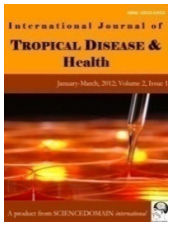

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Therapeutic Arsenal against Leishmaniases: A Review

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Leishmaniases are included in the group of reemerging neglected tropical diseases caused by flagellated parasites of the *Leishmania* genus. WHO recommends the use of chemotherapy as the main control measure for all the types of the disease. Antimonials are the first drug of choice, but they can be replaced by amphotericin B, mitelfosine, paromomycin and pentamidine isethionate, or combinations. Generally, all the medicines that contain these drugs have several drawbacks. In view of this, chemotherapy for Leishmaniasis is increasingly the object of study, searching for safer or more effective drugs or new drug delivery vehicles, where nanotechnology is crucial. Thus, the purpose of this review is to present the therapeutic arsenal available in the market and that has been investigated for this application.

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1. INTRODUCTION

Leishmaniasis is a parasite disease transmitted by the hematophagy of a *Phlebotominae* (*Phlebotomus* or *Lutzomyia* genus) which major reservoir are the humans [1,2,3]. It is caused by more than 20 different species of a protozoa of the *Leishmania* genus, which is subdivided into two different subgenera, *L. (Leishmania)* spp. And *L. (Viannia)* spp., of the *Kinetoplastida* order, *Tripanosomatidae* family. Depending on the lifecycle stage, these protozoa take one of the two forms: With flagellum, named promastigote and without flagellum, amastigote. Based on the species of the infectious agent and the spectrum of their clinical manifestation, Leishmaniases are classified by World Health Organization (WHO) [4] into Cutaneous Leishmaniasis (CL) and Visceral Leishmaniasis (VL).

According to the Drugs for Neglected Diseases initiative (DNDi), Leishmaniasis is one of the most important neglected diseases, as it occurs in 98 countries with 350 million people at risk. VL is endemic in 88 countries (across Asia, East Africa, South America and the Mediterranean region), of which 72 are low and middle income countries, but its impact on global public health is underestimated, since the notification of the disease is mandatory in only 32 of the affected countries. The most affected countries are Bangladesh, Brazil, India, Ethiopia, Kenya, Nepal and Sudan, which represent more than 90% of new cases; Whereas the countries most affected by CL are Afghanistan, Algeria, Colombia, Brazil, Iran, Syria, Ethiopia, Sudan, Costa Rica and Peru [5].

This disease appears as reemerging in several regions, already described in countries such as Brazil and India [6-8]. This panorama is attributed to several factors such as: The range of zoonotic *Leishmania* species of unknown etiology that can affect humans; The occurrence of infection in other secondary hosts; Human invasion of zoonotic foci; Changes in susceptibility to infection of the human host [9], as for example, the co-infection *Leishmania*-HIV [10].

However, regardless of the form of manisfestation of Leishmaniasis, WHO recommends chemotherapy as the primary form of control.

2. LEISHMANICIDAL DRUGS

2.1 Antimonials

According to the WHO, antimonials are the first drug of choice [4].

Antimony is classified as a metalloid, and is found in nature as the isotopes 121Sb (57.25%) and 123Sb (42.75%). Its most common oxidation states are SbIII and SbV [11].

The first global report on the use of antimonials in Leishmaniasis treatment was by the Brazilian doctor Gaspar Vianna in 1912, who described the use of tartar emetic or antimony III and potassium tartrate [12]. However, in view of its toxicity (it normally accumulates in vascularized organs and tissues, especially liver and kidney, besides having high affinity for the spleen and blood) other antimony compounds have been tested, arriving to pentavalent antimonials [13]. In both cases, the compounds with therapeutic action are the result of the formation of water soluble complexes between organic acids and derived carbohydrates (tartaric acids or gluconic acid) and antimony [14].

2.1.1 Pentavalent antimonials

The antimonials used in Leishmaniasis treatment are listed in Fig. 1 [13].

N-methylglucamine antimoniate (Glucantime®, Sanofi-Aventis, France) is the antimonial compound used in Southern European and Latin American countries; and sodium stibogluconate (Pentostam®, GlaxoSmithKline, United Kingdom) is used in English-speaking countries [15]. The difference in the choice of these drugs is not only due to the different *Leishmania spp.* species that cause the disease, but also to the inherent differences in sensitivity of the species to the available drugs and the site of the infection (CL, ML and VL forms), thus imposing different requirements on drug pharmacokinetics [16].

2.1.2 Dosage

Glucantime® is marketed in 5mL vials, containing 1.5gcrude antimony and 405 mg/SbV. Then, each mL contains 81mg of SbV. According to the WHO, the dosage must be calculated in mg of SbV/kg/day aiming at standardizing the treatment.

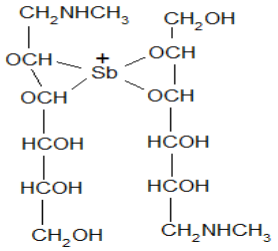
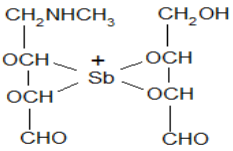
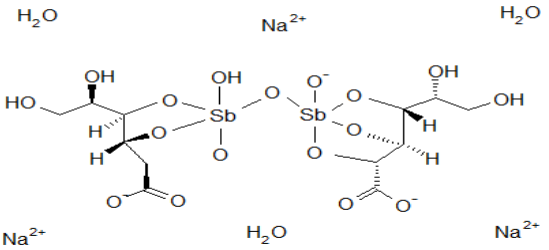
Proposed structural formula	Chemical name
 or  MW 507 MW 370	N-metthylglucamine antimonate
	Antimony gluconate (V) sodium or sodium stibogluconate

Fig. 1. Proposed chemical structure of antimonials [13,14]

In 1982,WHO suggested optimal dose of 20mg/kg/day, for both, CL and VL treatments, limited to a maximum dosage of 850mg/Sb/day for up to 28 days (usually 20 days for CL and 28 days for VL, with possible dose and/or therapy duration adjustments in individual cases – [5]). This dosage is well tolerated by children and malnourished people. The drug should be preferably intravenously administered by slow injection over 5 minutes, but gluteal intramuscular administration can also be used [17].

Marsden reports that conventional pentavalent antimonials cannot be orally administered due to poor absorption and/or inactivation in the stomach [18].

2.1.3 Mechanism of action

From the discovery of pentavalent antimonial drugs in 1940 until nowadays, they have been the first-line treatment in almost all geographic areas, through intramuscular, intravenous or intralesional administration for any infection by *Leishmania* [5,19]. However, the mechanism of action and the reason of the selective toxicity of these leishmanicidal agents remain little known [20].

According to Sereno et al. [21], there are indications that trivalent antimony is substantially more potent than its pentavalent oxidation state against both parasite forms (amastigotes and promastigotes) of, at least three *Leishmania* species. Some authors have reported that trivalent antimony is the toxic element to intracellular *Leishmania* [21,22]. Such results strengthen some hypotheses, as that from Miekeley et al. [23], who verified that after intramuscular administration of N-methylglucamine antimoniate in Leishmaniasis patients, the stibiate (organic compound) is converted *in vivo* to the ionic forms SbIII and SbV. This result indicates that SbV is bioreduced to its trivalent form, in accordance with other studies that show that the *in vivo* formation of SbIII is responsible for both the drug toxicity and its therapeutic activity. This theory was first proposed by Goodwin and Page, who believed that SbV could act as a prodrug, thus needing to be converted to its active form, SbIII [24]. Frézard et al. [25] pointed out the importance of the thiols as a reducing agent in this conversion, emphasizing four different ones: Glutathione (GSH), which is the main thiol in the cytosol of mammalian cells; Cysteine (Cys) and cysteinyl-glycine (Cys-Gly), which are the predominant thiols within lysosomes, and the glutathione-spermine conjugate, trypanothione ($T(SH)_2$), which is the predominant thiol within the parasite.

The *Leishmania* species present an organelle characteristic of the Trypanosomatidae family,

the glycosome, which contains nine enzymes involved in reactions of the glycolytic pathway, including glyceraldehyde 3-phosphate dehydrogenase (GAPDH). It acts both, in human hosts and in trypanosome, catalyzing the conversion of glyceraldehyde 3-phosphateto 1,3-diphosphoglycerate, in the presence of $NAD^+$and inorganic phosphate. This biochemical path is considered the main source of energy for promastigotes but it is also important for amastigotes. As a result, the depletion of intracellular ATP levels due to the interference of glycolysis and beta-oxidation of fatty acids in amastigotes is regarded as the main mechanism of action of antimonials against different *Leishmania* species [26].

Another possible mechanism of action of antimonials is the inactivation of a zinc-dependent metalloprotease in the amastigote form, through the substitution of antimony by zinc in that enzyme, thus inhibiting the development of the parasite [27].

2.1.4 Adverse effects

It is known that the pentavalent form does not bind to erythrocytes. Therefore, they reach higher concentration levels in plasma than the trivalent compounds. After consecutive administrations, there is an increase of the retention levels, transforming the trivalent form and accumulating in tissues such as spleen and liver, which causes the toxic effects related to antimony administration. The trivalent compounds bind in greater amount to the tissues and red blood cells, justifying their higher toxicity [28].

According to the systematic review by Oliveira et al., the most frequently reported adverse effects of antimonials are musculoskeletal pain, nausea, vomiting, diarrhea, abdominal pain, headache, anorexia, asthenia, fatigue, fever, pancreatitis, rash, erythema and hives; Besides frequently reported reactions, such as phlebitis and edema associated with intravenous administration [29]. Side effects such as cardiotoxicity, pancreatitis and nephrotoxicity [30-34], can lead to patient hospitalization and monitoring and interruption of antimonial treatment.

Clinical tests show elevation of serum levels of pancreatic enzymes associated with abdominal pain. In addition, increased creatine phosphokinase and alkaline phosphatase, renal failure, eosinophilia, leucopenia and thrombocytopenia have also been reported; Leading to treatment discontinuation in some cases [29].

2.1.5 Factors that lead to antimonial treatment substitution

The parenteral administration of these drugs requires a multiple dose regimen due to rapid renal clearance. When multiple consecutive doses are not administered, the dosage is not fulfilled, resulting in drug resistance [35]. Resistance can also occur because of the intrinsic differences among the different species that cause the disease in diverse geographic regions. Thus, reassessment of each type of treatment depending on VL and/or CL endemic areas is necessary [19].

When there is no satisfactory response and in some limited cases such as co-infection with HIV and treatment of pregnant women, treatment with pentavalent antimonials is not recommended and the adoption of second choice drugs is required [4].

2.2 Amphotericin B

In Bihar, an Indian state where VL is more endemic, the parasites are increasingly less susceptible to treatment with antimonials. This lead to failure of the general treatment therewith, resulting in changing Leishmaniasis treatment to conventional amphotericin B [19].

Amphotericin B is a polyene antibiotic (Fig. 2) naturally produced by the actinomycete *Streptomyces nodosus* widely used in the treatment of most systemic mycoses affecting immunocompromised patients [36]. It was introduced in the pharmaceutical market in 1950 [37] and the reports on its use for Leishmaniasis treatment in northern Bihar and districts adjacent to Nepal are from the 80s when the response to antimonials became less than 50% [38]. Nowadays, it is the first drug of choice for treating pregnant women and the second-line drug when there is no response to treatment with antimonials or when antimonials are not recommended such as cases of immunocompromised and HIV-VL co-infected patients [39].

Four formulations are currently marketed: amphotericin B deoxycholate (Fungizone®, Bristol Meyers Squibb, United States), liposomal amphotericin B (AmBisome®; Gilead Sciences,

United States), amphotericin B lipid complex (ABLC; Abelcet®, Enzon Pharmaceuticals, United States) and amphotericin B colloidal dispersion (ABCD; Amphotec™, Inter Mune Corp, Canada) [39]; However AmBisome® is the drug recommended by the WHO for Leishmaniasis treatment [4].

Amphotericin B liposomal formulation was introduced in the European pharmaceutical market in 1989 [40] and the combination of the drug with the nanocarrier (small unilamellar vesicles – SUVs) allowed a reduction of the adverse effects, due to a specific release to macrophages of the liver, spleen and bone marrow, affected by Leishmaniasis [39].

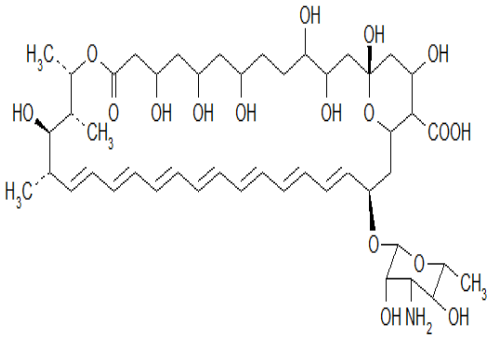


Fig. 2. Amphotericin B chemical structure

2.2.1 Dosage

For VL treatment it is also recommended the intravenous administration of 0.75-1 mg/kg of amphotericin B for 15–20 doses (daily or alternate days) and for VL treatment it is also recommended the administration of 0.7 mg/kg/day (intravenous infusion – 25/30 doses) or 0.7-1 mg/kg/day (intravenous infusion – 25/40 doses) [41].

In case of liposomal amphotericin B, WHO recommends a daily administration of0.75 to 1mg/kg of, which comes in vials containing 50mg of freeze dried amphotericin B sodium deoxycholate through intravenous infusion during14 to 20 days, with a 50mg minimum daily dose for VL treatment. For CL treatment it is also recommended the administration of 0.75to1mg/kg daily or on alternate days, limited to 50mg/day [5,42]. Treatment duration should be determined based on clinical evolution, considering the speed of the response and the presence of co-morbidities [43].

Immunocompromised patients present higher tolerance and, therefore require higher amphotericin B total doses. Currently, 3mg/kg liposomal amphotericin B is intravenously administered on days1 to 5, 14 and 21; Or 3 mg/kg/day during 7 to 10 days; Or 10 mg/kg/day for 2 days VL infected patients. Immunocompromised patients, HIV-VL co-infected patients receive 0.5 to 1.0 mg/kg/day until reaching a total dose of 1.5 to 2.0g amphotericin B and2 to 4 mg/kg/day liposomal amphotericin B in consecutive days or 4 mg/kg on alternate days, accumulating a total dose of 20 to 60 mg/kg [44].

The recommendations about the use of all current marketed formulations are presented in Table 1 [45].

2.2.2 Mechanism of action

According to Chattopadhyay and Jafurulla, *Leishmania* binding and internalization into host cells involves its interaction with the cellmembrane, thus requiring the presence of cholesterol to occur the pathogen-host interaction [46]. Then, cholesterol would play a key role in protein organization and function of membranes and receptors. Amphotericin B binds to the cell membrane causing a specific change to sterols, resulting in pore formation, which would allow the leakage of intracellular substances and the parasite's cell death [47]. Liposomal amphotericin B specifically interacts with membrane sterols of the parasite (in this case, macrophages) membrane, sequestering them, and consequently, effectively reducing the ability to interact with cholesterol [40,46].

2.2.3 Adverse effects

The use of amphotericin B in the clinic is limited due to the development of nephrotoxicity, the most common adverse effect that may manifest as an acute kidney injury and tubular injury, including renal failure, hypokalemia and hypomagnesemia [48,49]. However, side effects such as anemia, heart toxicity effect, nausea, vomiting, phlebitis, chills and fever are reported sometimes requiring treatment discontinuation [50]. Liposomal amphotericin B is preferred to conventional because it presents a milder toxicity profile [19], in addition to high potential and action spectrum, resulting in greater reliability of use [36]. The main advantage of the liposomal formulation is that it reduces renal and cardiac toxicity [36,51].

Table 1. Amphotericin B regimens currently in use and their recommendations

Drug	Treatment
Amphotericin B deoxycholate (Fungizone®, Bristol Meyers Squibb, United States)	Intravenous administration of 0.75–1 mg/kg for 15–20 doses (daily or alternate days); for VL treatment it is also recommended the administration of 0.7 mg/kg/day (intravenous infusion – 25/30 doses) or 0.7–1 mg/kg/day (intravenous infusion – 25/40 doses)
Liposomal amphotericin B (AmBisome®; Gilead Sciences, United States)	Daily administration of 0.75 to 1mg/kg through intravenous infusion during 14 to 20 days, with a 50mg minimum daily dose for VL treatment; 0.75 to 1mg/kg daily or on alternate days, limited to 50mg/day for CL treatment
Amphotericin B lipid complex (ABLC; Abelcet®, Enzon Pharmaceuticals, United States)	2 mg/kg; daily for 5 days, per infusion
Amphotericin B colloidal dispersion (ABCD; Amphotec™, InterMune Corp, Canada)	2 mg/kg; daily for 7 days, per infusion

2.3 Pentamidine

Pentamidine (Fig. 3) is also used for CL systemic treatment [19]. It was introduced in the pharmaceutical market in 1937 but it was used as leishmanicide only in 1973. It is the drug of choice in cases of resistance to antimonials, when they are contraindicated or when the patient shows signs of liver or cardiac toxicity as a consequence of its use [52].

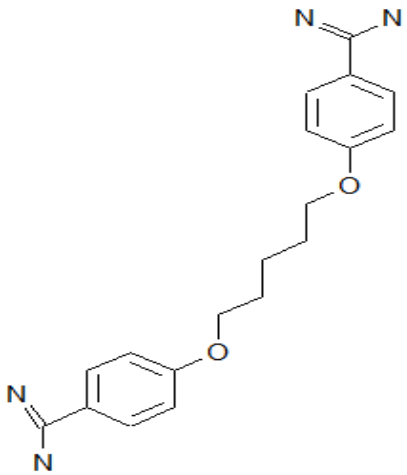


Fig. 3. Pentamidine chemical structure

2.3.1 Dosage

Usually, pentamidine isethionate (Pentcarinat®, Sanofi-Aventis, France) is administered at a dose of 4mg/kg on alternate days, with 20 intramuscular applications for CL treatment [53,54].

2.3.2 Mechanism of action

Its mechanism of action remains unknown. According to Basselin et al., amastigotes and promastigotes do not metabolize, but accumulate pentamidine against a concentration gradient through the cell membrane [55]. This accumulation results in polyamine transport inhibition, causing an imbalance between intra and extracellular polyamines, with consequent parasite growth inhibition.

2.3.4 Adverse effects

Pentamidine was the first drug to be used in patients resistant to antimony and this treatment was effective, although associated with adverse effects including pain at the injection site, nausea, fever, bitter taste, development of hypoglycemia, hypotension and type 1 diabetes [29]. Cardiac toxicity and nephrotoxicity have also been reported [50], besides effectiveness decreasing after consecutive administrations [39].

2.4 Paromomycin

Paromomycin (PM) or aminosidineis a broad spectrum antibiotic (Fig. 4), active against *Leishmania* (promastigotes and amastigotes), and some enteric protozoa and bacteria [44-56]. Its leishmanicide action was discovered in 1980 and has been successfully administered for VL treatment by parenteral route and as topical agent for CL treatment as will be discussed in the relevant chapter of this manuscript ("Topic Treatment of American Tegumentary

Leishmaniasis Lesions") [19]. It is indicated when the use of antimonials and amphotericin B is contraindicated [57], whether because of resistance to antimonials or because of adverse effects caused by them or amphotericin B. Paromomycin has fewer side effects than amphotericin B and is not sensitive to light [58].

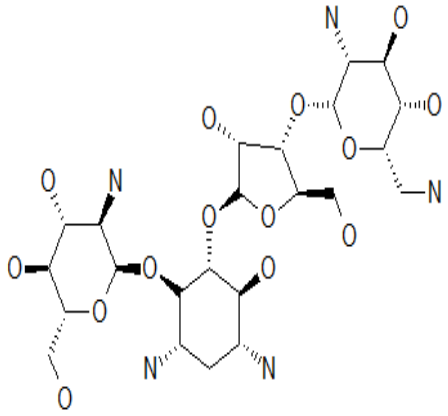


Fig. 4. Paromomycin chemical structure

2.4.1 Dosage

It is marketed as Humatin® (Pfizer, United States) in vials or capsules (parenteral or oral treatment). The former is indicated for Leishmaniasis treatment, with a dose of 14-16 mg/kg/day for up to 3 weeks [59]. However, some studies report that a smaller dose of 11 mg/kg/day intramuscular administered is also effective [39-60].

2.4.2 Mechanism of action

According to Davidson et al., paromomycin binds to the 30S ribosomal subunit, impairing protein synthesis of the parasite causing its death through inhibition of its metabolism and mitochondrial respiration [56].

2.4.3 Adverse effects

Adverse effects are not common, but include elevation of liver function enzymes, reversible ototoxicity and renal failure [44].

Resistance to the drug and its *in vitro* induction for *Leishmania* have been reported. However, unlike resistant bacteria, *Leishmania* species do not present mutations in the 30S ribosomal subunit (main mechanism leading to resistance), but a lower paromomycin absorption [56].

2.5 Miltefosine

Miltefosine, trade name Impavido® (Paladin Labs, Canada), is an alkylphosphocholine analog (hexadecylphosphocholine) (Fig. 5), which was originally developed as an antitumor agent, but that has proven to be clinically effective. It was recently introduced in the global pharmaceutical market for VL treatment [61].

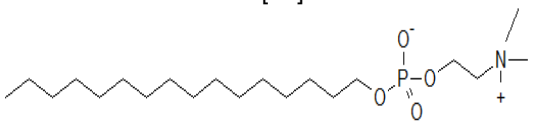


Fig. 5. Miltefosine chemical structure

Although the leishmanicidal biochemical mechanism of miltefosine remains uncertain, it shows good *in vitro* and *in vivo* efficiency against *Leishmania*, when orally administered to animals. In a study that investigated oral miltefosine tolerance for Indian VL, 29 out of 30 patients (97%) were healed with 100 mg miltefosine/day (an average of 2.5mg miltefosine/kg/day) during 28 days [62].

Taking into consideration, that the other drugs used in Leishmaniasis treatment are currently parentally administered, miltefosine is the only one with the possibility of oral administration [13-42].

2.5.1 Dosage

Miltefosine is formulated in capsules. A daily dose from 1.5 to 2.5 mg/kg is indicated for VL treatment of children above 3 years of age, adolescents, adults and the elderly; Whereas for CL treatment, the daily dose indicated for children above 12 years of age weighing at least 30 kg, adolescents and adults weighing less than 45 kg is 100mg miltefosine, and 150 mg/day for patients weighting more than 45 kg [63].

2.5.2 Mechanism of action

Miltefosine is a phospholipid that, despite not knowing exactly how it acts as leishmanicidal in the human body, interacts with the protozoan's cell membrane, causing its death. This is attributed to the close similarity between protozoan and human plasma membranes [64]. Miltefosine is not considered toxic to mammalian cells because it does not induce the activation of natural killer cells, of cytotoxic spleen cells, the phagocytic activity of macrophages, or a humoral response perse [65].

2.5.3 Adverse effects

The limitations of this drug are: High cost, long half-life thus becoming vulnerable to rapid resistance development, and the need for monitoring gastrointestinal and liver side effects besides nephrotoxicity [39]. Increased serum levels of aminotransferases and creatine phosphokinase were also reported [29]. In addition, it is potentially teratogenic [5,66].

3. COMBINATION THERAPY

According to van Griensven and Diro, combination therapy has been increasingly exploited, especially in highly endemic areas, aiming at identifying a short, inexpensive and well-tolerated combination regimen [67]. The combination of drugs with different mechanisms of action can also help to delay resistance emergence and increase the therapeutic life of drugs, bringing new therapies to current administration possibilities [68].

Several therapeutic regimens using this strategy have been reported [56,67,69]. Some examples are described as highly efficient and have been adopted and indicated by WHO: Combination of liposomal amphotericin B (5mg/kgper infusion, single dose) and miltefosine (2.5mg/kg/day during 7 days) and the combination of liposomal amphotericin B (5mg/kg per infusion, single dose) and paromomycin (11mg/kg during10 days) used for the treatment of VL caused by *L. donovani* in Bangladesh, Bhutan, India and Nepal. The combination between pentavalent antimonials (20mg/kg/day intramuscular or intravenously administered) and paromomycin (15mg/kg/day intramuscular administered) during17 days is also indicated by WHO for the treatment of VL caused by *L. donovani* in East Africa (Ethiopia, Eritrea, Kenya, Somalia, Sudan and Uganda) and Yemen [4].

4. UNDER STUDY

The long list of adverse effects described for all the drugs currently marketed, together with parenteral administration, except for miltefosine, leading to frequent treatment abandonment by the patients, thus favoring the development of resistant strains [70], explains that Leishmaniasis chemotherapy is increasingly being investigated. There are researches in literature on new chemical substances and plant extracts, as well as new formulations of known drugs, where nanotechnology is highlighted. These, then, are the object of the next topics of this paper.

4.1 Sitamaquine

Sitamaquine is an 8-aminoquinoline analog (Fig. 6) whose formulation is under development by GlaxoSmithKline (GSK) and is currently undergoing phase III clinical trials in India and Kenya. It is known that sitamaquine interacts with anionic phospholipids, such as phosphatidylinositol (PI) or phosphatidylglycerol (PG), while no interaction has been described with zwitterions or sterols. After sitamaquine initial interaction with the lipid monolayer, the hydrophobic interactions between the aromatic rings of the former and the acyl groups of the phospholipids of the latter, allow the complete insertion of the drug, with subsequent accumulation inside the parasite cells causing its death [71,72].

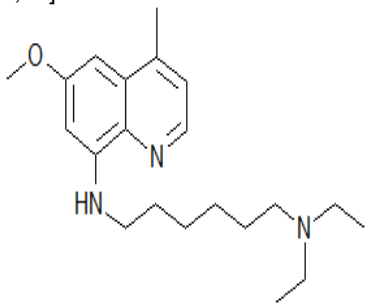


Fig. 6. Sitamaquine chemical structure

Sitamaquine, similar to miltefosine, is active by the oral route; However it has not shown to be efficient when topically administered for cutaneous Leishmaniasis treatment [66,72].

The side effects common to sitamaquine administration are: Vomiting, abdominal pain, headache, methemoglobinemia, cyanosis, nephrotic syndrome, glomerulonephritis and nephrotoxicity [73].

Sitamaquine has shown *in vitro* activity against a range of species causing CL, with ED_{50}values against amastigotes varying between 2.9-19.0μM [74]. Another study by Jha et al. [66], where 4 different sitamaquine doses (1.5; 1.75; 2.0 and 2.5mg/kg/day) were used during 28 days,for a total of 30 patients, concluded that the oral administration of sitamaquine is efficient for VL treatment under test conditions and that it was, in general, well tolerated. Similarly, the oral administration of 2mg/kg/day sitamaquine during 28 days has been efficient and well tolerated in the treatment of 61 Kenyan patients. However, further studies are necessary to define dosage and duration of the ideal administration of this

drug to better understand its adverse effects on the kidneys [73,75].

4.2 Natural Products

Plants are described as a potential source of new antiprotozoal drugs [76]. Many groups of natural products, including alkaloids, flavonoids, terpenoids and essential oils, have leishmanicidal activity already reported in the literature.

4.2.1 Alkaloids

Alkaloids are secondary metabolites, nitrogenated and generally alkaline (except colchicine, piperine and oximes), and, among other activities, their high leishmanicidal activity has been highlighted in scientific circles [77]. The literature reports were listed in chronological order in Table 2.

4.2.2 Flavonoids

Flavonoids are a group of polyphenolic compounds widely found throughout the plant kingdom and with known antioxidant activity [87].

Tasdemir et al. conducted a comprehensive study to assess the efficiency of several flavonoids and their analogs against *L. donovani* amastigotes [88]. The study showed that the most potent metabolites were fisetin (IC_{50} = 0.6µg/mL), 3-hydroxyflavone (IC_{50}= 0.7µg/mL), andluteolin (IC_{50}= 0.8µg/mL). The drug used as control, mitelfosine, had $IC_{50}$of 0.34µg/mL.

Vila-Novaet al. is olatedrutin from *Dimorphandra gardneriana* grains, a native plant of northeastern Brazil caatinga biome and tested it against *L. infantum chagasi* parasites [89]. The compound presented results that were not significantly different from the results of the control drug – EC_{50} of 30.3µg/mL and 43.3µg/mL for promastigotes and amastigotes, respectively, whereas for amphotericin B the results were: no effect and 19.75µg/mL, respectively.

Muzitano et al. [90] isolated quercitrin from *Kalanchoe pinnata* (Crassulaceae) and proved their leishmanicidal action by the reduction of parasitemia (57%) in BALB/c mice infected with *L. amazonensis* (murine model of CL), compared to Pentostam (8mg/kg twice a week). Some years before, Muzitano et al. had suggested the importance of quercetin aglycone-type structure and the rhamnosyl unit linked at C-3, in an unusual flavonoid (quercetin 3-*O*-α-L-arabinopyranosyl (1 → 2) α-L-rhamnopyranoside) isolated from the same plant, for the leishmanicidal activity. A IC_{50}≅ 45mg/mL (78mM) against *L. amazonensis* amastigotes was obtained [91].

Quercetin (3,3',4',5´-7-penta-hidroxy-flavone) stands out among the flavonoids, because its leishmanicidal activity was confirmed in several studies, as shown in Table 3 (in chronological order).

Several authors tried to elucidate quercetin's leishmanicidal activity. Fonseca-Silva et al. attributed it to a pro-oxidant action generating reactive oxygen species (ROSs), which would lead to mitochondria dysfunction and ultimate death of the parasite [96]. Quercitin can be seen as a non-cytotoxic drug. Da Silva et al. elucidated, through docking analyses, the interaction of quercetin, quercitrin and isoquercitrin with Asp129amino acid, which is involved in the formation of the bridge between the metal and Mn^{2+} A and B cofactors in arginase active site [94]. The three compounds interacted with Asp129, which may indicate a correlation between leishmanicidal action and arginase-flavones interaction. But, from the three compounds, only quercetin interacted with L-arginine substrate and the Mn^{2+}cofactor of *L. amazonensis*, at pH 9.6 and was thus considered a mixed inhibitor.

4.2.3 Terpenoids

Terpenoids represent one of the largest and most diverse classes of secondary metabolites among the natural compounds. Although terpenoids do not derive from isoprene, but from mevalonic acid (MVA) and/or methylerythritol phosphate (MEP), this class is usually classified according to the number of isoprene units [96]. Table 4 lists the studies found in the literature for this application in chronological order.

4.2.4 Essential oils

Essential oils are derived from aromatic plants and include highly concentrated lipophilic compounds. As they are widely used in medical applications and have reported virucidal and antimicrobial activity, their leishmanicidal properties have been investigated [103].

Table 2. Investigation of alkaloid leishmanicidal activity

Alkaloid	Type	Evaluated species	Type of test	Result	Reference
N-demethylconodurine	indole	*L. amazonensis* amastigotes	*In vitro*	IC_{50} = 25µg/mL	[78]
Dictylomide-A and dictylomide-B	quinoline	*L. amazonensis* promastigotes	*In vitro*	total lysis of promastigotes at 100 µg/mL	[79]
N-demethylholacurtine, 15α-hydroxyholaminee holacurtine	steroid	*L. donovani* promastigotes	*In vitro*	6.25 > IC_{50}> 1.56µg/mL	[80]
Holamine				1.56 > IC_{50} > 0.39µg/mL	
Dihydrocorynantheine	indole	*L. major* promastigotes	*In vitro*	IC_{50} = 1.65µM	[81]
Corynantheine				IC_{50} = 1.12µM	
Corynantheidine				IC_{50} = 2.81µM	
E-3-quinol-2-yl-prop-2-en-1-ol	quinoline	*L. amazonensis* amastigotes	*In vivo* administered by oral route in BALB/c mice	25mg/kg, during 15 days, reduced the parasite load in 54% and showed a significant reduction of the lesion weight of 22% Meglumine antimoniate (100mg/kg) reduced 98.4% and 87.3%, respectively	[82]
		L. infantum amastigotes		25 mg/kg, during 10 days, were able to reduce the parasite load by 83% Meglumine antimoniate (100mg/kg) reduced by 65%	
		L. donovani amastigotes		25 or 12.5mg/kg, during 10 days, were able to reduce the parasite load in 61% and 59%, respectively Mitelfosine (7.5mg/kg) reduced by 72%	
15,22-*O*-diacetyl-19-oxo-dihydroatisine	diterpenoid	*L. infantum* promastigotes	*In vitro*	IC_{50} = 12.8mg/L (after 72-hour culture), low toxicity (74.28mg/L)	[83]
azitine				IC_{50} = 10.12mg/L (after 72-hour culture), high toxicity (> 200mg/L)	
isoazitine				IC_{50} = 7.39mg/L (after 72-hour culture), high toxicity (> 300mg/L)	
O-methylmoschatolinee	isoquinoline	*L. braziliensis* promastigotes	*In vitro*	IC_{50} = 320.8µg/mL	[84]
liriodenine				IC_{50} = 58.5µg/mL	
Neolitsine	isoquinoline	*L. mexicana* promastigotes	*In vitro*	IC_{50} = 15µM	[85]
Cryptodorine				IC_{50} = 3µM	
Duguetine β-*N*-oxide	isoquinoline	*L. braziliensis* promastigotes	*In vitro*	IC_{50} = 0.11µM	[86]
Dicentrinone				IC_{50} = 0.01µM	

The activity of *Chenopodium ambrosioides* essential oil on *L. amazonensis* has been investigated. BALB/c mice were infected and were treated with *Chenopodium ambrosioides* essential oil administered by intraperitoneal, oral and intralesional routes. Its activity against the parasite amastigotes and promastigotes was confirmed except for the intralesional administration (3%), which had no effect – the size of the lesion did not decrease (P> 0.05). The treatments by intraperitoneal and oral routes with 30mg/kg of essential oil resulted in a leishmanicidal activity greater than the control, 1mg/kg amphotericin B (P> 0.05).However, lesion size increase was observed during oral treatment (P <0.01), and the authors concluded that the intraperitoneal route was the most efficient, although *Chenopodium ambrosioides* essential oil showed toxicity at that concentration causing a small abscess in the peritoneal cavity and death of 2 animals, after 25 injections. Healing was not observed in none of the animals treated [104].

In a recent study, which assessed the susceptibility of *L. infantum*, *L. tropica*and*L. major*to the essential oil *Cymbopogon citratus* andits main compounds mryceneand citral, *C. citratus*and citral exhibited a potential inhibition of *L. infantum, L. tropica*and *L major,* with IC_{50} between 25, 52 and 38, and 42, 34 and 36µg/mL, respectively. Mrycene proved to be the less active compound, with an IC_{50} value of 164 µg/mL for *L. infantum* and no effect on the other species evaluated [105].

According to Monzote et al., the essential oil linalol-rich, from *Croton cujacara* leaves has been successfully used against Leishmaniasis parasites [106]. The oil proved to be efficient against *L. amazonensis* promastigotes and amastigotes with IC_{50} of 8.3 and 22 ng/mL, respectively. No toxic effect on mammalian cells was observed.

4.2.5 Extracts

Plant extracts or compounds derived from plants are likely to provide a valuable source of new medicinal agents [107,108], including those with potential leishmanicidal activity [109,110]. The activity of these extracts largely depends on the solvent used for extraction, the partof the plant used and the presence of one or more natural products [111].

Moreira et al. demonstrated the leishmanicidal activity of *Stachytarpheta cayennensis* (Verbenaceae) hydroalcoholic extract [112]. In folk medicine, its crushed leaves and roots are already applied to the ulcerated lesion of Tegumentary Leishmaniasis. *L. braziliensis* and *L. amazonensis* amastigotes and promastigotes were used in the study. The leaf extract proved to be more efficient against *L. Braziliensis* (IC_{50} of 3.7 µg/mL) than against *L. amazonensis* (IC_{50} of 382.5 µg/mL).

The leishmanicidal activity of the methanol extract of *Tridax procumbens* (Asteraceae) whole plant has already been described [113]. The research was conducted on the amastigotes of *L. mexicana*, causative agent of CL. *In vitro* studies with this extract showed growth inhibition of that parasite, with IC_{50} of 3 µg/mL, whereas oxylipin (3*S*)-16,17-didehydrofalcarinol, a compound obtained from a *T. procumbens* exhibited greater inhibition, with IC_{50} of 0.478 µg/mL. Both, the extract and the compound were obtained through chromatographic methods.

García et al. [114] assessed the leishmanicidal activity of 48 extracts of 46 Cuban plants against *L. amazonensis* by *in vitro* bioassay. The best result against amastigotes of that parasite were obtained with ethanol extracts of *Hura crepitans* leaves, with IC_{50} (27.7 ± 0.6 µg/mL), followed by the extract from *Bambusa vulgaris* leaves and roots and the extract from *Simarouba glauca* leaves IC_{50} (41.5±0.6 µg/mL and 45.5±0.3 µg/mL, respectively).

Pontin et al. [115] assessed *in vitro* the leishmanicidal effect of different concentrations of Brazilian greenpropolis hydroalcoholic extract against *L. braziliensis* promastigotes (1, 10, 30, 50, 100, 250, 500 and 750 µg/mL) and amastigotes (10, 100 and 250µg/mL). The results showed that the extract was inactive against amastigotes and it caused the lysis of 79.3% amastigotes at 500 µg/mL, whereas amphotericin B used as control caused the lysis of 68.3% at the same concentration. *In vivo* effects were also evaluated using Brazilian green propolis extract in BALB/c mice infected with *L. braziliensis* promastigotes. For systemic evaluation, the infected animals were treated by the oral route. The extract concentration used was 1.5mg/kg/day and the lesion diameter was measured monthly. The intraperitoneal administration of 20mg/kg/day of Glucantime® was used as a control. The oral treatment with propolis extract caused a significant reduction of 78.6% of the lesion diameter over 90-day treatment. Glucantime® caused a 57.7% reduction of the size lesion.

Table 3. Leishmanicidal activity of the flavonoid quercetin

Evaluated species	Type of test	Result	Reference
L. donovani amastigotes	*In vitro*	IC_{50} = 45.5μM	[92]
	In vivo orally administered to golden hamster	14mg/kg of the compound was able to reduce the spleen parasite load in 90%	
L. donovani amastigotes	*In vitro*	IC_{50} = 1.0μg/L	[88]
	In vivo intraperitoneal administered to BALB/c mice	30mg/kg during 5 days, was able to reduce infection in 15.3%	
L. amazonensis promastigotes	*In vitro*	IC_{50} = 1.0μg/mL	[93]
L. infantum chagasi amastigotes	*In vitro*	EC_{50} = 10.6μg/mL(Amphotericin B: ED_{50}= 19.75μg/mL)	[89]
L. infantum chagasi promastigotes		EC_{50} = 26μg/mL(Amphotericin B: ED_{50} = no effect)	
L. amazonensis amastigotes	*In vitro*	IC_{50} = 4.30μM	[94]
L. amazonensis promastigotes	*In vitro*	IC_{50} = 31.4μM	[95]

Table 4. Leishmanicidal activities of terpenoids

Terpenoid	Type of terpenoid	Evaluated species	Type of test	Result	Reference
Dihydrobetulinic acid	triterpenoid	*L. donovani* amastigotes	*In vitro*	IC_{50} = 4.1μM	[97]
		L. donovani promastigotes		IC_{50} = 2.6μM	
		L. donovani promastigotes	*In vivo* orally administered togolden hamsters	10mg/kg during 6 weeks allowed to reduce the parasite load by 95.5% and 92.5% in spleen and liver, respectively; the compound did not present toxicity	
			In vivo intramuscular administered togolden hamsters	10mg/kg during 6 weeks allowed to reduce the parasite load by 97% and 99% in spleen and liver, respectively; the compound did not present toxicity	
Maesabalide III	triterpene saponins	*L. donovani* amastigotes	*In vivo* subcutaneously administered to golden hamsters	0.8mg/kg allowed to reduce 94.2% of the amastigotes in liver (7-day treatment) AmBisome® (5 mg/kg) reduced 99.4%	[98]
Nerolidol	sesquiterpene	*L. amazonensis*	*In vitro*	IC_{50} = 67μM	[99]

Terpenoid	Type of terpenoid	Evaluated species	Type of test	Result	Reference
		amastigotes			
		L. amazonensis promastigotes		IC_{50} = 85μM	
		L. braziliensis promastigotes		IC_{50} = 74μM	
		L. chagasi promastigotes		IC_{50} = 75μM	
		L. amazonensis amastigotes	*In vivo* intraperitoneally administered to BALB/c mice	100mg/kg during 12 days were able to reduce (P <0.05) the size of the lesion, but, in the long term the disease was not healed. No toxic effects were observed	
		promastigotas de *L. amazonensis*			
Ursolic acid	triterpenoid	*L. donovani* promastigotes	*In vitro*	IC_{50} = 3.7μg/mL (the drugs pentamidine and amphotericin B, were used as control and presented IC_{50} values of 1.9μg/mL and 0.7μg/mL, respectively)	[100]
Hautriwaic acid lactone				IC_{50} = 7.0μg/mL (idem)	
8,13-diacetyl-piptocarphol	lactone sesquiterpene	*L. amazonensis*		IC_{50} = 0.2μM	[101]
ursolic acid				IC_{50} = 0.99μM	
8-acetyl-13-O-ethyl-piptocarphol				IC_{50} = 0.37μM	
Cumanin	sesquiterpene	*L. amazonensis* promastigotes	*In vitro*	growth inhibition higher than 80% at 5μg/mL (19μM) for both evaluated species	[102]
		L. braziliensis promastigotes			

Ferreira et al. [116] investigated the water extract of same kind of propolis and concluded that oral doses of this were able to reduce parasite load of in the liver of mice infected with *Leishmania infantum*.

Lantana ukambensis (Verbenaceae) methanol extract showed leishmanicidal activity with IC_{50} of 6.9µg/m Lagainst *L. donovani* promastigotes [117].

4.3 Nanotechnology

The research and development in nanotechnology involve structures with one (lamellae), two (nanotubes and nanofilaments) or three (nanoparticles) dimensions in the nanometric scale [118]. The last group stands out in Pharmaceutical Technology, where liposomes, polymeric nanoparticles and cyclodextrins are the most investigated nanocarriers for Leishmaniasis chemotherapy, although metallic nanoparticles and lamellar clays have also been reported.

Liposomal amphotericin B is the sole example of drug based on nanotechnology, which has already been used in Leishmaniasis treatment, as already mentioned in the present paper (AmBisome®, Gilead Sciences, Inc, Foster City, USA). It is basically composed by phospholipid molecules (hydrogenated soy phosphatidylcholine and distearoyl phosphatidylglycerol) arranged in a lipid bilayer [119,120], where the drug is included. Although it has been the standard treatment for VL, there are several limitations in the use, including high cost and nephrotoxicity [121]. According to Filippin and Souza, the formation of liposomal complexes has been investigated as resource to reduce this nephrotoxicity characteristic of amphotericin Badministration [36].

Frézard et al. [35] encapsulated meglumine antimoniate in liposomes by dehydration-rehydration procedure and obtained a high degree of encapsulation efficiency (28-58%), besides low indices of lipid weight. Opposite to the formulations obtained through the conventional methods, this one can be stored lyophilized and reconstituted just prior to administration.

In other study, liposomal meglumine antimoniate reduced parasite load in liver and spleen of mice infected with *Leishmania infantum* [116]. Furthermore, the authors evaluated its association (single dose by intraperitoneal route) with water extract of green propolis (oral doses). Unexpectedly, there was no synergism in reducing the parasite load.

In an attempt to neutralize the damaging effects of the oxidative stress caused by the use of SbIII, Castro et al. [122] prepared liposomal nanoparticles of trivalent antimony associated with ascorbic acid. For evaluating their leishmanicidal action, BALB/c mice were infected with promastigotes of *L. infatum* by intravenous route. The results showed the preservation of leishmanicidal activity and confirmed the reduction of toxic effects on the liver and kidneys.

Amphotericin B was nanoencapsulated in poly (lactic-co-glycolic acid) (PLGA)/ dimercaptosuccinic acid (DMSA) and associated (Nano-D-AMB-MG) or not (Nano-D-AMB) to magnetic nanoparticles, and evaluated in C57BL mice infected. The Nano-D-AMB achieved a greater reduction in Leishmaniasis parasites if compared to the group treated with D-AMB (Fungizome®). Furthermore, a fewer frequency of dosages was required to achieve the same therapeutic levels. Regarding the Nano-D-AMB-MG, the formation of aggregates difficulted the deliveryof the drug at the targeted site, so that the authors suggest further investigation [120].

Demicheli et al. [123] assessed the complexation of meglumine antimoniate with β-cyclodextrin, which resulted in an increase of antimony absorption by the oral route ant turned it orally active in a murine model infected by *L. amazonensis*. The efficiency of the oral formulation was equivalent to that of meglumine antimoniate formulation intraperitoneally administered with a two times higher antimony dose. And in a more recent study, the authors also observed that lyophilization increased the oral absorption of Sb and contributed to a better controlled drug release [124].

Das et al. [25] developed quercetin conjugates with gold nanoparticle. Although quercetin shows leishmanicidal activity, it has a serious problem of systemic transportation due to its low water solubility. The nanostructured system proved to be efficient, with a complete absorption by macrophages with IC_{50} of 15, 40and 30µM against *L. donovani,* wild type, and strains resistant to sodium stibogluconate and paromomycin, respectively. These results were compared with quercetin (34; 150 and 75µM, resp.), amphotericin B (0.2; 0.4 and 0.35µM,

resp.), sodium stibogluconate (3.6; 130 and 115μM, resp.) and paramomycin (10; 380 and 330μM, resp.). The authors attributed the effect to synergism between quercetin and the nanocarrier, which can cause deficiencies in the oxygen metabolism of the parasite. The toxicity of those nanoparticles was considered low.

The effects of several metal and metal oxide nanoparticles were investigated against *L. major* in the presence of ultra-violet light (UV), infrared (IR) and in the dark. These nanomaterials have a known antibacterial effect, which mechanism is based on the production of reactive oxygen species (presence of UV), or on the generation of heat (IR), causing damage to the parasites. The results confirmed the leishmanicidal activity of all tested nanoparticles, especially the nanoparticles of silver and of titanium dioxide, which demonstrated the bestresults [126].

Menezes et al. [127] produced a nanocomposite containing SbIII and lamellar clay (Mg, Al HDL) as nanocarrier, which showed ability to be internalized by macrophages, although its leishmanicidal activity is still under investigation. Different from other nanocarriers, this one, which is inorganic, degrades at acidic pH and presents biocompatibility and thus, some authors considered it a potential nanocarrier in the treatment of intracellular diseases, such as Leishmaniasis.

5. TOPICAL TREATMENT OF AMERICAN TEGUMENTARY LEISHMANIASIS

American Tegumentary Leishmaniasis is caused by species of the *Leishmania* or *Viannia* sub-genera [41]. The high incidence and severity of this disease make an early diagnosis essential in order to immediately begin treatment to prevent further tissue damages or, when not treated, the death of the patient [128].

Following diagnosis, the choice between topic or systemic treatment is done according to the evidence degree, geographic distribution, clinical manifestation and *Leishmania*s peciesinvolved [129]. There is an increasingly number of studies on the efficiency of the topic treatment for one of the clinical manifestations of this type of Leishmaniasis, Cutaneous Leishmaniasis.

WHO recommends paromomycin (ointment composed by 15% paromomycin and 12% methylbenzethonium chloride) twice a day as the first drug of choice for CL (*L. major*, *L. tropica*, *L. aethiopica* and *L. infantum*) treatment. However, the benefits obtained with topic treatment, were considered modest when compared with the placebo and the antimonials [56]. The intralesional administration of 1 to 5mL of antimony is considered as the treatment of second choice [42].

Imiquimod (Aldara®, Bayer, Germany), an imidazoquinoline amineused for the treatment of skin lesions caused by the human papillomavirus (HPV), genital warts and pre-malignant conditions [130] has shown high leishmanicidal potential [131].It acts inducing the release of nitric oxide, which has a potent cytotoxic effect against *Leishmania* species, and subsequent macrophage activation [130].

Arevalo et al. provedits efficiency when associated to intramuscular administered meglumine antimoniate in Peruvian regions where the infection by *L. Peruvians* was endemic [132]. Twelve patients with active lesions which had not responded to previous intravenous or intramuscular treatment with antimonials (20mg/kg/day) were selected for imiquimod evaluation. 250mg of ointment (5% of drug) were spread on the lesions for 20 days, on alternate days, in conjunction with 20mg SbV/kg/day intramuscular administered during 20 consecutive days. The response to treatment was daily assessed by the same physician. The results suggest that the combined therapy of imiquimod andmeglumine antimoniate should be considered for CL patients who did not respond to the initial treatment with only meglumine antimoniate.

Another study observed that one patient who did not present a good response to liposomal amphotericin B was successfully treated with imiquimod. However, the authors are not sure whether this improvement was due to previous treatments received by the patient, including amphotericin or even to spontaneous healing of the disease [133].

6. CONCLUSION

Leishmaniasis is a neglected disease which can trigger mild, moderate or severe signs and symptomsin infected patients. This situation depends on the form of the disease, but in most cases a pharmacological treatment is required. However, the drugs currently used and available in the pharmaceutical marketare potentially toxic which may result on treatment interruption and

consequent resistance of the parasites that cause the disease or even on the death of the patient, depending on the species. Connected to this, the need for affordable treatments is today the most serious drawbacks of existing therapies. In addition, this review showed that most of the researches evaluate the effects of the compounds under test on the promastigotes. This suggests the need for further studies on the human infective form (amastigote), and a better knowledge of the vector and its behavior, the dynamics of the disease and different hosts and reservoirs involved in the biological cycle for the development and choice of the most adequate treatment (one or more chemotherapy agents, and its administration route) as well as conducting a better control of the disease. So, the development of non-profit organizations has helped to increase the research and development (R&D), but until the lack of commercial and political is overcomed, the situation of millions of people at risk of infection unfortunately has no perspectives to be improved.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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