Review Paper:

Mushroom and mushroom-derived compounds in the management of Leishmaniasis

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Abstract

Leishmaniasis is a zoonotically affected disease caused by the vector-borne parasite Leishmania and transmitted by an infected female Phlebotomine sandfly. It infects 2 million people every year causing more than 50000 deaths among the affected in nearly 100 endemic countries. Hepatosplenomegaly, musculoskeletal pain, kidney failure, chronic fever and a weakened immune system that is susceptible to some different diseases are the most prominent symptoms of the disease. Medical treatments are based on age-old antimonial and new therapies used in the treatment are the combination of drugs of liposomal amphotericin B, paromomycin and miltefosine. One of the principal challenges in these curatives is resistance, a long-term convoluted routine with a lot of disastrous side effects and high prices.

Naturally derived compounds can be one of the options in medical therapy and many anti-leishmanial compounds have been found from natural background till now with leishmanicidal properties. Mushrooms, one of the key members of nature, enriched with a plethora of bioactive molecules, play a vital role in the prevention of human diseases. To understand the progress in natural medicine, scientific reports published in ISI PubMed, Google Scholar, Scopus and Science Direct from 2006 to 2023 on mushrooms and mushroom-derived compounds having anti-leishmanial properties were summarized. In this study, we have tried to report the novel mushrooms and their potential to combat Leishmaniasis, revealing their mode of action which will be helpful in the future for the identification of bioactive molecules resourcing from mushrooms.

Keywords: Leishmaniasis, Vector-borne, Mushrooms, Bioactive Compounds.

Introduction

Leishmaniasis is a medical and public health emergency that affects 12 million people annually from approximately 100 endemic countries[15]. Every year, 900 000 to 1·3 million new cases and 20,000 to 30,000 deaths, are reported from these endemic areas. More than 50% of cases are only reported from three countries, India, Nepal and Bangladesh. Leishmaniasis has been classified as a neglected tropical disease (NTD) by the World Health Organization (WHO), highlighting the disease's significant effects on both health and society as a whole, particularly on those with severe economic burden[44]. There are mainly three types of Leishmaniasis present depending on the species variation of the parasite: *Leishmania* which is an obligate intracellular pathogen that invades phagocytic host cells and macrophages and causes fever, fatigue and weight loss to hepatomegaly and splenomegaly.

Since 1922, sodium stibogluconate has been the primary treatment option for leishmaniasis. Current medications for leishmaniasis include miltefosine, liposomal amphotericin B and glucantime but however, these drugs have a variety of side effects[43]. These drugs endanger the lives of patients due to their toxic side effects causing kidney failure, anaemia, anorexia, asthenia, erythema and urticaria. In addition, musculoskeletal pain, nausea, vomiting, diarrhea, abdominal pain and headache with lengthy regimens make the patients stop treatment. Therefore, it is necessary to search for some medicines with good therapeutic effects, fewer side effects and low cost with minimal cytotoxicity.

Natural medicines with high pharmacological activities and fewer side effects are new choices for the treatment of Leishmaniasis[30]. Nature-derived compounds have forever been an exceptional source of therapies for diseases with records, for example, quinine, broadly used for the treatment of malaria from *Cinchona pubescens*, artemisinin from *Artemisia annua*, well-established for the cure of Malaria with MDR strains, reserpine from *Rauwolfia serpentina* regularly utilized to treat hypertension, vinblastine and vincristine from *Catharanthus roseus* utilized for the improvement of anticancer medications including Paclitaxel from *Taxus brevifolia*.

Mushrooms, a significant member of the human diet due to their delicate taste, flavor and texture with a lot of health-promoting benefits, can be pharmacologically important in treating diseases due to the presence of different secondary metabolites[7]. Chinese traditional history reveals that mushrooms have been in use as an important medical strategy for the treatment of different diseases from ancient times. For example, pisosterol from *Pisolithus tinctorus* and astrakurkurone from *Astraeus hygrometricus* have been found to display anti-cancer, anti-diabetic and anti-leishmanial properties. Many compounds derived from mushrooms have been found to regulate T-cell differentiation and to promote the apoptosis of parasite cells by increasing ROS generation and pro-inflammatory

cytokine expression[3]. Therefore, this review will provide insight on the role of mushroom and mushroom-derived compounds in treating leishmaniasis.

Literature search: The literature search was made by gathering every significant manuscript present in electronic data sets like PubMed, Scopus, Google Scholar and Science Direct published from 2006 to 2023 focused on mushrooms and their anti-leishmanial property. Around 80 research works have been found focused on the impact of mushrooms on Leishmaniasis. Among them, 32 papers have been eliminated due to irrelevant content and duplication. Therefore, only 48 research papers have been studied thoroughly to identify the potential mushroom extracts, their isolated active compounds and how they worked to prevent *Leishmania* infection. Poisonous and endangered mushrooms have not been chosen in this review study.

The uniqueness of mushrooms over other natural alternatives: Humankind has forever been allured with nature and has researched natural products since ancient times[10]. Plants have been a wealthy source of bioactive constituents due to their unique and different biosynthetic pathways[6]. Recently, due to the ever-evolving nature of the diseases, exploration for curatives led to a more structured and target-specific and activity-directed chemical approach toward drugs. Although this approach sounded advantageous, the targeted synthetic drugs frequently affect the non-targeted zone of the physiological system, directly or indirectly, giving rise to several adverse side effects some of which offer newer symptoms and newer complications. Bioprospecting for new alternatives from natural background has increased global demand for medicinal plants which has caused the extinction of certain species, contributing to biodiversity loss and reduction of natural resources that are important for humanity.

Large-sized medicinal plants also take a longer time to get their flowering season and their cultivation is troubleshooting. Certain or little changes in soil pH, soil bacterial population, rainfall and other biotic factors hinder the production of active secondary metabolites in these plants. So, despite having a high number of secondary metabolites, plants are not the best choice because overexploitation of these plants is a driving factor in biodiversity loss and due to changes in biotic and abiotic conditions, the probability of getting desired metabolites is downscale. Hence, research of natural sources for healing present-day diseases has guided researchers to find bioactive compounds present in mushrooms[27]. Mushrooms represent a relatively untapped bioresource for novel natural drug discovery despite having different secondary metabolites.

Mushrooms, filamentous fungi with fruiting bodies, show a huge number of pharmacological and nutraceutical properties with low-fat content and unsaturated fatty acids along with high fiber content, triterpenes, phenolic compounds, sterols, eritadenine and chitosan. These secondary metabolites are bioactive and low molecular weight compounds that are produced in response to stress that support its survival but are not generally required by the fungi for their typical growth and reproduction. It is economical, rich in pharmacological properties, easy to cultivate, requires low finances and area and can be grown all over the world [5]. Mushrooms have several reports of having anti-cancer, anti-viral, anti-diabetic and anti-parasitic properties. So, we can consider the evolving role of mushrooms in global healthcare as a new alternative to human health medication.

Relevance of mushrooms in leishmaniasis: According to the study, there are 1,50,000-1,60,000 mushroom species available in nature of which only 10% have been explored. More than 700 edible pharmacologically active mushroom species are present [43]. Due to profound health benefits, mushroom consumption around the world in the last 50 years has increased up to 30% and they are not only used as food sources but also as supplements and medicinal sources. Mushrooms have been found to have the potential as anti-cancer, anti-diabetic, anti-parasitic, anti-inflammatory and immunomodulatory properties along with managing good metabolism[40]. Covid-19 can be treated with a few mushrooms according to several researchers. For effective antiparasitic applicants, mushrooms and mushroom-derived products are good choices for researchers due to minimal expense and fewer side effects.

Natural products literature states that a wide variety of mushroom extracts are in service for the treatment of parasitic diseases like Chagas disease, malaria and many complicated diseases. There are tons of mushrooms containing medicinal properties and are traditionally used in natural medication[24]. Mushrooms, for instance, *Agaricus, Calocybe, Cantharellus, Cordyceps, Coprinus, Cortinarius, Ganoderma, Grifola, Huitlacoche, Hydnum, Lentinus, Morchella, Pleurotus, Rigidoporus, Tremella, Trametes* sp, *Termitomyces sp, Rusulla sp, Volvaria volvacia, Laccaria laccata, Tricholoma crassum* etc. play an important role in protection against several diseases due to the presence of various metabolic constituents with biological activities like anti-oxidant and anti-inflammatory properties[31].

These days around the world mushroom extracts, mushroom tea[47] and mushroom cocktails are one of the sharp subjects of perception and exploration, particularly in the medication disclosure and nourishment field. The objective of this study is to offer thorough direction about mushrooms reported in treating leishmaniasis. Fig. 1 shows the mechanism of anti-leishmanial activity by mushrooms in a nutshell.

Pathogenesis of leishmaniasis and its complication: Leishmaniasis shows a spectrum of clinical manifestations like long-term fever, hepatosplenomegaly, weight loss, pancytopenia and hypergammaglobulinemia. This protozoan is known to cause chronic illnesses and infections by successfully evading, invading, altering the host's defense

mechanisms and modulating the host's metabolic processes for their survival[45]. For a human organism to function normally, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are essential because they help the host to show quick and effective response against parasites by making intracellular terrain vulnerable to parasite growth.

During host-pathogen interaction, several biochemical signaling processes get activated which synthesize several effector molecules like cytokines that are of two kinds- pro and anti-inflammatory cytokines and the balance between two cytokines finally decides either the host survival or parasite progression in the host cell[25]. Numerous researches on visceral leishmaniasis demonstrate that the host's anti-leishmanial mechanism is linked to a Th1-type immune response because Th1 cytokines stimulate the expression of iNOS and the subsequent generation of NO, which in turn causes macrophages to destroy the parasites[2].

On the contrary, progression of the disease is thought to be co-related with Th2 type cytokines which cause induction of host ARG that results in the formation of L-ornithine and polyamine which are crucial for the growth and differentiation of parasite cells.

Another intermediate, spermidine generated in the L-arginine pathway, combines with glutathione to form trypanothione [T(SH)2] which is crucial for maintaining thiol redox equilibrium, synthesizing deoxyribonucleic acids, developing drug resistance and protecting against chemical oxidative stress in parasites. Successful parasite invasion shows a range of clinical manifestations in Leishmaniasis which is crucially related to species of *Leishmania* parasite and the host's immune system response[15]. Fever, anorexia, weight loss, abdominal distension and weakness are symptoms of leishmaniasis that can appear between a week and a month after infection. Leishmaniasis is typically identified by the increase of mononuclear phagocytic cells within the invaded organs and subsequent hyperplasia of the reticuloendothelial cells, which are clinically present as non-tender splenomegaly, hepatomegaly and pallor.

Within two to three years, untreated symptomatic VL will lead to death due to severe multi-organ illness, haemorrhagic diathesis, low platelets and secondary infections[18]. Since ARG is the first enzyme in the polyamine pathway, therefore inhibiting it, can lower T(SH)2 levels, which affects the parasite's redox system and ultimately causes the parasite to die. On the other hand, due to reduced ARG activity, SOD levels fall which ultimately reduce the parasite's ability to fight off ROS and RNS. As a result, ARG activity is fundamental to understand the pathophysiology of leishmaniasis and its consequences.

The potential of mushrooms with antileishmanial properties: Around the world, mushrooms are not synthetic, they are herbal drugs. There is a large diversity of bioactive compounds tracked in mushrooms. They are well known by the name of secondary metabolites which are produced by them during various stress conditions. Secondary metabolites belong to several categories but among them, polysaccharides, terpenoids, sterols and alkaloids are predominant in most mushroom species[34]. 50 types of polysaccharides, 30 kinds of terpenoids and different sterol compounds are reported to be present in mushrooms[17]. Sesquiterpenes hypnophilin and panepoxydone isolated from the ethyl acetate extracts of the mushroom *Lentinus strigosus*, have been shown to have anti-leishmanial activity against *Leishmania major*, *L. infantum*, *L. donovani*, *Trypanosoma brucei*, *T. cruzi* and *T. gondii*.

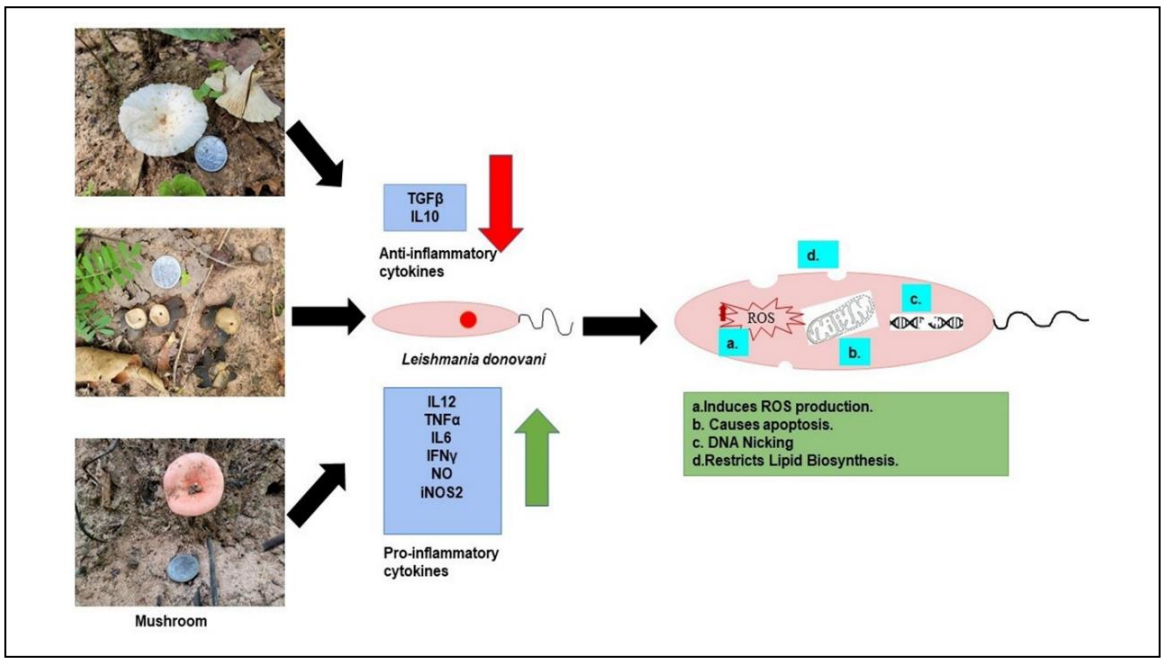

Fig. 1: The mechanism of anti-leishmanial activity by mushrooms in a nutshell.

There are many steroidal chemicals present in mushrooms. Ergosterol isolated from *Pleurotus salmoneostramineus* and *Pleurotus ostreatus* has been reported to have an anti-parasitic property[42]. A significant member of the class of secondary metabolites called alkaloids[46], is found in significant levels in many mushrooms. 6-hydroxyindole-3-carbaldehyde and 6-hydroxyindole-3-acetamide isolated from *Agrocybe cylindracea* exhibit free radical-scavenging properties that may aid in the treatment of the parasitic disease leishmaniasis. The frequently investigated mushrooms include *Astraeus hygrometricus, Agaricus blazei, Agrocybe aegerita, Flammulina velutipes, Grifola frondosa, Lentinus strigosus, Merulius incarnatus, Morchella importuna and Russula sp.*

All these mushrooms have been found to have anti-leishmanial properties by inducing apoptosis via increasing ROS, accumulating lipids in promastigotes, decreasing protein contents and inducing loss of Ψ_m causing oxidative damage. These mushrooms also target chemokine networks of parasites and prevent their progression and growth[12].

Biological mechanism of mushrooms on anti-leishmanial effects

1. Anti-Proliferative action: The presence of a high load of parasites in the infected area is the classical manifestation of the disease leishmaniasis. Reducing parasite load is one of the measures for anti-leishmanial treatment. All studied mushrooms showed a significant dose-dependent decrease of promastigotes and intracellular amastigotes replication in *L. donovani*-infected macrophages in comparison to the PBS-treated infected control cells at 48 hours. Among all the studied mushrooms, *Astraeus hygrometricus* is the most potent one with good anti-leishmanial potential[13]. 80% ethanol extract of *Astraeus hygrometricus* at a concentration of 250 µg/mL[20], made 16.80% promastigotes non-viable but in the case of amastigotes, water-soluble fraction of *Astraeus hygrometricus* was most effective at a concentration of 90.9 µg/mL.

Further studies on *Astraeus hygrometricus* led to the isolation of specific compounds responsible for anti-leishmanial properties[16]. The methanolic extract of powdered basidiocarps of the mushroom was subjected to silica-gel column chromatography with a hexane/AcOEt gradient followed by crystallization that ultimately yielded two pure compounds, astrakurkurone and astrakukurol. Further studies reported that astrakurkurone[20] was effective against *Leishmania donovani* promastigotes culture at a concentration of 10.0 µg/mL. It showed *in vitro* time-dependent growth inhibition of up to 95% within 144 hours.

Astrakukurone[21] has been found to inhibit the replication of *L donovani* AG83 promastigotes at a concentration of 37.5 µg/mL than reference drug sodium stibogluconate and amastigotes up to 50% at a concentration of 2.5µg/mL. Astrakurkurone at a concentration of 37.5 µg/mL showed better inhibition of *L donovani* AG83 promastigotes than reference drug sodium stibogluconate. Another mushroom *Agaricus blazei*,[39] second highest in the number of reports having anti-leishmanial properties, presented similar activity to amphotericin B at a concentration of 200 µg/mL. The inhibitory concentration of water extract of *Agaricus blazei* [40] was 67.5, 65.8 and 56.8 µg/mL for *L. amazonensis, L. chagasi* and *L. major* promastigotes respectively whereas the IC_{50} values for amastigote-like stages were little higher than promastigotes with 115.4,112.3 and 108.4 µg/mL for *L. amazonensis, L. chagasi* and *L. major* respectively. A detailed *in vivo* study revealed that oral administration of *Agaricus blazei* water extract at a concentration of 100 mg/kg/day, in BALB/c mice infected BALB/c mice with *L. amazonensis* reduced the footpad swelling infection within 20 days[42].

The effectivity of *Agaricus blazei* water extract [28] was comparable to AmpB therapy because both of them were able to reduce parasite burden in the spleen to a nearly similar extent. Both *in vivo* and *in vitro* studies of *Agaricus blazei* presented a good result having potent leishmanicidal properties. Purification of water extract through the amicon column had given 5 fractions, among them 2 fractions had the best activity against promastigotes of *L donovani* with IC_{50} values of 15.8 ± 1.2 and 13.0 ± 1.3 µg/mL respectively. Further studies on *Agaricus blazei* showed that polysaccharide-rich fractions of *Agaricus blazei* with recombinant antigen, LiHyp1 in association with saponin had presented a reduction in parasite load in the spleen in BALB/c mice.

Mushrooms are reservoirs of high amounts of polysaccharides. Fucogalactan, a heteropolysaccharide, isolated from the polysaccharide fraction of the medicinal mushroom *Agrocybe aegerita*[23] reduced parasite load at a concentration of 5.82 ± 0.57 µM. *Lentinus strigosus*, a subtropical mushroom has a wide range of therapeutic uses for treating a variety of ailments including bronchial inflammation, cancer, heart illness, infectious disease and parasitic infections. Terpenoids: hypnophilin and panepoxydone identified from this mushroom, in combination, presented typical dose-dependent inhibition of amastigotes of *Leishmania amazonensis* at a concentration of 10 µg/mL. At 1.25 µg/mL concentration, both hypnophilin and panepoxydone showed significant inhibition of amastigote cells without affecting normal PBMC cells[37].

Grifola frondosa, commonly known by the name of Hen of Woods, reduced the lifespan of *L. donovani* promastigotes, with an inhibitory concentration (IC_{50}) of 20.37 µg/mL[38]. It also provided efficacy against two additional *Leishmania* strains, L. *major* LV39 (MRHO/Sv/59/P strain) and *L. tropica* WR683 (MHOM/ SU/58/OD) with IC_{50} values of 46.08 µg/mL and 53.79 µg/mL respectively. Coral Pink Mushroom, *Merulius incarnatus*[14] high in polyphenolic chemicals, has been reported to contain polyphenol, 5-heptadeca-8'Z,11'Z,16-trienylresorcinol which reduced

parasite survival at IC_{50} concentration of 3.6 µg/mL without causing cytotoxicity in our normal cells.

Among the edible wild mushrooms, *Russula*, one of the largest genera of ectomycorrhizal mushrooms in the Rusullaceae family, is an economically and medicinally beneficial mushroom. In a dose-dependent manner, the water-soluble polysaccharide fraction and the polyphenolic fraction of *Russula laurocerasi*, *Russula albonigra* and *Russula delica* [19] inhibited the replication of intracellular amastigotes of *Leishmania donovani* in macrophages. The highly valued morel mushroom, or *Morchella importuna*, offers several health-enhancing qualities[29]. From the study, here we can conclude that the *Leishmania* parasite was most effectively killed by the mushroom's bioactive compound, sesquiterpenoid astrakurkurone which prevents the parasite's growth and replication at significantly low concentration. Other bioactive compounds like hypnophilin and panepoxydone and 5-heptadeca-8'Z,11'Z and 16-trienylresorcinol have actively taken part in showing good activity against *Leishmania* parasites. Figure 2 shows the structure of active compounds having anti-leishmanial potential.

2. Induction of Oxidative Stress: As soon as a sand fly bite occurs to a healthy host cell, neutrophils are the first line of defense cells to be contacted. By creating NETs, or neutrophil extracellular traps, or by inducing a powerful oxidative burst, it helps to kill the parasites that are invading the body. But in case of the successful onset of Leishmaniasis, parasites use the Trojan rabbit strategy to get away from apoptotic neutrophils and infect the macrophages[24].

Leishmania surface components such as LPG, proteophosphoglycan (PDF), glycosyl inositol phospholipids (GIPLs) and glycoprotein gp63 have troublemaking effects on the host by regulating the signaling pathways and changing the expression of numerous cytokines which promote the growth of parasites inside the infected macrophage's phagolysosome, that induces disease progression. In macrophages, two enzymes inducible nitric oxide synthase enzyme and arginase enzyme play pivotal roles in disease progression and disease elimination. The growth of *Leishmania* in macrophages is aided by the host's arginase activity in the conversion of L-arginine to ornithine and urea for polyamine biosynthesis which facilitates parasite growth and differentiation.

Another crucial enzyme is inducible nitric oxide synthase, responsible for NO production which helps in the elimination of invasive pathogens by generating a high amount of ROS and RNS and protects the host immune system against outside invaders[31]. One of the most important mechanisms in killing parasites by drugs available in the market is the robust elevation of ROS in the territory of the parasite causing lipid peroxidation of the parasite cell membrane and acting as a mediator of inflammation. Mushroom extracts have been found to elevate oxidative stress as one of the major paths in *Leishmania* parasite killing.

The carbohydrate fraction of *Astraeus hygrometricus* [13] has been found to restore host defense machinery by promoting iNOS2 mRNA expression and NO production. It also induced ROS generation in macrophages leading to the activation of several apoptotic signaling pathways or cell death.

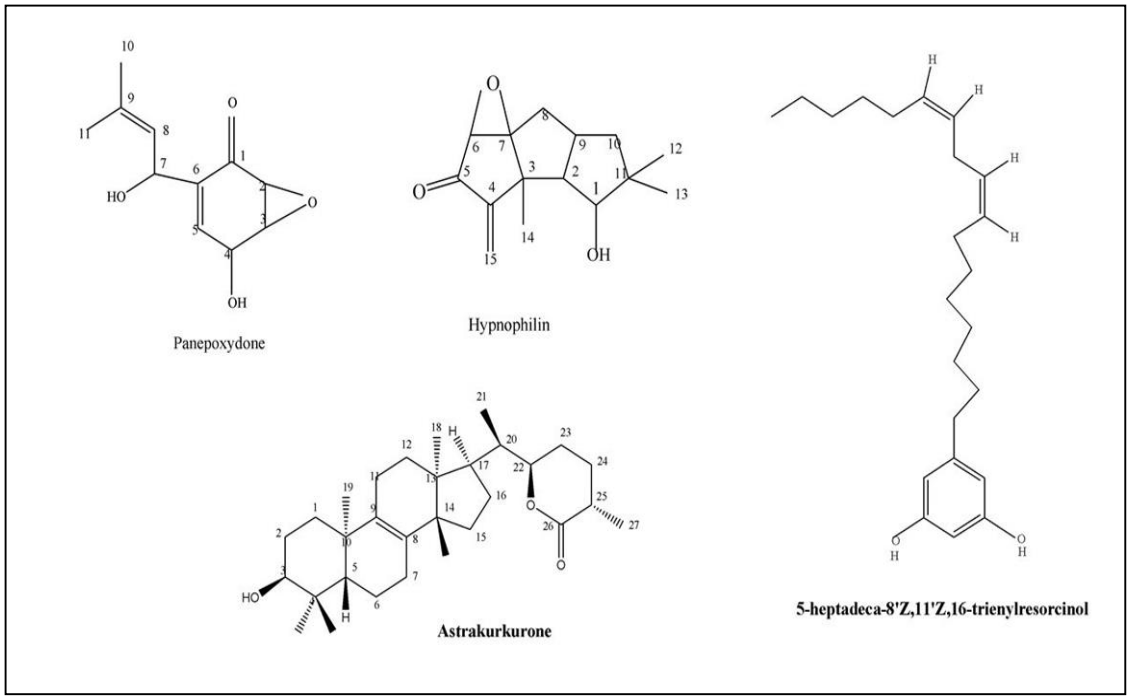

Fig. 2: The structure of active compounds having anti-leishmanial potential.

Thin layer chromatography, column chromatography, HPLC and 1H and 13C-NMR studies have proved the presence of two active terpenoids astrakurkuone and astrakurkurol[19] in *Astraeus hygrometricus* and among them, astrakurkurone had strong antileishmanial property. Astrakurkurone at a concentration of 2.5µg/mL prevented replication of promastigotes by upregulating iNOS2 expression and in the case of amastigotes, it induced NO production in macrophages which ultimately led to apoptosis.

H2DCFDA study and PI-hoechst study in promastigotes have exhibited that apoptosis and oxidative stress are interrelated in killing promastigotes by astrakurkurone at 50% inhibitory concentration of 37.5 µg/mL and in the presence of free radical scavengers NAC and GSH, promastigotes' growth continued[16]. Orally administrated *Agaricus blazei* water extract also shows leishmanicidal properties and its killing efficacy by elevating NO level[39]. But another report on *Agaricus blazei* water extract, at a concentration of 50µg/mL demonstrated no increase in NO level and no significant rise in iNOS expression in Western Blotting[40]. Therefore, oxidative stress is not the only pathway of parasite death by particular extract. Inhibition of arginase enzyme activity is one of the pathways to kill *Leishmania* parasites.

Polysaccharide fraction of macrofungi *Agrocybe aegerita* and its purification established the presence of Fucogalactan which was able to prevent parasite growth by inhibiting Arginase activity. It was also able to stimulate host cell responses by elevating NO production and iNOS expression.

So, the mushroom *Astraeus hygrometricus* is a potent mushroom that has good antiproliferative properties causing oxidative stress-mediated death in the *leishmania* parasite which is an important survival mechanism of host cells against the parasite. Fig. 3 shows the mechanism of induction of oxidative stress by mushroom derived bioactive compounds.

3. Immunomodulatory Action: Cells of the innate and adaptive immune systems are always involved in maintaining host survival. Non-specific immune cells including macrophages, basophils, eosinophils, neutrophils, mast cells and monocytes react immediately as soon as infection occurs. Adaptive immune cells, such as B cells and T cells, enter as the second line of defence after the initial line of defence and assist the innate immune system in eradicating external factors and creating a memory of the pathogen to prevent the reoccurrence of the disease[11]. B cells are responsible for secreting specific Y-shaped antigen-specific antibodies whereas T cells kill the extruders by activating its two subpopulations- TH (T Helper Cells) and TC Cells (T Cytotoxic Cells).

T helper cells work by activating several effector molecules like cytokines and T cytotoxic cells remove the affected cells by cytotoxic T lymphocytes[2]. Among the most important players in the immune system, macrophages are captains, responsible for killing intruders in assistance with dendritic cells. The complement system is another member which plays an important role in the eradication of parasites from host cells. In complement cascade, they follow three different pathways: Classical Complement Pathway, Alternative Complement pathway and Lectin Pathway depending on the cell wall component of an external organism.

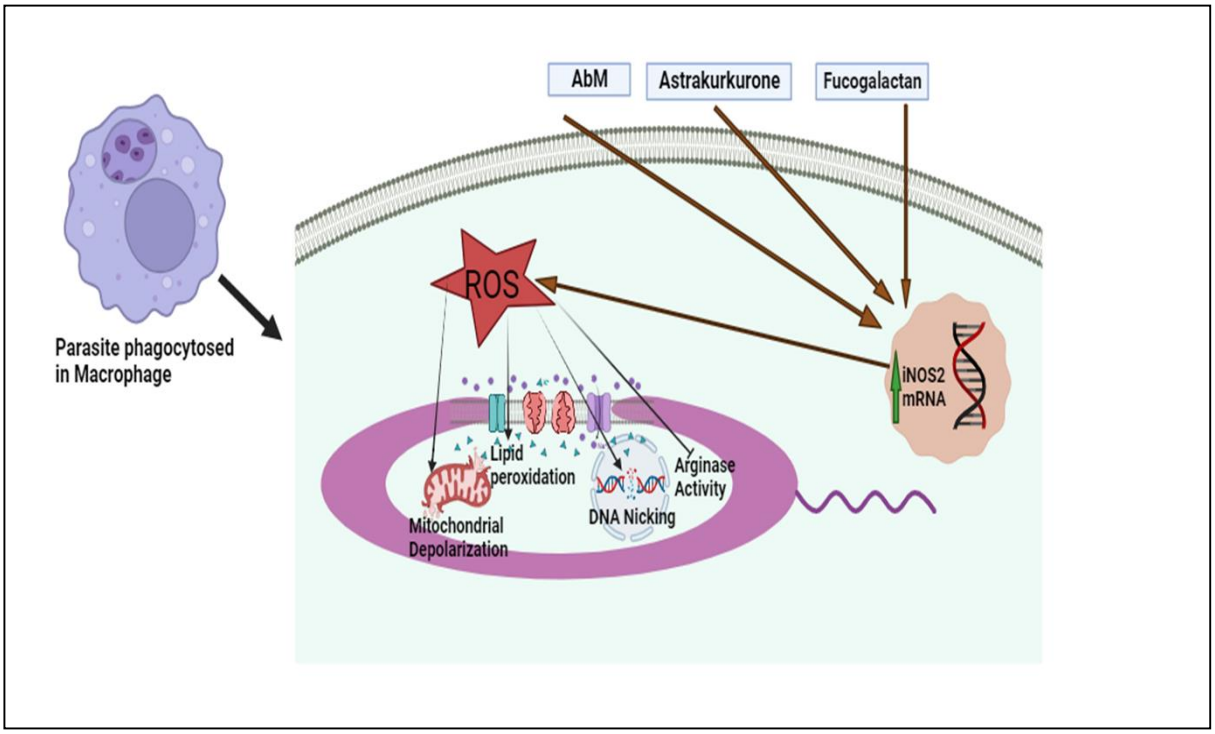

Fig. 3: The mechanism of induction of oxidative stress by mushroom-derived bioactive compounds.

But in *Leishmania* infection, first, they target the complement pathways by engaging different cell surface receptors including complement receptor, fibronectin receptor, TLR 2, 3 and 4 and mannose receptor and enter into the host cells. They recruit membrane protease Gp63 which cleaves C3b of the complement cascade and converts it to inactive C3bi facilitating the binding to CR3 receptor and mediating entry of promastigotes into macrophages. This strategy protects the parasites from lysis [22]. After engulfment by macrophages, promastigotes convert into amastigotes and block the anti-microbial cascade, preventing the activity of T Helper cells which are responsible for producing effector molecules like interleukin 12 (IL12).

IL12 is important in defense because it helps in creating oxidative stress via upregulation of iNOS2 mRNA expression and increased NO production. IL12 also induces IFNγ expression and CD4+ T cells, responsible for parasite elimination. *Leishmania* parasites in one way block the expression of IL12 but on the other hand, they promote the expression of IL10 and TGFβ favouring the parasite survival and disease promotion [26].

In the last few decades, experts from all over the world have become increasingly interested in mushrooms because of their exceptional immune-modulating qualities and variety of resources and structures. One of the most significant biological aspects of mushrooms is immunomodulatory action which is well known as a biological response modifier (BRM). Through interactions with immune cells such as dendritic cells (DCs), macrophages and NK cells, mushrooms and mushroom-derived compounds can activate intracellular cascade signaling and trigger immunological responses. A variety of immune cell receptors including dectin-1 and toll-like receptors (TLRs), are proposed to be involved in mushroom-induced immune responses including immune modulation and antileishmanial activity.

TLRs are pathogen recognition receptors that get activated by PAMP (Pathogen Associated Molecular Pattern) and induce the immune cells, specific effector molecules like interleukins and NO. In the successful establishment of leishmaniasis[36], TLR-2, TLR-7 and TLR-9, crucial for providing immunity to *L. donovani*-infected hosts, have been taken over by parasites and cause disease pathogenicity. The carbohydrate fraction of *Astraeus hygrometricus* has been found to have the potential to regain the expression of TLR2, TLR7 and TLR9 in infected cells which ultimately provide immunity to the cells by inducing pro-inflammatory cytokines IL-6, IL-1β, TNF-α, IL-12 and IFN-γ and enhancing Ly6C+ cells, immediate precursors of competent myeloid progenitor cells that differentiate into Ly6C+ inflammatory monocytes and dendritic cells.

It downregulates anti-inflammatory cytokines IL-10 and TGF-β[8]. This balance between pro and anti-inflammatory cytokines ultimately directs the infected cells towards a Th1-based response which stabilizes the anti-parasitic environment around the infection[9]. On the contrary, astrakurkurone, isolated from *Astraeus hygrometricus* induced amastigote killing by expressing TLR9 in both infected and uninfected macrophages but was unable to induce TLR2 and TLR7[21]. However, this compound was more active and specific towards reducing parasite burden and inducing Th1-mediated anti-amastigote activity. Water soluble polysaccharide extract of *Rusulla laurocerasi* and polyphenolic extract of *Rusulla albonigra* produced a significant amount of IL12 in infected macrophages to kill the intracellular amastigotes[19].

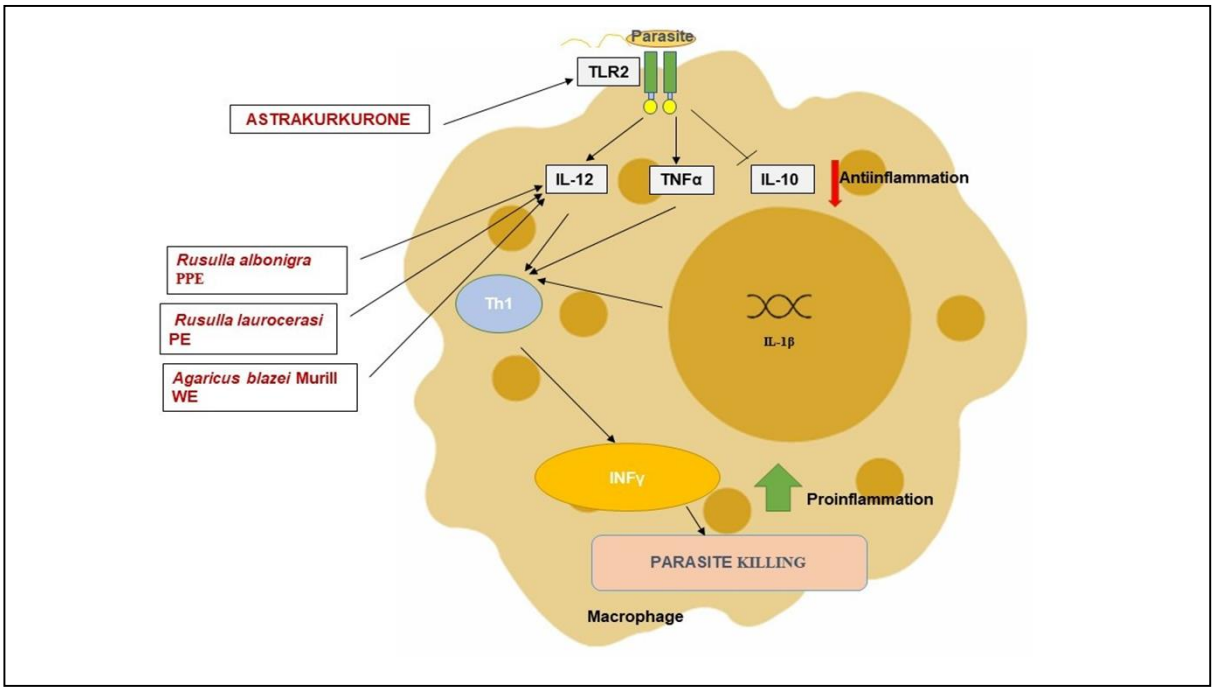

Fig. 4: Mechanism of immunomodulation by mushroom-derived bioactive compounds.

Agaricus blazei Murill, used in folk medicine, has the potential to modulate host immune response significantly. Water extract of *Agaricus blazei* Murill and rLiHyp1protein with *Agaricus blazei* Murill fraction was able to generate strong immunomodulation than control drug amphotericin B by producing a higher level of IFNγ and lower level of IL-4 and IL-10 leading towards Th1 immune response[28,41]. *In vivo* study in BALB/c mice with *Agaricus blazei* Murill water extract has shown its efficacy in the induction of pro-inflammatory cytokines and IgG2a-specific antibodies suggesting the dominance of Th1-specific immune response than the control mice treated with Amphotericin B. Fig. 4 presents mechanism of immunomodulation by mushroom derived bioactive compounds.

However, mushrooms reported with anti-leishmanial properties are more or less directly associated with immunomodulation. It has been proved that Th1-specific immune response is always responsible for the nitric oxide synthase metabolic pathway which stimulates the production of ROS and NO causing oxidative stress in parasites. Astrakurkurone and *Agaricus blazei* water extract take part actively in immunomodulation in *Leishmania* infection Therefore, there is a good possibility that astrakurkurone will prove to be a highly effective medication substitute for the treatment of leishmaniasis.

Conclusion

Leishmaniasis, one of the oldest neglected diseases, affects millions of patients around the world, particularly in the Middle East and Central Asia[32]. It should receive more time and attention from researchers worldwide since it poses a severe threat to public health. Along with this, medications available today for leishmaniasis have significant drawbacks including multidrug resistance[33]. The new issue to be resolved in the fight against any sort of parasite disease is multiple drug resistance. Therefore, it has become a hot research area for the development of novel options due to the lack of access to proper therapy and drug resistance globally.

Almost 90% of therapeutic and semisynthetic drugs yet discovered have come from natural resources, therefore, bioactive constituents from mushrooms can add vast resources to the repertoire of modern-day therapeutics. This review has highlighted the wide range of mushrooms and their secondary metabolites, a significant component of nature with the potential of being used in the production of antileishmanial medications. Carbohydrate fraction and an ethanolic fraction of *Astraeus hygrometricus* have potent anti-leishmanial activity which led to the isolation of pure sesquiterpenoid compound astrakurkurone which induced mitochondria-mediated apoptosis and caused oxidative stress in *Leishmania* parasites with good IC_{50} values ranging between 50-100 ug/mL. It has also the immunomodulatory potential of expressing Th1-specific anti-parasitic responses. Another mushroom *Agaricus blazei* has the second highest number of reports of having anti-leishmanial properties with its water extract which shows better activity than the control drug amphotericin B. More research should be done to isolate the particular compound responsible for the anti-leishmanial property of *Agaricus blazei*. From the cumulative study of different extracts, it has been found that arginase, an important enzyme required for the survival of parasites within macrophages, is a less explored target for anti-leishmanial drug development. It is significant to note that among the different types of secondary metabolites, most of the reports were from triterpenes and sesquiterpenes and over dominant genera are *Astraeus*, *Agaricus*, *Lentinus* and *Russula*. Among them, *Astraeus* is mostly and widely consumed by the Santal community of West Bengal, Jharkhand, and Chattishgarh. Thus, further studies including chemical trials for effective bioactive compounds of mushrooms especially for *Astraeus hygrometricus* should be done against the parasites of leishmaniasis. This study can therefore help any researcher to choose mushrooms with particular secondary metabolites that have leishmanicidal capabilities and aid in further research for developing effective therapies against the disease.

Future perspective

Edible mushrooms are a promising and relatively untapped source of material with potential medicinal applications. Mushrooms and their biologically active substances can in the future be promising starting materials for the pharmaceutical industry in the treatment of various diseases with as little toxicity as possible, unlike the drugs available today, which have serious side effects. Therefore, we should make concerted efforts in the research and development of mushroom cultivation and the bioactivity-guided isolation of compounds from potent mushrooms. To date, most studies have been conducted in animals or cell culture models and there are very few experimental studies in humans. Therefore, in different animal models, significant studies should be performed to support the *in-vitro* studies to develop a potent anti-leishmanial alternative.

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