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Leishmaniasis: Possible New Strategies for Treatment

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1. Introduction

1.1. Leishmaniasis

Leishmaniasis is a vector-born disease caused by protozoan parasites in the genus *Leishmania*. Leishmaniasis is a potentially lethal, neglected disease that mostly affects economically disadvantaged individuals in many developing countries. Human infection occurs through the bite of infected female phlebotomine sand flies followed by injection of promastigote forms into the skin, which penetrate the macrophages and/or other types of mononuclear phagocytic cells. The promastigotes then transform into amastigotes (tissue stage of the parasite) in these cells and replicate, resulting in the bursting of the host cell and infection of other mononuclear phagocytic cells. Sand flies become infected by ingesting amastigote-infected cells during blood meals. These evolutionary forms transform into promastigotes in the gut of the insect and migrate to the proboscis, infecting other people through the bite (Scheme 1).

Depending on the *Leishmania* species involved and the host immune response, infection can lead to tegumentary or visceral manifestations of the disease. The tegumentary form of leishmaniasis (TL) includes cutaneous (CL), mucocutaneous (MCL), and diffuse (DL) clinical manifestations. All tegumentary forms have a major impact on patient life, because the skin lesions can lead to disfigurement and social stigmatization [2, 3]. Visceral leishmaniasis (VL), or Kala-azar, affects organs and internal tissues such as spleen, liver, bone marrow, and lymph
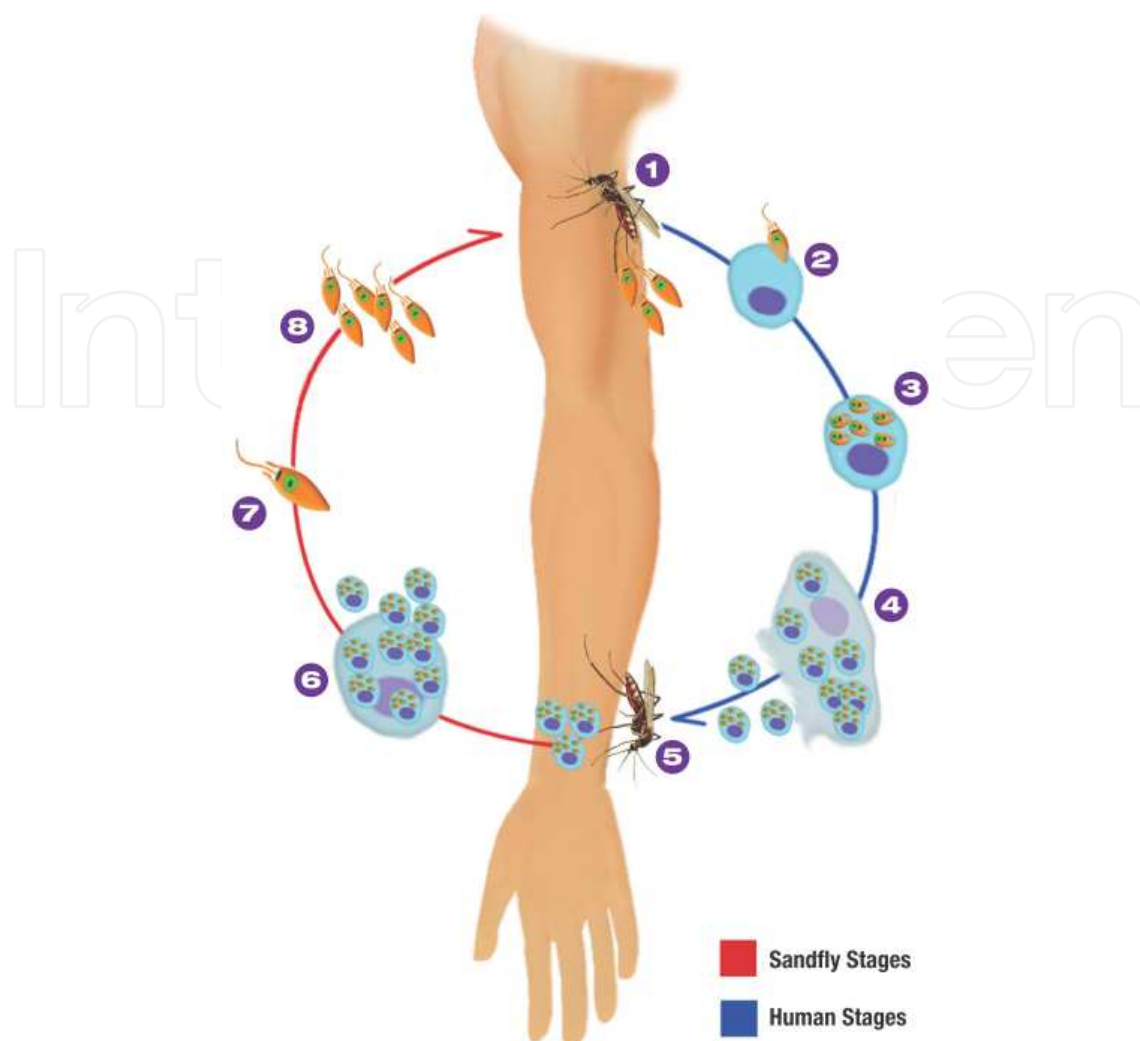
Scheme 1. Leishmaniasis life cycle. 1- sand flies inject promastigotes during blood meals ;2-promastigote infects macrophages and other types of mononuclear phagocytic cells;3-promastigotes transform into amastigotes; 4-amastigotes multiply. 5- sand flies become infected by ingesting macrophages infected with amastigotes during blood meals; 6- parasitized cells ; 7- in sand flies, amastigotes transform into promastigotes, in the gut; 8- promastigotes divide and migrate to proboscis based on CDC, 2013 [1].

nodes. With the progression of VL, splenomegaly and hepatomegaly lead to a distended abdomen and pain. Eventually, VL may lead to death caused by secondary infections, severe anemia, or organ failure [4]. Both TL and VL are endemic diseases in several countries. In fact, the number of cases of leishmaniasis may be underestimated because only 40 of the 88 countries where the disease frequently occurs report cases on a regular basis [5, 6].

The World Health Organization (WHO) estimates that 1.6 million new leishmaniasis cases occur annually, of which 500,000 correspond to VL (90% of them occurring in Bangladesh, Brazil, Ethiopia, India, Nepal, and Sudan) and 1.1 million to CL (90% of them occurring in Afghanistan, Algeria, Brazil, Iran, Peru, Saudi Arabia, Sudan, and Syria) [7]. Leishmaniasis currently affects an estimated 12 million people and approximately 350 million people live at risk of infection [1], whereas an estimated half a million people die annually of VL. In addition,

immunosuppressive conditions such as AIDS contribute to the emergence of severe clinical forms of the disease. To date, the greatest prevalence of *Leishmania*-HIV co-infection has been reported in the Mediterranean basin [8, 9]. In some east African regions, up to 40% of patients with VL are co-infected with HIV, which further complicates treatment [10].

2. Current drugs

Currently, most antileishmanial drugs can be considered orphan drugs. In fact, leishmaniasis, Chagas disease, are examples of tropical neglected diseases (TND) as they receive little attention from governments and the pharmaceutical industry. For instance, by the 2000s, global investment in new anti-parasitic drugs was only about 0.1% of global investment in research [11, 12]. Additionally, the lack of human vaccines for leishmaniasis makes chemotherapy the primary method used to control the disease. Despite the existence of several chemotherapics to treat human leishmaniasis, many of them are new formulations of ancient drugs [13]. The chemotherapeutic agents currently used in the treatment of VL and TL such as stibogluconate of sodium (Pentostam®), *N*-methylglucantime (Glucantime®), pentamidine (Pentacarinato®), and amphotericin B (Fungizone®) do not possess activity when orally administered and require parenteral administration for long periods [14]. In addition, those chemotherapeutic agents are very expensive and cause severe side effects due to their high toxicity [15].

Taken together, all these factors contribute to poor patient adherence or abandonment of treatment. In turn, treatment failure has a great impact on the spread of the disease and the emergence of drug-resistant strains. However, the introduction of new chemotherapeutic agents, including liposomal amphotericin B (AmBisome®), paramomycin, and miltefosin has certainly improved the current scenario for the treatment of leishmaniasis. AmBisome® has become the first-choice drug for treating VL in several countries. For instance, this treatment is currently used in Bihar, India, because pentavalent antimonials have become less effective against parasites [16, 17]. However, none of these drugs are free of severe side effects and the development of new strategies and/or alternative therapeutic agents remain crucial.

The incorporation of amphotericin B into lipid formulations has brought new perspectives to the treatment of leishmaniasis, resulting in the incorporation of several other drugs to different lipid formulations, including meglumine antimoniate [18], furazolidone [19], paromomycin sulfate [20] and miltefosin [21]. Conventional chemotherapeutics usually have difficulty reaching target tissues in therapeutic concentrations and are also associated with toxic effects on healthy organs and tissues. Thus, drug delivery approaches should improve the efficacy, specificity, tolerability, and therapeutic index of antiparasitic agents [12]. However, despite advances in the efficacy of existing drugs, the toxic potential of these substances must be considered. Thus, the search for new strategies and/or alternative therapeutic agents is crucial.

3. Natural products and drug delivery systems against leishmaniasis: state-of-the-art

The use of tools and materials at the nanoscale enabled the creation of nanoparticulate formulations such as liposomes, microemulsions, and microcapsules of great interest to the pharmaceutical industry. Drug delivery systems using liposomes are the ones most studied because of their high biocompatibility, ease of preparation, and chemical versatility [22]. Basically, liposomes are microscopic vesicles composed of one or more concentric lipid bilayers separated by aqueous media. Liposomes can encapsulate hydrophilic and lipophilic substances; the former stay in the aqueous compartment, whereas the latter are inserted into the membrane. Because liposomes are biodegradable, biocompatible, and non-immunogenic, they are highly versatile for research, therapeutic, and analytical applications [23]. These vesicles are primarily consisted of phospholipids (either synthetic or natural), sterols, and antioxidants [24]. Lipids with a cylindrical shape such as phosphatidylcholine, phosphatidylserine, phosphatidylglycerol, and sphingomyelin, which tend to form a stable bilayer in aqueous solution, are commonly used in liposomal formulations. Phosphatidylcholine is the most employed lipid in liposomal formulation studies due to its great stability against pH or salt concentration variations in the medium [25]. The pharmacokinetic properties of liposomes can be simply modified by changing the chemical composition of the bilayer components. Moreover, peptides, polysaccharides, or affinity ligands such as antibodies can be incorporated into liposomes [12].

Phospholipid-based liposomes are usually used to change the pharmacokinetics profile of several drugs, either natural or synthetic ones. Natural products such as crude extracts, fractions, or isolated phytocompounds have been incorporated into different colloidal carriers, including liposomes, with promising results. As expected in the incorporation of synthetic drugs, lipid formulations enhance the solubility and bioavailability of extracts and bioactive compounds derived from plants. Additional benefits of phytoformulations include: (i) protection from toxicity; (ii) enhanced pharmacological activity; (iii) enhanced stability; (iv) increased retention time; and (v) protection from physical and chemical degradation [26,22]. The ability of colloidal carriers to improve tissue macrophages distribution may have an impact on *Leishmania* infection. The tendency of nanoformulations, especially liposomes, to be captured by the mononuclear phagocyte system may be an additional advantage in the treatment of leishmaniasis. In fact, intraperitoneal and intravenous administration of liposomes proved to be a good biodistribution system for drugs in the treatment of VL, because it increased drug accumulation in macrophage-rich tissues such as liver and spleen, thus reducing the toxicity level to other tissues and organs [27].

Since ancient times, products from plant, mineral, and animal sources have been used in traditional medicine to fight many human diseases. In fact, for centuries traditional medicine has been the only health care system available for the prevention and treatment of several diseases in different cultures. Currently, the practice of traditional medicine still has a great impact on the health of people who have no access to modern health care practices. In fact, an estimated 80% of people living in developing countries rely almost completely on traditional medicinal practices to meet their primary medication needs [28-30]. Thus, the use of natural products as medicines has attracted the interest of research laboratories around the world

seeking new bioactive molecules. Among the most studied natural products, plants are a valuable source of compounds with antileishmanial activity. Active compounds derived from plant extracts have been described by several laboratories worldwide [31-34]. The antileishmanial activity of several crude extracts and fractions derived from plants has been attributed to compounds belonging to diverse chemical groups, including phenolic compounds (e.g., chalcones, flavonols, aurones, lignans, coumarins, quinines, tannins), terpenoids (monoterpenes, sesquiterpenes, diterpenoids, triterpenes), and alkaloids (indole alkaloids, isoquinoline alkaloids, quinoline alkaloids) [35-37]. In fact, phytoscience may be an important tool in the search for novel antileishmanial agents with fewer side effects and lower potential costs. Secondary metabolites isolated from plant extracts or essential oils can be used in several different ways for the development of drugs. Nanoformulation-based delivery systems are a promising approach to developing novel antileishmanial agents.

Species	Family	Geographical origin (country)	Part used	References
Aesculus hippocastanum	Hipocastanaceae	not cited	Seeds	[40]
Andrographis paniculata	Acanthaceae	(India)	Leaves	[41, 42]
Bacopa monniera	Scrophulariaceae (Plantaginaceae)	(India)	Leaves	[43, 44]
Camptotheca acuminata	Cornaceae	(China)	bark, stem	[45]
Curcuma longa	Zingiberaceae	(India)	rhizome	[46]
Fagopyrum esculentum	Polygonaceae	(India)	not cited	[39, 47]
Mimusops elangii	Sapotaceae	(India)	Seeds	[48]
Peganum harmala	Nitrariaceae	(India)	Seeds	[49, 50]
Piper aduncum	Piperaceae	(Brazil)	inflorescence	[51]
Piper nigrum	Piperaceae	(India)	Seeds	[52, 53]
Swertia chirata	Gentianaceae	Himalayan region (India)	aerial part	[54, 55]
Taxus baccata	Taxaceae	Peloponnese region (Greece)	needles, branches	[56]
Terminalia bellerica	Combretaceae	—	stem bark	[57]
Zanthoxylum chiloperone	Rutaceae	Cordillera (Paraguay)	stem bark	[58, 59]

Table 1. Natural sources of active substances used in nanoformulations against *Leishmania*.

Table 1 lists the natural sources used in nanoformulations to improve the bioavailability of antileishmanial drugs, thus increasing their expected therapeutic efficacy. However, the botanical species used for isolation of the active constituent have not been described in some

formulations such as those containing asiaticoside and acaciaside [38], whereas others have a plant origin but were purchased commercially [39]. It should be noted that the most studied species listed in Table 1 are native to developing countries, where the popular use of medicinal plants is widespread.

4. Phytocompound nanoformulations and antileishmanial activity

In the last decades, advances in nanoscience have enabled the development of nano-range materials approved for therapeutic use or in clinical development stage. In fact, many encapsulation matrices have been approved by the Food and Drug Administration (FDA) for use in humans, i.e., a wide range of naturally or chemically modified cyclodextrins that are extensively used in medicine and food fields [60, 61]. Similarly, vesicular systems such as liposomes, niosomes, nanoparticles, and microspheres are very useful and have many advantages in delivering drugs of natural origin, representing a promising approach for the treatment of several diseases, including leishmaniasis. Nanoformulations prepared with compounds from medicinal plants and their antileishmanial activity are summarized in Table 2. Liposomes, niosomes, and nanoparticles are the main formulations investigated.

Saponins (liposomes) and alkaloids (nanoparticles) are among the main plant constituents used to prepare nanoformulations. Liposomes are mainly formed by a mixture of phosphatidyl choline (PC), cholesterol (Chol), and phosphatidic acid (PA), usually in a 7:4:1 molar ratio [55, 38], whereas nanoparticle formulations have polylactide (PLA) as their main ingredient, providing greater stability, biocompatibility, and an efficient delivery system compared to liposomal structures [50, 57]. The antileishmanial properties of each nanoformulation are briefly cited below.

Flavonoids are among the most common phenolic compounds found in the human diet and a variety of members of this family has been described as bioactive agents. Significant antiprotozoal activity of flavonoids has been reported against *Trypanosoma* and *Leishmania* species. Quercetin (Fig. 1) is a widely studied food-derived flavonoid with several biological effects, including antioxidant, antihypertensive, anti-inflammatory, and antiprotozoal activities. Quercetin inhibits parasite arginase activity [62,63] and induces the production of superoxide anion, hydrogen peroxide, and other reactive oxygen species (ROS) by infected cells. Thus, ROS generation induced by quercetin could be crucial for maximal antiparasitic activity, because ROS are naturally generated by macrophages as a mechanism to kill intracellular parasites such as *Leishmania* [64, 65]. In fact, liposomal, niosomal, microspherulated, and nanocapsulated quercetin formulations have been tested to evaluate the best drug delivery system. In hamster models of *L. donovani* infection, all quercetin vesicular formulations reduced the parasite load compared to the free form of the drug. Nanocapsulated quercetin is more effective than non-capsulated quercetin in the control of leishmaniasis (87% reduction in spleen parasite burden) and its pronounced activity may be related to vesicular composition and size. Moreover, drug efficacy may be inversely correlated to the size of vesicular forms [39].

Similarly, the incorporation of terpenoids into nanoparticle carriers has also shown promising results. The search for active molecules to treat leishmaniasis is very laborious, because most molecules have low solubility. The incorporation of andrographolide (Fig. 2), a diterpenoid extracted from the herbaceous species *Andrographis paniculata* (Acanthaceae), with different poly (d,l-lactide-co-glycolide) (PLGA) nanoformulations enhanced the antileishmanial activity of andrographolide against axenic and intracellular amastigote forms of *L. donovani*. Among the formulations tested, the 175 nm andrographolide-loaded nanoparticles exhibited the best antileishmanial activity (IC$_{50}$ = 36 and 28μM for axenic and intracellular amastigotes, respectively) [66]. *Andrographis paniculata* can be considered an interesting source of antileishmanial agents. While 14-deoxy-11-oxoandrographolide (Fig. 2), an andrographolide-derived diterpenoid, reduced spleen parasite load in hamster models of *L. donovani* infection in 39%, liposomal, niosomal, and microspherulated formulations of this substance suppressed spleen parasite load by 78, 91, and 59%, respectively. In addition, the toxicity of 14-deoxy-11-oxoandrographolide to hepatic tissue also decreased after incorporation of 14-deoxy-11-oxoandrographolide into colloidal carriers, as demonstrated by the normal levels of serum alkaline phosphatase (ALP) and serum glutamate pyruvate transaminase (SGPT) in the blood [67]. Interestingly, particle size also proved to be an important factor for drug delivery efficacy. In fact, nanoparticles in a size range below 200 nm have been associated with increased phagocytosis by *Leishmania*-infected macrophages [68].

Formulations	Active ingredients	Administration Route	Target and Parasite reduction	% Entrapment efficiency	References
Liposomes Niosomes	Amarogentin	SC	*L. donovani* (LP 69% and NS 90%)	33 24	[55]
Mannose-coated Liposomes Liposomes	Piperine	SC	*L. donovani* (ML 90%; LP 77%)	22	[52]
Nanoparticles	2′,6′-dihydroxy-4′-methoxychalcone	IP	*L. amazonensis* (53%)	92	[51]
Liposomes	camptothecin	IP	*L. donovani* (55%)	2.7	[45]
Liposomes Niosomes Micropspheres Nanoparticles	Quercetin	SC	*L. donovani* (LP 51%; NS 68%; MS 44%; NP 87%)	40 50 09 -	[39]
Liposomes Niosomes Nanoparticles Microspheres	bacopasaponin C	SC	*L. donovani* (LP 81%; NS 86%; NP 79%; MS 91%)	30 30 30 50	[44]
Liposomes Niosomes Micropspheres	14-deoxi-11-oxoandrographolide	SC	*L. donovani* (LP 78%; NS 91%; MS 59%)	10 1 10	[42]
Liposomes	Harmine	SC	*L. donovani*	65	[50]

| Formulations	Active ingredients	Administration Route	Target and Parasite reduction	% Entrapment efficiency	References
Niosomes Nanoparticles			(LP 60%; NI 70%; NP 80%)	60 20	
Lipid nanospheres	Piperine	IV	*L. donovani* (52-90%)	100	[53]
Liposomes	asiaticoside	nr	*L. donovani* (LP 62%)	Nd	[38]
Liposomes	acaciaside	nr	*L. donovani* (LP 92%)	Nd	
Microemulsion Nanoparticles	bassic acid	SC	*L. donovani* (ME 62%; NP 78%)	100 50	[48]
Nanogels Nanoparticles	arjunglucoside I	SC	*L. donovani* (NG 79%; NP75%)	80 60	[57]
Nanoparticles	andrographolide	*in vitro* assay	*L. donovani* Axenic amastigotes (IC_{50} = 36 ± 4 µM/mL) Amastigotes in macrophage (IC_{50} = 28 ± 2 µM/mL)	80	[66]
Nanoparticles	β-aescin	*in vitro* assay	*L. infantum* Amastigotes (IC_{50} = 1.04 ± 0.23ug/ml)	2.8 – 31.9	[40]
Gold nanoparticles	quercetin	*in vitro* assay	*L. donovani* Axenic amastigotes (IC_{50} = 15 ± 3 µM/ml) Amastigotes in macrophage (IC_{50} 10 ± 2 µM/ml)	77	[69]

LP: liposomes; NS: niosomes; ML: manose-coated liposomes; NP: nanoparticles; MS: microspheres; ME: microemulsions; NG: nanogels.

SC: subcutaneous; IP: intraperitoneal; IV: intravenous.

nr: not reported; nd: not determined.

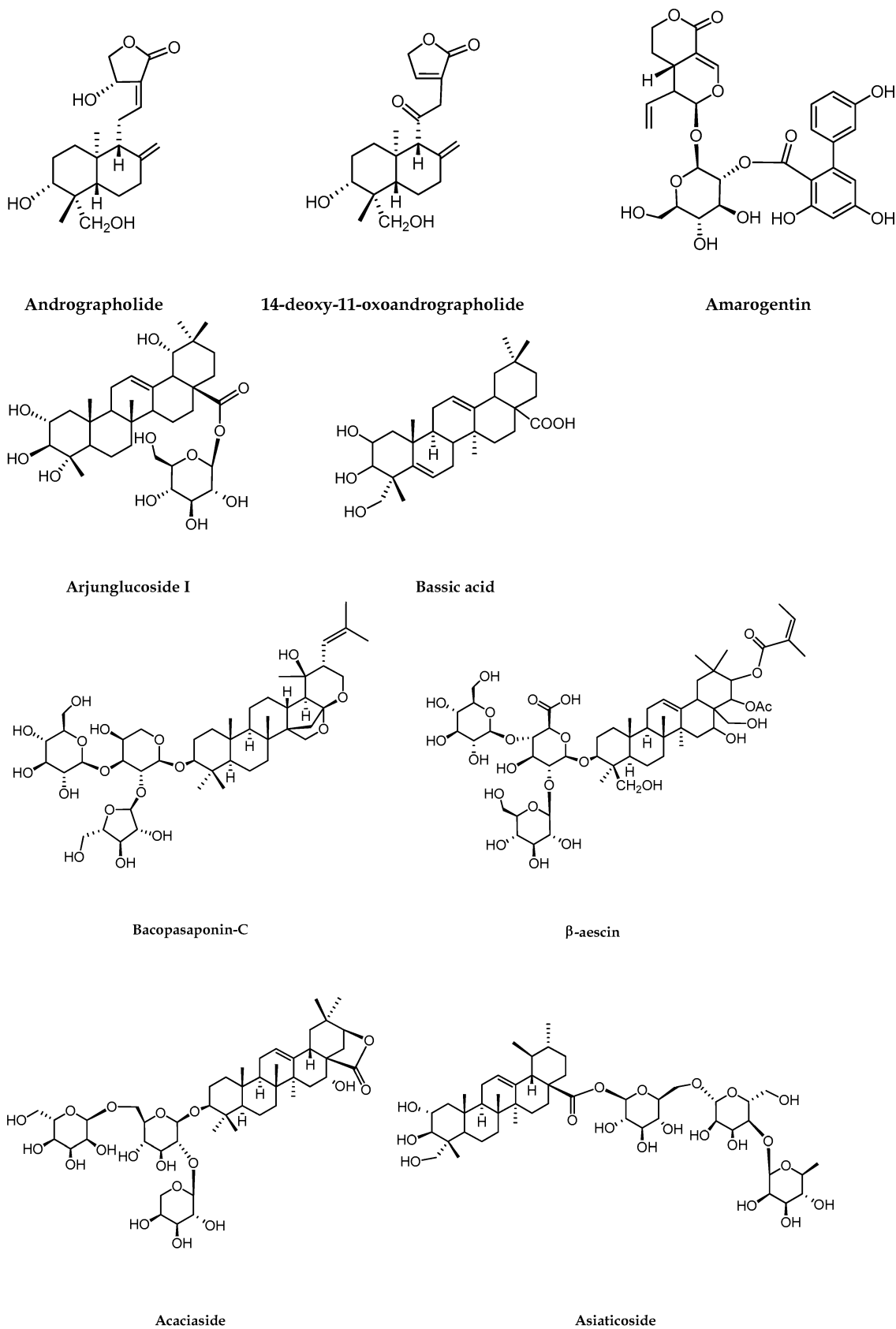**Table 2.** Phytocompound nanoformulations and antileishmanial activity.

Triterpenoidic or steroidal saponins directly reduce cell viability via membrane disruption. The mode of action of saponins is related to its aglycone portion, which binds to membrane sterols, leading to the formation of transmembrane pores and loss of intracellular content. This feature of saponins demonstrates the toxic potential of these molecules, hampering their use as antileishmanial agents. Conversely, polymeric nanoparticles composed of PLGA were successfully used to improve the efficacy of β-aescin (Fig. 2), the main saponin isolated from the seeds of horse-chestnut *Aesculus hippocastanumi* (Sapindaceae), lowering its cytotoxic effect for mammalian cells [40]. Bacopasaponin-C (Fig. 2) was firstly reported as an antileishmanial

2´, 6´-dihidroxy-4´-methoxychalcone   **Quercetin**
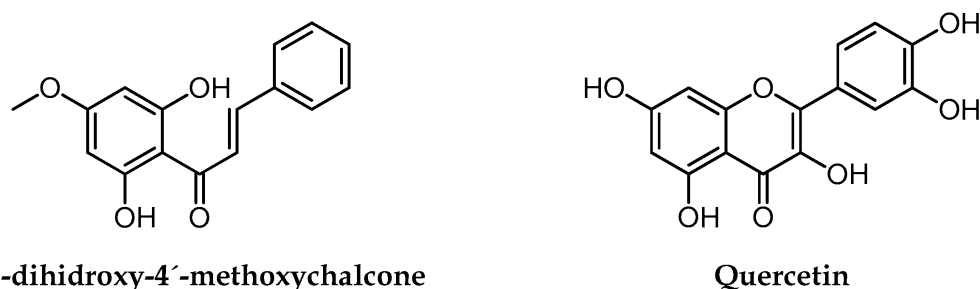
Figure 1. Structure of phenolic compounds loaded in nanoformulations.

agent in 2002, but its mechanism of action remains unclear. This glycoside extracted from *Bacopa monnieri* (Plantaginaceae) has glucose and rhamnose residues attached to the triterpe‐noid aglycone moiety. The glucose residue may be responsible for targeting bacopasaponin-C to glucose receptors on the cellular surface. Incorporation of bacopasaponin-C into various delivery carriers (niosomes, microspheres, nanoparticles, and liposomes) improved its antileishmanial activity. After six-day treatment with subcutaneous injections of liposomal, niosomal, microencapsulated, or nanocapsulated formulations of bacopasaponin-C, hamster models of *L. donovani* infection showed a significant reduction in spleen parasite burden (81, 86, 79, and 91%, respectively) compared to free drug-treated animals (40%). At the same dose (1.7 mg/kg), the smallest vesicles had the best efficacy, as follows: nanocapsules > niosomes > liposomes > microspheres [44]. Another glycoside with remarkable activity against *Leishma‐nia* parasites has been isolated from the indigenous plant *Swertia chirata* (Gentianaceae): amarogentin (Fig. 2) is a secoiridoid glycoside with the capacity to inhibit DNA-topoisomerase I, an essential enzyme related to *Leishmania* viability. Liposomal and niosomal formulations of amarogentin (2.5 mg/kg) reduced spleen parasite load by 69 and 90%, respectively, whereas free drug at equivalent dose reduced parasite load by 39%. In addition, both SPTG and ALP activity remained close to normal levels when liposomal or niosomal formulations of this iridoid were used in murine models [55].

The amide alkaloid piperine (Fig. 3) extracted from *Piper nigrum* (Piperaceae), an Indian species commonly used in traditional medicine, has been reported as a potent antileishmanial acting against both visceral (*L. donovani*) and tegumentary (*L. amazonensis*) leishmaniasis [70, 71]. *In vivo* tests have shown that piperine entrapped into liposomes and mannose-coated liposomes were effective against murine models of *L. donovani* infection. After 12-day treatment with liposomal and mannose-coated liposomal formulations of this alkaloid (four doses, 6 mg/mL) administered subcutaneously, a reduction in parasite burden of approximately 77 and 90%, respectively was achieved. Free piperine not only was less effective in reducing parasite burden (29%)but also had higher toxicity to liver compared to the colloidal carriers (ALP = 20.5 µmol of *p*-nitrophenol released/min/dL of sera and SGPT = 77.2 µmol of sodium pyruvate/min/L of sera) [52]. Nevertheless, better results can be achieved by using lipid nanospheres of piperine (LN-P). A single dose (5 mg/kg) of the lipid formulation composed of stearylamine (LN-P-SA) reduced parasite burden in liver and spleen of *L. donovani*-infected hamsters by 90% and 85% after 15 days post infection, respectively. Despite the size of the vesicles (about 884.6 nm), the efficacy of LN-P-SA may be related to the preferential uptake of stearylamine-bearing

Andrographolide   **14-deoxy-11-oxoandrographolide**   **Amarogentin**

Arjunglucoside I   **Bassic acid**

Bacopasaponin-C   **β-aescin**

Acaciaside   **Asiaticoside**

Figure 2. Structure of terpenoids loaded in nanoformulations.

positively charged liposomes (+ 24.2 mV) by peritoneal macrophages compared to neutral and negatively charged vesicles [53].

β-carboline alkaloids such as harmane, harmaline, and harmine were initially described as potent psychoactive and hallucinogenic agents. However, a wide range of pharmacological activities have been reported for those compounds, including those against *Leishmania* parasites [72]. Harmine (Fig. 3), isolated from *Peganum harmala* (Nitrariaceae), displays *in vitro* anti-*L. donovani* promastigote activity at 25 μg/mL. Recently, this alkaloid was incorporated into liposomes, niosomes, and nanoparticles at an equivalent dose of 1.5 mg/kg body weight, and after six doses administered subcutaneously to *L. donovani*-infected murine models, all harmine-entrapped vesicular formulations were able to reduce spleen parasite burden, especially nanosomes (a reduction of about 79%). Nevertheless, the mechanism of action of harmine against *Leishmania* remains unclear and may be related to necrotic membrane damage.

Piperine   **Camptothecin**   **Harmine**
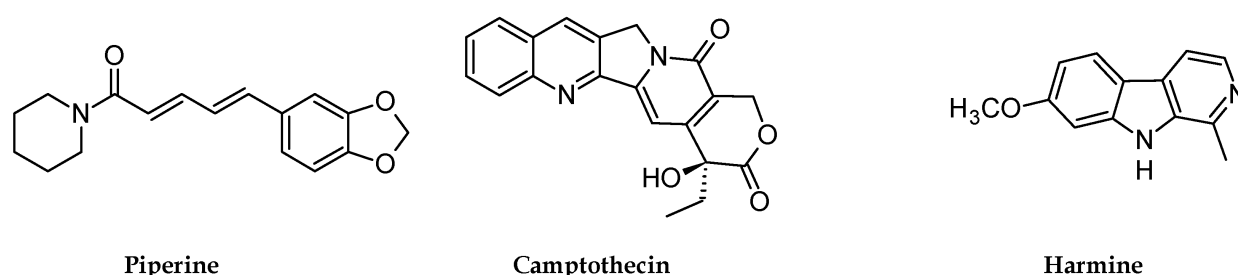
Figure 3. Structure of alkaloids loaded in nanoformulations.

The search for new compounds with antileishmanial activity in medicinal plants is an interesting strategy, however most studies are still limited to basic research and probably only a few compounds will reach stages of clinical trials and development of new drugs. The combination of phytochemistry and nanoformulations may open up new perspectives in the search for new antileishmanial drugs, because drug delivery systems based on nanoformulations can improve the effectiveness of natural compounds making them more attractive to the development of new therapeutic targets. Due to their structural versatility in terms of size, composition, and ability to incorporate hydrophilic or lipophilic substances, there are many possible applications of nanoparticulate formulations in the treatment and control of leishmaniasis.

5. Parasite targets for new drugs

Parasite resistance, cost, side effects, toxicity, and other therapy issues prompt an urgent need to identify and develop new drugs and alternative targets for leishmaniasis treatment [73, 74, 10]. Metabolic pathways such as glycolytic pathway [75, 76], polyamine biosynthesis [77, 78], glyoxalase pathway [79, 80], uptake and turnover of phospholipids/sphingolipids [81, 82], microtubule biosynthesis [83, 84], and folate metabolism [85] have been targeted.

The development of drugs directed at new targets such as parasite enzymes represents another approach in the search for new antileishmanial drugs. Arginase is a recently described target for the treatment of leishmaniasis. The enzyme is localized in the glycosome, a subcellular organelle found in some trpypanosomatids such as *Leishmania*. Inhibition of the arginase pathway causes inhibits the polyamine biosynthetic pathway, resulting in antileishmanial activity [86].

Other enzyme systems investigated as potential targets for antileishmanial drug action include nitric oxide synthase, DNA topoisomerase, trypanothione redutase, superoxide dismutase enzymes, and hypoxanthine-guanine phosphoribosyltransferase [74]; heme oxygenase-1 [87]; ribose 5-phosphate isomerase B [88]; dihydroorotate dehydrogenase [89]; ornitine decarboxylases [62]; Abl family kinases and phosphoinositide 3-kinase γ [90-93]; and spermidine synthase [94, 95].

A total of 154 peptidases were detected in the *Leishmania major* genome, including serine, cysteine, aspartic, threonine, and metallopeptidases. The cysteine peptidase-specific inhibitor K11777 has shown that these peptidases are necessary for parasite growth [96]. Even though some inhibitors have been developed but failed to kill parasites, peptidases are promising targets [97-99].

Carbonic anhydrases (CAs, EC 4.2.1.1) are a new target that are starting to be studied for *Leishmania*. CAs are metalloenzymes that catalyze CO_2 hydration to bicarbonate and protons, and five CA classes have been identified: α, β, γ, δ, and ζ. The reaction catalyzed by CAs is essential in the regulation of acid-base balance in organisms [100, 101]. A β-carbonic anhydrase was recently cloned and characterized from *Leishmania donovani chagasi*, and enzyme-specific inhibitors were tested against *Leishmania* [102].

The death of the parasite by inhibiting an enzyme or pathway essential for parasite survival and non-essential for the host requires the exploration of differences between these pathways or enzymes [103]. Thus, new *Leishmania* molecules should be studied and the possibility of developing rational and more effective drugs with less harmful side effects for the host investigated. Finally, new *Leishmania* chemotherapeutic targets and new approaches to the development of drugs should be considered [104, 93, 105].

6. Synthetic drugs

No vaccine candidates for leishmaniasis are currently under animal or clinical trials. Thus, new and effectives drugs should be investigated. In the last years, several drugs have been studied to find potential new drugs with desirable characteristics. Leishmaniasis is expanding in developed countries and in North America and Europe, which has alarmed health authorities worldwide [106]. Although leishmaniasis is treatable, it is difficult to control due to the absence of an effective vaccine, the adaptation of the vector and reservoir hosts to human environments, and the emergence of resistant lineages [107]. The first-line chemotherapy drugs available are pentavalent antimonials [108], whereas pentamidine and amphotericin B are second-line therapies, but these are associated with limited effectiveness, a long-term treatment, toxicity,

and significant side effects [109]. Consequently, there is an urgent need to discover new drugs that are effective against leishmaniasis [110].

Some compounds have been studied and regarded as promising new drugs. The stilbene trans-3,4',5-trimethoxy-3'-amino-stilbene (TTAS) showed a LD_{50} of 2.6 lg/mL against *Leishmania infantum* with low toxicity. The action mechanism is the disruption of the mitochondrial membrane potential and the ability to block *Leishmania* parasites during the G2/M phase of cell cycle [111].

N-butyl-1-(4-dimethylamino)phenyl-1,2,3,4-tetrahydro-β-carboline-3-carboxamide has been tested against *Leishmania amazonensis* and ultrastructural alterations, depolarization of the mitochondrial membrane with loss of cell membrane integrity, and increased formation of mitochondrial superoxide anions were detected, indicating that this compound induced mitochondrial dysfunction [111].

Besides the lack of an effective vaccine against leishmaniasis, in some East African regions, up to 40% of patients with visceral leishmaniasis are co-infected with HIV, which complicates the treatment. Peptidase inhibitors, used to treat HIV-infected individuals are a new route that needs more studies. HIV-1 protease inhibitors such as Indinavir, Saquinavir, and others have been tested to treat leishmaniasis and inhibition of parasite growth has been reported at high drugs concentrations [9].

Other synthetic antileishmanial compounds are currently being developed an evaluated for therapeutic use. β-carbolines from various natural and synthetic sources have shown diverse biological activities. A total of 22 compounds were synthesized and tested *in vitro* against *Leishmania donovani*, out of which six compounds (4, 5, 10, 11, 19, and 22) showed more activity than the standard miltefosine ($IC_{50} = 12.07 \pm 0.82$ lM), with compound 4 being the most potent ($IC_{50} = 2.16 \pm 0.26$ lM) [112]. Moreover, a semi-synthetic berberine analogue, 5,6-didehydro-8,8-diethyl-13-oxodihydroberberine chloride showed nanomolar level potency against *in vitro* models of leishmaniasis, malaria, and trypanosomiasis, as well as activity in an *in vivo* visceral leishmaniasis model [113]. Tamoxifen is a synthetic estrogen that has been successfully used to prevent recurrence of breast cancer in women who are estrogen-receptor positive. Miguel *et al.* (2008) [114] reported the leishmanicidal activity of tamoxifen *in vitro* using BALB/c mice infected with *L. amazonensis* and treated with this compound for 15 days.

Diospyrin, a bis-naphthoquinone isolate from the tree *Diospyros montana* and its semi-syntheitic derivatives showed inhibitory activity against *Leishmania* spp. The di-epoxide derivative of diospyrin (D17) was more effective against *L. donovani* promastigotes than diospyrin. The same derivative tested in *L.donovani* BHU1216 selectively inhibited intracellular amastigotes. Computational docking studies demonstrated that D17 could inhibit *L donovani* ornithine decarboxylase but not trypanothione reductase [115].

Therapeutic approaches using drugs that act on structures of vital importance to the parasite but absent or sufficiently different in their hosts have been explored by several research groups. Indotecan and AM13-55, are TopIB poisons with indenoisoquinoline structure. Both compounds were tested against *L infantum* and the results compared with paromomycin, a leishmanicidal drug. The tests were done on a murine BALB/c model of splenocytes infected with *L. infantum*. The results showed that Indotecam reduced more than 80% of the parasite

burden of the spleen and liver, indicating that this compound is a potential drug against visceral leishmaniasis [116].

TiO2@Ag nanoparticles (TiAg-Nps) produce reactive oxygen species (ROS), which have an antimicrobial effect, including antileishmanial effects on *Leishmania tropica* and *Leishmania infantum* promastigotes and amastigotes, mainly non-visible light-exposed TiAg-Nps [117]. Twenty-four porphyrin precursors and derivatives were evaluated against *Trypanosoma brucei*, *L. donovani*, and *Plasmodium* sp. The perforine 4i derivative showed the best activity against *T. brucei* with a MEC value of 6.25 mM, but the compound was not active against intramacrophage amastigotes of *L. donovani* [118].

Another approach used in studies is the combination of drug therapies aimed at finding the most effective and secure one. Pam3Cys (an in-built immunoadjuvant and TLR2 ligand) and miltefosine were combined and the resulting combination was evaluated. All experiments were done in BALB/c mouse. Parasitic inhibition significantly increased in groups treated with combinations of the drugs compared to groups receiving miltefosine and Pam3Cys separately. Moreover, increased production of Th1 cytokines, RNS, ROS, and H_2O_2, as well as increased phagocytosis were observed during the study of immunological alterations [119].

Several aromatic/heterocyclic sulfonamides and 5-mercapto-1,3,4-thiadiazoles were recently investigated against *L. donovani* CAs. The sulfonamides were medium potency-weak inhibitors, but some heterocyclic thiols inhibited the enzyme with KIs in the range of 13.4–52 nM. Microscopic studies revealed cell swelling and structural alterations on the flagellar pocket such as presence of vacuoles. Autophagic vacuoles that cause intracellular damages and parasite death, and accumulation of intracytoplasmic electron-dense granules were also induced by the inhibitors. These result suggest that β-CA from *Leishmania* is a potential new antileishmanial drug target [102].

7. Conclusions

Leishmaniasis is a neglected, potentially lethal infectious disease caused by parasites in the genus *Leishmania* that affects many developing countries. Infection can lead to tegumentary or visceral manifestations of the disease. The tegumentary form of leishmaniasis (TL) includes cutaneous, mucocutaneous, and diffuse clinical manifestations, whereas visceral leishmaniasis (VL), or Kala-azar, affects organs and internal tissues. The treatment of VL is challenging and long. Treated patients need monitoring and hospitalization. Therapeutic problems include toxicity and teratogenicity of the available drugs, and low response in human immunodeficiency virus (HIV)/*Leishmania* co-infections. In addition, drug resistance is increasing. Moreover, leishmaniasis received little attention from governments and the pharmaceutical industry until the last decade. The absence of either prophylactic or preventive vaccine candidates makes it further difficult to control the disease.

The development of new parasite targets and synthetic drugs along with the research on natural products represents a major strategy for the discovery of new compounds against Leishmania sp.

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