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Review Article

ROLE OF NANOEMULSION FOR SAFE AND COST-EFFECTIVE DELIVERY OF AMPHOTERICIN B

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ABSTRACT

Amphotericin B is a highly effective antifungal agent and its use has been surged drastically due to its effectiveness against a wide range of fungal infections. It is effective against both topical and systemic infections and even this is the only drug having high efficacy against Leishmania, Candida, Aspergillus and many more. Owing to this, the demand of Amphotericin B is gaining momentum. However, this drug possesses numerous drawbacks like toxicity, poor solubility to name a few, due to which its current use is dwindling. So far the numbers of formulations have been marketed in order to triumph over its toxicity and solubility related issues. Unfortunately, no single study conducted hitherto gain success in overcoming its lethal ramifications. Among all formulations, nanoemulsions are at the top to combat all issues related to this drug. Nanoemulsion has shown enhanced stability, reduced cost and toxicity. This review will assimilate complete information on the use of nanoemulsion based formulations of Amphotericin B, its developed formulations and also will enunciate the important considerations and future perspectives. The complete data have been composed from Google Scholar, ScienceDirect and PubMed using the following keywords.

Keywords: Toxicity, Stability, Nanoemulsion, Amphotericin B, Fungal infections

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INTRODUCTION

Fungal infection is an increasing cause of concern in today's era as a wide population is affected by it. More than a billion people of the world are suffering from a diversity of fungal infections. Leading international fungal education (LIFE) portal states that more than 80% of the human population are at the risk of fungal infections [1]. There can be two major categories of fungal infections affecting the human body i.e., Topical and systemic. The topical fungal infections are highly prevalent in the tropic and sub-tropic countries that are hot and humid. The topical infection mainly affects the skin, hair, nails and scalp. The second category i.e., Systemic fungal infection, is more fatal as it is the main cause for the increased rate of mortality and morbidity across the world. The systemic infections are widely prevalent in the countries like India, Africa and Brazil. Thus, for treating fungal infections variety of antifungal agents are developed to exhibit excellent therapeutic activity towards diseases like Aspergillosis, Candidiasis, Blastomycosis, Coccidioidomycosis, etc. [2].

For the past 30 y, Amphotericin B is considered as the choice of drug for the therapeutic treatment of severe systemic fungal diseases such as systemic candidiasis caused by yeast, overpowering the other newly synthesized antifungal agents. Another area associated with the use of Amphotericin B is in the effective treatment of visceral leishmaniasis, commonly known as kala-azar. So far, Amphotericin B is the only agent that has proven to be effective against visceral leishmaniasis. Amphotericin B can be administered into the body through different routes for procuring the desired results in treating the fungal infections. Topical and intravenous administrations are the major routes associated with the delivery of Amphotericin B into the body. In the case of topical fungal infections, topical delivery of the drug is the most efficient route for treatment. Topical application is preferred as the drug is in direct contact with the infected part of the body and easily penetrates into the stratum corneum of the skin, which shows fungicidal or the fungi-static activity as the desired cure for the infection [3]. Various marketed formulations available for topical treatment include Fungizone cream, Fungizone lotion, Fungizone ointment and Fungisome liposomal gel for the topical administration of amphotericin B.

Nowadays, for the safe and effective delivery of the Amphotericin B topical route is highly preferred in the treatment of fungal infections located on the local surface. As discussed above, an Amphotericin B gel (Fungisome) and a lotion (Fungizone) is available in the market for treating topically different fungal infections such as skin infections, fungemia, cutaneous leishmaniasis, etc. But there are some confines for these formulations that include very less skin permeability and the clinical efficacy limitation. However, in the case of systemic fungal infections and the visceral leishmaniasis, the preferred route of administration is parenteral. Parenteral delivery is preferred as the drug directly reaches the systemic circulation and kills the fungal agents or inhibits its growth. Different formulations like Ambiosome, Phosome, Abelcet, Amphocil, Anolip to name a few, have been marketed for parenteral administration of the Amphotericin B. These formulations suffer from the drawback of having high toxicity and high cost. Firstly, the drug itself has high nephrotoxicity and secondly, incorporation of high content of surfactants to develop a formulation further boosted up its toxicity issues. The use of surfactants is required to form the suitable formulation, which is the main hurdle due to the physiochemical properties of drug molecules.

Physiochemical properties of 'Amphotericin B'

The drug possesses high molecular weight and bulky structure, which are the main reasons that affect the formulation development. The structure of the molecule consists of a hydroxyl rich hydrophilic chain and a hydrophobic heptane chain, which shows decreased solubility effects in hydrophobic as well as aqueous systems resulting in decreased skin permeation effects. The drug is amphiphilic in nature, which is the foremost hurdle to be overcome in designing of an effective delivery system. Consequently, the need for modifying the existing dosage form is necessary to formulate a novel delivery system [4].

Evolution in amphotericin b delivery systems

Amphotericin B is amphiphilic in nature due to which the formulation development of the drug offers a number of hindrances. As the hydrophobicity of Amphotericin B is very high and the conventional parenteral formulation uses a very high concentration of sodium deoxycholate as a solublizer i.e., 65% w/w in the formulation, which offers some pitfalls as an intravenous drug delivery such as nephrotoxicity, anemia, cardiotoxicity, hypotension, anorexia, dyspnea, fever, nausea, chills, vomiting, pain at the injection site, electrolyte abnormalities and hepatotoxicity. Fungizone is the licensed formulation available in the market, which consists of the surfactant with Amphotericin B, phosphate buffer and sodium deoxycholate. As only parenteral administration of this drug is effective for the treatment of systemic infections due to its low bioavailability and solubility, the scientists emerged with the need to formulate such a dosage form. In the

succeeding years, various lipid-based formulations were developed in the form of colloidal dispersions or lipid complexes. Though these formulations claimed for the decreased toxicity than the previous formulation still were found to exhibit adverse effects over the phagocytes rich organs. Finally, in the year of 1998, a liposome based formulation also known by the name Ambisome was developed. This was claimed to be a better approach than the previous formulations because they added lipid bilayer stability, drug being entrapped for an increased time period, highly targeted, exterminate toxicity and reduced side effects. The increase in the administered dosage increases the adverse effects related to the drug; thus, the amount of the drug to be administered in the body must be optimized for its safe, efficacious and effective use.

Therefore, the most desirable modification was performed a decade ago on Amphotericin B where the physical characteristics of the drug were altered, which finally depicted enhanced efficacy when compared to Fungizone. From that point of time the emphasis was highly shifted towards the development of various lipoidal formulations with effective therapeutic activity in combination with the declined toxicity effects. The methods to achieve the desired results deal with the alteration of the drug molecule and its delivering models. The delivery of Amphotericin B has been further modified by combining it with other agents and determine its synergistic effects and interactions. Amphotericin B has also been administered as an aerosol preparation through the intranasal route for drug delivery [5].

Ehrlich gave the concept of "magic bullet" in drug delivery, which is the term he coined for medicine. According to the previously defined ideal characteristics of a dosage form, a delivery system should act in such a manner so as to target its desired site and show the maximum therapeutic effect and minimum adverse effects. Therefore, the researchers aimed at developing such a dosage form of amphotericin B that will successfully target the specified area and act on it with the least side effects. In lieu of this, Nanoemulsions of Amphotericin B were developed for topical and systemic delivery that have been altered to such an extent that they exhibit the minimum side effects, decreased toxicity, enhanced stability, safety and enhanced targeting therapeutic action [6]. Among all formulations developed so far, Nanoemulsions were only carriers, which have shown the best results with reduced cost.

Nanoemulsions

Nanoemulsions are identified as submicron emulsion, mini-emulsions and ultrafine emulsions that are nanosized colloidal particles. This dosage form is kinetically as well as thermodynamically stable dispersion, consisting of two non-miscible liquids (eg., Oil and water), an appropriate surfactant and co-surfactant that helps in stabilization of the film between the interface of the two liquids so as to form a single-phase formulation [7]. The size of the nanoemulsion droplet ranges between 5-100 nm. The modifications in the size of the nanoemulsion can lead to uncommon elastic behavior and optical transparency [8]. The components used in the formation of nanoemulsion must be categorized as "generally recognized as safe (GRAS)" by the United States Food and Drug Administration (FDA) [9]. The surfactants used in formulating the nanoemulsion must possess varied characteristics to enlist; some are non-toxic, compatible with other raw materials, non-irritant, etc. Surfactants widely used includes Sorbitan esters, polysorbates (nonionic), sodium lauryl sulfate, potassium laurate (anionic), quaternary ammonium halide (cationic) and some Zwitterionic surfactants. The site-specificity of the nanoparticles can be enhanced by magnetizing the delivery system. The major goal of a delivery system must target in excelling its therapeutic efficacy and causing declination of toxicity and side effects [10]. Depending on the composition three types of nanoemulsions are formed; First one is the, oil in water (O/W) nanoemulsion in which the aqua is the continuous phase and oil is the dispersed phase, second is the water in oil (W/O) nanoemulsion in which the water acts as the dispersed phase and the oil being continuous phase and lastly, bi-continuous nanoemulsion in which the oil and water are interspersed with each other in the system [11].

Composition of nanoemulsion

The major three components of a nanoemulsion include oils, surfactants/Co-surfactant and an aqueous phase. The nanoemulsion formulation is a colloidal dispersion consisting of an aqueous phase, surfactant, oil phase and a co-surfactant in appropriate ratios. Some of the oils, surfactants and co-surfactants combination for the development of nanoemulsions is shown in table 1.

Table 1: Various combinations used for the development of nanoemulsions

Oils	Surfactants	Co-surfactants	Outcome	Reference
Triacetin	Cremophor EL	Butanol	Decreased oral toxicity and depicted highly efficacious insecticidal activity.	[12]
Capryol 90: Vitamin E	Tween 80	Transcutol-HP	Naringenin nanoemulsion development for nose to brain drug delivery system. Efficacious anti-Parkinson therapeutic effects with enhanced bioavailability and efficacy.	[13]
Mineral oil and medium-chain triglycerides	POE-40 hydrogenated castor oil	Sorbitan iso-stearate	Enhanced stability and sustained release of Lapachol were observed.	[14]
Tocotrienols and oleic acid	Tween 80	Ethanol and propylene glycol	The outcome of this study resulted in successfully exhibiting the antioxidant activity present in the Tocotrienols and enhanced stability for a large time period.	[15]
Oleic acid	Labrasol	PEG 400	Enhancement of bioavailability with effective and efficient targeting to the brain.	[16]
Capryol 90	Poloxamer 188	Tween 80	Enhanced bioavailability, Accurate dosing and decreased toxicity of Itraconazole were obtained.	[17]
Nigella sativa seed essential oil	Tween 80	Polysorbate 80	Decreased cell viability with targeted drug delivery depicting anti-cancerous activity.	[18]
Miglyol 812	Tween 80	Labrasol	The nanoemulsion formulated depicted enhanced bioavailability of Fisetin with decreased Labrasol toxicity, improved anti-tumoral and anti-angiogenic activity.	[19]
Flaxseed oil	Egg phosphatidylcholine	Deoxycholic acid	Efficacious anti-tumor and anti-cancer therapeutic effects with enhanced bioavailability and decreased acute toxicity.	[20]
Peppermint oil	Tween 80	Ethanol	This depicted the enhanced bioavailability of Carvedilol in the body, increasing its solubility in the respective oil.	[21]
Lauroglycol-90	Transcutol-HP	Isopropyl alcohol	The nanoemulsion formulated depicted enhanced anti-cancerous effects.	[22]
Capmul MCM	Tween 80	Polyethylene glycol 400	Decreased nasal cilio-toxicity and increased diffusion were obtained from the developed nanoemulsion.	[23]
Sefsol-218	Tween 80	Carbitol	Development of stable nanoemulsion with increased bioavailability of Ramipril.	[24]
Peanut oil	Tween 80 and Cremophor EL	Transcutol P and PG	It depicted high bioavailability of Acetazolamide in the eye successfully treating glaucoma with the associated prolonged effect on the eye.	[25]

The HLB value of formulated nanoemulsion is an important parameter of consideration. HLB value stands for hydrophilic-lipophilic-balance which determines the ratio of the lipophilic and hydrophilic regions present in the moiety and helps in defining the oil or water affinity with the surfactant. The nanoemulsion formulation is an advantageous one as it shows decreased interfacial tension than the coarse emulsions [26]. Co-surfactants are the main components that help with the resultant low interfacial tension. Thus, forms a nanoemulsion which is stable thermodynamically. Droplet size is below 140 nm forming transparent formulation. A variety of administration routes are known, but a large emphasis is towards the topical administration of the nanoemulsions. Drug-vehicle mobility, drug release and the drug permeation through the skin are the three major aspects to determine transdermal drug permeation. Therefore, nanoemulsions depict enhanced transdermal delivery. The skin permeation of nanoemulsion gels is decreased due to its viscosity and in such comparisons nanoemulsion formulations are more effective for the permeation into the skin. Due to the increase in lipophilic and hydrophilic drug's solubilisation potential, the transdermal flux of nanoemulsion formulation has gained superiority and hence, increasing the thermodynamic action on skin and drug permeability. In such scenarios, the nanoemulsion constituents act as enhancers of permeation. By altering the stratum cornea's structure, the permeation through the skin can be improved. As depicted in fig. 1, Nanoemulsions possess various merits and demerits as drug delivery systems.

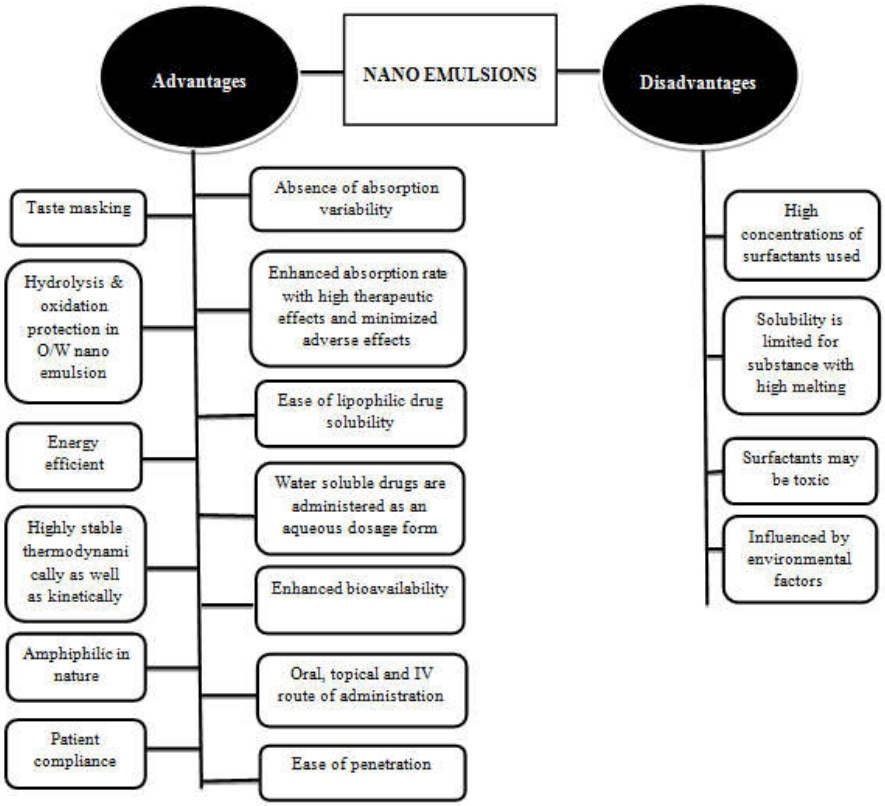

Fig. 1: Nanoemulsion formulation pros and cons

Factors to be considered during the formulation of nano-emulsions

Primarily, the most essential element of nanoemulsion is the surfactant that is unable to develop lyotropic liquid crystalline "microemulsions" phases. Short-chain alkanes, water, surfactants and alcohols when used along with co-surfactant forms such phases in a solitary system. Secondly, Ostwald ripening must be avoided using the appropriate amount of components. The dispersion medium and dispersed phase must not be soluble in one another and lastly, the coalescence must be removed by using high amounts of surfactants to enable rapid coating in the process of emulsification [10].

Nanoemulsion for delivery of amphotericin b

Topical delivery via nanoemulsion

In recent years, a bulk of a topical dosage form of Amphotericin B has been developed in different forms, such as creams, lotions, gels. The demand for the topical dosage form has been increased for delivering Amphotericin B in the treatment of various fungal infections.

In year 2012, topical nanoemulsion of amphotericin B was developed. In this article, the topical delivery of the amphotericin B was tested by loading it in nanoemulsion. The permeation studies were conducted *in vitro* on dermal models. The nanoemulsion developed to depict low particle size and enhanced encapsulation efficiency. The Amphocil provided the best ever results of the skin penetration and hence, the activity towards fungal infections [27].

In the year 2014, another topical formulation was developed that was cost-effective and showed sustained release of the drug. By varying the composition of the components and using the titration method the nanoemulsions were formulated. Different evaluations of particle size, permeability, zeta potential, stability and skin deposition were performed. The final results stated that the formulation developed can be used as an effective, safe and efficient approach in the fungal infection treatment [28].

In the year 2015, a vitamin E TPGS nanogel of amphotericin B was developed. It was highly beneficial in the treatment of cutaneous fungal infections. The developed nanoemulsion was similar in strength to the marketed formulations and was efficiently optimized by the pseudo-ternary phase diagrams. The S_{mix} ratio, co-surfactant, surfactant, size distribution, viscosity, rheology and percent transmittance are the parameters that were optimized. This formulation exhibited increased deposition on the skin. Confocal laser scanning microscopy (CLSM) determined the distribution of the formulation deeper into the skin [4].

In the same year, another research for nanoemulsion development was carried out for treating topical fungal infection. Chalcones were the main components that were used in their development. These are very less water-soluble compounds. The full factorial design was used to optimize the dosage form in the effective treatment of cutaneous leishmaniasis. The formulation was developed using the process of spontaneous emulsification. Stability studies were performed using an antileishmanial assay against different strains of *Leishmania*. The final results confirmed its activity against the cutaneous leishmaniasis [29].

Till this time, the research towards the nanoemulsion formulation has reached unprecedented levels and more to be accomplished. Further, in the year 2016 an amphotericin B gel was formulated depicting antifungal activity and further testing it for its *in vivo* and *in vitro* activity. The gel formulated in this attempt was made using different ratios of the polymer. The results of this article depicted that the dosage form formulated shows no interactions and contains a pure drug. The formulation was also tested for pH, drug content, particle size and drug release profiles. The best formulation was screened and found to be stable with the drug content of 98.12% [30].

In the same year further, another nanoemulsion gel was prepared by the following components sefsol-218, Transcutol and tween 80 using slow spontaneous titration processes. Further the nanoemulsion was incorporated into the gel and the particle size, rheology, charge, release profile, hemolytic and permeability studies were performed. Confocal laser scanning microscopy determined the irritability and permeability studies. It was concluded that it shows decreased toxicity and can be effectively used in the treatment of fungal skin infections [28].

Further, in the same year, a nanoemulsion gel was formulated and evaluated for its topical antifungal activity. The components for developing this nanoemulsion include Capmul, Labrasol, and Polyethylene glycol-400 and drug amphotericin B. The nanoemulsion was evaluated for particle size, zeta potential, polydispersity index and zone of inhibition. The release studies were performed using Franz diffusion cell. Fungisome is the marketed formulation of nanoemulsion gel of amphotericin B. The optimal formulation was safe, stable, sustained and effective [28].

Further in the year 2017, a topical nanoemulsion of amphotericin B was formulated for curing the skin infections such as candidiasis and aspergillosis. In this article, the nanoemulsion formulated consists of those components which show desired solubility and compatibility studies. Further, by the use of pseudo-ternary phase diagram the best formulation was screened. Finally, after formulating the dosage form various studies, like stability, permeation in the skin, release of the drug, antifungal effect and skin retention capacity was performed. The results determined that the formulation developed is optimized for topical usage [31].

In the same year, another research was progressing, nanoemulsion formulation of amphotericin B was developed using lipids and surfactants. The process of slow and spontaneous titration was used for its formulation. Different evaluation parameters were tested, which included zeta potential, polydispersity index, zone of inhibition, skin permeation and deposition studies, particle size and histopathology. When it was compared to the conventional dosage form it exhibited activity without toxicity. A zone of inhibition values is a good method of showing increased safety and efficacy for the treatment of fungal infections [32].

In the year 2018 another nanoemulsion for topical administration of amphotericin B was developed. Amphotericin B exhibits a positive action as a polyene antifungal agent. The mechanism of action followed by this includes the fungal cell wall disruption by binding to sterols leading to pore formation and, thus, leakage of the components of the cell. The nanoemulsion gel was formulated by altering the ratio of the polymer. The different analysis test proved the drug purity, no interactions between chemicals, pH, drug content, release profiles and particle size. The best-optimized formulation depicted stability with the drug content 93.92% [7].

Intranasal delivery via aerosols

Varying routes are available for the administration of the drug-using different types of drug delivery systems. In the year 2012, lipid nanoemulsion of amphotericin B was developed for delivery through pulmonary route. There are two already available lipid nanoemulsions i.e., Intralipid and Clinoleic. The formulation was finally evaluated for zeta potential, particle size, loading efficiency, drug content and particle fraction. This article determines that the lipid nanoemulsion of amphotericin B is an excellent modification for intranasal delivery in the form of a nebulizer [33].

Intravenous delivery via nanoemulsion

Till date different lipid formulations of amphotericin B are available under various marketed names, Ambiosome being the most popular one.

In the year 2003, a lipid nano-sphere emulsion was developed that was shown to exhibit low dose therapeutic action for delivering amphotericin B to its site of action. It was a homogenous emulsion which showed high plasma concentration and was much more effective than Fungizone in the treatment of systemic infections. Thus, it was an effective dosage form for the low dose delivery to the desired site of action [34].

In the year 2013, the first approach towards the intravenous treatment of visceral leishmaniasis was practiced. In this article, the Chitosan nanocapsules incorporating Amphotericin B were developed with the help of polymer deposition technique. They depicted good stability as Chitosan helped in preventing the destabilization. It was less toxic than the Fungizone and Ambisome. The final result suggested that it is an efficient dosage form to be administered with chemotherapy and is low in cost [35].

Intravenous delivery of drugs is a preferred dosage form for treatment of systemic fungal infection. In the year 2015, Amphotericin B nanoemulsions were formulated consisting of a lipophilic amine, cholesterol and the drug. The formulation developed was optimized by testing it for stability, retention of drug, absorption studies, toxicity studies and circular dichroism data. The product finally formulated shows the desired *in vitro* anti-leishmanial action [36].

In the year 2018, the article main focus was to develop a nanoemulsion and optimize it to give efficacious results. The developed formulation was further tested for hemolytic toxicity and the anti-leishmanial activity. The formulation was evaluated for the particle size, polydispersity index and encapsulation efficiency. The formulation was tested against the strains that cause visceral leishmaniasis. The final results showed a decrease in the hemolytic toxicity and positive anti-leishmanial activity, thus characterizing the formulation to be effective in the treatment of visceral leishmaniasis [37].

Toxicity associated with amphotericin b and alternatives to reduce its toxic levels

Amphotericin B is the only drug known to be effective towards leishmaniasis. Therefore, it is a drug of great significance. Nonetheless, the drug expresses a large number of adverse effects on the human body. The acute toxicities observed by the drug-deoxycholate complex include fever, nausea, vomiting, rigors, hypertension, hypotension, hypoxia, flank and urticaria. Further, some of the chronic toxicities related to the drug are cardiotoxicity and nephrotoxicity, which resulted in the increased blood urea nitrogen and serum creatinine levels, which when treated with

liposomal formulation of Amphotericin B exhibited alleviation of azotemia. Hyperphosphatemia, anemia and thrombocytopenia are the other minute toxicities seen with Amphotericin B. The various alternatives to reduce the drug toxicity includes combining it with the liposomes to reduce the chronic toxicity (the first-ever liposomal formulation of Amphotericin B was developed in the year 1990), administering sodium chloride hours before administration of Amphotericin B results in decreased levels of nitrogen, Conjugating Amphotericin B with Arabinogalactan and regular monitoring of electrolytes, phosphates and magnesium [38, 39, 40].

Future prospects

The nanoemulsion seems to be a holistic approach for delivery of Amphotericin B. The development of antifungal nanoemulsion is proliferating and will reach out to various advantageous prospects. The nanoemulsion employed as a carrier and if to emphasize with further research deemed to be an efficient carrier with cheaper cost than the already available marketed Amphotericin B formulations. The cost-effectiveness of this emulsion-based system would act as one of the factors for it is the preferred medication for treatment of severe fungal infections as the majority of the population cannot afford such costly medications. Another advantage of the nanoemulsion is its stability as it is highly stable than the available marketed liposomal liposomal Amphotericin B formulation Furthermore, these systems tend to plunge the toxicity issues associated with this drug. So, in future this formulation would act as the standard to treat severe fungal infections.

CONCLUSION

To conclude, Amphotericin B is highly hydrophobic in nature, resulting in decreased solubility of the drug and its bioavailability. This review has targeted all the available dosage forms of Amphotericin B and the difficulties crossed during the development of any dosage form. This review is mainly pointed towards the development of such a dosage form, which would be cost-effective, safe and highly stable, exhibiting similar actions as that of the other dosage forms with the best results. The area of interest here leads towards the nanoemulsion development of Amphotericin B. Though the drug shows numerous adverse effects the development of nanoemulsion would result in diminishing the reactions as it uses a small and specified amount of surfactants which, when used in high amounts was the reason causing the urticaria, fever, hypoxia, hypertension, etc. As Amphotericin B is the only standard drug for the treatment of leishmaniasis, there arises a high need to develop a formulation which is cheaper at cost, patient compliant, easily affordable and effective towards the treatment of the disease.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally.

CONFLICT OF INTERESTS

The authors have no conflict of interest to declare.

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