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Cutaneous Leishmaniasis: Physiopathology, Molecular Diagnostic, and Therapeutic Approaches

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Abstract: This in-depth study explores various aspects of cutaneous leishmaniasis, shedding light on the physiopathology of the infection, advances in molecular diagnostic techniques, and therapeutic approaches currently in development. Our investigation seamlessly integrates fundamental and clinical research data to provide a comprehensive perspective on this debilitating disease. Cutaneous leishmaniasis, primarily manifested through skin lesions, poses a significant diagnostic challenge. We delve into molecular diagnostic methods, especially PCR, emphasizing their crucial role in accurately detecting the infection. Simultaneously, we examine the implications of the physiopathology of cutaneous

leishmaniasis, unveiling the complex mechanisms underlying this disease. In terms of treatment, we scrutinize current therapeutic approaches, highlighting limitations associated with using antimonials. Our study also explores alternative solutions, investigating the potential benefits of flavonoids and other natural compounds, thus offering innovative therapeutic perspectives. In conclusion, this research aims to enhance the understanding of cutaneous leishmaniasis from a diagnostic and therapeutic standpoint. This review is an essential resource for healthcare professionals and researchers, laying the groundwork for an integrated and holistic approach to better comprehend and treat this complex disease.

Keywords: Leishmaniasis; cutaneous leishmaniasis; diagnosis; immunology; therapeutic strategies

1. Introduction

The skin, the largest organ of the human body, holds crucial importance as a physical barrier against external assaults. Comprising distinct layers, including the epidermis, dermis, and hypodermis [1, 2], it provides multifunctional protection ranging from thermal regulation to infection prevention [3, 4]. However, despite its sophisticated defense mechanisms, the skin remains vulnerable to various conditions [5–7], among which parasitic infections are significant. This intricate interface between the host and the environment can be the site of various skin diseases, with cutaneous leishmaniasis emerging as a significant concern.

Cutaneous leishmaniasis is a set of parasitic diseases caused by flagellated protozoa belonging to the genus Leishmania and transmitted by vectors [8, 9]. Many species of mammals, including humans, can be affected by these parasites, which are transmitted through the bite of a vector insect called a sandfly [10, 11]. Leishmania parasites multiply in the skin cells, causing cutaneous ulcers [12]. The disease is primarily transmitted through sandfly bites, and although it is not directly contagious between people, it can also spread through blood transfusions or exchanges of biological material [13, 14].

Cutaneous leishmaniasis, widespread worldwide, initially encompasses an intertropical geographic area. However, its presence extends well beyond temperate regions, including Southern Europe, North Africa, Asia, and the Americas [15, 16]. This disease is endemic in four continents, affecting 98 countries or territories. It is estimated that nearly 350 million people are at risk of contracting leishmaniasis, and the annual number of newly diagnosed cases, encompassing all clinical forms, is estimated between 1.5 and 2 million [17, 18].

The current clinical management of cutaneous leishmaniasis relies on several approaches, including early diagnosis, appropriate treatment, and implementing preventive measures. Recent advances in understanding the pathogenesis of the disease have led to significant improvements in clinical management. Cutaneous leishmaniasis exhibits notable clinical variability in this context, ranging from localized forms to more diffuse manifestations. Factors influencing the clinical presentation include host-related aspects and the specificity of the parasite strain, resulting in "wet" or "dry" ulcers depending on the species involved [19, 20]. Cutaneous leishmaniasis is characterized by the tendency of skin lesions to spontaneously heal over a period of 2 to 10 months, with an incubation period ranging from 1 to 4 months. Symptoms often begin with the appearance of an erythematous papule, which may transform into a plaque or ulcer as the disease progresses.

Historically, the diagnosis of leishmaniasis relied on the isolation, visualization, and culture of the parasite from infected tissues [21, 22]. However, recent advances in PCR techniques specific to various Leishmania species have allowed for a rapid and more sensitive direct diagnosis [23, 24].

The decision to medically treat leishmaniasis is complex and depends on multiple factors, requiring a careful evaluation of the benefit-to-risk balance. Current therapeutic options remain limited, with pentavalent antimonial compounds remaining the first-line drugs in many endemic countries since their introduction in the 1930s [25–27]. However, the high toxicity and prohibitive cost of conventional drugs, along with the emergence of resistant strains, underscore the need to explore alternatives, especially among endemic plants, which may offer anti-leishmanial efficacy with reduced toxicity [28–30]. Recent studies have highlighted the significant activity of natural molecules against leishmaniasis, paving the way for new treatment perspectives [31–34].

This review aims to delve into the multiple facets of cutaneous leishmaniasis, with a particular focus on the pathophysiology of the infection, recent advances in molecular diagnostic techniques, and various therapeutic approaches currently under development. By integrating data from both fundamental and clinical research, our goal is to provide a comprehensive perspective on the current state of knowledge and identify potential gaps that require future exploration.

2. Overview of cutaneous Leishmaniasis

Leishmaniasis is an infectious disease transmitted by vectors caused by obligate intracellular parasitic protozoa that belong to the Leishmania genus [35]. It remains a

significant health challenge observed across four eco-epidemiological regions globally: North Africa, Southeast Asia, East Africa, West Asia, and the United States of America. The disease is characterized by three major clinical forms, including cutaneous, visceral, and mucocutaneous leishmaniasis, which are caused by different species of Leishmania[36]. C. Leishmaniasis is the most prevalent form, leading to skin lesions primarily on exposed areas such as the face, arms, and legs [37].

Mucocutaneous leishmaniasis manifests several years after the initiation of cutaneous leishmaniasis, leading to disfiguring lesions on the face due to the destruction of oral nasal and pharyngeal cavities [38]. The initial symptoms present mildly, beginning with nasal inflammation, gradually progressing to ulceration and septal perforation. These lesions do not resolve spontaneously and tend to appear several months or even years after the initial episode of cutaneous leishmaniasis. This manifestation occurs when macrophages within the mucous membrane of the naso-oropharynx become colonized [39]. Over 90% of mucocutaneous leishmaniasis cases occur in Bolivia, Brazil, Ethiopia, and Peru. *L. amazonensis, L. panamensis, L. braziliensis, L. guyanensis* are the most identified species causing this form [40].

Visceral leishmaniasis (VL) is marked by sporadic episodes of fever, weight loss, spleen and liver enlargement, and anemia. In contrast to the cutaneous form, VL affects internal organs. The incubation period for VL can vary from a few months to several years [39]. In 2018, Visceral Leishmaniasis (VL) was deemed endemic in 83 countries, with over 95% of new cases reported in 10 specific countries: Brazil, China, Ethiopia, India, Iraq, Kenya, Nepal, Somalia, South Sudan, and Sudan [41]. This form is represented by two species *L. infantum* and *L. donovani* [37]. The first species is widespread in Mediterranean countries (Europe and North Africa), Central and South America (Brazil, Venezuela, Bolivia, Mexico), Southeast Europe, the Middle East, Central Asia, and North [36, 42–44]. Meanwhile, *L. donovani* is found in South Asia, the Middle East, Central Africa, India, and China [42, 45–48]. The transmission cycle of VL differs between *L. infantum* and *L. donovani*. *L. donovani* is usually considered to be anthroponotic [48], but there are suspicions of zoonotic transmission, given the detection of parasites in wild mammals in East Africa [37] and in domestic animals on the Indian subcontinent [49]. Visceral leishmaniasis caused by *L. infantum* is primarily considered a zoonotic disease; however, cases of congenital transmission have been documented, even within Europe [50]. In summary, Leishmaniases, including different forms, are endemic in large areas of the tropics, subtropics, and the Mediterranean basin.

In our review, we were interested in Cutaneous leishmaniasis (CL), which is the most widespread. *Cutaneous leishmaniasis* leads to the formation of skin lesions, which have the potential to last for months or even years. These skin lesions tend to appear several weeks or months after exposure, although, in rare cases, they may not manifest until years later. While this form of leishmaniasis can be self-healing, it often results in atrophic scarring that may persist throughout a person's life. The process typically leads to the formation of scars, which may leave permanent marks on the skin [37, 39].

The importance of this form in the world is illustrated by the increase of cases either in its geographical distribution or in the annual number of new cases recorded. According to the World Health Organization (WHO), 253,435 new Cutaneous Leishmaniasis (CL) cases were recorded in 2018. CL is endemic in 87 countries globally, with an estimated 500,000 to 1,000,000 new cases reported each year [36]. The majority of cases occurred in two regions (Eastern Mediterranean Region and America Region), mostly in seven countries: Afghanistan [51, 52], Algeria [53, 54], Brazil [55, 56], Iran [57, 58], Iraq [59, 60], Pakistan [61, 62] and Syria [63]. The transmission cycle of the infection involves various species of leishmania, some of which are zoonotic, with reservoir hosts that can vary between domestic and wild mammals. In contrast, some Leishmania species are anthroponotic, demonstrating human-to-human transmission facilitated by the vector [46]. Various Leishmania species are responsible for Old World and New World cutaneous leishmaniasis [36, 37], The causative agents of Leishmaniasis in the Old World encompass a spectrum of species, including *L. tropica, L. major, L. aethiopica, L. infantum, L. donovani, and L. killicki* [64]. These species exhibit prevalence across an extensive geographical expanse, spanning Central and North Africa, Mediterranean countries, the Middle East, and Central Asia [40, 54, 64–69]. *L. aethiopica* is confined to the Ethiopian and Kenyan highlands [70].

The primary Leishmaniasis species in the New World belong to either the *L. mexicana* species complex (*L. venezuelensis, L. mexicana*, and *L. amazonensis*) or the subgenus Viannia (*L. guyanensis, L. braziliensis, L. peruviana*, and *L. panamensis*). Additionally, *L. infantum/ L. chagasi* also causes cutaneous leishmaniasis in the New World [37]. Firstly, *L. mexicana* is a widely identified species in the United States of America, Ecuador, Peru, and Venezuela [71, 72]. Also, *L. amazonensis* is a species with wide geographical distribution, especially in South America (Bolivia, Brazil, and Venezuela) [73]. Then, *L. venezuelensis,* concerned Northern South America and Venezuela [74, 75]. *L. braziliensisis* most identified in Guatemala, the Western Amazon basin, South America, Bolivia, Brazil, and Peru [76]. *L. panamensis* has a wide geographical distribution and mainly concerns Brazil, Panama, Guatemala and Colombia, Central and South America, Venezuela, Equator [77–79]. The *L.*

peruviana is limited to Peru and Bolivia [80, 81]. Furthermore, *L. guyanensis* and *L. laisoni* extend mostly in Northern South America, Bolivia, Brazil, French, Guiana, and Surinam [82, 83]. Finally, *L. shawi* and *L. naiffi* are present only in Brazil and French Guyana [84–86].

Eco-epidemiological studies conducted in Morocco have identified the presence of three distinct noso-geographic forms of leishmaniasis. These forms are characterized by their geographical distribution, clinico-epidemiological aspect, causal agent, and potential vectors and reservoirs [87–89]. The primary cause of cutaneous leishmaniasis (CL) in Morocco is *L. major*, which is mainly concentrated in the middle, southern, and southeastern regions of the Atlas Mountain range. Previous research has also indicated that this form of CL is widespread in arid and Saharan regions [65, 90–93]. In contrast, cases of *L. infantum* in Morocco were previously limited to sporadic occurrences in Sidi Kacem and Taounate [90]. Recently, it was suggested to be responsible for non-sporadic CL cases in several regions in our country, such as Sefrou, Taza, Larache, Tetouan, Al Hoceima, Chefchaouen, and Taounate and Ouazzane provinces [67, 68, 94–96]. Finally, *L. tropicais* is characterized by a wide geographical extension throughout the country from North to South [92, 97–99] and recently, it has been identified in areas previously known as foci of CL due to *L. major*, such as Errachidia and Ouarzazate provinces [99, 100].

Although its incidence is rising worldwide, cutaneous leishmaniasis is considered a neglected disease due to its low fatality rate. This has resulted in a lack of interest from public health authorities and professionals to conduct research or implement measures for the prevention and control of the disease. Therefore, it is important to understand the latest diagnostic and treatment methods for CL.

3. Pathophysiology of cutaneous leishmania

3.1. Immune response and Leishmania

The early response to Leishmania infection is affected by a variety of host- and parasite-derived factors, including the genetics of the host, inoculation site, the number of parasites, the species of Leishmania, and components of sand fly saliva [101, 101–103]. The innate immune response, which is the first line of defense characterized by the ability of the host to recognize conserved features of pathogens that are not present in an uninfected or undamaged host, and the adaptive immune response, which is the second line of defense specific to the encountered pathogen, both play a role in immune defense against Leishmania. The ability of *Leishmania* parasites to modulate and resist the innate and adaptive systems in patients determines their persistence. Certain types of immune response, such as strong T cell response characterized by delayed-type hypersensitivity, lead to reduced parasite load in the lesions, while high antibody levels and predominantly humoral response fail to control the parasite load [103]. Notably, many of the cells and molecules involved in the response during

Leishmania infection can have a protective or a pathogenic role. The equivalence of studies in mice and men also remains somewhat uncertain at times. For example, loss of TNF-α in mice leads to a fatal *L. major* infection, but this cytokine has also been implicated in pathogenesis in patients infected with *L. braziliensis* [104–106]. Similarly complex is the role of IFNγ dendritic cells (DC) [104]; neutrophil and macrophage/monocyte function can also vary in leishmaniasis, especially depending on the infecting *Leishmania* species/strain. Below, we discuss aspects of humoral and cellular immune responses related to Leishmania infection.

3.2. Complement and Leishmania

The complement system consists of several plasma proteins, including proteases that are activated by proteolytic cleavage and react with one another to opsonize pathogens, recruit and activate phagocytes to induce inflammatory responses, and create membrane attack complexes (MAC) or pores to lyse the pathogen. In the classical pathway, C1q either directly binds the surface of the pathogen or binds antibody:antigen complexes to activate the cascade. Complement can also be activated by binding the mannan-binding lectin to mannose-containing carbohydrates on the surface of the pathogen. Finally, a spontaneously activated complement can bind to the surface of the pathogen in what is known as the alternative pathway. Leishmania-dependent complement activation can be *via* the alternative pathway [107–109] or the classical pathway [110, 111]. In *in vitro* experiments, promastigotes in the logarithmic phase of growth were more readily lysed by alternative activation of complement, while the lysis of promastigotes in the stationary phase depended on the species of Leishmania [112]. Leishmania promastigotes have several mechanisms to escape or deactivate the host complement system, including prevention of MAC insertion into their membrane [113, 114]. Enhanced phagocytotic uptake of Leishmania by macrophages due to complement activation leading to increased Leishmania survival has also been noted during infection in animal models [113, 114].

3.3. Macrophage and monocytes

Macrophages serve as the primary host for *Leishmania* parasites. Early stages of Leishmania infection is characterized by uptake of the parasite by phagocytic cells, including skin-resident macrophages [115]. Once the macrophage phagocytoses the promastigote, the promastigote develops into its replicative form – the amastigote – within the phagolysosome[103]. Although the reactive oxygen species (ROS) generated during the respiratory burst associated with phagocytosis can kill Leishmania, the respiratory burst in non-activated macrophages cannot kill this parasite [116,117]. If IFNγ already activates macrophages, their respiratory burst is enhanced to levels sufficient to kill Leishmania parasites [118]. By contrast, monocytes are efficient at producing high levels of ROS and ROS-dependent killing of Leishmania without undergoing prior activation [118, 119].

3.4. Neutrophils

The primary cells responsible for eradicating Leishmania in the body are neutrophils, swiftly mobilized to the infection site. Regarded as the primary antimicrobial effector cells,

neutrophils effectively kill Leishmania. [120]. Paradoxically, neutrophils can also contribute to better survival of *Leishmania* [120]. For example, on the one hand, *L. amazonensis* promastigotes are killed by Neutrophil Extracellular Traps (NETs) [121, 122]. Depletion of neutrophils in BALB/c mice using RB6-8C5 mAb enhanced *L. braziliensis* numbers [123]. In vitro co-culture experiments have shown that neutrophils can diminish the parasite load in macrophages infected with *L. braziliensis.* A comparable effect was observed with *L. amazonensis* and *L. chagasi* [123].On the other hand, the depletion of neutrophils by NIMP-R14 mAb led to partial resolution of footpad lesions induced by *L. major* [124]. Additionally, Genista mice are resistant to *L.mexicana* or the Seidman strain of *L. major* [125, 126]. Thus, the role of neutrophils in *Leishmania* infection is complex.

Neutrophil function during Leishmania infection is likely influenced by the host's genetic background, the parasite strain, and the parasite's developmental stage [120]. For example, the depletion of neutrophils using RB6-8C5 mAb enhanced *L. braziliensis* load but did not affect *L. major* [123]. Reduced activation of macrophages and DCs following the uptake of apoptotic neutrophils and better parasite survival were observed in the case of L. *major* infection [127].

3.5. NK cells

The roles of NK cells are significant in immunological processes, particularly in the initial defense against pathogens such as Leishmania. NK cells have been reported to be present in lesions of patients diagnosed with local cutaneous leishmaniasis, disseminated cutaneous leishmaniasis, mucocutaneous leishmaniasis, and diffuse cutaneous leishmaniasis [128–130]. In a patient with disseminated cutaneous leishmaniasis lesions, monthly BCG along with monovalent PH8 and M2903 anti-Leishmania vaccines induced healing associated with the two-fold increase in the frequency of $CD16^+$ $CD56^+$ NK cells [131]. Based on *in vitro* proliferation and IFN γ and IL-6 production by peripheral blood mononuclear cells (PBMCs) from non-exposed healthy Swedish donors when challenged with *L. aethopia* antigen, it was proposed that $CD3^-$ $CD16/56^+$ NK cells, which constituted the major responding PBMC population, have a role in the protective response [132]. Similar results were obtained with PBMC from Ethiopia, where *L. aethopia* is endemic [133]. NK cells can be activated directly by *Leishmania* promastigotes or their cell surface glycolipid lipophosphoglycan (LPG) [134, 135]. The recruitment and activation of NK cells in mouse models of leishmaniasis is well-characterized [136]. Activated NK cells in the paracortex produce IFNγ [137]. This, in turn, enhances the production of IL-12 by DCs. It has been reported that at low activated NK cell immature DC ratio (1:5), DC maturation and cytokine production were enhanced in a cell-cell contact-dependent manner [138]. By contrast, at a high activated NK cell immature DC ratio (5:1), DC function was drastically reduced, potentially due to the killing of autologous DCs by NK cells [138].

3.6. Dendritic cells

Dendritic cells (DC) are the professional antigen-presenting cells. Immature DCs are avid phagocytes, while their maturation after antigen uptake results in migration, antigen-presentation to T cells, and TLR-driven cytokine production. Much like that described for other innate immune cells, the response of DCs to *Leishmania* can vary widely. DCs have been implicated in activating both $CD8^+$ T cells and $CD4^+$ T cell response against *Leishmania* [139]. Different DC subsets, including cross-priming $Baft3^+$ DCs or cDC1s, have been linked to $CD8^+$ *versus* $CD4^+$ T cell activation [140, 141]. Dermal dendritic cells are a highly motile subset of skin DCs that continuously crawl through interstitial spaces [142]. Within minutes of contact with Leishmania, these cells become immobile and incorporate multiple parasites within their vacuoles, suggesting that these cells are essential for surveillance against Leishmania [142]. Another subset of skin-resident DCs, the Langerhans cells, are also known to internalize Leishmania parasites [143]. Activation was associated with the internalization of *Leishmania major* amastigotes, as evidenced by the upregulation of MHC class I and II, increased expression of CD80 and CD86 costimulatory molecules, and production of IL-1 [143]. Monocytes recruited to the site of *Leishmania* infection have also been demonstrated to differentiate into dermal monocyte-derived DCs, which subsequently migrate to the draining lymph nodes [144]. In addition, monocytes recruited to the draining lymph nodes determined into lymph node monocyte-derived DCs [144]. These DC subsets also produce IL-12 and activate *L. major*-specific T-cell response[144]. However, controversy exists as to which specific type of DC holds the key to anti-*Leishmania* adaptive immunity [145], various DC subsets may contribute to initiating the T_H1 response against *Leishmania*. Interestingly, not all species of *Leishmania* induce DC activation, suggesting that DC response and adaptive immunity can vary depending upon the species of the Leishmania parasite. Infection with *L. mexicana* amastigotes does not result in enhanced expression of CD80 or MHC class II [146].

While DCs are linked to an anti-*Leishmania*T_H1 response against *L. braziliensis*, these cells may also be linked to a T_H2-type immune response in *L. amazonensis* patients. Here, the secretion of IL-4, IL-5, and IL-13 facilitates parasite replication and increased susceptibility to infection [147–150]. Similarly, Leishmania-induced apoptosis of neutrophils may also generate an immunosuppressive environment. Inoculation of the Leishmania parasite into the skin is followed by a massive recruitment of neutrophils that engulf the parasite. During this process, neutrophils die by apoptosis. These dead or dying neutrophils are captured by dermal DCs [151]. Depletion of neutrophils led to enhanced activation and function of *L. major* infected DCs, indicating that the engulfment of apoptotic neutrophils can suppress DC maturation and inhibit the development of adaptive immunity to Leishmania [151].

3.7. T Cells

DCs initiate the adaptive response to Leishmania. IL-12, produced by DCs in response to NK cell-derived IFN γ, is essential for the development of T_H1 $CD4^+$ cells that have anti-*Leishmania* immune resistance [152, 153]. T_H1 $CD4^+$ cells, recruited to the cutaneous

lesions, produce IFNγ that activates macrophages and promotes the killing of *Leishmania* parasites [103]. While the initial source of IFNγ is the NK cells [154], $CD8^+$ cells can also produce IFN γ. $CD8^+$ cell-produced IFN γ is critical for the TH1 $CD4^+$ cell response when the Leishmania parasite load is low [155, 156]. By contrast to IFN γ, other cytokines can favor the *Leishmania* parasite. IL-10, produced primarily by regulatory T (T_{regs}) cells, can lead to immunosuppression and the persistence of a low number of parasites even after lesion resolution [155]. An IL-4-rich environment favors TH2 CD4+ cell development and Leishmania susceptibility [157]. A TH17 response has been associated with severe disease [158, 159]. The extent of inflammatory response in *L. braziliensis* cutaneous in patients correlates with IL-17 levels [160, 161].

Long-lasting immunity to reinfection is dependent on $CD4^+$ T cells [162]. A role of both short-lived $CD4^+LY6C^+Tbet^{hi}$cells, as well as long-lived $CD44^+CD62L^{hi}CCR7^+$central memory (T_{CM}) cells, are involved in this process, and perhaps also $CD44^+CD62L^{lo}IL7R^+$ effector memory (T_{EM}) cells [163–165].

Apart from IFNγ production and TH1 CD4+ cell response induction at low parasite burden, other functions have also been ascribed to $CD8^+$ T cells during Leishmania infection. After treatment, a strong CD8+ T cell response was also reported in patients recovering from *L. mexicana* infection. $CD8^+$ T cells associated with Leishmania lesions are cytotoxic and express granzymes [105, 166–168]. *Cd8*$^{-/-}$ mice or the depletion of T cells in mice leads to a failure in controlling *L. major* [155]. However, cytolytic $CD8^+$ T cells, when recruited in large numbers, can be pathogenic and increase lesion development [103]. $CD8^+$ T cell numbers increase from early, non-ulcerated lesions to late, ulcerated lesions in patients infected with *L. braziliensis*, skewing the $CD8^+$:$CD4^+$ T cell ratio [105]. Thus, $CD8^+$ T cells can have both a protective and a pathogenic function during primary *Leishmania* infection. Nonetheless, $CD8^+$ T cells have a crucial role in protection during secondary challenge. $CD8^+$ T cell numbers in the spleen and lymph nodes expand up to 50-fold after re-infection, and this expansion correlates with increased production of IFNγ [169, 170].

3.8. B Cells

Albeit that much less is known about B cells in leishmaniasis, a similar pattern of apparently paradoxical roles of these cells in pathogenesis *versus* protection has been described. IL-7-mediated B cell expansion correlated with disease exacerbation in BALB/c mice, indicating that B cells might promote pathogenesis in Leishmania infection [171]. Similarly, BALB/c MT was partially resistant to *L. major* infection, but the adoptive transfer of naive B cells restored susceptibility [172]. In agreement with this hypothesis, the absence of B cells or IgG in JhD mice decreased disease progression [173]. However, B cell deletion enhanced *L. major* disease pathogenesis in normally resistant C3H/HeN and C57BL/6 mice [174]. Antibodies produced by B cells can coat parasites and help DCs prime $CD4^+$ T cells, increase IFN γ and IL-10, and enhance disease pathogenesis [175]. Notwithstanding, B cell-mediate immunity is unlikely to have a significant anti-Leishmania effect since disseminated

cutaneous leishmaniasis patients with high antibody titer but decreased or absent $CD4^+$ T cells were reported [176].

4. Diagnosis of cutaneous leishmaniasis

To diagnose CL, laboratory testing is typically combined with examining clinical symptoms. Several methods are available for diagnosing the disease, including direct parasitological tests using microscopy, histopathology, and parasite culture. Indirect testing methods, such as serology and molecular diagnostics, are employed. [177]. The diagnostic method utilized often depends on the resources available at the diagnostic facility and the existing infrastructure rather than the diagnostic efficiency. The following sections will discuss the most commonly used methods for diagnosing CL (Figure 1).

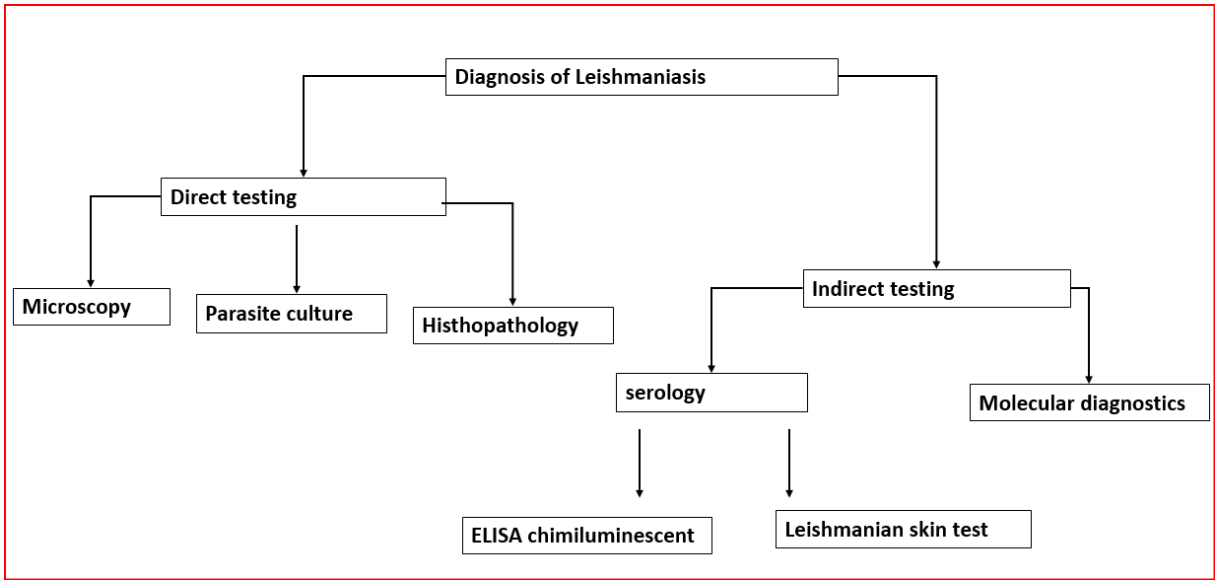

Figure 1. Diagnostic tests for Leishmaniasis.

4.1. Direct microscopy, histopathology and culture

Parasitologic diagnosis is a particular and effective method for diagnosing leishmaniasis. It is typically accomplished through the demonstration of amastigotes in skin lesions via microscopy on skin biopsy or through the culture of these specimens[178]. While microscopy may be the simplest method to achieve this, its deficiency is evident by the high rate of undiagnosed cases associated with this method [111, 179, 180].

Indeed, the effectiveness of the examination varies according to the clinical form and decreases with the age of the lesions [181, 182]. The sensitivity of direct test varies; the study by Reithingeren 2007 showed that the sensitivity of direct examination is around 89.3%. The intrinsic qualities of this examination are closely linked to the reader's experience.

In resource-limited settings, culture-based testing continues to be a practical diagnostic approach. Leishmania can be cultured from biopsy and aspirate samples, with promastigotes grown in several culture systems, including the Novy, MacNeal, Nicolle (NNN) medium and Schneider's insect medium [183], or they can be injected into a susceptible animal, such as hamsters for parasite recovery [177].

The use of the microculture method has significantly increased the sensitivity for detecting promastigotes. The high sensitivity of this method can be attributed to the use of capillary tubes, which concentrate the sample material and create favorable microaerophilic conditions for the transformation of amastigotes to promastigotes [184]. High levels of CO_2 may act as a trigger for amastigote-to-promastigote transformation and promote the survival of promastigotes [185]. Recently, a modified method known as Press-imprint-Smear has been developed [186]. Previous studies have shown this method is also more sensitive than histopathology and skin scraping [187].

4.2. Serology

The serological diagnosis of leishmaniasis relies on detecting either a cell-mediated immune response for cutaneous and mucocutaneous leishmaniasis or a specific humoral response for visceral leishmaniasis [188]. Current serologic tests for cutaneous leishmaniasis employ various formats, including Western blot, lateral flow assay, direct agglutination test, indirect fluorescent antibody, and enzyme-linked immunosorbent assay (ELISA). However, these techniques have limitations, such as reduced serum antibody levels due to the infection, leading to lower sensitivity [177, 189]. Below are descriptions of some of the more commonly used techniques.

4.2.1. Enzyme-linked immunosorbent assay

The Enzyme-Linked Immunosorbent Assay (ELISA) is an essential technical in the immune diagnosis of leishmaniasis. The test is useful for laboratory tests in addition to field applications. However, ELISA's specificity and sensitivity are greatly influenced by the antigen used. The incorporation of recombinant Leishmania antigens or specific purified antigen preparations for serologic diagnosis would develop the operational characteristics of

these tests[187]. This technique showed a vital result for the serodiagnosis of VL. It depends on the utilization of recombinant kinesin antigen (RK39) [190]. Studies have indicated that individuals with cutaneous leishmaniasis, other infections, or self-resolving *L. donovani* or *L. chagasi* infections typically do not have detectable antibodies to rK39 [183]. Conversely, in the assessment of anti-α-galactosyl antibodies in human sera using a chemiluminescent ELISA, individuals infected with *L. tropica* or *L. major* exhibited significantly elevated levels (up to 9-fold higher) of anti-α-Gal IgG compared to the healthy individuals used as controls [191].

4.2.2. Leishmanin skin test

The Leishmania Skin Test (LST), commonly known as the Montenegro test, is an immunological skin test employed to measure delayed-type hypersensitivity to Leishmania antigen. It is a diagnostic tool for cutaneous leishmaniasis [192, 193]. The Montenegro test was first introduced by Brazilian physician João Montenegro in 1926 and is recognized for its simplicity and high sensitivity, ranging from 86.4% to 100% [194].

To conduct the Montenegro skin test, 0.1 ml of Leishmania antigen is injected into the forearm, with the species and antigen preparation varying across countries and laboratories. A result is considered positive if the local induration is 5 mm or greater after 48-72 hours, with positivity detected four months after the appearance of lesions [177]. A delayed-type hypersensitivity skin reaction to LST is considered positive if it measures 5mm or greater and negative if it measures less than 5mm. The intradermal leishmania (Montenegro) skin test is generally positive in individuals with asymptomatic, self-resolving *L. donovani, L. infantum*, and *L. i chagasi* infections, as well as in those with cutaneous or mucosal leishmaniasis [183]. Research indicates that incorporating data on the production of antigen-specific interferon-c (IFN-c) with LST results can improve the diagnosis of exposure to Leishmania infection in suspected cases [195].

4.3. Molecular diagnostic

Several molecular diagnostic tests have been developed for the diagnosis of cutaneous leishmaniasis (CL). These tests are believed to offer improved sensitivity and specificity compared to traditional diagnostic methods and necessitate less invasive sampling [196, 197].

Polymerase Chain Reaction (PCR) is a promising tool, offering the potential advantage of utilizing blood specimens instead of conventional invasive procedures such as splenic aspirate, bone marrow, and liver biopsy [198, 199]. PCR is a method that enables the

direct and accurate detection of parasites with high sensitivity and specificity. Its sensitivity is reported to be between 86% and 95% in individuals with acute lesions, but it may decrease to 45.5% in those with chronic cases [187]. Numerous tests have been developed, targeting various gene sequences, including the ribosomal DNA internal transcribed spacer one sequence, the tubulin gene, the gp63 gene locus, multilocus microsatellite DNA [177], or sequences within the kinetoplast DNA of the Leishmania genus as the primary targets [200]. Despite its potential, the use of PCR for diagnosing Leishmania infection is currently limited to research settings, and field deployment remains challenging due to high costs. There have also been reported instances where PCR failed to detect the presence of parasites in blood samples from asymptomatic blood donors who were culture-positive for the infection. Despite these limitations, PCR has demonstrated an ability to see as few as 10 parasites/mL [201].

4.4. Genetic diagnosis

New methods of typing that rely on the genetic traits of the parasite are being developed. These include PCR-based techniques, which can accurately distinguish between different species when combined with restriction fragment length polymorphism analysis or sequencing. Researchers have identified several target genes for this purpose in the last decade, with the mini-exon gene being one of the most widely used. The mini-exon gene is involved in the process of nuclear messenger RNA trans-splicing and is present in 100-200 tandem repeats per nuclear genome. These repeats consist of three primary segments, making the mini-exon gene an exceptional marker for genotyping [202, 203]. The mini-exon PCR-RFLP genotyping method has been tested and confirmed using cultured Leishmania strains recognized as references by the World Health Organization. Additionally, cultured isolates obtained from patients have also been utilized to validate this method [202]. The mini-exon PCR-RFLP genotyping technique has gained popularity due to its high accuracy, sensitivity, and specificity. It is widely recognized as a powerful tool for the identification of all clinically significant species of Leishmania. As a result, this method has become a widely utilized approach for high-resolution diagnosis [204–206]. In addition to the mini-exon gene, other genes have also been considered potential genotyping markers. For example, the HSP70, hexokinase, and phosphoglucomutase genes have been investigated for several Old World Leishmania species. Meanwhile, the HSP70 gene region has been studied for New World species as a potential candidate for genotyping [207]. Species identification tools based on the HSP70 gene have great global application potential in various clinical and sampling settings. This method could become the gold standard for identifying Leishmania species in clinical specimens [208].

5. Different therapies developed against cutaneous leishmaniasis

Recent years have seen a pivotal shift in cutaneous leishmaniasis treatment. This is evident by advancements in oral medication options and an increased focus on patient-centric therapies. Similarly, clinical trials are essential and decisive parts of the drug discovery and new treatment process to ensure the safety and efficacy of any new medication and comparison with the already available treatments [209, 210]. In this paper, we discuss clinical trials of drugs against cutaneous leishmaniasis.

5.1. Old drugs and drugs in current use for the treatment

The medications used to treat cutaneous leishmaniasis and pentavalent antimonials were introduced 60 years ago [211].

5.1.1. Medical therapy

The cornerstone of cutaneous leishmaniasis treatment lies in drugs such as pentavalent antimonials. Specifically, the utilization of intralesional antimonials aims to mitigate adverse effects in comparison to their systemic administration. However, antimonials are renowned for their high incidence of reversible side effects [212]. Other drugs, such as amphotericin B, pentamidine isethionate, paromomycin, and antifungals, are employed in the treatment [213].

Historically, pentavalent antimonials, such as meglumine antimoniate and sodium stibogluconate, have been widely used to treat leishmaniasis. Studies have demonstrated that sodium stibogluconate has an efficacy rate of 83% in the Old World. In comparison, intralesional pentavalent antimony showed an efficacy rate of 75%, which increased further when combined with cryotherapy. Meglumine antimoniate, on the other hand, had an efficacy rate of 68%. In the New World, intralesional pentavalent antimony was 77% effective, while sodium stibogluconate showed an efficacy rate of 61%. Meglumine antimoniate, however, had the highest efficacy rate at 82%. [214]. We must consider that this treatment requires the infiltration of each lesion, so it is not for all cases of cutaneous leishmaniasis. The reversible side effects reported were local irritation, edema, pain, erythema, and pruritus [215].

Amphotericin B is regarded as a second-line treatment for leishmaniasis. A review of liposomal amphotericin B (L-AmB) treatment for Old World cutaneous leishmaniasis (OWCL) indicated an efficacy rate of 85% in immunocompetent patients with OWCL. However, the review also noted limitations in the reported data due to variations in treatment dosages [47, 216].

The pentamidine can be administered intravenously or intramuscularly in leishmaniasis. Lai *et al.* [217] emphasized that both pentamidine mesylate and pentamidine isethionate exhibit comparable safety, efficacy, and side effects in the treatment of cutaneous leishmaniasis in Surinam.

Moreover, a study conducted in Panama revealed that the paromomycin-gentamicin cream achieved a cure rate of 79% against New World Leishmania species, with minimal local adverse events [218, 219].

The azole molecules such as ketoconazole, itraconazole, and fluconazole have been studied for antileishmanial therapy in several investigations with different cure rates and fewer side effects [220, 221].

5.1.2. Cryotherapy

Cryotherapy is a physical and localized treatment that involves exposing lesions to subzero temperatures. It is an effective treatment for leishmaniasis because all Leishmania species are thermosensitive. During cryotherapy, liquid nitrogen is directly applied to the lesion, causing direct damage by inducing a lethal temperature. Additionally, this treatment has been shown to stimulate an immune response. [222]. Cryotherapy is a cost-effective and low-risk treatment that is well-tolerated by patients with minimal side effects. Research has indicated that combining cryotherapy with antimony intralesional infiltration is more effective than utilizing each technique alone. Several studies have confirmed these findings. [214, 215, 223].

5.1.3. Thermotherapy

Thermotherapy is a recommended treatment for lesions caused by all species of Leishmania. This treatment can be administered using infrared light, laser, or direct electrical stimulation [224]. Heat therapy is safe compared with other traditional treatments and simple to use in rural zones without electricity, and the cure rate is about 87 to 98% [225]. Nevertheless,, it is an expensive technique [226].

5.1.4. Laser therapy

Laser treatment may be a viable alternative to traditional methods for treating cutaneous leishmaniasis. It utilizes a specific thermolysis effect on infected tissue and has minimal impact on normal skin, resulting in few side effects [227].

Different types of lasers are available for cutaneous leishmaniasis treatment, such as carbon dioxide laser, argon laser, erbium glass laser, neodymium-doped yttrium aluminum garnet laser, pulsed dye laser [227–229].

5.1.5. Drugs in clinical development for the treatment

The goal of numerous studies is to optimize the development of an immunotherapeutic for leishmaniasis to be used to reinforce chemotherapy. This could be achieved by demonstrating the efficacy of this vaccine in patients with drug-refractory leishmaniosis or by decreasing the amount of chemotherapy needed to heal [230]. Information on the immune response of patients and its progression during the leishmaniasis disease course is essential.

An immunotherapeutic antigen preparation was developed for patients with a history of treatment failure. Following administration, 90% of patients experienced complete clinical remission. Additionally, all patients remained asymptomatic during a 5-year follow-up examination [230]. Several studies have been conducted to develop vaccines against Leishmania. Five first-generation vaccines have been approved, two of which are suitable for human administration. These vaccines use the entire parasite in live or attenuated form [215].

6. Natural products for the treatment

Research is underway to develop new candidate drugs using natural compounds [231–237]. It is estimated that there are approximately 250,000 medicinal plant species worldwide. However, the biological activities of only about 6% of these species have been tested [238, 239].

Given the current lack of an effective vaccine and increasing resistance to traditional drug treatments, research is focused on exploring alternative compounds and therapeutic agents, including natural botanical agents and their compounds [240, 241] having anti-leishmanial or leishmanicidal potency is one of the critical challenges, and combining low cost, low incidence of toxicity and good efficacy [242–244]. Unfortunately, most of these studies have been taken only in in vitro studies and only a few in vivo, and they are still in the preclinical trials [245, 246].

The choice of second-line drugs is minimal. Medicinal plants are essential sources of promising new compounds, and for this reason, their valorization seems to be crucial. Several investigations have described the screening of species of plants tested in Leishmania treatment, as summarized in Table 1.

Table 1. Effects of medicinal plants on *Leishmania* parasite.

Plant name	Common name	Family name	Main component or plant part used	Effects	References
Allium sativum	Garlic	Amaryllidaceae	Bulbe/ Allicin, Alliin	Destroyed Promastigote form	[247]
Aloe latex	Aloe	Asphodelaceae	Aloe-emodin /leaf vs. Bark	Inhibited the growth of amastigotes and induced apoptosis in promastigotes.	[248]
Artemisia annua	Wormwood/ and wash	Asteraceae	Endoperoxide	Effective against the promastigote form	[249]
Capsicum annuum	Kapsa	Solanaceae	Capsaicin	Effective against the promastigote form	[250]
Cassia fistula	Golden shower	Fabaceae	Flowers	Destroyed the Promastigote form	[251]
Coriandrum sativum	Coriander	Apiaceae	Seeds	Iron-dependent enzymes and membrane lysis of the parasite	[244]
Cymbopogon citratus	Lemon grass	Poaceae	Aerial parts/ citral	Inhibiting the parasites growth by apoptosis without cytotoxicity induction	[252]
Dalbergia ecastaphyllum	Liane à barrique bord de mer	Fabaceae	Formononetin, biochanin A, daidzein and pinocembrin	Effective against the promastigote form	[253]
Eucalyptus camaldulensis	Blue gum	Myrtaceae	1,8-cineole	Reduced cutantaneous lesions	[254]
Glycyrrhiza glabra	Chalcones	Fabaceae	Glycyrrhizic acid	Inhibiting the promastigotes and intracellular amastigotes	[255]
Haplophyllum bucharicum	Sadaap	Rutaceae	Flowers/ Diphyllin	Inhibited growth of amastigote form	[256]

Matricaria chamomilla	Camomila	Asteraceae	Flowers	Inhibitory effect on parasitic growth	[257]
Mimosa tenuiflora	Jurema	Fabaceae	Flowers	Reduced parasitic growth	[258]
Mikania glomerata Spreng	Guaco	Asteraceae	Leaf	Inhibitory effect on parasitic growth	[257]
Nigella sativa	Blach seed	Ranunculaceae	Seeds	Reduce the parasitic growth	[259]
Origanum onites	Greek oregano	Lamiaceae	Carvacrol (70.6%), linalool (9.7%), p-cymene (7%), γ-terpinene (2.1%), and thymol (1.8%)	Reduced parasitic growth	[260]
Peganum harmala	Harmal	Nitrariaceae	Seeds	Inhibitory effect on promastigote and amastigote forms	[261]
Piper aduncum	Pariparoba	Piperaceae	Peperine	Inhibitory effect on promastigote	[262]
Piper marginatum	*Piper marginatum*	Piperaceae	Phenolic compounds, terpenoids, and 3,4-methylenedioxy propiophenone	Antipromastigote and antiamastigote	[263]
Prunus domestica	European plum	Rosaceae	Leaf	Inhibitory effect on parasite growth	[257]
Ricinus communis	Castor	Euphorbiaceae	Leaf	Iron-dependent enzymes and membrane lysis of the parasite	[244]
Stachys lavandulifolia	Wood betony	Lamiaceae	Aerial parts	Inhibitory effect on promastigotes	[264]
Strychnos Pseudoquina	Quina	Strychnos genus	Flavonoids of quercetin 3-Omethyl ether and strychnobilavonethyl purified from acetate extract	Inhibitory effect on promastigotes	[265]
Stryphnodendron Obovatum	Mimosoid clade	Fabaceae	Gallic acid, gallocatechin, epigallocatechin,	Destroyed the Promastigote form	[266]

Tanacetum parthenium	Feverfew	Asteraceae	Aerial parts	Inhibitory effect on parasite growth	[257]
Tanacetum vulgare L	Tenaceto	Asteraceae	Leaf	Inhibitory effect on parasite growth	[257]
Thymus capitellatus Hoffmanns. and Link	Thym	Lamiaceae	Aerial parts/ (1,8-cineole and borneol)	Induction of apoptosis without significant cytotoxic effects	[267]
Urtica dioica	Burn nettle, stinging nettle	Urticaceae	Aerial parts	Reducing amastigote and promastigote growth	[268]
Zajuria multiflora Boiss	Thyme	Lamiaceae	Aerial parts	Reduce the parasitic growth	[269]
Vitis vinifera L.	Grape vine	Vitaceae	Leaf/ anthocyanin	Destructed promastigotes	[270]

7. *Streptomyces*: bacterial heroes in the battle against cutaneous leishmaniasis

The *Streptomyces*, members of the Gram-positive, aerobic, filamentous, and saprophytic bacterial genus belonging to the Actinomycetaceae family, constitute a fascinating phenomenon within the soil ecosystem [271–279]. Their ubiquity and pivotal role in the decomposition of organic matter make them key players in environmental dynamics. Beyond their contribution to biodegradation, these microorganisms have gained significant recognition due to their exceptional ability to produce a wide range of bioactive compounds, thereby conferring an impressive functional diversity [280, 281]. The biological properties of these compounds make *Streptomyces* an invaluable source for pharmacological research. Their portfolio of activities includes antimicrobial [279, 282], antifungal [3, 4], antioxidant [271, 283], antiviral [284, 285], anticancer [283, 286], and antiparasitic [287–289] activities. These versatile capabilities stem from the secondary metabolites produced by these bacteria, offering myriad possibilities for drug discovery.

In the specific context of parasitic diseases, *Streptomyces* have garnered attention as potential antiparasitic agents, particularly in the fight against leishmaniasis. Studies have revealed that *Streptomyces* show promising efficacy against various parasites responsible for leishmaniasis [287, 290–295]. At the core of these investigations, cutaneous leishmaniasis (CL) provides a fertile ground for discovering innovative treatments. In this context, the work of Awada *et al.* [293] highlights the significant effectiveness of *Streptomyces* sp. HAS1 in the treatment of cutaneous leishmaniasis caused by *Leishmania tropica*. Cultivated in a specific

production medium called INA, *Streptomyces* sp. HAS1 produced a crude extract demonstrating notable potency against a clinical isolate of *Leishmania tropica*. This therapeutic potential was confirmed through bio-guided fractionation, during which the bioactive compound was isolated from the crude extract. The structure of this compound was elucidated using advanced techniques such as nuclear magnetic resonance (NMR) and high-resolution mass spectrometry coupled with liquid chromatography (LC-HRMS) [293]. These results suggest that *Streptomyces* sp. HAS1 could represent a promising source of bioactive compounds for treating cutaneous leishmaniasis, paving the way for developing new therapies against this neglected tropical disease.

8. Conclusions

This comprehensive review of cutaneous leishmaniasis highlights significant breakthroughs in understanding its pathophysiology, molecular diagnosis, and therapeutic strategies, paving the way for targeted perspectives in disease management. The clarified pathophysiological mechanisms create opportunities for more precise interventions, while advancements in molecular diagnosis enhance the rapid identification of cases, improving clinical responsiveness and epidemiological surveillance. The exploration of novel therapeutic approaches, particularly bioactive compounds, presents concrete opportunities for more effective treatments. However, ongoing in-depth research on molecular mechanisms, pharmacokinetic parameters, and safety remains crucial. Emphasizing the importance of practical applications, this review underscores the need to focus research efforts on prevention, diagnosis, and treatment methods, bringing us closer to more effective management and sustainable control of cutaneous leishmaniasis. Through collaborative efforts, envisioning a future where this disease is no longer a significant threat to global health becomes a tangible possibility.

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