Green Approach: “A Forwarding Step for Curing Leishmaniasis—A Neglected Tropical Disease”

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The present review focuses on a dreaded vector-mediated leishmaniasis, with the existing therapeutic approaches including a variety of drugs along with their limitations, the treatment with natural compounds, and different types of metal/metal oxide nanoparticles (NPs). As evidenced, various metallic NPs, comprising silver, silver oxide, gold, zinc oxide, titanium, lead oxide, etc., played a curative role to treat leishmaniasis, are also presented. Keeping in view the advance success of vaccines against the prevalent dread diseases in the past and the present scenario, efforts are also being made to develop vaccines based on these NP formulations.

Keywords: leishmaniasis, natural sources, nanobiotech, green route, nanoformulations

INTRODUCTION

Neglected tropical diseases (NTDs) are contagious diseases that cause substantial illness in more than one billion people globally (Maheshwari and Bandyopadhyay, 2020). Various parasite-mediated diseases comprising giardiasis, Chagas disease, Babesiosis, toxoplasmosis, leishmaniasis, etc., befall in animals and further spread to human population (Oryan, 2015; Hotez et al., 2020). Leishmaniasis is one of the NTDs considered as imperative parasitic diseases, commonly caused by an etiologic agent Leishmania, a genus of trypanosomes. Leishmaniasis is located in the ninth place of the global burden of disease among individual infectious diseases. More than 22 species of infectious Leishmania have been reported (Maheshwari and Bandyopadhyay, 2020). Leishmania are transmitted to mammals through the bite of infected female sand flies belonging to Lutzomyia and Phlebotomus (Oryan and Akbari, 2016). It is endemic in 98 nations of the world, where more than 350 million people are at risk and more than 12 million cases of infection have been reported (Verma and Dey, 2004; McGwire and Satoskar, 2014). Based on the species and intensity of infection to the host, it has been classified into cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), and visceral leishmaniasis (VL) forms. Considering these classes, CL caused by L. aethiopica is most commonly found in human population and reported to infect 6,00,000 to one million people annually all around the world. It causes severe symptoms like ulcers, serious disabilities, and life-long marks (Surur et al., 2020).

Previously, various chemical drugs including liposomal amphotericin B, amphotericin B, pentamidine, pentavalent antimonials, miltefosine, and paromomycin have been practiced against Leishmania. Among these drugs, pentavalent antimonials (sodium stibogluconate and meglumine) are existing chemical drugs, and they are a major therapeutic source to treat Leishmania infection. However, current treatment practices are associated with certain side effects like high toxicity, high cost, and most importantly, development of drug resistance. Hence, there is an instantaneous necessity to invent new, harmless, and efficient prevention therapies to overcome these limitations (Mitropoulos et al., 2010). Currently, various approaches are involved to control the elevated level of infection, including nanoformulations and targeted drug
delivery using nanocarriers as well as with the aid of particular bioactive compound obtained from plants (Javed et al., 2020; Santana et al., 2020). Inventions in nanoscience greatly contribute to overcome the problems allied with the treatment of infectious diseases. Owing to the small size of NPs (1–100 nm), the ability to penetrate easily into the cells, extensive circulation within the body, and the efficient targeted drug delivery system, they can be reflected as a better medication to treat endemic leishmaniasis (Ebrahimi et al., 2017). Further, plant-based nanoparticles have been reported as a successful approach for the preclusion of microbial infections as well as in the treatment of leishmaniasis (Alti et al., 2020). Owing to the green and eco-friendly nature, cost effectiveness, less hazardous nature, and involvement of phytoconstituents for capping and stabilization, plant-based metal oxide nanoparticles (NPs) (silver, zinc, nickel, iron, etc.) have been in use to cure leishmanial infection (Ismail et al., 2019; Alti et al., 2020). The present review focuses on the use of plant-based natural products, the phytosynthesized NPs, to cure *Leishmania*.

**LIFE CYCLE**

The life cycle of *Leishmania* parasite begins with the bite of infected female phlebotomine sandflies and is completed in two different morphological forms, that is, promastigote and amastigote. Flagellated metacyclic promastigote, formed in the infected sandflies firstly enter into the macrophages via phagocytosis and formed phagosome. Resultant phagosome enter into the stages of maturation (membrane transformation) and formed a new structure: the parasitophorous vacuole. In this organelle, promastigotes metamorphose into amastigote, followed by its multiplication until explosion of the cells of the macrophage system (4–6°days) spreads infection further. The parasite either initiates infection superficial cells or visceral cell depending on its tropism characteristics. The life cycle of *Leishmania* into the host is completed when another uninfected sandfly sucks blood as a source of meal. The sensitivity of infection is based on the sandfly species, ecology, epidemiology, and pathogenicity (Bañuls et al., 2007). Further, detailed description of its infection period and transmission is clearly shown in Figure 1.

**EXISTING THERAPEUTIC APPROACHES AND LIMITATIONS**

Leishmaniasis is one of the most important NTDs coupled with various adverse as well as life-threatening factors, including substantial morbidity, early death, and long-term infirmity. Treatment comprises control of disease spreading and use of existing parameters, while the currently used therapies including chemical drugs necessitate long-duration therapy and low efficiency with numerous toxic effects. Although no relevant therapies have been developed to prevent the infection which is extensively spread among human population, only few prevention methods are available (Gharirvand Eskandari et al., 2020). Among them, some kind of clinically approved drugs are
found to treat this endemic disease, including meglumine antimoniate (glucatime), sodium stibogluconate (pentostan), amphotericin B, and miltefosine. However, the excessive use of these chemotherapeutic sources is associated with antagonistic effects (Ghobakhloo et al., 2016). This has led to the search of some natural methods to treat leishmaniasis.

Chemical-Based Drugs for the Treatment of Leishmaniasis

Since last several years, various kinds of pharmaceutical drugs including amphotericin B, pentamidine, miltefosine, and paromomycin were involved in the treatment of leishmaniasis. None of the clinically approved drugs could be deliberated as the ultimate source of treatment due to their time-taking process and high toxicity combined with severe adverse effects. In addition, the most often used medicines do not eradicate the parasites entirely from all infected entities (De Menezes et al., 2015). Further, applications of some of these medicines with their limitations are described below:

• Pentavalent antimonials can be administered by the intravenous, intramuscular, and intralymphatic routes with the optimum dosage of 20 mg/kg/day (28–30 days) and exhibited 35–95% potentiality. Continuous and excessive use of this drug causes toxicity like nephrotoxicity, hepatotoxicity, severe cardiotoxicity, and pancreatitis (De Menezes et al., 2015).

• Oral administration of miltefosine not only showed inhibitory effects on the growth of Leishmania but also affected adversely and created severe infecting symptoms comprising nephrotoxicity, teratogenicity, vomiting and diarrhea, and hepatotoxicity (Sundar et al., 2011).

• Paromomycin, also being used as a therapeutic agent to treat leishmaniasis, reported to show some toxic effects during its treatment phase, like severe nephrotoxicity, hepatotoxicity, and ototoxicity (Jhingran et al., 2009).

• Pentamidine with the prime dosage of 3 mg/kg/day can potentially involve in the retardation of Leishmania growth with some severe antagonistic effects such as hypotension, elevated rate of hyperglycemia, tachycardia, pancreatic damage, and electrocardiographic changes (De Menezes et al., 2015).

The existing chemotherapies have a list of short comings comprising high cost, higher toxicity, and acquired resistance toward parasitic strain, and other side effects during their prevention mechanism insisted scientists and medical practitioners to evolve a new therapeutic system to treat NTDs. During the last decades, green therapies involving plant extracts, bioactive compounds, and secondary metabolites derived from particular plant species and different kinds of NPs synthesized using plant extract become promising as well as safer prevention therapies.

Natural Methods

From ancient times, plant-based traditional methods are being used in the therapeutics against various infectious ailments. Currently, plant extract and particular bioactive compound extracted from plants are either directly used as a therapeutic source or as derived herbal drugs for the treatment of leishmaniasis as well as other microbial infection (Oryan, 2015).

Involvement of Plant Extracts and Plant-Derived Secondary Metabolites

The consumption of herbal drugs derived from plants is being used from centuries as a prevention source for NTDs as well as other diseases including bacterial (and their vectors), helminth (and their vectors), fungal, ectoparasitic, protozoan (and their vectors), and viral infections (and their vectors). The use of medicinal plants becomes more advantageous over other chemotherapies due to their nontoxic, environment-friendly, and cost-effective properties. Further natural compounds obtained from plants are considered as a reliable therapeutic source to treat leishmaniasis (Cheuka et al., 2017).

Agaratum conyzoides, Bidens pilosa, and Eugenia uniflora showed efficient leishmanicidal effects. Bidens pilosa (root) has been reported for its antileishmanial properties (against L. amazonensis, promastigote) with the least IC_{50} value (1.5 μg/ml) as compared to other plant species (Table 1). Essential oils from Eugenia uniflora potentially inhibit the growth of both the parasitic forms, that is, promastigote and amastigote, of L. amazonensis, and Agaratum conyzoides has been reported to treat infection caused by L. donovani (amastigote form) (Silveira et al., 2021). E-caryophyllene, the main component of Melampodium divaricatum and Casarea sylvestris essential oil, has been reported for its promising antileishmanial response against L. amazonensis (IC_{50} values of 10.7, 10.7, and 14.0 μg/ml) (Moreira et al., 2019). Moreover, 1,8-cineole, a-pinene, and p-cymene active constituents of Protium alstonii and P. hebetatum (Burseraceae) exhibited dose-dependent amastigote inhibition with IC_{50} of 48.4, 37, and 46 μg/ml, respectively (Santana et al., 2020). Butanol fraction of K. odoratissima with 154.1 μg/ml IC_{50} value showed antileishmanial properties against L. major promastigote and amastigote (Mirzaei et al., 2020).

Role of Plant-Based Nanoparticles in the Treatment of Leishmaniasis

Methods in controlling infectious diseases have modernized translational sciences to develop a better controlling method for infectious diseases. The field of nanomedicine has shown enormous potential in developing highly sensitive diagnostic tools with excellent drug delivery properties. Recently, nanoparticle-conjugated drugs have increasingly been studied as an alternative, cost-effective therapy with increased effectiveness. However, toxicity is a major barrier that needs to be encountered. Several reports have shown the effective antimicrobial activities of various metal/metal oxide nanoparticles as well as against the Leishmania causing organism through their wide surface area and unique properties.

Nanoparticles synthesized using crude as well as various solvent-fractionated extracts of medically important plants are considered as efficient agents for the delivery of specific phytoconstituents into the cells. Keeping in view the effective antimicrobial activities of silver metal, silver/silver oxide NPs
| S. no | Plant used                  | Plant part used for extract preparation | Bioactive compound involved | Mode of study and optimum dosages | Organism tested | Structural formula | Mechanism of action                                                                 | References                          |
|-------|----------------------------|-----------------------------------------|-----------------------------|----------------------------------|-----------------|-------------------|-------------------------------------------------------------------------------------|-------------------------------------|
| 1     | *Baccharis uncinella* (groundsel) | Leaves                                  | Ursolic acid                | *In vivo* 1.0 mg/kg or 2.0 mg/kg (body weight) | *L. infantum*   | ![image](image1.png) | Treatment with ursolic acid causes a remarkable reduction in liver as well as splenic parasitism | Jesus et al. (2017)                 |
| 2     | *Allium sativum* (garlic)     | Bulb                                    | Allicin                     | *In vitro and in vivo* 50 μM for *in vitro* studies | *L. major*      | ![image](image2.png) | -                                                                                 | Metwally et al. (2016)              |
| 3     | *Eremurus persicus* (desert candles) | Root extract                            | Aloesaponol III 8-methyl ether | *In vitro* IC50 73 μg/ml | *L. infantum* | ![image](image3.png) | After treatment with isolated compound, mitochondrial potential and few structural alterations in the promastigote form of tested organism were observed | Rossi et al. (2017)                 |
| 4     | *Olea europaea* (wild olive, Indian olive, and brown olive) | Air-dried, pulverized leaves            | Oleuropein                  | *In vitro and in vivo* 128.4 μM (69.4 μg/ml), for *in vitro* studies | *L. donovani* | ![image](image4.png) | Oleuropein responsible to raise ROS production, upregulation of host antioxidant enzymes, and downregulation of other enzymes of the parasite. Furthermore, *in vivo* model delayed-type hypersensitivity and elevation of IgG2a/IgG1 ratio (*leishmania-specific*) were observed | Kyriazis et al. (2016); Sharma et al. (2019) |
| 5     | *Zingiber zerumbet* (awapuhi and bitter ginger) | Fresh rhizome                           | Zerumbone                   | *In vitro* 10 μM                | *L. donovani*   | ![image](image5.png) | Zerumbone extracted from *Zingiber zerumbet* causes apoptosis in promastigotes by affecting ROS production coupled with reduction of intracellular amastigotes in infected macrophages | Mukherjee et al. (2016)             |
### TABLE 1 | (Continued) Role of plant-based natural products in the treatment of leishmaniasis.

| S. no | Plant used                      | Plant part used for extract preparation | Bioactive compound involved | Mode of study and optimum dosages | Organism tested | Structural formula | Mechanism of action                                                                                     | References                                      |
|-------|---------------------------------|----------------------------------------|----------------------------|-----------------------------------|-----------------|-------------------|--------------------------------------------------------------------------------------------------------|------------------------------------------------|
| 6     | Morinda lucida (brimstone tree) | -                                      | Molucidin                  | In vitro IC\textsubscript{50} 4.24 μM for Leishmania hertigi and anti-010 activity with MIC of 4.167 μM | L. hertigi and field strain-010 | ![Molucidin](image) | Molucidin: Normal cells have single set of nucleus and kinetoplast, that is, 1N/1K, but molucidin stimulates two different sets of kinetoplast and nucleus in the cells of parasite. After division of both the sets, this compound obstructs the cytokinesis and causes cell cycle arrest which leads to death of parasites | Amoa-Bosompem et al. (2016); Sharma et al. (2019) |
| 7     | Artemisia annua (sweet annie, annual mugwort, sweet sagewort, or annual wormwood) | -                                      | Artemisin | In vivo and in vitro 100 μg/ml for in vivo studies | L. major | ![Artemisin](image) | -                                                                                                       | Ghaftiarifar et al. (2015); Sharma et al. (2019) |
| 8     | Hypericum Carinatum (St John’s wort) | Flowering aerial parts | Cariphenone A (1), isouliginosin B (2), and uliginosin B (3) | In vitro IC\textsubscript{50} values of 10.5, 17.5, and 11.3 μM for compound 1, 2, and 3, respectively. | L. amazonensis | ![Cariphenone](image) | Inhibition of parasites mediated by oxidative stress (ROS production) and alteration in mitochondrial potential-like hyperpolarization condition | Dagnino et al. (2018) |
| 9     | Euphorbia peplus (radium weed) | Peplus aerial parts | Simiarenomol | In vitro IC\textsubscript{50} values of 20.24, 34.87, and 32.05 μg/ml | L. donovani | ![Simiarenomol](image) | -                                                                                                       | Moawad et al. (2016)                           |
| S. no | Plant used                                      | Plant part used for extract preparation | Bioactive compound involved | Mode of study and optimum dosages | Organism tested | Structural formula | Mechanism of action                                                                 | References                  |
|-------|-----------------------------------------------|-----------------------------------------|-----------------------------|----------------------------------|----------------|-------------------|-------------------------------------------------------------------------------------|-----------------------------|
| 10    | *Strychnos pseudoquina*                       | -                                       | Strychnobiflavone           | In vitro 5.4 and 18.9 μM        | *L. amazonensis* | -                 | Mechanism of action will be allied with alteration in mitochondrial membrane potential in parasitic cells | Lage et al., 2015            |
| 11    | *Melampodium divaricatum* and *Casearia sylvestris* | Essential oils (aerial parts of *Melampodium divaricatum* and leaves of *Casearia sylvestris*) | E-caryophyllene (22.2%), germacrene D (19.6%), and bicyclogermacrene (12.2%) | In vitro 24.2, 29.8, and 49.9 μg/ml | *L. amazonensis* | -                 | -                                                                                   | Moreira et al. (2019)        |
| 12    | *Handroanthus species*                        | -                                       | Lapachol                    | In vitro and in vivo (IC50 = 79.84 ± 9.10 μM, SI = 42.65) for *L. amazonensis* and (IC50 = 135.79 ± 33.04 μM, SI = 25.08) for *L. infantum* 25 mg/kg for in vivo model | *L. infantum* and *L. amazonensis* | -                 | In the in vivo model, lapachol will be able to reduce the parasitic load in the spleen, liver, and skin lesions | Araújo et al. (2019)          |
| 13    | *Ifluga spicata* (I. spicata) (aj al arza, aj al ghazal, and hasal) | Whole plant (leaves, flowers, stem, and roots) | 3,4-Dihydroxybenzoate (compound 1) and benzoate (compound 2) | In vitro LD50 values of 10.40 ± 0.09 and 14.11 ± 0.11 μg/ml for compound 1 and compound 2, respectively. | *L. tropica* | -                 | Both the isolated compounds showed great affinity with gp63 (leishmanolysin) receptor of leishmania parasite. Binding of these compounds with their receptors resulted in the smooth entry of parasites into the cells, and after injection, it binds with DNA and causes apoptosis | Shah et al. (2019)            |
| S. no | Plant used | Plant part used for extract preparation | Bioactive compound involved | Mode of study and optimum dosages | Organism tested | Structural formula | Mechanism of action | References |
|-------|------------|-----------------------------------------|-----------------------------|----------------------------------|-----------------|-------------------|---------------------|------------|
| 14    | Protium altsonii (PaEO) and P. hebetatum (PhEO) | Essential oil | In vitro PaEO IC\(_{50}\) were 14.8 μg/ml and 7.8 μg/ml and PhEO IC\(_{50}\) were 0.46 μg/ml and 30.5 μg/ml | L. amazonensis | - | Mitochondrial membrane potential associated with NO production could be an effective mechanism of leishmaniasis | Santana et al. (2020) |
| 15    | Artemisia aucheri | Whole plant extract | In vitro and in vivo IC\(_{50}\) 90 μg/ml | L. major | - | - | KarimiPourSaryazdi et al. (2020) |
| 16    | Clerodendrum myricoides (blue-flowered tinder wood) and Salvadora persica (arak, jhak, pliu, Salvadora indica, toothbrush tree, mustard tree) | Aqueous extract of stem | In vitro MIC = 625 μg/ml | L. major | - | - | Maina et al. (2020) |
| 17    | Croton blanchetianus Baill | Ethanolic extract | In vitro IC\(_{50}\) values of 208.6 and 8.8 μg/ml for Leishmania infantum and IC\(_{50}\) values of 73.6 and 3.1 μg/ml for Leishmania amazonensis and L. infantum | L. amazonensis and L. infantum | - | Ethanolic extract of Croton blanchetianus targets a significant depolarization of mitochondrial membrane potential and leads to mitochondrial dysfunction | Pereira et al. (2020) |
| 18    | Prunus armeniaca (Armenian plum) | Leaf extract | In vitro anti-promastigote activity with IC\(_{50}\) 11.48 ± 0.82 μg/ml and anti-amastigote activity with IC\(_{50}\) 21.03 ± 0.98 μg/ml | L. tropica | - | - | Shaheen et al. (2020) |
| 19    | Urtica dioica (common nettle, stinging nettle) | Aqueous extract | In vivo and in vitro 3,500 and 6,000 μg/ml for promastigotes and amastigotes, respectively. | L. major | - | It proficiently killed the amastigote form of L. major; additionally, remarkable reduction of parasite load, skin lesion size, and IL-4, and significant increase of NO and IFN-γ were observed | Badrzadeh et al. (2020) |
| 20    | Piper marginatum (cake bush, anesi wiwiri, marigold pepper) | Leaves (ethanolic extract) | In vivo | L. amazonensis | - | - | Macedo et al. (2020) |
| Plant used | Plant part used for extract preparation | Bioactive compound involved | Mode of study and optimum dosages | Organism tested | Structural formula | Mechanism of action | References |
|------------|----------------------------------------|-----------------------------|----------------------------------|-----------------|------------------|---------------------|-------------|
| Kelussia odoratissima (kelus celery and wild celery) | Dried leaves (butanol fraction) | - | In vitro half (IC\textsubscript{50}) 264.1 and 154.1 µg/ml for promastigotes and amastigotes, respectively. | L. major | - | - | Mirzaei et al. (2020) |
| Tabernaemontana coronaria (milkwood) | Dried powder of stem bark | Voacamine | In vivo IC\textsubscript{50} value was found to be 14.702 ± 0.101 mM | L. donovani | Voacamine suppresses the relaxation potential of LdTop1B (L. donovani topoisomerase IB) and makes the cleavable complex steady | Chowdhury et al. (2017) |
| Picramnia gracilis (bitterbush) | Powder of dried leaves | 5,3′-hydroxy-7,4′-dimethoxyflavanone | In vitro and in vivo EC\textsubscript{50} 17.0 ± 2.8 mg/ml, 53.7µM for in vitro and 2 mg/kg/day for in vivo studies | L. braziliensis | - | Robledo et al. (2015) |
| Lindera aggregate (spice bush) | Leaves/bark | Boldine | In vitro 600 µg/ml | L. amazonensis | - | Salama et al. (2017) |
| Hypericum andinum | Dried and powered materials of aerial parts | Ulginosin B | In vitro (IC\textsubscript{50}) of 36.1 µg/ml | L. amazonensis | - | Dagnino et al. (2015) |
| Amphilophium crucigerum (monkey’s comb) | Aerial parts | Ipolamide | In vitro IC\textsubscript{50} = 100 µM | L. amazonensis | - | Vendruscolo et al. (2018) |

(Continued on following page)
### TABLE 1 | Role of plant-based natural products in the treatment of leishmaniasis.

| S. no | Plant used | Plant part used for extract preparation | Bioactive compound involved | Mode of study and optimum dosages | Organism tested | Structural formula | Mechanism of action | References |
|-------|------------|-----------------------------------------|----------------------------|----------------------------------|----------------|-------------------|--------------------|------------|
| 27    | Valeriana jatamansi (Indian Valerian or Tagar) | Rhizomes | Valepotriates | In vitro $IC_{50} = 2.96 \mu M$ | L. major | - | - | Glaser et al. (2015) |
| 28    | Nymphoides indica (banana plant, robust marshwort, and water snowflake) | Leaves | 3-O-methylquercetin-7-O-β-glucoside | In vitro $IC_{50} 32 \mu M$ | L. infantum | - | - | Amin et al. (2016) |
| 29    | Vitex grandiflora (black plum, chocolate and berry tree) | Air-dried leaves | Bartioside | In vitro $IC_{50} 27.51 \mu M$ | L. donovani | - | - | Bello et al. (2018) |
| 30    | Scrophularia sylvica (figworts) | Aerial parts | 6-O-a-lrhamnopyranosylcatalpol | In vitro $EC_{50} 100 \mu M$ | L. major | - | - | Alkhaldi et al. (2020) |

**Section 2: Natural drugs for treating leishmaniasis**

| S. no | Compound(s) | Company/originator | Country | Year | Mode of studies | Probable mechanism involved | Patent and IPC | Ref. |
|-------|-------------|-------------------|--------|------|----------------|-----------------------------|----------------|-----|
| 31    | Ethyl 3-(2-chloroacetamido)benzoate, dihydroquercetin, and bisabolol | Auclair et al., AC Bioscience SA | Switzerland | 2019 | In vitro and in vivo studies | Inhibition of some important parasitic enzymes with tryparedoxin peroxidase and tubulin | WO2019043212 and A61K A61P | Hajaji et al. (2018) |
| 32    | Diterpenoid membranolides | Baker et al., the University of South Florida | United States of America | 2016 | In vitro | Impedes lipid synthesis | US20160030388 and A61K | Baker et al. (2016) |
| 33    | Withaferin-A and miltefosine | Maurya et al., the University of Hyderabad | India | 2017 | In vitro and in vivo studies | Inhibits pteridine reductase-1 enzyme, and phosphatidylcholine synthesis, and cytochrome c oxidase | WO201704677Band A61K A61P | Maurya and Chandrasekaran (2017) |
synthesized using a variety of medicinally important plant species, including Mentha arvensis L., Ficus benghalensis, Cuminum cyminum, Moringa oleifera, Silybum marianum, and Sechium edule, at a dosage of 10, 300, 0.5, 246, and 51.88 μg/ml tested against L. tropica, L. donovani, L. tropica, L. major, L. tropica, and Leishmania donovani, respectively (Baranwal et al., 2018; El-Khadragy et al., 2018; Hameed et al., 2019; Ismail et al., 2019; Bagirova et al., 2020; Javed et al., 2020). Gold and silver bimetallic NPs synthesized by using medically important plants have also been reported to be used for the prevention of this disease (Alti et al., 2020). However, Cannabis sativa–based Au–NPs accomplished virtuous antileishmanial activity against amastigote forms (IC50: 171–0.0 ± 2.28 μg/ml) (Hameed et al., 2020). 7, 8-dihydroxyflavone, a type of flavonoid found abundantly in plants used to produce gold nanoparticles, has also been reported to preclude leishmaniasis (Prasanna et al., 2020).

Zno-NPs were also reported to show dose-dependent cytotoxicity against L. tropica (IC50: 8.30 μg/ml) (Iqbal et al., 2019). Rod-shaped zinc oxide NPs produced by using Lilium ledebourii tuber extract potentially inhibited the growth of L. major with the IC50 value of 0.001 mg ml⁻¹ (Khatami et al., 2020). Saleh (2019) also concluded that green TiO2 nanoparticles have shown effective roles to counter noxiousness of Leishmania tropica in male rats. Hematite (Fe₂O₃) NPs fabricated with the Rhus punjabensis extract played an efficient role in the treatment of leishmaniasis (Naz et al., 2019). Khalil et al. (2020) prepared lead oxide NPs (PbO-NPs) by green route using aqueous leaf extracts of Sageretia thea. The experimental data showed that PbO-NPs were significantly active in arresting the growth of promastigote and amastigote forms of Leishmania tropica, with 14.7 μg/ml and 11.95 μg/ml IC50 values, respectively.

Plant-mediated (Trigonella foenum-graecum) iron oxide nanoparticles have been reported to exhibit significant inhibitory effects on L. tropica (Ain et al., 2019). Further, Abbasi et al. (2019) also stated the antileishmanial efficacy of Nio-NPs fabricated by using Geranium wallichianum against L. tropica.

Besides, the nanostructured drug delivery system was also reported in ameliorate NTDs including leishmaniasis. Furthermore, crude plant extracts and precise phytoconstituents obtained from plant which is involved in the prevention mechanism were also loaded in the nanostructured drug delivery system and used as a therapeutic source to cure leishmaniasis, and the mechanism is depicted below:

- Liposome NPs consisting of phospholipids are assisted as a transport system for the delivery of hydrophilic as well as lipophilic pharmaceutical drugs (Momeni et al., 2013). They provide improved pharmacokinetic assets along with target diligence which offers a foremost advantage (Kaye and Scott, 2011). Liposome can spear the macrophages through phagocytosis and offers direct delivery of the drugs at their targeted sites. Different drug formulations including AmB colloidal formulations, liposomal AmB, and AmB lipid network can overwhelm the toxic effects of conventional drugs (Moreno et al., 2015). Liposome-encapsulated Curcuma longa and Combretum leprosum extracts were also reported for their antileishmanial properties (Aditya et al., 2012; Barros et al., 2013).
- Beta-lapachone extracted from Lapacho tree with the use of lecithin-chitosan NP encapsulation method has been reported in the treatment of leishmaniasis (Moreno et al., 2015).
- 8-hydroxyquinoline with the polymeric micelle encapsulation method has been used to treat Leishmania (Duarte et al., 2016). Berberin, an isoquinoline alkaloid extracted from medicinal plants, has been reported to possess various biologic properties, including antileishmanial properties. A previous study addressed the preparation of BER-loaded liposomes with the aim to prevent its rapid liver metabolism and improve the drug selective delivery to the infected organs in visceral leishmaniasis (VL) (Calvo et al., 2020).

As per the literature survey, plant-based nanoparticles contribute efficient roles in the treatment of leishmaniasis as compared to other existing practices. Phytosynthesized NPs revealed an identical effect on the inhibition of parasitic growth at a comparatively lesser concentration than the prescribed dose of Amp B to cure this disease. Additionally, bimetallic nanoparticles including Au–Ag, Zn–Ag, and Ti–Ag were synthesized using the green approach and proficiently used as a therapeutic source to treat leishmaniasis (Alti et al., 2020). NPs are preferred over other therapeutic sources to treat this dreaded disease because of their nontoxic, harmless, and efficient delivery system for vaccine. Currently, with the advancement of nanosciences, there is a new method of synthesizing vaccines using NPs as carriers of antigen preparation. Solid lipid nanoparticles can assist as an effective tool to produce leishmanial vaccine (Saljoughian et al., 2013). However, any kind of NP-based vaccine is not accessible, and it needs more consideration.

RESTORATIVE MECHANISM OF NANOFORMULATIONS AGAINST LEISHMANIASIS

*Leishmania* sp. are protozoal parasites which result in cutaneous and visceral leishmaniasis. Different clinical studies exhibit the development of self-curable to detrimental conditions, depending upon the immune responses triggered by the affected host (Noormehr et al., 2018). Chemotherapy with pentavalent antimonials (like sodium stiboglucone or meglumine antimoniate) and other antileishmanial drugs (amphotericin B, fluconazole, pentamidine, and miltefosine) are optimal for leishmanial therapy. However, due to adverse effects, high cost, difficult infusion routes, low cure, and increasing resistance are of significant concern in developing more efficient ways in leishmaniasis therapy. Moreover, the efficacy of the drug used in the treatment also varies for different leishmanial sp. (Noormehr et al., 2018; Alti et al., 2020; Calvo et al., 2020). In self-treatment, the innate immune cells (phagocytes) detect and engulf the causal agents, which induces *Leishmania* assassination by producing reactive oxygen species, nitric oxide, and tumor necrosis factors.
After innate immune responses, respective activation and production of CD8⁺, NK, and IFN cells by TH1 immunity results in killing of Leishmania parasites (Noormehr et al., 2018). In susceptible conditions, the defense system fails to overcome infections, and follows incorrect TH2 immune responses along with antibody response, which is the key factor to generate new ways of parasite elimination. Metal nanoparticles inhibit proliferation and viability of infected cells, which is contingent with the NP strength and time of exposure (Rosas-Hernández et al., 2009; Fanti et al., 2018).

Several in vitro as well as in vivo findings suggest leishmanicidal effects of bio–Ag-NPs by direct (exclusive of inflammatory mediators) or indirect (immunomodulatory) mechanisms (Fanti et al., 2018; Calvo et al., 2020). In the direct method, metal-NPs kill the parasitic cells by causing vacuolation inside parasites and damage to the cellular membrane without generating immunomodulatory intermediaries, that is, reactive oxygen species (ROS), nitric oxide (NO), and apoptotic and necrotic factors (Fanti et al., 2018). In situations when Leishmania parasites override the oxidative burst inside phagocytic cells and reside in phagolysosomes, nanoformulations assist site-specific delivery and accumulation of drugs, which is responsible for parasite killing (Shoaib Sarwar et al., 2020). According to Fanti et al. (2018), Ag-NPs after diffusion through the cellular membranes get oxidized due to acidic conditions within the phagolysosomes, and eventually, the release of free Ag⁺ ions causes parasite assassination.

On the other hand, the indirect method involves immunomodulatory response generation at infection sites. Other ways to provide leishmanicidal effects are through activating immune response mediators in which the cell viability and proliferation get declined as an effect of metallic nanoparticles. NPs basically induce different morphological abrasions such as distorted membranal integrity, cytotoxicity, mitochondrial destruction, cell cycle arrest (G1), increased/decreased ROS and NO generation, affected enzymatic activities, and release of apoptotic or necrotic factors (Park et al., 2010; Kruszewski et al., 2011; Zahir et al., 2015). As a result of mitochondrial disintegration, ATP generation gets influenced, which causes cytotoxic effects, and ultimately affects the infection growth (AshaRani et al., 2009). Moreover, NP exposure exhibits decreased parasitic load and reduction in an essential parasitic enzyme trypanothione reductase system (Fanti et al., 2018).

CONCLUSION AND FUTURE PROSPECTIVE

Chemotherapy due to lack of effective therapies till date has become the only choice in treating leishmaniasis, as these therapies exhibit higher toxicity levels, treatment cost, and resistance development against leishmanial parasites, and encourage other side effects. In addition, it is evident that the efficiency of drugs varies from species to species due to leishmanial antigen variants and different immunological responses against the drug. To overcome these challenges, biogenic nanomaterials being nontoxic, bio-compatible, cost effective, and having high targeted drug-loading potentials have been indicated as beneficial alternatives to formulate nanovaccines. Targeted drug delivery barriers can be conquered by using nanoformulations for enhanced parasiticidal efficiencies. Also, various studies have demonstrated leishmanicidal activities of plant-derived natural compounds (such as berberine, 7, 8-dihydroxyflavone, E-caryophyllene, essential oil constituents, α-terpineol, glycosides, tannins, and anthraquinone flavonoids), which can further integrate beneficial outcomes. Besides, most of the studies conducted on leishmanicidal activities revealed only the basic outcomes like assessment of the effect of test drugs (crude extract, isolated bioactive compounds, essential oil, and purified fraction) on the parasite growth. Few of them identify the proper formulation as well as the effect on the promastigote stage, found in the sandflies (vector). As widely conferred in the literature, plants possess a variety of bioactive compounds, and most of them have been reported for their pharmaceutical properties. Thus, the standardization may conclude the identification of particular compound responsible for leishmanicidal activities. Biosynthesized nanoparticles majorly eliminate the infection either by triggering the immunomodulatory response of the host or sometimes directly by resulting in vacuolization of parasitic cells, leading to parasite killing. Nanovaccines are a relatively new concept in treating Leishmania although no vaccine is yet available, but studies are ongoing to find efficient nanovaccines. Although nanotechnology has provided a hope toward improved and successful eradication of neglected tropical diseases, the accurate molecular mechanism responsible still needs thorough transparency to bring utmost benefits.

AUTHOR CONTRIBUTIONS

LS: writing original draft and editing; MD: writing, review, and editing; AS: conceptualization and validation; MS: supervision, conceptualization, and validation.

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SUPPLEMENTARY MATERIAL

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Saljoughian, N., Zahedifard, F., Doroud, D., Doustdari, F., Vasei, M., Papadopoulou, B., et al. (2013). Cationic Solid-Lipid Nanoparticles Are as Efficient as Electroporation in DNA Vaccination against Visceral Leishmaniasis in Mice. *Parasite Immunol.* 35, 397–408. doi:10.1111/pim.12042

Santana, R. C., Rosados, A. d. S. S., Mateus, M. H. d. S., Soares, D. C., Atella, G., Guimarães, A. C., et al. (2020). In Vitro leishmanicidal Activity of Monoterpenes Present in Two Species of Protium (Burseraceae) on Leishmania Amazonensis. *J. Ethnopharmacology* 259, 112981. doi:10.1016/j.jep.2020.112981

Shah, S., Ullah, F., Ayaz, M., Sadiq, A., Hussain, S., Ali Shah, A.-U.-H., et al. (2019). Benzoic Acid Derivatives of *Ifloga* Spicata (Forssk.) Sch.Bip. As Potential Anti-leishmanial against Leishmania Tropica. *Processes*, 7, 208. doi:10.3390/pr7040208

Shaheen, N., Qureshi, N. A., Ashraf, A., Hamid, A., Iqbal, A., and Fatima, H. (2020). In Vitro anti-leishmanial Activity of Prunus Armeniaca Fractions on Leishmania Tropica and Molecular Docking Studies. *J. Photochem. Photobiol. B: Biol.* 213, 112077. doi:10.1016/j.jphotobiol.2020.112077

Sharma, R., Gupta, V., Chawla, V., and Chawla, P. (2019). Exploring the Possible Role of Nature in Curing Leishmaniasis. *Jamps*, 20(3), 1–21. doi:10.9734/jamps/2019/v20i330114

Shoaib Sarwar, H., Varikuti, S., Farhan Sohail, M., Sarwar, M., Akhtar, S., Satoskar, A. R., et al. (2020). Oral Delivery and Enhanced Efficacy of Antimonial Drug through Macrophage-Guided Multifunctional Nanocargoes against Visceral Leishmaniasis. *Eur. J. Pharmaceutics Biopharmaceutics* 152, 307–317. doi:10.1016/j.ejpbef.2020.05.029

Silveira, E. S., Castro Rodrigues, N. L. D., Machado, N. J., Marciano Fonseca, F. R., Teixeira, M. J., and Almeida Moreira Leal, L. K. (2021). Medicinal Plants Containing Coumarin or Essential Oils from the Brazilian Biome May Be New Option for Treating Leishmaniasis. *Phrev* 14, 53–61. doi:10.5330/phrev.2020.14.9

Sundar, S., Sinha, P. K., Rai, M., Verma, D. K., Nawin, K., Alam, S., et al. (2011). Comparison of Short-Course Multidrug Treatment with Standard Therapy for Visceral Leishmaniasis in India: An Open-Label, Non-inferiority, Randomised Controlled Trial. *Lancet* 377(9764), 477–486. doi:10.1016/S0140-6736(10)62050-8

Surur, A. S., Fekadu, A., Makonnen, E., and Hadu, A. (2020). Challenges and Opportunities for Drug Discovery in Developing Countries: The Example of Cutaneous Leishmaniasis. *ACS Med. Chem. Lett.* 11, 2058–2062. doi:10.1021/acsmedchemlett.0c00446

Vendruscolo, M., das Neves, G. M., Kagami, I. P., Rodrigues Junior, L. C., Nunes Diehl, M. L., Gnoatto, S. C. B., et al. (2019). In Vitro and In Silico Activity of Iridoids against Leishmania Amazonensis. *Cddt* 16, 173–183. doi:10.2174/1570163814666171002102058

Verma, N. K., and Dey, C. S. (2004). Possible Mechanism of Miltefosine-Mediated Death of Leishmania Donovani. *AAC* 48, 3010–3015. doi:10.1128/AAC.48.8.3010-3015.2004

Zahir, A. A., Chauhan, I. S., Bagavan, A., Kamaraj, C., Elango, G., Shankar, J., et al. (2015). Green Synthesis of Silver and Titanium Dioxide Nanoparticles Using Euphorbia Prostrata Extract Shows Shift from Apoptosis to G0/G1Arrest Followed by Necrotic Cell Death in Leishmania Donovani. *Antimicrob. Agents Chemother.* 59, 4782–4799. doi:10.1128/AAC.00098-15

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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