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Utilization of Fungal Proteins in Increasing Bioavailability and Stability of Hydrophobic Drugs

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Abstract:- Hydrophobins are a group of small, low molecular weight cysteine-rich fungal proteins found in the hyphae walls of fungi. Due to their composition, hydrophobins have the surface-modifying ability to form an amphiphilic membrane at the hydrophobic-hydrophilic interface in an aqueous solution which can be used to coat hydrophobic surfaces and change their nature. This property has applications in increasing the bioavailability of low aqueous solubility hydrophobic compounds by preparation of nanosuspensions. The low solubility hinders their efficacy in being used as therapeutic drugs. The objective of this review is to explore the medical application of hydrophobins in the preparation of hydrophobin-drug nanosuspension in order to increase the bioavailability of hydrophobic drugs and to explore the stability of these nanosuspensions using five hydrophobic therapeutics - curcumin, nifedipine, cyclosporine, docetaxel, and amphotericin.

Keywords:- *Hydrophobin nano suspensions, Hydrophobic drugs, Amphotericin, Docetaxel, Nifedipine, Cyclosporine, Curcumin, Bioavailability.*

I. INTRODUCTION

Hydrophobic molecules are those that show extremely poor solubility in an aqueous medium but are most likely soluble in organic solvents. Hydrophobic molecules tend to be non-polar and do not dissolve in polar solvents such as water, they generally prefer non-polar solvents. The insolubility of these molecules caters to unintended pharmacokinetic properties that decrease their efficacy. Solubility refers to the ability of a substance, which can be solid, liquid, or gas, to dissolve in a solvent and produce a homogenous solution. When a solution is in a stable soluble condition, the dissolution and precipitation rates of the solute inside the solvent are in a dynamic equilibrium [1].

Drugs with low water solubility will precipitate in physiological settings, lowering permeability and absorption in the biological system. This may necessitate a greater drug dose or more frequent drug dosing to achieve a therapeutic concentration, as well as an increase in negative effects [2]. Almost 60-70% of the drugs are poorly soluble in water and show decreased permeability for adsorption through the GIT (Gastrointestinal Tract). Additional treatments or aided methods of drug delivery systems must be used to work the drug to its full potential [3]. Over 40% of all medications on the market are insoluble, while roughly 90% of all compounds identified as possible innovative therapeutics are water-insoluble [2].

The Absorption, Distribution, Metabolism, and Excretion (ADME) properties of a drug determine how efficient the drug is in its mechanism. Enhancement in these methods increases the potential of the drug [4]. One factor that improves the efficacy of a hydrophobic drug is the increase in the solubility of that drug. The solubility of a medicine is a crucial factor that determines its bioavailability, or the ability of a drug to reach its target area without compromising its efficacy. Increasing the bioavailability of hydrophobic drugs by the preparation of nanosuspension systems helps in their working action and therapeutic effects. These nanosuspensions can be stabilized by fungal proteins called hydrophobins.

Hydrophobins are a group of small, low molecular weight cysteine rich proteins. These proteins are produced from cells and are secreted into liquid media or remain at the surface of mycelia [5]. They were first discovered in Schizophyllum commune in 1991 [6]. The name hydrophobin was first given by Wessels and colleagues in 1984, who examined genes that are expressed during fruiting body formation in Schizophyllum commune [7]. As these proteins contain hydrophobic amino acids, that is where the name ‘hydrophobin’ originates [8]. Most of the hydrophobins were found during certain stages of fungal development without knowing anything about the encoded proteins, which were expressed by sequencing complementary deoxyribonucleic acid (cDNAs) representing messenger ribonucleic acid (mRNAs) [7]. Since the initial discovery of SC3 hydrophobin, extracted from Schizophyllum commune, other hydrophobins have been isolated from other fungi such as hydrophobin DewA from the fungus Aspergillus nidulans [9].

The following is the review of studies conducted to explore the use of various hydrophobins in the preparation of hydrophobin-drug nanosuspensions and the effectiveness of these nanosuspensions in increasing the bioavailability of hydrophobic drugs like Curcumin (Cur) - potent anti-inflammatory and antioxidant, nifedipine - calcium channel blocker, Cyclosporine A (CyA) - calcineurin inhibitor, Docetaxel (DTX) - cytotoxic chemotherapy drug and Amphotericin B (AmpB) - antifungal agent.

II. BACKGROUND

A. Properties of Hydrophobins

Hydrophobins are exceptional surface tension reducing protein, produced by fungi which develop during different fungal development stages [10,11]. They are small secreted proteins that are produced by filamentous fungi belonging to the ascomycetes and the basidiomycetes, and may also be produced by zygomycetes [12]. The size of these molecules is about 10kDa. They are highly insoluble and

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hence strong chemicals such as Trifluoroacetic acid make them detectable on SDS-PAGE ([13]. Hydrophobins contain eight conserved cysteine residues which form four disulphide bridges. Reductions in cysteine residue seem to show increased agglomeration in an aqueous medium. The cysteine rich nature of hydrophobins is what keeps them in the soluble state [14]. They reduce the water surface tension and cover aerial structures like spores and fruiting bodies (e.g., mushrooms) to make them hydrophobic, allowing fungi to escape from the watery environment into the air. They also affect the design of cell walls and facilitate the adhesion of hyphae (fungi filamentous cells) to hydrophobic surfaces [15].

This characteristic property of hydrophobins to form an amphipathic membrane on contact with a hydrophilic–hydrophobic interface allows them to change the nature of a surface [16]. This means hydrophobins can be used to change hydrophobic surfaces into hydrophilic and vice versa. Hydrophobins have many industrial applications such as agents for enhancing bioavailability of water insoluble drugs, food stabilizers, antifouling agents for biomedical devices like catheters, fusion partner for recombinant proteins for purification, low friction coatings on biomaterials, immobilizing enzymes in biosensors, etc [16]. Hydrophobic surfaces of liquids (e.g., oil droplets) or solids (e.g., Teflon) can be made hydrophilic by suspending or submerging them into a solution of hydrophobin [17]. The hydrophobin in the solution coats the surfaces of the submerged solid or liquid and changes the nature of the substance from hydrophobic to hydrophilic. Hydrophobins also do not seem to be toxic because they are ingested by humans upon consumption of mushrooms and fungus-fermented foods and form an immune-suppressive barrier in drug formulations [19]. The fungal strains that are used to extract hydrophobins are Generally Regarded As Safe (GRAS) [20]. This means, they do not cause adverse effects to the human body and can be used in medical applications. Some strains that are GRAS include Pleurotus ostreatus [21,22]. Aspergillus niger, Aspergillus oryzae, and Trichoderma reesei [20]. Usually, commonly used surfactants for hydrophobic drug formulations like Tween80 and Cremophor EL tend to show immunogenic effects. Cremophor is a nonionic solubilizer and emulsifier produced by a reaction of ethylene oxide with castor oil [23]. The pharmacokinetic behavior of Cremophor EL is dose-independent and highly influenced by the duration of the infusion, whereas hydrophobin nanosuspension depends on the protein-drug ratio [24]. Tween 80 is a nonionic surfactant [25]. Tween 80 showed large areas of macroemulsion indicating lack of stability, whereas hydrophobin-drug nanosuspensions showed variance in stability based on protein-drug interaction [26].

B. Types of Hydrophobin

Hydrophobins are divided into two classes depending on various properties. These properties include compound solubility, hydropathy plots and the kind of layer they form [27].

The Class I hydrophobins have a membrane that is highly insoluble. These require treatment with harsh chemicals such as formic acid and trifluoroacetic acid (TFA) to dissolve the monolayer rodlets of these proteins [15].

Class II hydrophobins are devoid of the rodlet layer and hence show properties less stubborn than those of Class I and can be dissolved in organic solvents and detergents like 60% ethanol and 2% Sodium Dodecyl Sulfate (SDS). Class II hydrophobins can be dissociated merely by applying pressure as well [27]. Class I hydrophobins are found in both basidiomycetes and ascomycetes, whereas the Class II hydrophobins are found only in ascomycetes [13]. (Refer Table I).

Table 1: Types of hydrophobin [13]

Name of Hydrophobin	Class of Hydrophobin
DewA	Class I
SC3	Class II
HFBI, HFBII	Class II
Vmh2	Class I
EAS	Class I

C. Source of Hydrophobin

Since the isolation of SC3, many diverse types of hydrophobins have been found. Most hydrophobin sources are fungal although there are bacteria that produce hydrophobin. They are mainly isolated from phylum ascomycetes, and basidiomycetes, although they may also be present in zygomycetes. However, there is no evidence of the presence of hydrophobins in chytridiomycetes [28] [Refer Table II]. Many species of the genera Aspergillus, Trichoderma, Fusarium, Penicillium, and Neurospora are already known for industrial production of acids, enzymes, proteins, specialty products etc [16].

Table 2: Sources of Hydrophobins

Name of Hydrophobin	Isolated From	Phylum
SC3	Schizophyllum commune	Basidiomycetes
DewA	Aspergillus nidulans	Ascomycetes
DewY	Aspergillus nidulans	Ascomycetes
HGFI	Grifola frondosa	Basidiomycetes
Hydrophobin HPB	Trichoderma reesei	Ascomycetes
Vmh2	Pleurotus ostreatus	Basidiomycetes
BslA	Bacillus subtilis (Bacterial)	Firmicutes

D. Application of Hydrophobins

Hydrophobins fulfill a broad spectrum of functions in fungal growth and development. This is due to the property of hydrophobins to self-assemble at hydrophilic-hydrophobic interfaces into amphipathic films [29]. As a result, hydrophobins have various applications. They can be used in surface modification to change the nature of a surface from hydrophobic/hydrophilic to hydrophilic/hydrophobic, they can be used to stabilize foams, and they can be used in immobilization of

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antibodies, enzymes, cells, peptides and inorganic molecules. They can also be used to coat medical equipment ([30]. [Refer Figure 1].

Surface modification properties of hydrophobins have been proven to be very useful in many industries. To give an example, hydrophobin can be used to change hydrophilic materials to hydrophobic and vice versa in the textile industry [31]. Hydrophobins have a great foam stabilizing property and have an upper hand over other small proteins because of their highly surface-active nature and self-assembling properties. [33,34,35]. Immobilization of various molecules such as enzymes, cells, and antibodies caters to various applications. Hydrophobins can be used to create biofunctional layers for self-immobilization of these molecules [36]. Enzymes fused with appropriate hydrophobins have been experimented on to immobilize enzymes like laccase onto hard surfaces [37]. In the food industry, the use of formation of emulsification and hydrophobin-catered emulsifications showed an advantage over other agents [38]. Air filled emulsifications in the food industry used as fat replacements employ the surface elasticity property of hydrophobin [39]. There are various highly efficient molecules in the industrial and scientific world that suffer a setback in costs, and increased toxicity due to the use of organic solvents or certain processing [39]. Hydrophobin even at low concentrations provides surface modification of particles and promotes dispersion of these particles [40]. This is useful not only in these fields but also in medical applications such as nanoparticle mediated drug delivery [41].

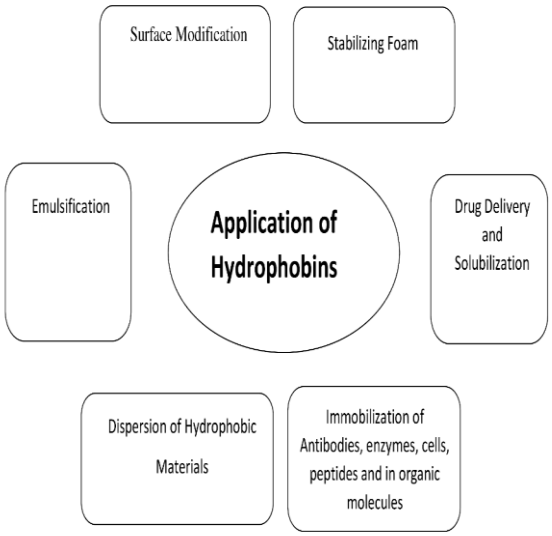

Fig. 1: Applications of Hydrophobins

III. EXTRACTION OF HYDROPHOBIN

There are two classes of hydrophobins, class I and class II, where class I is more stable [42]. In the extraction of class II hydrophobins, such as Trichoderma reesei, harsh chemicals such as TFA are required [16]. First, the mycelia is grown in potato dextrose extract, glucose, and yeast media. The mycelia is then harvested and the cells are broken by a sonication bath. After the sonication bath, the mycelia is washed with 2% SDS in NaH2PO4 solution. Washes with distilled water are given to remove the soluble proteins from the fungi. A final wash is given with 60% ethanol. The mycelia is then lyophilized. It is then treated with TFA to disassociate the hydrophobin. The mycelia dipped in TFA is given a sonication bath for 14 minutes [16]. The TFA is extracted in tubes and dried with a stream of high pressure air until only the pellet remains. The pellet contains the hydrophobin. The pellet is stored in 60% ethanol which dissolves the hydrophobin and stops it from self-assembling. [Refer Figure 2].

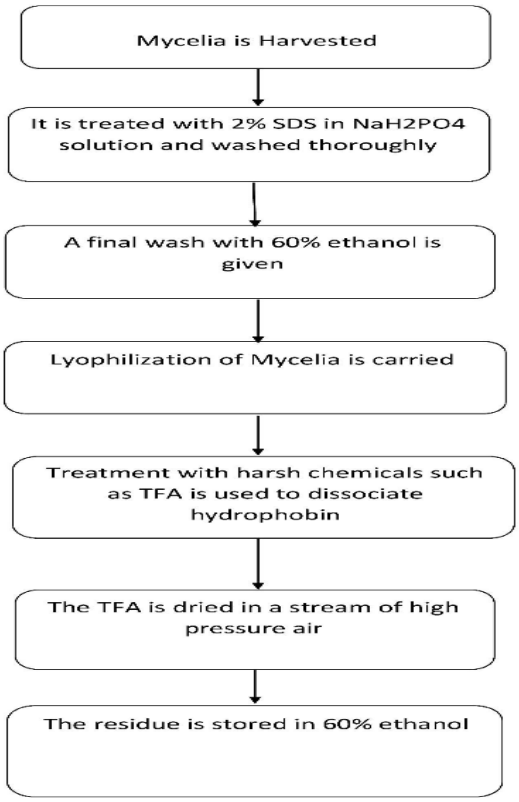

Fig. 2: Flow Process of Extraction Method for Hydrophobins

IV. NANOSUSPENSION OF DRUGS

Nanosuspensions are submicron colloidal dispersions containing nano sized drug particles which are poorly soluble in water and hence they are stabilized by surfactants [43]. Nanosuspensions increase the exposed surface area of a drug particle in an aqueous medium [44]. Surface modification of the drug particles aids in increased stability by use of surfactants by decrease of surface free energy. Low surface free energy makes the system thermodynamically stable [45]. Hydrophobins act as surfactants which enhance stability of these drug particles. The cysteine rich nature of hydrophobins reduces the agglomeration in an aqueous medium and keeps it in a soluble emulsion state with decreased particle size [14]. These properties of surfactants are important factors to consider for scaled up production of nanosuspensions. The preparation of drug nanosuspensions increases their bioavailability and help in medical treatment applications. [Refer Table III].

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A. Curcumin

Curcumin (Cur) is derived from a rhizomatous herbaceous perennial plant (Curcuma longa) of the ginger family called turmeric [46]. Turmeric has been used in India for more than 4000 years, where it was used as a spice and also for its medicinal properties [47]. According to Sanskrit medical treatises and Ayurvedic and Unani systems, turmeric has a long history of medicinal use in South Asia. Susruta's Ayurvedic Compendium, dating to 250 BC, recommends an ointment containing turmeric to relieve the effects of poisoned food [47]. The medicinal properties of turmeric are linked to curcumin, a hydrophobic phenol found in turmeric [48]. In Ayurvedic medicine, curcumin is used as a treatment for a variety of health conditions, including respiratory illness, liver disorders, inflammatory disorders, and diabetic wounds [49]. Curcumin has been tested for safety, tolerability, and nontoxicity at high doses in the human body by human clinical trials. It was found to be well tolerated by the body [50,51].

Curcumin has antioxidant and anti-inflammatory properties and can be used in the treatment of osteoarthritis which affects over 250 million people worldwide [52]. The therapeutic efficacy of curcumin against various human diseases, including cancer, cardiovascular diseases, diabetes, arthritis, neurological diseases, and Crohn's disease, has also been documented [53]. Curcumin has been shown to target multiple signaling molecules while also demonstrating activity at the cellular level, which has helped to support its multiple health benefits [52]. Curcumin and its derivatives have been shown to have a variety of biological impacts on health promotion and illness prevention. Curcumin derivatives can be used as an antioxidant, anti-inflammatory, neuroprotective, cardioprotective, hepatoprotective as well as an anticancer compound [54]. However, a major barrier to curcumin's clinical efficacy is its poor bioavailability. Efforts have therefore been dedicated to developing curcumin formulations with greater bioavailability and systemic tissue distribution [55].

Hence, a novel method of coating curcumin with rHGFI to enhance the solubility and dissolution rate of drugs was analyzed. To verify the influence of rHGFI on the solubility of curcumin, the wettability of Cur before and after modification was investigated with contact angle measurement. Curcumin, by itself, is hydrophobic. However, after adsorption of rHGFI, the contact angle of the rHGFI-Cur complex pellet surface decreased dramatically. This indicates that the rHGFI-Cur complex is hydrophilic [56]. The increased dissolution rate of rHGFI-Cur complex increases the bioavailability of the drug. Increase in dissolution rate makes the drug particles soluble in the gastrointestinal (GI) tract fluids. This enhances diffusion through the GI membrane into the bloodstream showing concentration of the absorbed complexed drug particles greater than uncomplexed ones [57].

The instability is another major challenge for curcumin absorption in the body, which could be improved by micelles [58]. An experiment was conducted to observe the dispersion stability of curcumin and rHGFI-Cur complexes. It was observed that cur began to settle within five minutes and a noticeable yellow precipitate appeared at the bottom of the bottle after 2 h in water and PBS. However, most of the rHGFI-Cur was still uniformly dispersed in the two solvents after 12 hours [56]. This observation suggests that coating curcumin with rHGFI increases not only the solubility, but also the stability of curcumin. Hence, the nanosuspensions formed will stay in their dispersed state without occurrence of precipitation with the passage of time. This broadens the scope of medical application of curcumin [59]. Considering the multiple benefits of curcumin and the general acceptance of turmeric products, increasing bioavailability could be very beneficial [60].

B. Nifedipine

Nifedipine is a revolutionary drug for cardiovascular diseases. It is a highly specific drug acting on calcium channels and aids in relaxing muscle cells to reduce blood pressure. Nifedipine came into action after several discoveries about treatments that could cater to cardiovascular diseases. The appearance of nifedipine in the picture began with the ground laying discovery of the availability of extracellular calcium ions. These ions could be manipulated using drugs for cardiac contraction [61]. Nifedipine is also known to increase the efficacy of some antineoplastic agents [62].

Nifedipine is a largely accepted drug for hypertension or high blood pressure. It has been found effective in lowering the b.p of patients with diastolic pressure over 120 mm of Hg or systolic pressures over 200 mm of Hg [63]. It is a calcium channel blocker and helps lower the blood pressure by hindering the entrance of calcium ions in the vascular smooth muscle and myocardial cells. Muscles need calcium to contract, so when you block the calcium, it makes the muscle cells relax. In spite of its profound therapeutic effect, it suffers a setback due to its poor and inconsistent bioavailability after oral administration [64]. Various methods have been tested to cater to this issue. Use of solid dispersions has been shown to effectively increase the bioavailability of Nifedipine [65].

SC3 hydrophobin, extracted and purified from Schizophyllum commune has shown to increase the bioavailability of nifedipine by almost 6±2 folds in comparison to its negative control of directly administering the drug without any supplements. SC3 decreased the particle size of the drug, making it more homogenous. Without hydrophobin, large aggregates of the drug are formed in the solvent [66] There is an increase in bioavailability with the reduction in the particle size, as the smaller particle size increases the surface area of the particle and increases the dissolution rate [44]. Increased dissolution rate corresponds to better absorption of the drug in the body through the GI membrane [57]. Hence SC3 has been shown to enhance this property of nifedipine and make it effective in its treatment.

C. Cyclosporine

Cyclosporine A (CyA) is a lipophilic, cyclic undecapeptide with a molecular weight of 1202 Daltons [67]. It was discovered in the lab of Sandoz in Switzerland in 1972, while searching for novel antifungal agents. Cyclosporine A was found to have immunologic properties and since then it has played a significant role in the advancement of transplant medicine [68]. Cyclosporine is an immunosuppressant which has had a tremendous impact upon organ transplantation [69]. Calcineurin is a calcium/calmodulin-dependent serine threonine protein phosphatase. Cyclosporine inhibits calcineurin by binding to the immunophile, cyclophilin. This step prevents the dephosphorylation of Nuclear Factor of Activated T- cells (NFAT) and its subsequent translocation from the cytoplasm to the nucleus in an IL-2-mediated process. Inhibition at this level thereby prevents activation of promoters of T-cell activation and the overall immune response [68].

There are differences in the bioavailability of cyclosporine in large part due to significant inter individual variability in intestinal absorption, a process that is further influenced by food ingestion, diabetes, gastric motility problems, and diarrhea among other things [44]. The metabolism of CyA occurs predominantly in the liver and is affected by several drugs known to alter hepatic metabolism [69]. However, CyA is hydrophobic, which hampers the bioavailability of the said drug. As cyclosporine is an effective immunosuppressant, many methods of increasing the bioavailability of cyclosporine have been researched.

Large visible aggregates of the drugs are formed when the drug solutions are diluted in water. In contrast, in the presence of SC3, stable suspensions of small particles are formed [66]. There is an increase in bioavailability with the reduction in the particle size, as the smaller particle size increases the surface area. This increases the dissolution rate of the particle [44]. Increased dissolution rate corresponds to better absorption of the drug in the body through the GI membrane [57].

Therefore, in vivo uptake with CyA shows that the formulation with SC3 hydrophobin results in an increase of the bioavailability of this drug. In addition, the SC3 hydrophobin formulation of CyA shows an interesting pharmacokinetic property, and the use of SC3 results in a reduced but longer lasting peak concentration of the drug in the blood [66]. Thus, hydrophobin formulation can be used as a non-toxic, generic method to increase in vivo uptake of CyA, thereby increasing the bioavailability of CyA.

D. Docetaxel

Docetaxel (DTX) is an antimicrotubular agent and acts as a cytotoxic taxane. It is mostly used to treat patients with breast cancer amongst a wide variety of cancers it can treat [70]. It has been used as an effective therapeutic drug for cancer and has been Food and Drug Administration (FDA) approved since 1996 [71].

It is a compelling semisynthetic derivative of paclitaxel. It is derived from the leaf extracts of Taxus baccata [71]. The mode of action of docetaxel is dual in nature. It suppresses the microtubule assembly and disassembly dynamically, which in turn leads to apoptosis. The other mode of action is to block Bcl-2 which causes a cascading effect on cell death [72]. All these effects lead to antiproliferative effects in cancer cells which is the cause of tumor formation.

Sparse bioavailability of anticancer drugs is mainly due to firstly, cytochrome P450 (CYP) activity in the gut wall and liver, and secondly, drug transporters, such as P-gp in the gut wall and liver. Shared substrate drugs are affected by the combined activity of these systems [72].

Docetaxel is a widely used and well-known chemotherapeutic drug used for breast cancer. It has cytotoxic properties and is an antimicrotubular agent [70]. It is also useful for other cancers like non-small cell lung cancer, ovarian cancer, and head and neck cancer. However, it suffers some setbacks in its bioavailability due to its poor aqueous solubility, poor permeability, and hydrophobicity [73].

DTX-Hydrophobin (HPB) complexes form a structure with the core consisting of the drug and the outer structure of HPB. These DTX-HPB- nanostructures have smaller size and greater stability than that of DTX administered alone intravenously [74]. Novel hydrophobin HPB shows low toxicity and decreases immunogenic responses in cancer patients as well [19]. Increased bioavailability is observed due to all effects of HPB coated DTX particles. Regarding its drug efficacy, more research is required to be carried out to make a revolution in the chemotherapy effects of docetaxel.

E. Amphotericin

Amphotericin B (AmpB) was initially designed for the treatment of local mycotic infections and later approved for the treatment of progressive and potentially fatal fungal infections [75]. Amphotericin is a widely used polyene macrolide antifungal agent which plays a significant role in the treatment of systemic fungal infections. It is effective in treating cryptococcal meningitis. It is even listed as an essential drug on the World Health Organization's List of Essential Medicines [76].

After 60 years of investigation, the mechanisms of antifungal action are not fully elucidated. However, there is ample consensus and evidence that AmpB affects cells in two ways: via ergosterol binding and via oxidative damage [75]. In ergosterol binding, the drug interacts with the lipid bilayer of the membrane through its hydrophobic domains resulting in multimeric pores that increase the permeability of ions and cause intracellular loss and consequent cell death [77]. In oxidative damage, AmpB induces the expression of stress genes as confirmed through genome-wide expression analysis [78]. However, the lipophilic nature of AmpB makes it highly insoluble [79]. This results in poor bioavailability of AmpB. Hence, to improve solubility, hydrophobin DewY is used to coat the drug.

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AmpB is very poorly soluble in aqueous solutions but highly soluble in Dimethysulfoxide (DMSO) [80]. Hence, DewY-AmpB nanosuspensions are made in DMSO. DewY-AmpB formulations are more stable than AmpB alone in water. The long-term stability and solubility of DewY-AmpB suspensions are dependent on the protein and drug ratio. There were no great changes in the complex formed even after a week which was analyzed through absorbance studies [80]. This in turn shows that DewY-AmpB nanosuspensions enhance the bioavailability by increasing the stability and solubility of AmpB, which can help in increasing drug efficacy.

Table 3: Hydrophobic Drugs and their Application

Name of Drug	Medical Application
Curcumin	Antioxidant, anti-inflammatory, neuroprotective, cardioprotective, hepatoprotective, anticancer compound
Nifedipine	Calcium channel blocker, Hypertension medication, increases efficacy of some antineoplastic agents
Cyclosporine A	Immunosuppressant, calcineurin inhibitor
Docetaxel	Cytotoxic chemotherapy drug
Amphotericin	Antifungal

Following is an overview of the nanosuspension preparation of the above-mentioned drugs. (Refer Figure 3).

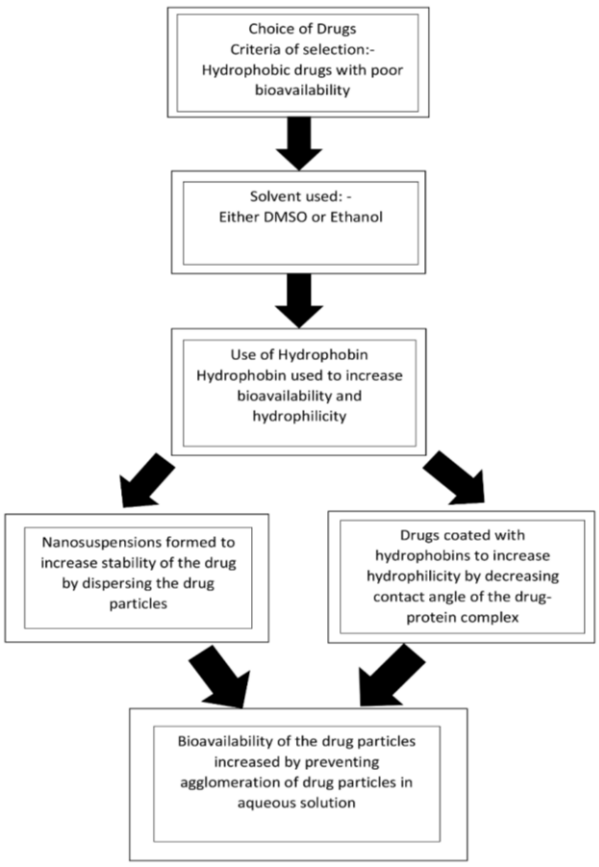

Fig. 3: Overview of Nanosuspension Preparation

V. CONCLUSION

Hydrophobins are surface active proteins which show remarkable properties of modifying the surface nature of molecules when they encounter them through hydrophobic-hydrophilic interactions. Due to such properties these molecules prove to be useful in biomedical applications such as increasing the bioavailability of hydrophobic drugs. One such method of increasing the bioavailability of a drug is by the preparation of nanosuspension in which the drug nanoparticles are coated by the amphiphilic hydrophobin membrane.

Many hydrophobic drugs have great applications in medicine but face a setback due to their poor solubility or stability which reduces their bioavailability and hampers their efficacy. Hydrophobin-drug nanosuspensions increase the hydrophilicity of that particle thereby increasing the bioavailability and allowing the drug to show its full effects in an efficient manner. Hydrophobin coating also prevents agglomeration of the particles and helps in dispersion. Therefore, the drug particles are sustained as nanoparticles for a longer duration after the preparation of the nanosuspension. Due to the smaller size of the drug nanoparticles there is an increase in the exposed surface area which increases the dissolution rate. Due to this the body can absorb and utilize the drug efficiently. The increase in bioavailability also resulted in reduced drug dosage and hence reduced side effects of the same too.

Long-term stability and solubility of the Hydrophobin-drug nanosuspension depend on the protein to drug ratio. Hydrophobin reacts with the drug to reduce its hydrophobicity and in some cases unexpected interactions with certain drugs were noted such as in the longer lasting peak concentration of CyA in blood. However, in each case, the drugs encapsulated showed greater nanoparticle dispersion and as Hydrophobin is considered to be non-toxic and shows no immunogenic response.

The advantage of using hydrophobin over a surfactant such as Cremophor El is that the effectiveness of the Hydrophobin-drug nanosuspension is dependent on the protein to drug ratio whereas Cremophor El is highly influenced by the duration of infusion. Hydrophobin is also more advantageous when compared to Tween 80 as Tween 80 shows a lack of stability as indicated by the formation of large macroemulsion. Therefore, hydrophobin is a viable candidate for reducing the hydrophobicity of a hydrophobic drug nanosuspension intended for human use. The fungal strains from which hydrophobin extracted are GRAS, making them safe to be used over other above-mentioned surfactants.

VI. LIST OF ABBREVIATIONS

- ADME- Absorption Distribution Metabolism Excretion
- AmpB- Amphotericin B
- cDNA- complementary deoxyribonucleic acid
- Cur- Curcumin
- CyA- Cyclosporin A

- CYP- cytochrome P540
- DMSO- Dimethyl Sulfoxide
- DTX- Docetaxel
- FDA- Food and Drug Administration
- GI- Gastrointestinal
- GRAS- Generally Regarded As Safe
- HPB- Hydrophobin
- mRNA- messenger ribonucleic acid
- NFAT- Nuclear factor of activated T-cells
- rHGFI- recombinant class I hydrophobin
- SC3- Schizophyllum commune hydrophobin 3
- SDS- Sodium dodecyl sulfate
- TFA- Trifluoroacetic acid

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