

## Therapeutic arsenal against leishmaniases: a review

### 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, miltefosine, paromomycin and pentamidine isethionate, or combinations. Generally, all the medicines that contain these drugs have several drawbacks. In view of this, chemotherapy for leishmaniasis treatment 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.

**Keywords:** neglected tropical diseases (NTD); leishmaniasis; chemotherapy; leishmanicidal; nanotechnology.

### INTRODUCTION

Leishmaniasis is a parasitic 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, Tripanosomatida 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 manifestation of leishmaniasis, WHO recommends chemotherapy as the primary form of control.

### LEISHMANICIDAL DRUGS IN THE MARKET

#### 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  $^{121}\text{Sb}$  (57.25%) and  $^{123}\text{Sb}$  (42.75%). Its most common oxidation states are Sb(III) and Sb(V) [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].

### Pentavalent antimonials

The antimonials used in leishmaniasis treatment are listed in figure 1 [13].

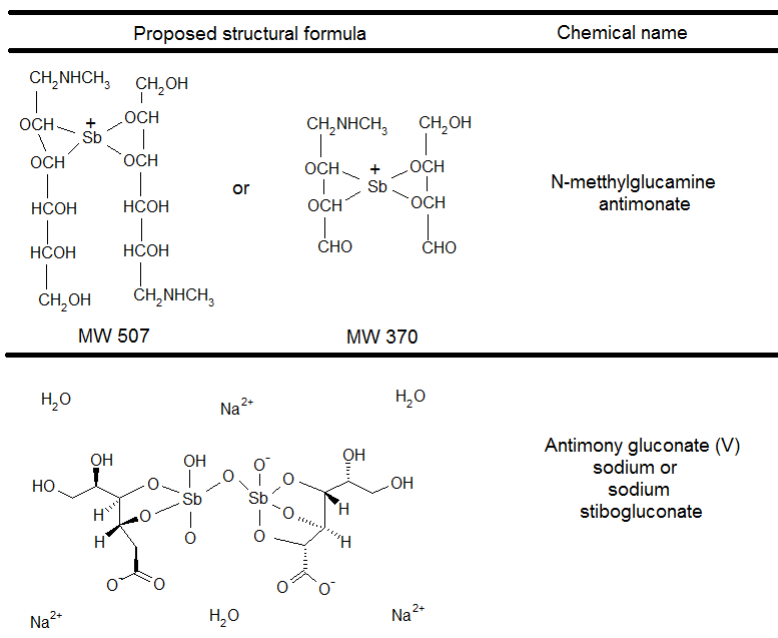


**Figure 1.** Proposed chemical structure of antimonials [13, 14]

N-methylglucamine antimoniate (Glucantime®, Sanofi-Aventis, France) is the antimonial compound used in Latin countries; and sodium stibogluconate (Pentostam®, GlaxoSmithKline, United Kingdom) is used in English-speaking countries. 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 [15].

### Dosage

Glucantime® is marketed in 5mL vials, containing 1.5g crude 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. 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 [16].

Marsden reports that conventional pentavalent antimonials cannot be orally administered due to poor absorption and/or inactivation in the stomach [17].

### 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; 18]. However, the mechanism

of action and the reason of the selective toxicity of these leishmanicidal agents remain little known[19].

According to Sereno et al.[20], 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* [20, 21]. Such results strengthen some hypotheses, as that from Miekeley et al.[22], 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 bio-reduced to its trivalent form, in accordance with other studies that show that *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 pro-drug, thus needing to be converted to its active form, SbIII [23]. Frézard et al. [24] 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)<sub>2</sub>), 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-phosphate to 1,3-bisphosphoglycerate, in the presence of NAD<sup>+</sup> 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 [25].

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 [26].

#### 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 [27].

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 [28]. Side effects such as cardiotoxicity, pancreatitis and nephrotoxicity [29-33], 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 [28].

#### 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 [34]. 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 [18].

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].

## Amphotericin B

In Bihar, an Indian state where VL is more endemic, the parasites are increasingly less susceptible to treatment with antimonials. This led to failure of the general treatment therewith, resulting in changing leishmaniasis treatment to conventional amphotericin B [18].

Amphotericin B is a polyene antibiotic (Figure 2) naturally produced by the actinomycete *Streptomyces nodosus* widely used in the treatment of most systemic mycoses affecting immunocompromised patients [25]. It was introduced in the pharmaceutical market in 1950 [36] 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% [37]. 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 [38].

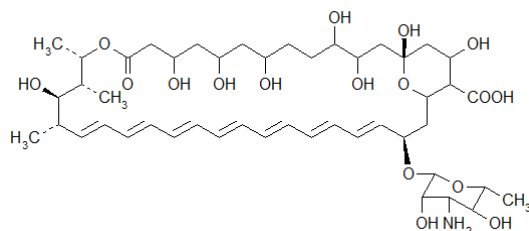


**Figure 2. Amphotericin B chemical structure**

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™, InterMune 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 [35] 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 [38].

## 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 CL 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) [40].

In case of liposomal amphotericin B, WHO recommends a daily administration of 0.75 to 1 mg/kg of, which comes in vials containing 50 mg of freeze dried amphotericin B sodium deoxycholate through intravenous infusion during 14 to 20 days, with a 50 mg minimum daily dose for VL treatment. For CL treatment it is also recommended the administration of 0.75 to 1 mg/kg daily or on alternate days, limited to 50 mg/day [5; 41]. Treatment duration should be determined based on clinical evolution, considering the speed of the response and the presence of co-morbidities [42].

Immunocompromised patients present higher tolerance and, therefore, require higher amphotericin B total doses. Currently, 3 mg/kg liposomal amphotericin B are intravenously administered on days 1 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 2.0 g amphotericin B and 2 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 [43].

## Mechanism of action

According to Chattopadhyay and Jafurulla, *Leishmania* binding and internalization into host cells involves its interaction with the cell membrane, thus requiring the presence of cholesterol to occur the pathogen-host interaction [44]. 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 [45]. 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 [38-44].

### **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 [46-47]. However, side effects such as anemia, heart toxicity effect, nausea, vomiting, phlebitis, chills and fever are reported sometimes requiring treatment discontinuation [48]. Liposomal amphotericin B is preferred to conventional because it presents a milder toxicity profile [18], in addition to high potential and action spectrum, resulting in greater reliability of use [35]. The main advantage of the liposomal formulation is that it reduces renal and cardiac toxicity [35-43].

### **Pentamidine**

Pentamidine (Figure 3) is also used for CL systemic treatment [18]. 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 [54].

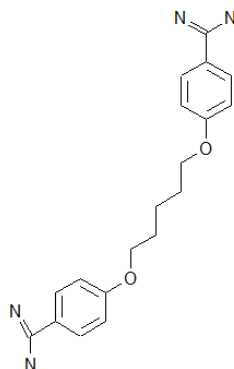


**Figure 3. Pentamidine chemical structure**

### **Dosage**

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

### **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.

### **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 [23]. Cardiac toxicity and nephrotoxicity have also been reported [49], besides effectiveness decreasing after consecutive administrations [56].

## Paromomycin

Paromomycin (PM) or aminosidine is a broad spectrum antibiotic (Figure 4), active against *Leishmania* (promastigotes and amastigotes), and some enteric protozoa and bacteria [33, 34]. Its leishmanicidal 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") [18]. It is indicated when the use of antimonials and amphotericin B is contraindicated [35], 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 [36].

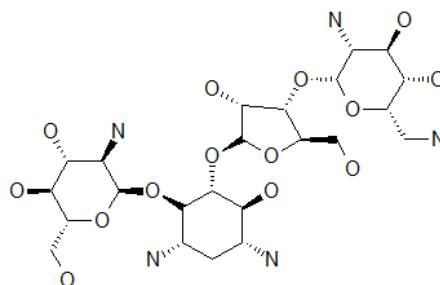


**Figure 4.** Paromomycin chemical structure

## 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 [37]. However, some studies report that a smaller dose of 11 mg/kg/day intramuscular administered is also effective [38, 34, 39].

## 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 [37].

## Adverse effects

Adverse effects are not common, but include elevation of liver function enzymes, reversible ototoxicity and renal failure [37].

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 [34].

## Miltefosine

Miltefosine, trade name Impavido® (Paladin Labs, Canada), is an alkylphosphocholine analog (hexadecylphosphocholine) (Figure 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 [40].

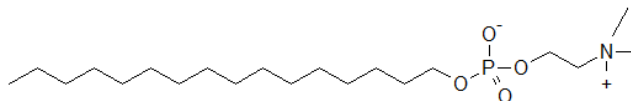


**Figure 5.** Miltefosine chemical structure

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 2.5 mg miltefosine/kg/day) during 28 days [41].

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; 41].

### **Dosage**

Miltefosine is formulated in capsules. A daily dose from 1.5 to 2.5mg/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 30kg, adolescents and adults weighing less than 45kg is 100mg miltefosine, and 150mg/day for patients weighing more than 45kg [61].

### **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 [62]. 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 per se [63].

### **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 [64]. Increased serum levels of aminotransferases and creatine phosphokinase were also reported [28]. In addition, it is potentially teratogenic [5; 64].

## **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 [65]. 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 [66].

Several therapeutic regimens using this strategy have been reported [64, 65, 67]. Some examples are described as highly efficient and have been adopted and indicated by WHO: combination of liposomal amphotericin B (5mg/kg per 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 during 10 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) during 17 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].

## **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 [68], 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.

## **Sitamaquine**

Sitamaquine is an 8-aminoquinoline analog (Figure 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 [69, 70].

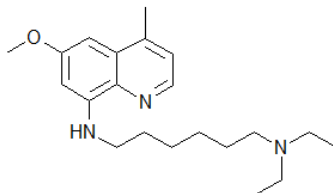


**Figure 6. Sitamaquine chemical structure**

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 [64, 70].

The side effects common to sitamaquine administration are: vomiting, abdominal pain, headache, methemoglobinemia, cyanosis, nephrotic syndrome, glomerulonephritis and nephrotoxicity [71].

Sitamaquine has shown *in vitro* activity against a range of species causing CL, with  $ED_{50}$  values against amastigotes varying between 2.9-19.0  $\mu$ M [72]. Another study by Jha et al., where 4 different sitamaquine doses (1.5; 1.75; 2.0 and 2.5 mg/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 [73]. Similarly, the oral administration of 2 mg/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 [74, 75].

### 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.

### 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 [76]. The literature reports were listed in chronological order in table 1.

**Table 1. Investigation of alkaloid leishmanicidal activity**

| Alkaloid                                                | Type        | Evaluated species                   | Type of test                                             | Result                                                                                                                                                                                             | Reference |
|---------------------------------------------------------|-------------|-------------------------------------|----------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| N-demethylconodurine                                    | indole      | <i>L. amazonensis</i> amastigotes   | <i>in vitro</i>                                          | IC <sub>50</sub> = 25µg/mL                                                                                                                                                                         | [76]      |
| dictylomide-A and dictylomide-B                         | quinoline   | <i>L. amazonensis</i> promastigotes | <i>in vitro</i>                                          | total lysis of promastigotes at 100 µg/mL                                                                                                                                                          | [77]      |
| N-demethylholacurtine, 15α-hydroxyholaminee holacurtine | steroid     | <i>L. donovani</i> promastigotes    | <i>in vitro</i>                                          | 6.25 > IC <sub>50</sub> > 1.56µg/mL                                                                                                                                                                | [78]      |
| holamine                                                |             |                                     |                                                          | 1.56 > IC <sub>50</sub> > 0.39µg/mL                                                                                                                                                                |           |
| dihydrocorynantheine                                    | indole      | <i>L. major</i> promastigotes       | <i>in vitro</i>                                          | IC <sub>50</sub> = 1.65µM                                                                                                                                                                          | [79]      |
| corynantheine                                           |             |                                     |                                                          | IC <sub>50</sub> = 1.12µM                                                                                                                                                                          |           |
| corynantheidine                                         |             |                                     |                                                          | IC <sub>50</sub> = 2.81µM                                                                                                                                                                          |           |
| E-3-quinol-2-yl-prop-2-en-1-ol                          | quinoline   | <i>L. amazonensis</i> amastigotes   | <i>in vivo</i> 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%<br>Meglumine antimoniate (100mg/kg) reduced 98.4% and 87.3%, respectively | [80]      |
|                                                         |             | <i>L. infantum</i> amastigotes      |                                                          | 25 mg/kg, during 10 days, were able to reduce the parasite load by 83%<br>Meglumine antimoniate (100mg/kg) reduced by 65%                                                                          |           |
|                                                         |             | <i>L. donovani</i> amastigotes      |                                                          | 25 or 12.5mg/kg, during 10 days, were able to reduce the parasite load in 61% and 59%, respectively<br>Mitelfosine (7.5mg/kg) reduced by 72%                                                       |           |
| 15,22-O-diacetyl-19-oxo-dihydroatisine                  | diterpenoid | <i>L. infantum</i> promastigotes    | <i>in vitro</i>                                          | IC <sub>50</sub> = 12.8mg/L (after 72-hour culture), low toxicity (74.28mg/L)                                                                                                                      | [81]      |

|                       |              |                                         |                 |                                                                                 |      |
|-----------------------|--------------|-----------------------------------------|-----------------|---------------------------------------------------------------------------------|------|
| azitine               |              |                                         |                 | IC <sub>50</sub> = 10.12mg/L (after 72-hour culture), high toxicity (> 200mg/L) |      |
| isoazitine            |              |                                         |                 | IC <sub>50</sub> = 7.39mg/L (after 72-hour culture), high toxicity (> 300mg/L)  |      |
| O-methylmoschatolinee | isoquinoline | <i>L. braziliensis</i><br>promastigotes | <i>in vitro</i> | IC <sub>50</sub> = 320.8µg/mL                                                   | [82] |
| liriodenine           |              |                                         |                 | IC <sub>50</sub> = 58.5µg/mL                                                    |      |
| neolitsine            | isoquinoline | <i>L. mexicana</i><br>promastigotes     | <i>in vitro</i> | IC <sub>50</sub> = 15µM                                                         | [83] |
| cryptodorine          |              |                                         |                 | IC <sub>50</sub> = 3µM                                                          |      |
| duguetine β-N-oxide   | isoquinoline | <i>L. braziliensis</i><br>promastigotes | <i>in vitro</i> | IC <sub>50</sub> = 0.11µM                                                       | [84] |
| dicentrinone          |              |                                         |                 | IC <sub>50</sub> = 0.01µM                                                       |      |

## Flavonoids

Flavonoids are a group of polyphenolic compounds widely found throughout the plant kingdom and with known antioxidant activity [35].

Tasdemir et al. conducted a comprehensive study to assess the efficiency of several flavonoids and their analogs against *L. donovani* amastigotes [36]. The study showed that the most potent metabolites were fisetin ( $IC_{50} = 0.6 \mu\text{g/mL}$ ), 3-hydroxyflavone ( $IC_{50} = 0.7 \mu\text{g/mL}$ ), and luteolin ( $IC_{50} = 0.8 \mu\text{g/mL}$ ). The drug used as control, miltefosine, had  $IC_{50}$  of  $0.34 \mu\text{g/mL}$ .

Vila-Nova et al. isolated rutin from *Dimorphandra gardneriana* grains, a native plant of northeastern Brazil caatinga biome and tested it against *L. infantum* chagasi parasites [37]. The compound presented results that were not significantly different from the results of the control drug –  $EC_{50}$  of  $30.3 \mu\text{g/mL}$  and  $43.3 \mu\text{g/mL}$  for promastigotes and amastigotes, respectively, whereas for amphotericin B the results were: no effect and  $19.75 \mu\text{g/mL}$ , respectively.

Muzitano et al. [38] 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- $\alpha$ -L-arabinopyranosyl (1  $\rightarrow$  2)  $\alpha$ -L-rhamnopyranoside) isolated from the same plant, for the leishmanicidal activity. A  $IC_{50} \cong 45 \text{mg/mL}$  (78mM) against *L. amazonensis* amastigotes was obtained [39].

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 2 (in chronological order).

**Table 2. Leishmanicidal activity of the flavonoid quercetin**

| Evaluated species                        | Type of test                                               | Result                                                                              | Reference |
|------------------------------------------|------------------------------------------------------------|-------------------------------------------------------------------------------------|-----------|
| <i>L. donovani</i> amastigotes           | <i>in vitro</i>                                            | $IC_{50} = 45.5 \mu\text{M}$                                                        | [90]      |
|                                          | <i>in vivo</i> orally administered to golden hamster       | 14mg/kg of the compound was able to reduce the spleen parasite load in 90%          |           |
| <i>L. donovani</i> amastigotes           | <i>in vitro</i>                                            | $IC_{50} = 1.0 \mu\text{g/L}$                                                       | [36]      |
|                                          | <i>in vivo</i> intraperitoneal administered to BALB/c mice | 30mg/kg during 5 days, was able to reduce infection in 15.3%                        |           |
| <i>L. amazonensis</i> promastigotes      | <i>in vitro</i>                                            | $IC_{50} = 1.0 \mu\text{g/mL}$                                                      | [91]      |
| <i>L. infantum chagasi</i> amastigotes   | <i>in vitro</i>                                            | $EC_{50} = 10.6 \mu\text{g/mL}$ (Amphotericin B: $ED_{50} = 19.75 \mu\text{g/mL}$ ) | [37]      |
| <i>L. infantum chagasi</i> promastigotes |                                                            | $EC_{50} = 26 \mu\text{g/mL}$ (Amphotericin B: $ED_{50} = \text{no effect}$ )       |           |
| <i>L. amazonensis</i> amastigotes        | <i>in vitro</i>                                            | $IC_{50} = 4.30 \mu\text{M}$                                                        | [92]      |
| <i>L. amazonensis</i> promastigotes      | <i>in vitro</i>                                            | $IC_{50} = 31.4 \mu\text{M}$                                                        | [93]      |

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 [34]. Quercetin can be seen as a non-cytotoxic drug. Da Silva et al. elucidated, through docking analyses, the interaction of quercetin, quercitrin and isoquercitrin with Asp129 amino acid, which is involved in the formation of the bridge between the metal and  $Mn^{2+}$  A and B cofactors in arginase active site [92]. The

429 three compounds interacted with Asp129, which may indicate a correlation between  
430 leishmanicidal action and arginase-flavones interaction. But, from the three compounds, only  
431 quercetin interacted with L-arginine substrate and the  $Mn^{2+}$  cofactor of *L. amazonensis*, at pH 9.6  
432 and was thus considered a mixed inhibitor.

433

#### 434 **Terpenoids**

435 Terpenoids represent one of the largest and most diverse classes of secondary  
436 metabolites among the natural compounds. Although terpenoids do not derive from isoprene,  
437 but from mevalonic acid (MVA) and/or methylerythritol phosphate (MEP), this class is usually  
438 classified according to the number of isoprene units [94]. Table 3 lists the studies found in the  
439 literature for this application in chronological order.

**Table3. Leishmanicidal activities of terpenoids**

| Terpenoid             | Type of terpenoid   | Evaluated species                      | Type of test                                                       | Result                                                                                                                                                                    | Reference |  |
|-----------------------|---------------------|----------------------------------------|--------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|--|
| dihydrobetulinic acid | triterpenoid        | <i>L. donovani</i> amastigotes         | <i>in vitro</i>                                                    | IC <sub>50</sub> = 4.1µM                                                                                                                                                  | [95]      |  |
|                       |                     | <i>L. donovani</i> promastigotes       |                                                                    | IC <sub>50</sub> = 2.6µM                                                                                                                                                  |           |  |
|                       |                     | <i>L. donovani</i> promastigotes       | <i>in vivo</i> orally administered <b>togolden</b> 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                    |           |  |
|                       |                     |                                        | <i>in vivo</i> intramuscular administered <b>togolden</b> 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 | <i>L. donovani</i> amastigotes         | <i>in vivo</i> 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%                                                          | [96]      |  |
| nerolidol             | sesquiterpene       | <i>L. amazonensis</i> amastigotes      | <i>in vitro</i>                                                    | IC <sub>50</sub> = 67µM                                                                                                                                                   | [97]      |  |
|                       |                     | <i>L. amazonensis</i> promastigotes    |                                                                    | IC <sub>50</sub> = 85µM                                                                                                                                                   |           |  |
|                       |                     | <i>L. braziliensis</i> promastigotes   |                                                                    | IC <sub>50</sub> = 74µM                                                                                                                                                   |           |  |
|                       |                     | <i>L. chagasi</i> promastigotes        |                                                                    | IC <sub>50</sub> = 75µM                                                                                                                                                   |           |  |
|                       |                     | <i>L. amazonensis</i> amastigotes      | <i>in vivo</i> 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 <i>L. amazonensis</i> |                                                                    |                                                                                                                                                                           |           |  |
| ursolic acid          | triterpenoid        | <i>L. donovani</i> promastigotes       | <i>in vitro</i>                                                    | IC <sub>50</sub> = 3.7µg/mL (the drugs pentamidine and amphotericin B, were used as control and presented IC <sub>50</sub> values of 1.9µg/mL and 0.7µg/mL, respectively) | [98]      |  |

|                                      |                          |                                         |                 |                                                                                  |     |
|--------------------------------------|--------------------------|-----------------------------------------|-----------------|----------------------------------------------------------------------------------|-----|
| hautriwaic acid<br>lactone           |                          |                                         |                 | IC <sub>50</sub> = 7.0µg/mL (idem)                                               |     |
| 8,13-diacetyl-<br>piptocarphol       | lactone<br>sesquiterpene | <i>L. amazonensis</i>                   |                 | IC <sub>50</sub> = 0.2µM                                                         | 99  |
| ursolic acid                         |                          |                                         |                 | IC <sub>50</sub> = 0.99µM                                                        |     |
| 8-acetyl-13-O-ethyl-<br>piptocarphol |                          |                                         |                 | IC <sub>50</sub> = 0.37µM                                                        |     |
| cumanin                              | sesquiterpene            | <i>L. amazonensis</i><br>promastigotes  | <i>in vitro</i> | growth inhibition higher than 80% at 5µg/mL (19µM)<br>for both evaluated species | 100 |
|                                      |                          | <i>L. braziliensis</i><br>promastigotes |                 |                                                                                  |     |

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## 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 [101].

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 [102].

In a recent study, which assessed the susceptibility of *L. infantum*, *L. tropica* and *L. major* to the essential oil *Cymbopogon citratus* and its main compounds *myrcene* and *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 36  $\mu\text{g/mL}$ , respectively. *Myrcene* proved to be the less active compound, with an  $IC_{50}$  value of 164  $\mu\text{g/mL}$  for *L. infantum* and no effect on the other species evaluated [103].

According to Monzote et al., the essential oil linalol-rich, from *Croton cujacara* leaves has been successfully used against leishmaniasis parasites [104]. 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.

## Extracts

Plant extracts or compounds derived from plants are likely to provide a valuable source of new medicinal agents [105–106], including those with potential leishmanicidal activity [107–108]. The activity of these extracts largely depends on the solvent used for extraction, the part of the plant used and the presence of one or more natural products [109].

Moreira et al. demonstrated the leishmanicidal activity of *Stachytarpheta cayennensis* (Verbenaceae) hydroalcoholic extract [110]. 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  $\mu\text{g/mL}$ ) than against *L. amazonensis* ( $IC_{50}$  of 382.5  $\mu\text{g/mL}$ ).

The leishmanicidal activity of the methanol extract of *Tridax procumbens* (Asteraceae) whole plant has already been described [111]. 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  $\mu\text{g/mL}$ , whereas oxylipin (3S)-16,17-didehydrofalcarninol, a compound obtained from a *T. procumbens* exhibited greater inhibition, with  $IC_{50}$  of 0.478  $\mu\text{g/mL}$ . Both, the extract and the compound were obtained through chromatographic methods.

García et al. assessed the leishmanicidal activity of 48 extracts of 46 Cuban plants against *L. amazonensis* by *in vitro* bioassay [112]. The best result against amastigotes of that parasite were obtained with ethanol extracts of *Hura crepitans* leaves, with  $IC_{50}$  (27.7  $\pm$  0.6  $\mu\text{g/mL}$ ), followed by the extract from *Bambusa vulgaris* leaves and roots and the extract from *Simarouba glauca* leaves ( $IC_{50}$  (41.5  $\pm$  0.6  $\mu\text{g/mL}$  and 45.5  $\pm$  0.3  $\mu\text{g/mL}$ , respectively).

Pontin et al. assessed *in vitro* the leishmanicidal effect of different concentrations of Brazilian green propolis hydroalcoholic extract against *L. braziliensis* promastigotes (1, 10, 30, 50, 100, 250, 500 and 750  $\mu\text{g/mL}$ ) and amastigotes (10, 100 and 250  $\mu\text{g/mL}$ ) [113]. The results showed that the extract was inactive against amastigotes and it caused the lysis of 79.3% amastigotes at 500  $\mu\text{g/mL}$ , 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 1.5 mg/kg/day and the lesion diameter was measured monthly. The intraperitoneal administration of 20 mg/kg/day of Glucantime<sup>®</sup> 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.

Ferreira et al. 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* [134].

*Lantana ukambensis* (Verbenaceae) methanol extract showed leishmanicidal activity with  $IC_{50}$  of 6.9 µg/mL against *L. donovani* promastigotes [135].

## Nanotechnology

The research and development in nanotechnology involve structures with one (lamellae), two (nanotubes and nanofilaments) or three (nanoparticles) dimensions in the nanometric scale [136]. 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 [137, 138], 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 [139]. According to Filippin and Souza, the formation of liposomal complexes has been investigated as resource to reduce this nephrotoxicity characteristic of amphotericin B administration [35].

Frézard et al. 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 [34]. 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* [140]. 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. [120] prepared liposomal nanoparticles of trivalent antimony associated with ascorbic acid. For evaluating their leishmanicidal action, BALB/c mice were infected with promastigotes of *L. infantum* 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 diffculted the delivery of the drug at the targeted site, so that the authors suggest further investigation [119].

Demicheli et al. assessed the complexation of meglumine antimoniate with β-cyclodextrin, which resulted in an increase of antimony absorption by the oral route and turned it orally active in a murine model infected by *L. amazonensis* [121]. 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 [122].

Das et al. developed quercetin conjugates with gold nanoparticles [123]. 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, 40 and 30 µM against *L. donovani*, wild type, and strains resistant to sodium stibogluconate and paromomycin, respectively. These results were compared with quercetin (34; 150 and 75 µM, resp.), amphotericin B (0.2; 0.4 and 0.35 µM, resp.), sodium stibogluconate (3.6; 130 and 115 µM, resp.) and paramomycin (10; 380

and 330 µM, 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 best results [124].

Menezes et al. 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 [125]. 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.

## TOPIC TREATMENT OF AMERICAN TEGUMENTARY LEISHMANIASIS LESIONS

American tegumentary leishmaniasis is caused by species of the *Leishmania* or *Viannia* sub-genera [40]. 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 [126].

Following diagnosis, the choice between topic or systemic treatment is done according to the evidence degree, geographic distribution, clinical manifestation and *Leishmania* species involved [127]. 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 [34]. The intralesional administration of 1 to 5 mL of antimony is considered as the treatment of second choice [34].

Imiquimod (Aldara®, Bayer, Germany), an imidazoquinoline amine used for the treatment of skin lesions caused by the human papillomavirus (HPV), genital warts and pre-malignant conditions [128] has shown high leishmanicidal potential [129]. It acts inducing the release of nitric oxide, which has a potent cytotoxic effect against *Leishmania* species, and subsequent macrophage activation [123].

Arevalo et al. proved its efficiency when associated to intramuscular administered meglumine antimoniate in Peruvian regions where the infection by *L. Peruviana* was endemic [130]. Twelve patients with active lesions which had not responded to previous intravenous or intramuscular treatment with antimonials (20 mg/kg/day) were selected for imiquimod evaluation. 250 mg of ointment (5% of drug) were spread on the lesions for 20 days, on alternate days, in conjunction with 20 mg 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 and meglumine 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 [131].

## CONCLUSIONS

Leishmaniasis is a neglected disease which can trigger mild, moderate or severe reactions in infected patients. A small number of cases can be resolved by the patient's immune system, but the vast majority of cases require a pharmacological treatment. However, the drugs currently used and available in the pharmaceutical market are 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

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

## REFERENCES

- 1.NgurePK, Kimutai A, Ng'ang'a ZW, Rukunga G, Tonui WK.A review of Leishmaniasis in Eastern Africa.Journal of Nanjing Medical University. 2009;23:79-86.
- 2.PavliA, Maltezou HC.Leishmaniasis, an emerging infection in travelers.International Journal of Infectious Diseases. 2010;14:1032–1039.
- 3.Control of the leishmaniasis: report of a meeting of the WHO Expert Committee on the Control of Leishmaniasis, Geneva, *World Health Organ Tech Rep Ser.* 1990;793:1-158.
- 4.CDC – Centers for Disease Control and Prevention (2013) Parasites –Leishmaniasis. Accessed 23 June 2014.  
Available: <http://www.cdc.gov/parasites/leishmaniasis/>.
- 5.DNDi- Drugs for Neglected Diseases initiative. Accessed 14 April 2014.  
Available: <http://www.dndi.org/diseases-projects/diseases/vl/current-treatment/current-treatment-cl.html>.
- 6.Arias JR, Monteiro PS, Zicker F.The reemergence of visceral leishmaniasis in Brazil.Emerging Infectious Diseases. 1996;2:145-146.
- 7.Sharma U, Redhu NS, Mathur P, Singh S. Re-emergence of visceral leishmaniasis in Gujarat, India. Journal of Vector Borne Diseases. 2007;44:230-232.
8. Arce A, Estirado A, Ordobas M, Sevilla S, García N, Moratilla L et al. Re-emergence of leishmaniasis in Spain: community outbreak in Madrid, Spain, 2009 to 2012. Eurosurveillance. 2013;18:1-9.
- 9.Ashford RW. The leishmaniasis as emerging and reemerging zoonoses.International Journal for Parasitology. 2000;30:1269-1281.
- 10.Ostfeld RS, Glass, GE, Keesing F. Spartial epidemiology: an emerging (or reemerging) discipline. Trends in Ecology & Evolution. 2005;20:328-336.
- 11.YanS, Jin L, Sun H. Antimony in Medicine. In: Gielen M, Tiekink ER, editors.Metallotherapeutic Drugs and Metal-Based Diagnostic Agents: The Use of Metals in Medicine. John Wiley & Sons; 2005:441-461.
- 12.Vianna G.Tratamento da Leishmaniose Tegumentar com Injeções Intravenosas de Tártaro Emético. In: Anais do 7º Congresso Brasileiro de Medicina e Cirurgia. 1912;4:426-428.
- 13.Rath S, Trivelin LA, Imbrunito TR, Tomazela DM, Jesús MN, Marzal PC. Antimoniais empregados no tratamento da leishmaniose: estado da arte. Química Nova. 2003;26:550-555.
- 14.Roberts WL, McMurray WJ, Rainey PM. Characterization of the antimonial antileishmanial agent meglumine antimonate (glucantime). Antimicrobial Agents and Chemotherapy. 1998;42:1076-1082.
- 15.Croft SL, Coombs GH. Leishmaniasis – current chemotherapy and recent advances in the search for novel drugs.Trends in Parasitology. 2003;19:502-508.
- 16.Davies CR, Kaye P, Croft SL, Sundar S. Leishmaniasis: new approaches to disease control. BMJ. 2003;326:377-382.
- 17.Marsden PD. Pentavalent antimonials: old drugs for new diseases.Revista da Sociedade Brasileira de Medicina Tropical. 1985;18:187-198.

18. Dorlo TPC, Balasegaram M, Beijnen JH, Vries PJ. Miltefosine: a review of its pharmacology and therapeutic efficacy in the treatment of leishmaniasis. *Journal of Antimicrobial Chemotherapy Advance*. 2012;67:2576-2597.
19. Soares-Bezerra RJ, Leon L, Genestra M. Recentes avanços da quimioterapia das leishmanioses: moléculas intracelulares como alvo de fármacos. *Brazilian Journal of Pharmaceutical Sciences*. 2004;40:139-149.
20. Sereno D, Cavaleira M, Zemzoumi K, Maquaire S, Ouaisi A, Lemesre JL. Axenically grown amastigotes of *Leishmania infantum* used as an in vitro model to investigate the pentavalent antimony mode of action. *Antimicrobial agents and chemotherapy*. 1998;42:3097-3102.
21. Gil ES, Cunha LC, Paula JR, Bezerra JCB, Aguiar FA. Leishmaníase: arsenal terapêutico e alvos moleculares. *Vita et Sanitas*. 2007;1:90-115.
22. Miekeley N, Mortari SR, Schubach AO. Monitoring of total antimony and its species by ICP-MS and on-line ion chromatography in biological samples from patients treated for leishmaniasis. *Analytical and Bioanalytical Chemistry*. 2002;372:495-502.
23. Goodwin LG, Page JE. A study of the excretion of organic antimonials using a polarographic procedure. *The Biochemical Journal*. 1943;37:198-209.
24. Frézard F, Demicheli C, Ribeiro RR. Pentavalent Antimonials: New Perspectives for Old Drugs. *Molecules*. 2009;14:2317-2336.
25. Balaña-Fouce R, Reguera RM, Cubria JC, Ordonez D. The pharmacology of leishmaniasis. *General Pharmacology*. 1998;30:435-443.
26. Bangs JD, Ransom DA, Nimick M, Christie G, Hooper NM. Cytocidal effects on *Trypanosoma brucei* and inhibition of *Leishmania major* GP63 by peptidomimetic metalloprotease inhibitors. *Molecular and Biochemical Parasitology*. 2011;114:111-117.
27. Brasil. Ministério da Saúde (2011) Manual de recomendações para diagnóstico, tratamento e acompanhamento de pacientes com a coinfeção Leishmania-HIV. Secretaria de Vigilância em Saúde. Brasília. Editora do Ministério da Saúde, 2011. 106 p. – ISBN 978-85-334-1771-7. Accessed 6 December 2013.  
Available: [http://portal.saude.gov.br/portal/arquivos/pdf/leishmania\\_hiv\\_web\\_25\\_01\\_11.pdf](http://portal.saude.gov.br/portal/arquivos/pdf/leishmania_hiv_web_25_01_11.pdf).
28. Oliveira LF, Schubach AO, Martins MM, Passos SL, Oliveira RV, Marzochi MC et al. Systematic review of the adverse effects of cutaneous leishmaniasis treatment in the New World. *Acta Tropica*. 2011;118:87-96.
29. Gasser JRRA, Magill AJ, Oster CN, Franke ED, Grogl M, Berman JD. Pancreatitis induced by pentavalent antimonial agents during treatment of leishmaniasis. *Clinical Infectious Diseases*. 1994;18:83-90.
30. Zaghloul IY, Al-Jasser M. Effect of renal impairment on the pharmacokinetics of antimony in hamsters. *Annals of Tropical Medicine and Parasitology*. 2004;98:793-800.
31. Matoussi N, Ameer HB, Amor SB, Fitouri Z, Becher SB. Cardiotoxicity of N-methyl-glucamine antimoniate (Glucantime). A case report. *Médecine et Maladies Infectieuses*. 2007;37(suppl. 3):257-259.
32. Shahian M, Alborzi A. Effect of meglumine antimoniate on the pancreas during treatment of visceral leishmaniasis in children. *Medical Science Monitor*. 2009;15:290-293.
33. Freitas-Junior LH, Chatelain E, Kim HA, Siqueira-Neto JL. Visceral leishmaniasis treatment: What do we have, what do we need and how to deliver it? *International Journal for Parasitology: Drugs and Drug Resistance*. 2012;2:11-19.

749 34. Frézard F, Michalick MSM, Soares CF, Demicheli C. Novel methods for the encapsulation of  
750 meglumine antimoniate into liposomes. Brazilian Journal of Medical and Biological Research.  
751 2000;33:841-846.

752

753 35. Filippin FB, Souza LC. Eficiência terapêutica das formulações lipídicas de anfotericina B.  
754 Revista Brasileira de Ciências Farmacêuticas. 2006;42:167-94.

755

756 36. Gallis HA. Amphotericin B: a commentary on its role as an antifungal agent and as a  
757 comparative agent in clinical trials. Clinical Infectious Diseases. 1996;22:145-147.

758

759 37. Sinha PK, Bhattacharya S. Single-dose liposomal amphotericin B: an effective treatment for  
760 visceral leishmaniasis. The Lancet Global Health. 2013;2:7-8.

761

762 38. Sundar S, Chakravarty J. Liposomal amphotericin B and leishmaniasis: dose and response.  
763 Journal of Global Infectious Diseases. 2010;2:159–160.

764

765 39. Brajtburg J, Bolard J. Carrier effects on biological activity of amphotericin B. Clinical  
766 Microbiology Reviews. 1996;9:512–531.

767

768 40. Goto H, Lindoso JAL. Cutaneous and Mucocutaneous Leishmaniasis. Infectious Disease  
769 Clinics of North America. 2012; 26:293–307.

770

771 41. WHO – World Health Organization (2014b) Box 1. Recommended treatment regimens for  
772 visceral leishmaniasis, ranked by preference. Accessed 14 March 2014.  
773 Available: [http://who.int/leishmaniasis/research/978924129496\\_pp67\\_71.pdf](http://who.int/leishmaniasis/research/978924129496_pp67_71.pdf).

774

775 42. Murray HW, Berman JD, Davies CR, Saravia NG. Advances in leishmaniasis. The Lancet.  
776 2005;366:1561-1570.

777

778 43. Bern, C. Treatment of visceral leishmaniasis. UpToDate (2013). Accessed 23 April 2014.  
779 Available: <http://www.uptodate.com/contents/treatment-of-visceral-leishmaniasis>.

780

781 44. Chattopadhyay A, Jafurulla MD. A novel mechanism for an old drug: amphotericin B in the  
782 treatment of visceral leishmaniasis. Biochemical and Biophysical Research Communications.  
783 2011;416:7-2.

784

785 45. Foglia F, Lawrence MJ, Demè B, Fragneto G, Barlow D. Neutron diffraction studies of the  
786 interaction between amphotericin B and lipid-sterol model membranes. Scientific reports.  
787 2012;2(778):1–6.

788

789 46. Laniado-Laborín R, Cabrales-Vargas MN. Amphotericin B: side effects and toxicity. Revista  
790 Iberoamericana de Micología. 2009;26:223-227.

791

792 47. Ulozas E. Amphotericin B-Induced Nephrotoxicity. Comprehensive Toxicology. 2010;7:347-  
793 357.

794

795 48. Pimentel MIF, Baptista C, Rubin EF, Vasconcellos ECF, Lyra MR, Salgueiro MM. American  
796 cutaneous leishmaniasis caused by *Leishmania (Viannia) braziliensis* resistant to meglumine  
797 antimoniate, but with good response to pentamidine: a case report. Revista da Sociedade  
798 Brasileira de Medicina Tropical. 2011;44:254-256.

799

800 49. Cohen BE. Amphotericin B toxicity and lethality: a tale of two channels. International Journal  
801 of Pharmaceutics. 1998;162:95-96.

802

803 50. Lai A Fat EJ, Vrede MA, Soetosenojo RM, Lai A Fat RF. Pentamidine, the drug of choice for  
804 the treatment of cutaneous leishmaniasis in Surinam. Tropical Medicine Rounds. 2002;41:796–  
805 800.

806

807 51. Liegl U, Bogner JR, Goebel FD. Insulin-dependent diabetes mellitus following pentamidine  
808 therapy in a patient with AIDS. Clinical Investigation. 1994;72:1027-1029.

809  
810 52. Costa JML, Moraes MSC, Saldanha ACR, Barral A, Burattini MN. Diabetes melitus  
811 associated with pentamidine isethionate in diffuse cutaneous leishmaniasis. Revista da  
812 Sociedade Brasileira de Medicina Tropical. 1995;28:405-407.  
813  
814 53. Basselin M, Lawrence F, Robert-Gero M. Pentamidine uptake in *Leishmania donovani* and  
815 *Leishmania amazonensis* promastigotes and axenic amastigotes. Biochemical Journal.  
816 1996;315:631-644.  
817  
818 54. Davidson RN, Den Boer M, Ritmeijer K. Paromomycin. Transactions of the Royal Society of  
819 Tropical Medicine and Hygiene. 2009;103:653-660.  
820  
821 55. Santos A, Noronha E, Ferreira L, Carranza-Tamayo C, Cupolillo E, Romero G. Efeito de uma  
822 formulação hidrofílica de paromomicina tópica na leishmaniose cutânea em pacientes com  
823 contra-indicações de tratamento com antimonial pentavalente. Revista da Sociedade Brasileira  
824 de Medicina Tropical. 2008;41:444-448.  
825  
826 56. Wiwanitkit V. Interest in paromomycin for the treatment of visceral leishmaniasis (kala-azar).  
827 Therapeutics and Clinical Risk Management. 2012;8:323–328.  
828  
829 57. Singh S, Sivakumar R. Challenges and new discoveries in the treatment of leishmaniasis.  
830 Journal of Infection and Chemotherapy. 2004;10:307-315.  
831  
832 58. Seaman J, Boer C, Wilkinson R, de Jong J, de Wilde E, Sondorp E et al. Liposomal  
833 amphotericin B (AmBisome) in the treatment of complicated kala-azar under field conditions.  
834 Clinical Infectious Diseases. 1995;21:188–193.  
835  
836 59. UKMi – UK Medicines Information (2013) New Drugs Online. Accessed 18 February 2014.  
837 Available: [http://www.ukmi.nhs.uk/applications/ndo/record\\_view\\_open.asp?newDrugID=2877](http://www.ukmi.nhs.uk/applications/ndo/record_view_open.asp?newDrugID=2877).  
838  
839 60. Sundar S, Jha TK, Thakur CP, Engel J, Sindermann H, Fischer C. Oral miltefosine for Indian  
840 visceral leishmaniasis. The New England Journal of Medicine. 2002;347:1739-1746.  
841  
842 61. Paladin Labs Inc. Application for Inclusion of Miltefosine on WHO Model List of Essential  
843 Medicines (2010). Accessed 17 April 2014.  
844 Available:  
845 [http://www.who.int/selection\\_medicines/committees/expert/18/applications/Miltefosine\\_applicati](http://www.who.int/selection_medicines/committees/expert/18/applications/Miltefosine_application.pdf)  
846 [on.pdf](http://www.who.int/selection_medicines/committees/expert/18/applications/Miltefosine_application.pdf).  
847  
848 62. Croft SL, Engel J. Miltefosine – discovery of the antileishmanial activity of phospholipid  
849 derivatives. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2006;100S:S4-  
850 S8.  
851  
852 63. Perez-Victoria FJ, Sánchez-Cañete MP, Seifert K, Croft SL, Sundar S, Castanys S, Gamarro  
853 F. Mechanisms of experimental resistance of *Leishmania* to miltefosine: Implications for clinical  
854 use. 2006;9:26–39.  
855  
856 64. Jha TK, Sundar S, Thakur CP, Felton JM, Sabin AJ, Horton J. A phase II dose-ranging study  
857 of sitamaquine for the treatment of visceral leishmaniasis in India. The American Journal of  
858 Tropical Medicine and Hygiene. 2005;73:1005–1011.  
859  
860 65. Van Griensven J, Diro E. Visceral leishmaniasis. Infectious Disease Clinics of North America.  
861 2012;26:309-22.  
862  
863 66. Sundar S, Sinha PK, Verma DK, Kumar N, Alam S, Pandey K. Ambisome plus miltefosine for  
864 Indian patients with kala-azar. Transactions of the Royal Society of Tropical Medicine and  
865 Hygiene. 2011;105:115–117.  
866  
867 67. Thakur CP, Oliaro P, Gothoskar S, Bhowmick S, Choudhury BK, Prasad S et al. Treatment  
868 of visceral leishmaniasis (kala-azar) with aminosidine (=paromomycin)-antimonial combinations,

869 a pilot study in Bihar, India. Transactions of the Royal Society of Tropical Medicine and  
870 Hygiene.1992;86:615-616.

871

872 68.Laguna F, Videla S, Jiménez-Mejías ME, Sirera G, Torre-Cisneros J, Ribera E et al.  
873 Amphotericin B lipid complex versus meglumine antimoniate in the treatment of visceral  
874 leishmaniasis in patients infected with HIV: a randomized pilot study. Journal of Antimicrobial  
875 Chemotherapy. 2003;52:464–468.

876

877 69.Dueñas-Romero AM, Loiseau PM, Saint-Pierre-Chazalet M. Interaction of sitamaquine with  
878 membrane lipids of *Leishmania donovani* promastigotes. Biochimica et Biophysica Acta.  
879 2007;1768:246–252.

880

881 70.Coimbra ES, Libong D, Cojean S, Saint-Pierre-Chazalet M, Salgadi A, Le Moyec L.  
882 Mechanism of interaction of sitamaquine with *Leishmania donovani*. The Journal of  
883 Antimicrobial Chemotherapy. 2010;65:2548–2555.

884

885 71.Loiseau PM, Cojean S, Schrével J. Sitamaquine as a putative antileishmanial drug  
886 candidate: from the mechanism of action to the risk of drug resistance. Parasite. 2011;18:115-  
887 119.

888

889 72.Garnier T, Brown MB, Lawrence MJ, Croft SL. *In vitro* and *in vivo* studies on a topical  
890 formulation of sitamaquine dihydrochloride for cutaneous leishmaniasis. Journal of Pharmacy  
891 and Pharmacology. 2006;58:1043–1054.

892

893 73.Wasunna MK, Rashid JR, Mbui J, Kirigi G, Kinoti D, Lodenyo H et al. A phase II dose-  
894 increasing study of sitamaquine for the treatment of visceral leishmaniasis in Kenya. The  
895 American Journal of Tropical Medicine and Hygiene. 2005;73:871-876.

896

897 74.Tiuman TS, Santos AO, Ueda-Nakamura T, Dias Filho BP, Nakamura CV. Recent advances  
898 in leishmaniasis treatment. International Journal of Infectious Diseases. 2011;15:525-532.

899

900 75.Mishra BB, Kale RR, Singh RK, Tiwari VK. Alkaloids: Future prospective to combat  
901 leishmaniasis. Fitoterapia. 2009;80:81-90.

902

903 76.Munoz V, Morretti C, Sauvain M, Caron C, Porzel A, Massiot G et al. Isolation of bis-indole  
904 alkaloids with antileishmanial and antibacterial activities from *Peschiera van heurkii* (syn.  
905 *Tabernaemontana van heurkii*). Planta Medica. 1994;60:455-459.

906

907 77.Lavaud C, Massiot G, Vasquez C, Moretti C, Sauvain M, Balderrama L. 4-Quinolinone  
908 alkaloids from *Dictyoloma peruviana*. Phytochemistry. 1995;40:317-320.

909

910 78.Kam T, Sim K, Koyano T, Toyoshima M, Hayashi M, Komiyama K. Cytotoxic and  
911 leishmanicidal aminoglycosides and aminosteroids from *Holarrhena curtisii*. Journal of  
912 Natural Products. 1998;61:1332-1336.

913

914 79.Staerk D, Lemmich E, Christensen J, Kharazmi A, Olsen CE, Jaroszewski JW.  
915 Leishmanicidal, antiplasmodial and cytotoxic activity of indole alkaloids from *Corynanthe*  
916 *pachyceras*. Planta Medica. 2000;66:531-536.

917

918 80.Nakayama H, Loiseau PM, Bories C, Torres de Ortiz T, Schinini A, Serna E et al. Efficacy of  
919 orally administered 2-substituted quinolines in experimental murine cutaneous and visceral  
920 leishmaniasis. Antimicrobial Agents and Chemother. 2005;49:4950-4956.

921

922 81.Gonzalez P, Marin C, Rodriguez-Gonzalez I, Hitos AB, Rosales MJ, Reina M et al. In vitro  
923 activity of C20-diterpenoid alkaloid derivatives in promastigotes and intracellular amastigotes of  
924 *Leishmania infantum*. International Journal of Antimicrobial Agents. 2005;25:136-141.

925

926 82.Costa EV, Pinheiro MLB, Xavier CM, Silva JRA, Amaral ACF, Souza ADL et al. A pyrimidine-  
927  $\beta$ -carboline and other alkaloids from *Annona foetida* with antileishmanial activity. Journal of  
928 Natural Products. 2006;69:292-294.

929  
930 83. Correa JE, Ríos CH, Castillo ADR, Romero LI, Ortega-Barría E, Coley PD et al. Minor  
931 alkaloids from *Guatteria dumetorum* with antileishmanial activity. *Planta Medica*. 2006;72:270-  
932 272.  
933  
934 84. Da Silva DB, Tulli EC, Militão GC, Costa-Lotufo LV, Pessoa C, de-Moraes MO et al. The  
935 antitumoral, trypanocidal and antileishmanial activities of extract and alkaloids isolated from  
936 *Duguetia furfuracea*. *Phytomedicine*. 2009;16:1059.  
937  
938 85. Lewin G, Cojean S, Gupta S, Verma A, Puri SK, Loiseau PM. In vitro antileishmanial  
939 properties of new flavonoids against *Leishmania donovani*. *Biomedicine and Preventive*  
940 *Nutrition*. 2011;1:168-171.  
941  
942 86. Tasdemir D, Kaiser M, Brun R, Yard V, Schmidt TJ, Tosunc F et al. Antitrypanosomal and  
943 antileishmanial activities of flavonoids and their analogues: in vitro, in vivo, structure-activity  
944 relationship, and quantitative structure-activity relationship studies. *Antimicrobial Agents and*  
945 *Chemotherapy*. 2006;4:1352-1364.  
946  
947 87. Vila-Nova NS, Morais SM, Falcão MJC, Bevilacqua CML, Rondon FCM, Wilson ME et al.  
948 Leishmanicidal and cholinesterase inhibiting activities of phenolic compounds of *Dimorphandra*  
949 *gardneriana* and *Platymiscium floribundum*, native plants from Caatinga biome. *Pesquisa*  
950 *Veterinária Brasileira*. 2012;32:1164-1168.  
951  
952 88. Muzitano MF, Falcão CAB, Cruz EA, Bergonzi MC, Bilia AR, Vincieri FF et al. Oral  
953 Metabolism and Efficacy of *Kalanchoe pinnata* Flavonoids in a Murine Model of Cutaneous  
954 Leishmaniasis. *Planta Medica*. 2009;75:307-311.  
955  
956 89. Muzitano MF, Tinoco LW, Guette C, Kaiser CR, Rossi-Bergmann B, Costa SS. The  
957 antileishmanial activity assessment of unusual flavonoids from *Kalanchoe*  
958 *pinnata*. *Phytochemistry*. 2006;67:2071-2077.  
959  
960 90. Mitra B, Saha A, Chowdhury AR, Pal C, Mandal S, Mukhopadhyay S et al. Luteolin, an  
961 abundant dietary component is a potent anti-leishmanial agent that acts by inducing  
962 topoisomerase II-mediated kinetoplast DNA cleavage leading to apoptosis. *Molecular Medicine*.  
963 2000;6:527-541.  
964  
965 91. Muzitano MF, Cruz EA, de Almeida AP, Da Silva SAG, Kaiser CR, Guette C et al. Quercitrin:  
966 an antileishmanial flavonoid glycoside from *Kalanchoe pinnata*. *Planta Medica*. 2006;72:81-83.  
967  
968 92. Da Silva ER, Maquiaveli Cdo C, Magalhães PP. The leishmanicidal flavonols quercetin and  
969 quercitrin target *Leishmania (Leishmania) amazonensis* arginase. *Experimental Parasitology*.  
970 2012;130:183-188.  
971  
972 93. Fonseca-Silva F, Inacio JD, Canto-Cavalheiro MM, Almeida-Amaral EE. Reactive oxygen  
973 species production and mitochondrial dysfunction contribute to quercetin induced death in  
974 *Leishmania amazonensis*. *Plos One*. 2011;6(2:e14666):1-7.  
975  
976 94. Phillips MA, Leon P, Boronat A, Rodriguez-Concepcion M. The plastidial MEP pathway:  
977 unified nomenclature and resources. *Trends in Plant Science*. 2008;13:619-623.  
978  
979 95. Chowdhury AR, Mandal S, Goswami A, Ghosh M, Mandal L, Chakraborty D et al.  
980 Dihydrobetulinic acid induces apoptosis in *Leishmania donovani* by targeting DNA  
981 topoisomerase I and II: implications in antileishmanial therapy. *Molecular Medicine*. 2003;9:26-  
982 36.  
983  
984 96. Maes L, Germonprez N, Quirijnen L, Van Puyvelde L, Cos P, Vanden Berghe D.  
985 Comparative activities of the triterpene saponin maesabalide III and liposomal amphotericin B  
986 (AmBisome) against *Leishmania donovani* in hamsters. *Antimicrobial Agents and*  
987 *Chemotherapy*. 2004;48:2056-2060.  
988

989 97. Arruda DA, D'Alexandri FL, Katzin AM, Uliana SRB. Antileishmanial activity of the terpene  
 990 nerolidol. *Antimicrobial Agents and Chemotherapy*. 2005;49:1679-1687.  
 991  
 992 98. Da Silva Filho AA, Resende DO, Fukui MJ, Santos FF, Pauletti PM, Cunha WR et al. In vitro  
 993 antileishmanial, antiplasmodial and cytotoxic activities of phenolics and triterpenoids from  
 994 *Baccharis dracunculifolia* D. C. (Asteraceae). *Fitoterapia*. 2009;80:478-482.  
 995  
 996 99. Odonne G, Herbette G, Eparvier V, Bourdye G, Rojas R, Sauvaine M et al. Antileishmanial  
 997 sesquiterpene lactones from *Pseudelephantopus spicatus*, a traditional remedy from the  
 998 Chayahuita Amerindians (Peru). Part III. *Journal of Ethnopharmacology*. 2011;137:875-879.  
 999  
 1000 100. Sülsen VP, Cazorla SI, Frank FM, Laurella LC, Muschietti LV, Catalán CA et al. Natural  
 1001 terpenoids from ambrosia species are active in vitro and in vivo against human pathogenic  
 1002 trypanosomatids. *PLoS Neglected Tropical Diseases*. 2013;7:1-10.  
 1003  
 1004 101. Rohloff J, Hymete A, Tariku Y. Studies in natural products chemistry. In: Atta-ur-Rahman,  
 1005 editor. *Plant-derived natural products for the treatment of leishmaniasis*. Elsevier. 2013;381-429.  
 1006  
 1007 102. Monzote L, Montalvo AM, Scull R, Miranda M, Abreu J. Activity, toxicity and analysis of  
 1008 resistance of essential oil from *Chenopodium ambrosioides* after intraperitoneal, oral and  
 1009 intralesional administration in BALB/c mice infected with *Leishmania amazonensis*: A  
 1010 preliminary study. *Biomedicine & Pharmacotherapy*. 2007;61:48-153.  
 1011  
 1012 103. Machado M, Pires P, Dinis AM, Santos-Rosa M, Alves V, Salgueiro L et al. Aldheides  
 1013 monoterpenes as potential anti-Leishmania agents: Activity of *Cymbopogon citratus* and citral  
 1014 on *L. infantum*, *L. tropica* and *L. major*. *Experimental Parasitology*. 2012;130:223-231.  
 1015  
 1016 104. Monzote L, Alarcón O, Setzer WN. Antiprotozoal Activity of Essential Oil. *Agriculturae*  
 1017 *conspectus scientificus*. 2012;4:167-175.  
 1018  
 1019 105. Carvalho PB, Ferreira EI. Leishmaniasis phytotherapy. Nature's leadership against an  
 1020 ancient disease-review. *Fitoterapia*. 2001;72:599-618.  
 1021  
 1022 106. Kayser O, Kiderlen AF. In vitro leishmanicidal activity of naturally occurring chalcones.  
 1023 *Phytotherapy Research*. 2001;15:148-152.  
 1024  
 1025 107. Chan-Bacab MJ, Peña-Rodríguez LM. Plant natural products with leishmanicidal  
 1026 activity. *Natural Product Reports*. 2001;18:674-688.  
 1027  
 1028 108. Rocha LG, Almeida JRGS, Macedo RO, Barbosa-Filho JM. Review of natural products with  
 1029 antileishmanial activity. *Phytomedicine*. 2005;12:514-535.  
 1030  
 1031 109. Adebayo OL, Suleman D, Samson AA. Natural products in antileishmanial drug discovery:  
 1032 a review. *Journal of Asian Scientific Research*. 2013;3:157-173.  
 1033  
 1034 110. Moreira RCR, Costa GC, Lopes TC, Bezerra JL, Guerra RNM, Rebêlo JMM et al. Efeito  
 1035 leishmanicida in vitro de *Stachytarpheta cayennensis* (Rich.) Vahl (Verbenaceae). *Revista*  
 1036 *Brasileira de Farmacognosia*. 2007;17:59-63.  
 1037  
 1038 111. Martín-Quintal Z, Moo-Puc R, González-Salazar F, Chan-Bacab MJ, Torres-Tapia LW,  
 1039 Peraza-Sánchez SR. In vitro activity of *Tridax procumbens* against promastigotes of *Leishmania*  
 1040 *mexicana*. *Journal of Ethnopharmacology*. 2009;122:463-477.  
 1041  
 1042 112. García M, Monzote L, Scull R, Herrera P. Activity of Cuban plants extracts against  
 1043 *Leishmania amazonensis*. *ISRN Pharmacology*. 2012;2012(104540):1-7.  
 1044  
 1045 113. Pontin K, Da Silva Filho AA, Santos FF, Andrade e Silva ML, Cunha WR, Nanayakkara  
 1046 NPD et al. In vitro and in vivo antileishmanial activities of a Brazilian green propolis extract.  
 1047 *Parasitology Research*. 2008;103:487-492.  
 1048

1049 114. Ferreira FM, Castro RAO, Batista MA, Rossi FMO, Silveira-Lemos D, Frézard F et al.  
 1050 Association of water extract of green propolis and liposomal meglumine antimoniate in the  
 1051 treatment of experimental visceral leishmaniasis. *Parasitology Research*. 2014;113:533-543.  
 1052  
 1053 115. Sawadogo WR, Le Douaron G, Maciuk A, Bories C, Loiseau PM, Figadère B et al. In vitro  
 1054 antileishmanial and antitrypanosomal activities of five medicinal plants from Burkina  
 1055 Faso. *Parasitology Research*. 2012;110:1779–1783.  
 1056  
 1057 116. ISO/TS 27687:2008 Nanotechnologies – Terminology and definitions for nano-objects –  
 1058 Nanoparticle, nanofibre and nanoplate.  
 1059  
 1060 117. Patel PA, Patravale VB. AmbiOnp: solid lipid nanoparticles of amphotericin B for oral  
 1061 administration. *Journal of Biomedical Nanotechnology*. 2011;7:632–639.  
 1062  
 1063 118. Carvalho RF, Ribeiro LF, Miranda-Vilela AL, Filho JS, Martins OP, Silva DOC et al.  
 1064 Leishmanicidal activity of amphotericin B encapsulated in PLGA–DMSA nanoparticles to treat  
 1065 cutaneous leishmaniasis in C57BL/6 mice. *Experimental Parasitology*. 2013;135:217–222.  
 1066  
 1067 119. Lima SAC, Silvestre R, Barros D, Cunha J, Baltazar MT, Dinis-Oliveira RJ et al. Crucial  
 1068 CD8+ T-lymphocyte cytotoxic role in amphotericin B nanospheres efficacy against experimental  
 1069 visceral leishmaniasis. *Nanomedicine: Nanotechnology, Biology and Medicine*. 2014;10:1021-  
 1070 1030.  
 1071  
 1072 120. Castro RAO, Silva-Barcellos NM, Licio CSA, Souza JB, Souza-Testasica MC, Ferreira FM  
 1073 et al. Association of Liposome-Encapsulated Trivalent Antimonial with Ascorbic Acid: An  
 1074 Effective and Safe Strategy in the Treatment of Experimental Visceral Leishmaniasis. *Plos One*.  
 1075 2014;9:e104055.  
 1076  
 1077 121. Demicheli C, Ochoa R, Silva JBB, De Melo AL, Falcão CAM, Rossi-Bergmann Bet al. Oral  
 1078 delivery of meglumine antimoniate-beta-cyclodextrin complex for treatment of leishmaniasis.  
 1079 *Antimicrobial Agents and Chemotherapy*. 2004;48:100-103.  
 1080  
 1081 122. Frézard F, Martins PS, Bahia APCO, Moyec LL, Melo AL, Pimenta AMC et al. Enhanced  
 1082 oral delivery of antimony from meglumine antimoniate/ $\beta$ -cyclodextrin nanoassemblies.  
 1083 *International Journal of Pharmaceutics*. 2008;347:102–108.  
 1084  
 1085 123. Das S, Roy P, Mondal S, Bera T, Mukherjee A. One pot synthesis of gold nanoparticles and  
 1086 application in chemotherapy of wild and resistant type visceral leishmaniasis. *Colloids and*  
 1087 *surfaces. B, Biointerfaces*. 2013;107:27-34.  
 1088  
 1089 124. Jebalia A, Kazemib B. Nano-based antileishmanial agents: A toxicological study on  
 1090 nanoparticles for future treatment of cutaneous leishmaniasis. *Toxicology in Vitro*.  
 1091 2013;27:1896-1904.  
 1092  
 1093 125. Menezes JB, da Silva T, dos Santos J, Catari E, Meneghetti M, da Matta Cet al. Layered  
 1094 double hydroxides (LDHs) as carrier of antimony aimed for improving leishmaniasis  
 1095 chemotherapy. *Applied Clay Science*. 2014;91-92:127-134.  
 1096  
 1097 126. Garcia AL, Parrado R, De Doncker S, Bermudez H, Dujardin JC. American tegumentary  
 1098 leishmaniasis: direct species identification of *Leishmania* in non-invasive clinical samples.  
 1099 *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 2007;101:368-371.  
 1100  
 1101 127. WHO – World Health Organization. Control of leishmaniasis. Report of a meeting of the  
 1102 WHO Expert Committee WHO, Geneve. WHO Technical Report Series. 2010;949:1–186.  
 1103  
 1104 128. Buates S, Matlashewski G. Treatment of experimental leishmaniasis with the  
 1105 immunomodulators imiquimod and S28463: efficacy and mode of action. *Journal of Infectious*  
 1106 *Diseases*. 1999;179:1485-1494.  
 1107

- 1108 129. Aguado M, Espinosa P, Romero-Maté A, Tardío JC, Córdoba S, Borbujo J. Outbreak of  
1109 cutaneous leishmaniasis in Fuenlabrada, Madrid. *Actas Dermo-Sifiliográficas (English Edition)*.  
1110 2013;104:334-342.  
1111  
1112 130. Arevalo I, Ward B, Miller R, Meng TC, Najar E, Alvarez E et al. Successful treatment of  
1113 drug-resistant cutaneous leishmaniasis in humans by use of imiquimod, an immunomodulator.  
1114 *Clinical Infectious Diseases*. 2001;33:1847-1851.  
1115  
1116 131. Hervás JA, Martín-Santiago A, Hervás D, Rojo E, Mena A, Rocamora V et al. Old world  
1117 *Leishmania infantum* cutaneous leishmaniasis unresponsive to liposomal amphotericin B  
1118 treated with topical imiquimod. *The Pediatric Infectious Disease*. 2012;31:97-100.