver, with low toxicity.	
Artesunate + diminazene	Artemisinin	Significant reduction of the parasitic load in the spleen.	Mutiso *et al*., 2011
Dihydroartemisinin	Artemisinin	More than 70% reduction in the parasite load in the spleen at 25 and 50mg/kg and more than 80% in the liver at the same dosages.	Ma *et al*., 2004
Isoxazole containing heteroretinoid and its amide derivatives	Retinoic acid	Reductions in the parasite load. Compounds: 2: inactive 3: more than 70% 4: more than 45% 5a: more than 70% 5b: more than 60% 5c: more than 50% 5d: more than 70% 5e: inactive 5f: not done 5g: not done 5h: inactive 5i: more than 50% 5j: more than 60% 5k: more than 70% 5l: more than 70%	Suryawanshi *et al*., 2012

Table 4. Main findings of the actions of terpenes associated with carriers compared to their free forms in the treatment of leishmaniasis

Free compound	Used carriers	Main findings	Reference
14-deoxy-1-oxoandrographolide	Microsphere	More than 50% reduction in the parasitic load in the spleen.	Lala *et al*., 2003
	Liposome	More than 70% reduction in the parasitic load in the spleen.	
	Niosome	More than 90% reduction in the parasitic load in the spleen.	
Bassic acid	Oil-in-water microemulsion	Reduction of more than 60% of the parasite load in the spleen, with verified toxicity.	Lala *et al*., 2006
	Poly-DL-lactide (PLA) nanoparticles	Reduction of more than 70% of the parasite load in the spleen, without presenting toxicity.	
Oleanolic acid	Nanoparticles of succinate D-alpha-tocopherol polyethylene glycol 1000 (TPGS)	Reduction of more than 95% of the parasite load in the spleen, with negligible toxicity.	Ghosh *et al*., 2016
Ursolic acid	Nanostructured lipid carrier + chitosan	Reduction of more than 95% of the parasitic load in the spleen.	Das *et al*., 2017a
Amarogentin	Liposome	Reduction of more than 60% of the parasite load in the spleen, without showing toxicity.	Medda *et al*., 1999
	Niosome	Reduction of more than 90% of the parasite load in the spleen, without any toxicity.	
Andrographolide	Intercalated lipossome	Reduction of more than 60% of the parasite load in the spleen, without presenting toxicity.	Sinha *et al*., 2000
	Intercalated liposome grafted with mannose	Reduction of more than 80% of the parasite load in the spleen, without presenting toxicity.	
Arjunglucoside	Nanogel and PLA nanoparticles	Reduction of more than 70% of the parasite load in the spleen, without presenting toxicity.	Tyagi *et al*., 2005

Table 4. Main findings of the actions of terpenes associated with carriers compared to their free forms in the treatment of leishmaniasis (continuation)

Free compound	Used carriers	Main findings	Reference
Artemisinin	Nanoliposome	Normalized NO levels; increase in Th1 cytokines and decrease in Th2 cytokines; reduction of more than 70% of the parasite load in the spleen and more than 80% in the liver, without presenting toxicity.	Want *et al*., 2017
	Poly-lactic co-glycolic acid (ALPLGA) nanoparticles	Increase in Th1 cytokines and decrease in Th2 cytokines; reduction of more than 80% of the parasite load in the spleen and liver, without presenting toxicity	Want *et al*., 2015
Bacosaponin-c	Liposome, niosome, microsphere and nanocapsule	Reduction in the parasite load ranged from more than 70% to more than 90%, with no toxicity.	Sinha *et al*., 2002
Cedrol	Nanostructured lipid	Reduction of more than 95% of the parasite load in the spleen and liver	Kar *et al*., 2017

3.3 Main Findings

Nine studies reported that the administration of compounds in their free forms was not toxic to experimental animals. Two studies highlighted that the toxicity was reduced or minimal for BALB/c mice, whereas six articles did not provide information about the toxicity of free compounds (Table 2). In studies with terpene derivatives, a thymol derivative (benzoyl-thymol) showed toxicity, albeit low, to macrophages (n = 1). One study reported that concentrations higher than 20 µmol x L^{-1} of 18β-glycyrrhetinic acid were toxic to BALB/c mice. Conversely, another study evaluating the same compound did not report any toxicological effect. Three studies did not provide information about the toxicity of terpene derivatives (Table 3). Moreover, studies evaluating terpene compounds associated with a carrier described either no toxic effect (n = 7) or negligible toxicity (n = 1). The formula using PLA nanoparticles showed no toxicity, whereas the bassic acid in an oil-in- water microemulsion was toxic to golden hamsters (n = 1). Two studies did not mention any toxicity of the compounds carried (Table 4).

From the 34 eligible studies, twelve evaluated the effect of terpenes on body and organs weight. Specifically, eight reported a reduction in the spleen and liver weights and four documented no significant alteration in animals' body weight (Supplementary Table 3). In addition, six studies cited that no animals' dead during the experimental period, whereas one study reported that one golden hamster died during the experiment that used maesabalide III at 0.8 mg/kg^{-1} as treatment. Other studies did not report this information (Supplementary Table 3).

In general, terpenes exhibited antileishmanial activity, either by direct action on the parasites or by indirect action through modulation of the immune response. In the latter, terpenes promoted a pro-inflammatory response profile and activation of microbicidal mechanisms that, in turn, lead to the production of reactive oxygen species (ROS) with consequent reduction of parasite load. According to the studies, terpenes act on parasites by

damaging their plasma membranes (n = 5) and controlling cytokine production (n = 5), modulating immune system pathways, and stimulating NO production (n = 5) by infected animals. Three works reported inhibition of DNA topoisomerase in *Leishmania* after treatment. Eight studies comment on the unique actions of terpenes, such as N-H binding to the macromolecular target in the parasite, generation of a molecule other than NO, the possibility of the phenomenon of cell targeting, among others. Three studies report that the mechanisms of action of terpenes are not known. Five studies do not suggest and do not provide any information about the mechanisms of action of terpenes.

Eleven studies observed high NO levels from macrophages after animal treatment with the compounds lupeol, artemisinin, glycyrrhizic acid, 18β-glycyrrhetinic acid, and asiaticoside (Tables 2–4). Infected animals treated with lupeol, ursolic acid, glycyrrhizic acid, asiaticoside, and artemisinin presented high expression levels of iNOS and iNOS2 mRNA. Only one study using 18β-glycyrrhetinic acid reported an increase in NO levels in a dose-dependent manner (Tables 2 and 3). Fourteen studies, in turn, measured cytokines production in infected animals treated with terpenes. They studies documented higher levels of Th1 cytokines, responsible by parasite elimination, and lower levels of Th2 cytokines, in animals treated with ursolic acid, a combination of vitamin D3+ retinoic acid and retinoic acid + chenodeoxycholic acid, lupeol, glycyrrhizic acid, 18β-glycyrrhetinic acid, artemisinin and asiaticoside (Tables 2–4).

With respect to the parasite, 13 studies observed a reduction between 45% and 89% of the parasite load in golden hamsters treated with isoxazole containing heteroretinoid and its amide derivatives, andrographolide, dihydroartemisinin, arjunglucoside and bassic acid (Tables 3 and 4); in BALB/c mice treated with artesunate, α-bisabolol, glycyrrhizic acid, lupeol, and retinoic acid combined with vitamin D3 and chenodeoxycholic acid, and BALB/c mice and golden hamsters treated with artemisinin (Tables 2–4). Moreover, 17 studies

described a reduction in parasite load equal to or above 90% in infected BALB/c mice treated with lupeol, cedrol, oleanolic acid, glycyrrhizic acid, 18β-glycyrrhetinic acid (Tables 2–4); in golden hamsters treated with clerodane, ursolic acid, dihydrobetulinic acid, 14-deoxy-1-oxoandrographolide, bacosaponin-c, and amarogentin, and BALB/ mice and golden hamsters treated with ursolic acid, maesabalide, and asiaticoside (Tables 2–4). One study reported no presence of parasites in the spleen of BALB/c mice after treatment with thymol derivative, whereas another one described a complete reduction of parasite load in BALB/c mice after treatment with 18β-glycyrrhetinic acid (Table 3). A study reported a moderate and mild presence of parasites in the spleen of animals treated with thymol and carvacrol, respectively (Table 2). The liver of these animals, in turn, presented a significant reduction of parasites after thymol treatment (Table 2). One study did not evaluated this parameter (Table 2).

3.4 Risk of bias

The results from bias analysis are shown in Fig. 2. No studies followed all the methodological criteria analyzed in this review. Regarding selection bias, no studies reported a random sequence generation (n = 34). Regarding selection bias, 29 studies (85.20%) reported baseline animal characteristics, whereas all 34 studies (100%) reported no information on allocation concealment. Regarding performance bias, only seven articles mentioned random accommodation (20.58%), and none reported the researchers being blinded (100%). With respect to the detection bias, only two studies (5.88%) reported evaluation of random results. None of the 34 studies reported being blinded by the evaluator (100%). Concerning attrition bias, 33 studies (97.05%) showed complete outcome data. Thirty-two studies (94.11%) exhibited complete results, exhibiting a low risk of reporting bias. In addition, 11 studies (32.35%) did not present other potential sources of bias, considered here as research approval by the Ethics Committee and details of mouse care, such

as *ad libitum* provision of feed and water. The analysis of individual study is detailed in Fig. S1.

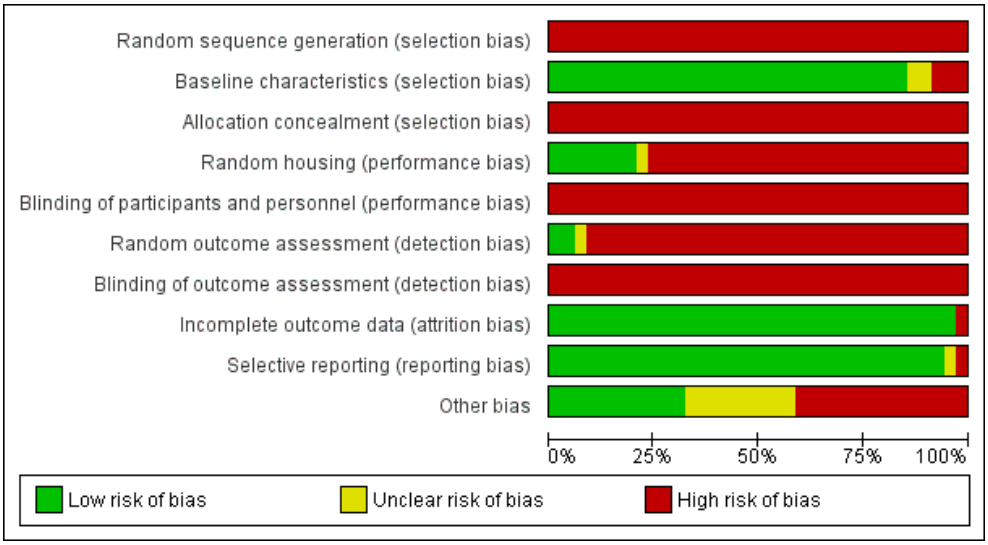


Figure 2 – Risk of bias chart of methodological quality and reporting of results of studies included in the systematic review. According to the answers to the SYRCLE tool's flagging questions, there is a possibility of a low risk of bias, high risk, or unclear risk, so that the answer "yes" to each question indicates "low risk of bias", "no" indicates "high risk of bias" and uncertainty of bias means "unclear" bias. Figure generated in Review Manager software version 5.4.

4. Discussion

Our findings revealed that terpenes modulate some immune system pathways by activating microbicide pathways. The compounds also acted directly on VL-causing protozoa in animal models. Here, we observed that terpenes improved NO production by macrophages and directed the production of Th1 profile cytokines. In addition, terpenes showed little or no toxicity to rodents experimentally infected with VL causing species. These results support the idea that these compounds have promising therapeutic effects against VL. Thus, we propose terpenes as potential candidates for clinical trials to improve the treatment of this disease. Undoubtedly, the use of conventional chemotherapy to treat VL has led to an increase in the parasites' resistance to drugs, resulting in a decrease in its effectiveness [30], and any effort to improve its treatment is relevant.

Most of the studies included in this review were carried out in India. VL is endemic in this country, as well as Brazil, Kenya, Ethiopia, Sudan [8], and its population traditionally

uses medicinal plants for treating several diseases [31]. However, the search for articles considering the use of terpenes to treat kala-azar did not recover studies developed in Ethiopia and Sudan, or the quantity was minimal (in the case of Brazil and Kenya). This fact may be related to the consolidated knowledge about the use of herbs in Asia, reflected in most studies of ethnobotany and ethnopharmacology, which end up predominating in this continent [32].

The animals predominantly used in the studies were BALB/c mice and golden hamsters. These animal models are used widely due to their susceptibility to leishmaniasis infection rather than C57BL/6, CBA/J, C3H, and BIOD2 [33]. Hamsters can better simulate human VL infection under experimental conditions [34,35]. Thus, their use is preferred in experiments testing treatments focused on human therapy. Moreover, few articles reported animal age and sex. Studies have reported differences in parasite load and immune response associated with sex, indicating that leishmaniasis progresses differently in male and female animals [36]. In addition, the evolution of leishmaniasis and immune responses can vary among distinct age groups [37]. Thus, the authors must report this information in future publications.

Terpenes constitute the largest and most diverse structural group of secondary metabolites derived from natural sources [38]. Herein, we showed a variety of terpenes used in the VL treatment and their potential mechanism of action. The structural diversity of this group seems to lead to multiple cellular targets [39]. Among them, the plasma membrane of parasites is one of the main targets in which terpenes act as spacers, increasing the membrane fluidity [40,41]. This mode of action is associated with the ability of terpenes to insert themselves between the fatty chains present in the lipid bilayers from the membrane. Due to their lipophilic profile, they interrupt the lipid packaging [42,43]. Also, isoprenoids may affect membrane integrity by modulating the MAP-kinase pathway [44]. Some terpenes can cause reactions with the ergosterol from the *Leishmania* membrane and increase cell

permeability. This effect was mainly observed in treatments using thymol derivatives [45]. Glycosylation inhibition with a consequent decrease in membrane stability and blocking of fatty acid biosynthesis are mechanisms of action suggested to α-bisabolol [46]. The possible mechanisms exerted by the compounds basic acid and lupeol, in turn, are related to the apoptosis of the parasite involving membrane damage and high membrane depolarization [47,48]. Apoptosis can also be triggered through the generation of iron-artemisinin adducts after treatment with artemisinin, leading to a reduction in intracellular amastigotes [49].

Furthermore, terpenes can also interfere with *Leishmania* DNA topoisomerase. This enzyme plays an essential role in the DNA topology modulation during the replication, transcription, recombination, and repair processes. Inhibition of DNA-topoisomerase ends up causing DNA fragmentation and cell death of the parasite [50]. This effect was described in studies evaluating amarogentin [51], dihydrobetulinic acid [52], and clerodane [53]. So, these compounds bind to the topoisomerase I enzyme and prevent the formation of a binary complex between this enzyme and DNA [54].

Our results evidenced an indirect action of terpenes on the parasites by modulation of the immune response, stimulating the Th1 response. In LV, an elevation in Th1 cytokines is essential to eliminate intracellular amastigotes and develop resistance to infection. In this helper response profile, the cytokine IL-12 triggers the differentiation and activation of the subset of $CD4^+$ T lymphocytes that secrete IFN-γ. This cytokine, in turn, helps in the activation of infected macrophages, which start to produce NO and ROS, factors responsible for the elimination of parasites present in phagocytic vacuoles [55]. The role of glycyrrhizic acid in restoring the pro-inflammatory response, for example, may be through the regulation of DUSP4, a key protein known to provide protection during VL [56]. Glycyrrhizic acid has also been implicated in inhibiting myeloid lineage-derived suppressor cells (MDSCs), which inhibit Th1 cytokines [57]. Its derivative, 18β-glycyrrhetinic acid, also appears to restore the

Th1 response [58]. When combined, glycyrrhizic acid and SAG interrupted the expression of IL-4, TGF-β, and IL-10 and suppressed the expression of MRP1 and P-gp, transporters present in macrophages responsible for antimony efflux. This effect allowed the retention of this drug in the intracellular environment [59]. Treatments performed with ursolic acid [60] and artemisinin [61] also caused increased Th1 response. Therefore, terpenes seem to exhibit an immunomodulatory activity capable of producing pro-inflammatory responses, with a Th1 profile. This is yet another effect that places this group among promising compounds for VL treatment.

The generation of NO is a critical mechanism in macrophages involved in controlling the replication of the parasite in rodents [62]. NO plays a fundamental role in the control of the infection, being able to interrupt *Leishmania's* mitochondrial respiration, inactivate its metabolism, and limit its proliferation [63]. However, this parasite can modulate some pathways during infection [64]. For example, *L. donovani* can inactivate NF-κB [65,66], a transcription factor that regulates the expression of the gene encoding the iNOS enzyme, responsible for the NO production in infected macrophages [67]. Furthermore, the inhibition of NF-κB by the parasite negatively regulates CD4+ T cells and the production of pro-inflammatory cytokines, responsible for the production of NO and ROS by phagocytes. In this way, the parasite manages to survive in the host [68]. Therefore, treatments that interrupt these modulating actions caused by the parasite are relevant.

The results found in this review demonstrated that treatment with some terpenes leads to NO production by infected macrophages. This fact may result from the regulation of the transcription factor NF-κB by the action of the compounds since this factor allows the expression of macrophage activating cytokine genes, such as IFN-γ and TNF-α, which trigger the production of NO in infected macrophages [55]. Particularly, these effects were observed *in vivo* in treatments with 18β-glycyrrhetinic acid and lupeol [68,69], asiaticoside [31], and

cedrol [70]. Another mechanism that led to the production of NO in infected macrophages was the inhibition of Cox-2, with a consequent decrease in PGE2 biosynthesis after treatment with glycyrrhizic acid [71]. The effect of this terpene is interesting once PGE2 can harm the microbicidal mechanisms of macrophages and inhibit Th1 cytokines. With the Th1 profile impaired by the parasite, the Th2 immune response can prevail, allowing the parasite to survive in the organism [72].

Furthermore, terpenes can reduce the infection by acting on several molecules essential to the parasite. For example, trypanothione reductase is an enzyme found in trypanosomatids that helps the parasite escape oxidative stress [73,74]. Cysteine proteases are related to the parasite's virulence, and their inhibition results in cell differentiation blockade and consequent elimination of parasitemia in animals infected with *Leishmania* spp. [75]. Adenosine ribosyl transferase, in turn, is a major component of the purine rescue pathway in parasites [76]. Lastly, microtubules are crucial to the cell cycle progression from G2 to M phases [77].

Other targets reached by terpenes in trypanosomatids are the *Leishmania* membrane Ca^{+}-ATPases after treatment with clerodane. The activation of these enzymes leads to an increase in calcium in the intracellular environment, which can induce the production of ROS [78]. Collavic acid, due to its alkylating activity, is capable of inhibiting cysteine proteases, which have an active site that can undergo alkylation. Oxidative stress caused by clerodane, as a consequence of glutathione depletion within the parasites causing mitochondrial depolarization, is another effect of a terpene on *L. donovani* [78]. On the other hand, in *L. mexicana*, the species that causes the cutaneous form of leishmaniasis, the depletion of endogenous glutathione by the action of psilostaquin and mexicanin I cause the production of ROS [79], helping to kill the parasite. These data reinforce evidence of the wide spectrum of leishmanicidal actions that terpenes can perform.

Unique mechanisms of action of terpenes have been proposed. In a study of isoxazole containing heteroretinoid and its amide derivatives, researchers suggested that the N–H bond present in the secondary amide might form a hydrogen bond with the macromolecular target in the parasite [80]. Another study evaluating combinations of vitamin D3 + retinoic acid and retinoic acid + chenodeoxycholic acid showed that, in *in vitro* assays, the use of these combinations negatively regulates the expression of mRNA of the coat gene containing tryptophan-aspartate (TACO) in the host. This gene helps the parasite enter the host, and its downregulation inhibits the parasite load [81]. A work with oleanolic acid suggested a direct effect of this acid on amastigote forms of *L. donovani*, although the leishmanicidal mechanism is not fully understood [82]. Other studies have not suggested mechanisms of action of terpenes in the parasites causing VL or have highlighted that they are still unknown [83–85].

Considering the progression of VL, it is noteworthy that the liver, spleen, and bone marrow usually suffer severe histological damage, as they are rich in macrophages, the target cells parasitized by *Leishmania* [55,86]. Histological damage resulting from the infection in these organs ameliorated after treatment with most of the terpenes. In the liver and spleen of treated animals, a study reported the formation of granulomas anatomically circumscribed and functional structures in which it is possible to limit the infection [87], contributing to the reduction of the parasite load. Furthermore, the spleen red and white pulps undergo hypertrophy and disorganization, respectively, during VL infection [55]. However, treatment with some terpenes can restore the white pulp and red pulp regions of the spleen, providing regression of the white pulp, expansion of the red pulp to levels considered normal, and a decrease in infiltrating monocytes in the venous sinusoids [83,88–90]. Maesabalide III performed satisfactorily, almost reducing the parasite load in the liver, especially in the

chronic phase of the disease, although this is not true for the spleen and bone marrow, where amastigotes persisted even after treatment [91,92].

5. Review Limitations

The design adopted in the present study followed the generally accepted procedures for performing systematic reviews. Overall, the methodologies used in the eligible studies were heterogeneous. The authors reported incomplete information about animal handling, which hinders the faithful reproduction of the experiment by other researchers. The risk of bias analysis highlighted the importance of improving the experimental design to ensure a high level of scientific evidence in preclinical trials. These trials, in turn, serve as a guide to design clinical studies with greater precision and reliability. Through extensive searches, it became evident that the literature still lacks more accurate information regarding the molecular mechanisms that concern the target of terpenes in the parasite. Nevertheless, immunomodulation was the most frequent mechanism described by the studies. Studies only reported general actions of terpenes, such as anti-inflammatory, antioxidant, antiviral, and anticancer. There is also a need to elucidate the structure-activity relationship for the various existing terpenes, considering the species causing VL.

6. Perspectives

According to this systematic review, the leishmanicidal potential of terpenes opens opportunities for extracting and using terpenes from plant sources and evaluating their role against VL-causing parasites. Further studies can detect the anti-leishmanial activity in other terpenes, such as hemiterpenes, sesterterpenes, tetraterpenes, polyterpenes, and irregular terpenes. Despite being a neglected disease, recent research aimed at combating leishmaniasis had a significant improvement due to the advances in technology. From this advanced

technology, we can mention the production of derivatives from original compounds, the use of more effective drug delivery methods compared to the compounds alone, and the analysis of molecular docking [48,53]. The latter is an in silico method used to predict the interaction between ligand and its target or even to know the structure-activity relationship of compounds with therapeutic potential [93]. However, molecular docking was not recurrent in the studies selected for this review. It is surprising because this analysis helps identify a more accurate treatment based on the relationship between a compound and its ligand. Therefore, it is expected that, in the coming years, the use of this technique will increase.

Derivative compounds emerge as a relevant therapeutic approach. Modifications in the molecular structure of parent drugs may enhance the activity of their derivatives, for example, reducing toxicity [45,94] and improving antiparasitic activity [95], as observed with the terpene derivatives discussed in this review [45,58,69,80,96,97]. The performance of derivative compounds may even be better than reference drugs or parent compounds [98,99]. It makes the derivatives more effective weapons due to the chemical changes to which they are subject, with an increment of their antimicrobial repertoire. In addition, the use of carriers is interesting because they increase bioavailability [100] and enhance pharmacokinetic properties, such as drug absorption, therapeutic concentration, and stability, resulting in effective drug targeting [101]. Moreover, they can efficiently deliver the drug to the infection site, which ameliorates the VL therapy. In this review, terpenes combined with the carries liposomes, niosomes, microspheres, nanogels, and nanoparticles exhibited better performance than the terpenes administered in their free form [47,83,90].

Thus, therapies based on plant-derived medicines, such as terpenes, for VL treatment may replace conventional chemotherapy in the future. We may include terpene derivatives as a treatment since the derivatization of compounds tends to improve their activities. It is relevant to emphasize the importance of conducting studies focused on the association

between terpenes and other structures able to enhance their effectiveness and increase the bioavailability of the compounds in the body.

7. Conclusions

We concluded that triterpenes are the most recurrent type of terpene used in treatment against kala-azar. Terpenes exhibited anti-leishmanial activity acting directly on parasites and indirectly by modulating the immune response and promoting a pro-inflammatory response through the release of cytokines. The activation of microbicidal mechanisms produces NO and ROS capable of annihilating the parasites, culminating in low parasite load. It is worth mentioning that treatments with terpenes were not toxic and exhibited equivalent or superior performance to the standard drugs. The use of nanostructures as a drug delivery system allows more accurate interactions between terpenes and target cells, increasing the drug bioavailability in the body and preventing its fast excretion by kidneys. Altogether, these findings evidenced the potential use of terpenes on a larger scale for other pre-clinical and human trials.

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Credit authorship contribution statement

The authors responsibilities were as follows - MM-N, VDR and RPRC: designed the study; VDR and RPRC: search strategy, and studies reviewed; VDR: wrote and revised the manuscript; EAMS and MM-N: read and approved the final version.

Declaration of interest statement

No conflict of interest to declare.

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Supplementary Archives

Supplementary Table 1. Identification of articles selected for systematic review

Reference	Title	Journal	DOI	Country
Bilbao-Ramos *et al*., 2020	Evaluating the potential of ursolic acid as bioproduct for cutaneous and visceral leishmaniasis	Molecules	10.3390/molecules25061394	Spain
Gogulamudi *et al*., 2019	Vitamins (A&D) and isoprenoid (chenodeoxycholic acid) molecules are accompanied by Th1 immunostimulatory response and therapeutic cure *in vivo*: possible antileishmanial drugs	Scientific Reports	10.1038/s41598-019-44630-4	India
Kaur *et al*., 2019	Lupeol induces immunity and protective efficacy in a murine model against visceral leishmaniasis	Parasitology	10.1017/S0031182019000659	India
Youssefi *et al*., 2019	*In vitro* and *in vivo* effectiveness of carvacrol, thymol and linalool against *Leishmania infantum*	Molecules	10.3390/molecules24112072	Iran
Das *et al*., 2017a	Oral delivery of ursolic acid-loaded nanostructured lipid carrier coated with chitosan oligosaccharides: development, characterization, *in vitro* and *in vivo* assessment for the therapy of leishmaniasis	International Journal of Biological Macromolecules	10.1016/j.ijbiomac.2017.04.098	India
Das *et al*., 2017b	Antileishmanial and immunomodulatory activities of lupeol, a triterpene compound isolated from *Sterculia villosa*	International Journal of Antimicrobial Agents	10.1016/j.ijantimicag.2017.04.022	India
Jesus *et al*., 2017	Therapeutic effect of ursolic acid in experimental visceral leishmaniasis	International Journal for Parasitology: Drugs and Drug Resistance	10.1016/j.ijpddr.2016.12.002	Brazil

Supplementary Table 1. Identification of articles selected for systematic review (continuation)

Reference	Title	Journal	DOI	Country
Kar *et al*., 2017	Development and evaluation of a cedrol-loaded nanostructured lipid carrier system for *in vitro* and *in vivo* susceptibilities of wild and drug resistant *Leishmania donovani* amastigotes	European Journal of Pharmaceutical Sciences	10.1016/j.ejps.2017.03.046	India
Want *et al*., 2017	Nanoliposomal artemisinin for the treatment of murine visceral leishmaniasis	International Journal of Nanomedicine	10.2147/IJN.S106548	Saudi Arabia
Ghosh *et al*., 2016	Oleanolic acid loaded poly lactic co-glycolic acid-vitamin E TPGS nanoparticles for the treatment of *Leishmania donovani* infected visceral leishmaniasis	International Journal of Biological Macromolecules	10.1016/j.ijbiomac.2016.09.014	India
Bandyopadhyay *et al*., 2015	Glycyrrhizic acid-mediated subdual of myeloid-derived suppressor cells induces antileishmanial immune responses in a susceptible host	Infection and Immunity	10.1128/IAI.00729-15	India
Bhattacharjee *et al*., 2015	Co-administration of glycyrrhizic acid with the antileishmanial drug sodium antimony gluconate (SAG) cures SAG-resistant visceral leishmaniasis	International Journal of Antimicrobial Agents	10.1016/j.ijantimicag.2014.10.023	India
Corpas-López *et al*., 2015	(−)-α-bisabolol, a promising oral compound for the treatment of visceral leishmaniasis	Journal of Natural Products	10.1021/np5008697	Spain
Want *et al*., 2015	Therapeutic efficacy of artemisinin-loaded nanoparticles in experimental visceral leishmaniasis	Colloids and Surfaces B: Biointerfaces	10.1016/j.colsurfb.2015.04.013	China
Morais *et al*., 2014	Thymol and eugenol derivatives as potential antileishmanial agents	Bioorganic & Medicinal Chemistry	10.1016/j.bmc.2014.08.020	Brazil

Supplementary Table 1. Identification of articles selected for systematic review (continuation)

Reference	Title	Journal	DOI	Country
Bhattacharjee *et al*., 2012	Glycyrrhizic acid suppresses Cox-2-mediated anti-inflammatory responses during *Leishmania donovani* infection	Journal of Antimicrobial Chemotherapy	10.1093/jac/dks159	India
Bhaumik *et al*., 2012	Asiaticoside induces tumour-necrosis-factor-a-mediated nitric oxide production to cure experimental visceral leishmaniasis caused by antimony-susceptible and -resistant *Leishmania donovani* strains	Journal of Antimicrobial Chemotherapy	10.1093/jac/dkr575	India
Suryawanshi *et al*., 2012	Chemotherapy of leishmaniasis. Part XI: synthesis and bioevaluation of novelisoxazole containing heteroretinoid and its amide derivatives	Bioorganic & Medicinal Chemistry Letters	10.1016/j.bmcl.2012.09.024	India
Mutiso *et al*., 2011	*In vitro* and *in vivo* antileishmanial efficacy of a combination therapy of diminazene and artesunate against *Leishmania donovani* in BALB/C mice	Revista do Instituto de Medicina Tropical de São Paulo	10.1590/S0036-46652011000300003	Kenya
Ukil *et al*., 2011	Curative effect of 18b-glycyrrhetinic acid in experimental visceral leishmaniasis depends on phosphatase-dependent modulation of cellular MAP kinases	Plos One	10.1371/journal.pone.0029062	India
Misra *et al*., 2010	16a-hydroxycleroda-3,13 (14)Z-dien-15,16-olide from *Polyalthia longifolia*: a safe and orally active antileishmanial agent	British Journal of Pharmacology	10.1111/j.1476-5381.2009.00609.x	India
Sen *et al*., 2010	Efficacy of artemisinin in experimental visceral leishmaniasis	International Journal of Antimicrobial Agents	10.1016/j.ijantimicag.2010.03.008	India
Lala *et al*., 2006	Critical evaluation of the therapeutic potential of bassic acid incorporated in oil-in-water microemulsions and poly-D,L-lactide nanoparticles against experimental leishmaniasis	Journal of Drug Targeting	10.1080/10611860600649765	India

Supplementary Table 1. Identification of articles selected for systematic review (continuation)

Reference	Title	Journal	DOI	Country
Germonprez *et al*., 2005	*In vitro* and *in vivo* anti-leishmanial activity of triterpenoid saponins isolated from *Maesa balansae* and some chemical derivatives	Journal of Medicinal Chemistry	10.1021/jm031150y	Belgium
Tyagi *et al*., 2005	Targeted delivery of arjunglucoside I using surface hydrophilic and hydrophobic nanocarriers to combat experimental leishmaniasis	Journal of Drug Targeting	10.1080/10611860500046732	India
Ukil *et al*., 2005	18 -glycyrrhetinic acid triggers curative Th1 response and nitric oxide up-regulation in experimental visceral leishmaniasis associated with the activation of NF-kB	The Journal of Immunology	10.4049/jimmunol.175.2.1161	India
Maes *et al*., 2004a	Comparative activities of the triterpene saponin maesabalide III and liposomal amphotericin B (AmBisome) against *Leishmania donovani* in hamsters	Antimicrobial Agents and Chemoterapy	10.1128/AAC.48.6.2056-2060.2004	Belgium
Maes *et al*., 2004b	*In vitro* and *in vivo* activities of a triterpenoid saponin extract (PX-6518) from the plant *Maesa balansae* against visceral leishmania species	Antimicrobial Agents and Chemoterapy	10.1128/AAC.48.1.130-136.2004	Belgium
Ma *et al*., 2004	Activity of dihydroartemisinin against *Leishmania donovani* both *in vitro* and *in vivo*	Chinese Medical Journal	10.3760/cma.j.issn.0366-6999.2004.08.129	China
Chowdhury *et al*., 2003	Dihydrobetulinic acid induces apoptosis in *Leishmania donovani* by targeting DNA topoisomerase I and II: implications in antileishmanial therapy	Molecular Medicine	-	India

Supplementary Table 1. Identification of articles selected for systematic review (continuation)

Reference	Title	Journal	DOI	Country
Lala *et al*., 2003	Delivery *in vivo* of 14-deoxy-11-oxoandrographolide, an antileishmanial agent, by different drug carriers	Indian Journal of Biochemistry & Biophysics	-	India
Sinha *et al*., 2002	Bacopasaponin c: critical evaluation of antileishmanial properties in various delivery modes	Drug Delivery	10.1080/107175402753413181	India
Sinha *et al*., 2000	Targeting of liposomal andrographolide to *L. donovani*-infected macrophages *in vivo*	Drug Delivery	10.1080/107175400455137	India
Medda *et al*., 1999	Evaluation of the in-vivo activity and toxicity of amarogentin, an antileishmanial agent in both liposomal and niosomal forms	Journal of Antimicrobial Chemotherapy	10.1093/jac/44.6.791	India

Supplementary Table 2. Description of the main characteristics of the experimental models used and information about the induction of leishmaniasis

Experimental model	**Sex**	**Age**	***Leishmania* species**	**Quantity and forms of the parasite for infection**	**Inoculation route**	**Reference**
BALB/c mice	?	6 to 8 weeks	*Leishmania donovani*	100 µl (10^{-7} promastigotes)	Intravenous	Gogulamudi *et al*., 2019
BALB/c mice	Both sexes	6 to 8 weeks	*Leishmania donovani*	1 x 10^7 promastigotes	Intravenous	Kaur *et al*., 2019
BALB/c mice	?	?	*Leishmania donovani*	10^7 promastigotes	Intracardiac	Das *et al*., 2017a
BALB/c mice	?	4 to 6 weeks	*Leishmania donovani*	1 x 10^7 promastigotes	Intravenous	Das *et al*., 2017b
BALB/c mice	Both sexes	6 weeks	*Leishmania donovani*	10^7 promastigotes	Intravenous	Kar *et al*., 2017
BALB/c mice	Female	6 to 8 weeks	*Leishmania donovani*	2,5 x 10^7 promastigotes	Intravenous	Want *et al*., 2017
BALB/c mice	Both sexes	Approximately the same age	*Leishmania donovani*	10^7 promastigotes	Intravenous	Ghosh *et al*., 2016
BALB/c mice	?	6 to 8 weeks	*Leishmania donovani*	2 x 10^7 promastigotes	Intravenous	Bandyopadhyay *et al*., 2015
BALB/c mice	?	4 to 6 weeks	*Leishmania donovani*	1 x 10^7 promastigotes	Intravenous	Bhattacharjee *et al*., 2015

Supplementary Table 2. Description of the main characteristics of the experimental models used and information about the induction of leishmaniasis (continuation)

Experimental model	**Sex**	**Age**	***Leishmania* species**	**Quantity and forms of the parasite for infection**	**Inoculation route**	**Reference**
BALB/c mice	Female	4 to 6 weeks	*Leishmania infantum*	10^7 promastigotes	Intraperitoneal	Corpas-López *et al*., 2015
BALB/c mice	Female	6 to 8 weeks	*Leishmania donovani*	2,5 x 10^7 promastigotes	Intravenous	Want *et al*., 2015
BALB/c mice	Male	21 days	*Leishmania infantum chagasi*	10^7 promastigotes	Intraperitoneal	Morais *et al*., 2014
BALB/c mice	?	4 to 6 weeks	*Leishmania donovani*	1 x 10^7 amastigotes	Intracardiac	Bhattacharjee *et al*., 2012
BALB/c mice	Both sexes	6 to 8 weeks	*Leishmania donovani*	1 x 10^6 promastigotes	Unknown	Mutiso *et al*., 2011
BALB/c mice	Female	?	*Leishmania donovani*	10^7 promastigotes	Intravenous	Ukil *et al*., 2011
BALB/c mice	?	4 to 6 weeks	*Leishmania donovani*	1 x 10^7 promastigotes	Intraperitoneal	Sen *et al*., 2010
BALB/c mice	Male	?	*Leishmania infantum*	10^7 amastigotes	Intravenous	Germonprez *et al*., 2005
BALB/c mice	?	?	*Leishmania donovani*	10^7 promastigotes	Intravenous	Ukil *et al*., 2005
BALB/c mice	Male	?	*Leishmania donovani*	10^7 amastigotes	Intravenous	Maes *et al*., 2004b

Supplementary Table 2. Description of the main characteristics of the experimental models used and information about the induction of leishmaniasis (continuation)

Experimental model	**Sex**	**Age**	***Leishmania* species**	**Quantity and forms of the parasite for infection**	**Inoculation route**	**Reference**
BALB/c mice and golden hamsters	Male	?	*Leishmania infantum*	1 x 10^7 promastigotes	Intracardiac	Bilbao-Ramos *et al*., 2020
BALB/c mice and golden hamsters	Female	?	*Leishmania donovani*	10^7 promastigotes	Intracardiac	Bhaumik *et al*., 2012
Golden hamsters	Male	?	*Leishmania infantum*	1 x 10^6 promastigotes	Intraperitoneal	Youssefi *et al*., 2019
Golden hamsters	?	8 weeks	*Leishmania infantum*	2 x 10^7 promastigotes	Intraperitoneal	Jesus *et al*., 2017
Golden hamsters	Both sexes	?	*Leishmania donovani*	1 x 10^7 amastigotes	Intracardiac	Suryawanshi *et al*., 2012
Golden hamsters	Male	?	*Leishmania donovani*	1 x 10^7 amastigotes	Intracardiac	Misra *et al*., 2010
Golden hamsters	Same sex	Same age	*Leishmania donovani*	2 x 10^6 amastigotes	Intracardiac	Lala *et al*., 2006
Golden hamsters	Same sex	Same age	*Leishmania donovani*	2 x 10^6 amastigotes	Intracardiac	Tyagi *et al*., 2005
Golden hamsters	Both sexes	?	*Leishmania donovani*	10^7 amastigotes	Intracardiac	Maes *et al*., 2004a
Golden hamsters	?	?	*Leishmania donovani*	5 x 10^6 amastigotes	Intraperitoneal	Ma *et al*., 2004

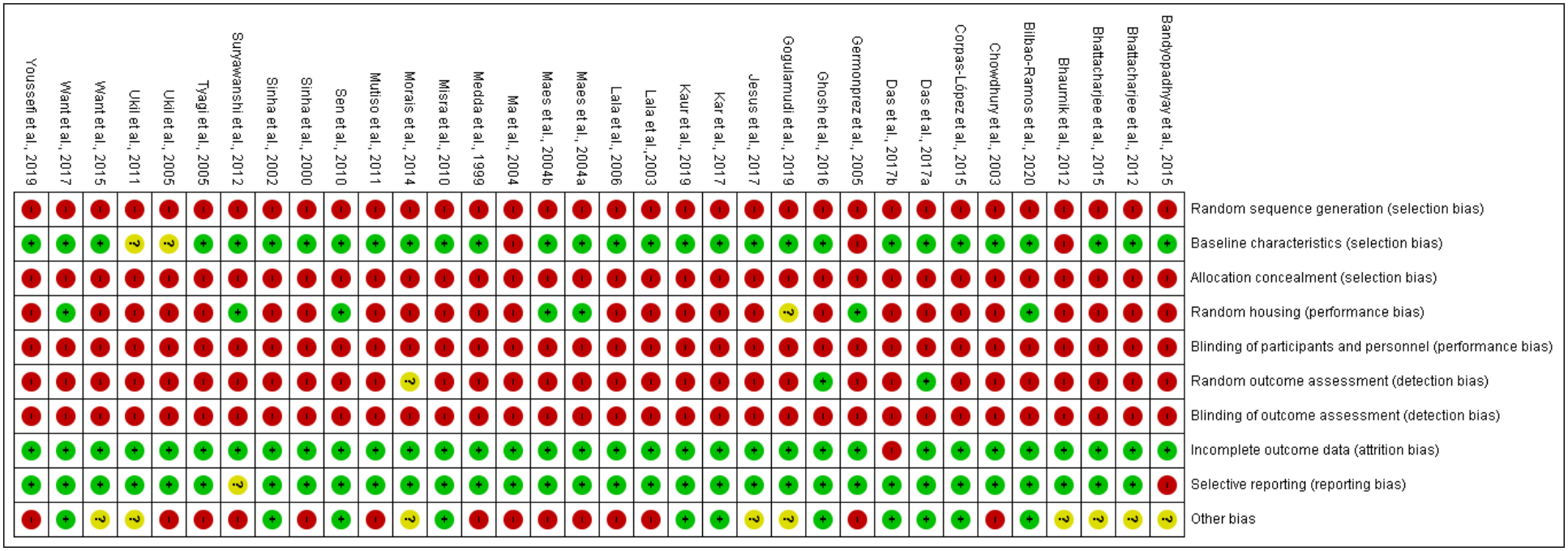
Supplementary Table 2. Description of the main characteristics of the experimental models used and information about the induction of leishmaniasis (continuation)

Experimental model	**Sex**	**Age**	***Leishmania* species**	**Quantity and forms of the parasite for infection**	**Inoculation route**	**Reference**
Golden hamsters	Male	6 weeks	*Leishmania donovani*	2-week established infection	Unknown	Chowdhury *et al*., 2003
Golden hamsters	?	?	*Leishmania donovani*	2 x 10^6 amastigotes	Intracardiac	Lala *et al*., 2003
Golden hamsters	?	?	*Leishmania donovani*	2 x 10^6 amastigotes	Intracardiac	Sinha *et al*., 2002
Golden hamsters	?	?	*Leishmania donovani*	2 x 10^6 amastigotes	Intracardiac	Sinha *et al*., 2000
Golden hamsters	?	?	*Leishmania donovani*	2 x 10^6 amastigotes	Intracardiac	Medda *et al*., 1999

Supplementary Table 3. Effects of terpene treatments in the animal weight and the occurrence of animal death during the experimental period (n = 34 studies).

References	Weigth	Animal death
Bilbao-Ramos *et al*., 2020; Ghosh *et al*., 2016; Chowdhury *et al*., 2003	No weight loss.	Not reported
Gogulamudi *et al*., 2019	Reduction in splenic and hepatic weight	Not reported
Want *et al*., 2017	Significant reduction in splenic and hepatic weight	Not reported
Das *et al*., 2017a	Significant reduction in splenic weight.	Not reported
Want *et al*., 2015	Significant reduction in splenic and hepatic weight	Not reported
Bhaumik *et al*., 2012	No marked effect on body weight.	Not reported
Sen *et al*., 2010	Significant reduction in splenic weight.	Not reported
Ukil *et al*., 2005; Maes *et al*., 2004b	No marked effect on body weight.	Not reported
Morais *et al*., 2014; Mutiso *et al*., 2011; Lala *et al*., 2006; Tyagi *et al*., 2005; Lala *et al*., 2003; Medda *et al*., 1999	Not reported	No animals died in the treatments.
Maes *et al*., 2004a	No marked effect on body weight.	One animal died during treatment
Kaur *et al*., 2019; Youssefi *et al*., 2019; Das *et al*., 2017b; Jesus *et al*., 2017; Kar *et al*., 2017; Bandyopadhyay *et al*., 2015; Bhattacharjee *et al*., 2015; Corpas-López *et al*., 2015; Bhattacharjee *et al*., 2012; Suryawanshi *et al*., 2012; Ukil *et al*., 2011; Misra *et al*., 2010; Germonprez *et al*., 2005; Ma *et al*., 2004; Sinha *et al*., 2002; Sinha *et al*., 2000	Not reported	Not reported

Supplementary Figure - Bias analysis of individual studies. The green "+" sign indicates "low risk of bias"; the sign "-", in red, means "high risk of bias"; the sign "?", in yellow, indicates "unclear bias.



CONCLUSÕES GERAIS

Com base nas informações apresentadas, é possível afirmar que os terpenos exibem potencial antileishmania, conforme evidenciado por suas ações diretas sobre os parasitos e também por suas ações indiretas, modulando a resposta imunológica. Isso torna esses compostos candidatos a testes em humanos, visando a descoberta de um tratamento eficaz da LV alternativamente à quimioterapia atual, que vem se tornando ineficaz por ser tóxica e porque os parasitos também têm apresentado resistência a ela.

Apesar das altas taxas de redução da carga parasitária mostradas, muitos trabalhos não trouxeram informações acerca da toxicidade, enquanto alguns revelavam que os compostos não eram tóxicos nas concentrações testadas, considerando-se os modelos experimentais utilizados. Dentre os trabalhos que fizeram observações sobre a toxicidade, um destacou que ela foi reduzida na dosagem de 50 μg/mL de ácido glicirrízico; outro trabalho comentou sobre toxicidade mínima a 40 mg/kg; um terceiro trabalho relatou toxicidade apenas em concentrações acima de 20 μmol x L^{-1} ao utilizar o ácido 18β-glicirretínico, sendo estas as concentraçõess que eliminaram a carga parasitária de forma eficiente.

Também é digno de nota que o uso de derivados de terpenos ou terpenos associados a carreadores parece ser mais vantajoso. Isso porque a literatura relata que compostos derivados reduzem a toxicidade, e os compostos associados a carreadores entregam a droga de forma direcionada ao sítio da infecção, além de permitirem sua permanência por mais tempo no organismo em comparação aos terpenos livres, enquanto estes são passíveis de rápida metabolização e excreção do organismo. O ácido 18β-glicirretínico, derivado do ácido glicirrízico, foi capaz de zerar a carga parasitária nos órgãos avaliados em um dos trabalhos. Estas informações tomadas em conjunto lançam luz às pesquisas por drogas contra LV utilizando os terpenos e pode encorajar o uso de compostos derivados e associados a carreadores em estudos que buscam por drogas eficazes no tratamento de diversas doenças, sobretudo as leishmanioses.