Review Article

A note on the current status of the trans ungual delivery of poorly soluble drugs at a glance: Formulation perspective

ABSTRACT

The Trans ungual drug delivery (TUDD) system involves the delivery of drugs through the hard keratinized nail plate. Topical treatment is ideal for treating nail diseases because it has localized effects, resulting in fewer systemic side effects and perhaps better adherence. Conventional formulations have failed to promote nail adhesion and medication absorption due to the nail's highly keratinized hydrophilic nature, which obstructs drug absorption. Systemic therapy has substantial adverse effects and a high rate of recurrence, whereas surgery is painful and invasive. As a result, there is a definite need to design an efficient TUDD system that allows antifungals to penetrate the nail and reach the infection site at a sufficient concentration and for a longer period of action to eliminate the infection. The system also avoids the oral toxicity of drugs. Several techniques, including physical, chemical, and mechanical, are employed to increase medication penetration over the nail plate. However, the nail remains a barrier to providing a complete cure. To increase nail adherence, therapeutic action, and bioavailability, several innovative formulation techniques are being researched, including the use of penetration enhancers in traditional dosage forms (gel, solution), novel dosage forms such as films, nail lacquer, and nanotechnology.

Keywords: Trans ungual drug delivery, Human nail, Anti-fungal, Permeation, Novel formulations, Nanotechnology, Bioavailability.

1. INTRODUCTION

The human nail is an organ that protects the tips of the fingers and toes from injury, enhances delicate touch sensations, and assists individuals in picking up and manipulating items. The nail, as a cosmetic organ, is also utilized for scratching and grooming [1-2]. Microorganisms such as bacteria and fungus can thrive on the dead tissues of hair and nails. Further, the moist and warm environment favors the infection. To combat these nail infections, topically the delivery of the drug is targeted through the nail plate and is called the trans ungual ("Trans" means "through" and "Unguis" means "Nail") system. Hence, the nail plate acts as a principal route for drug penetration. Because of its impermeability and hardness, drug administration by the nail is an unpromising route, resulting in just a portion of the medication reaching the target site and inability to obtain the appropriate therapeutic concentration [3-6]. The TUDD minimizes the systemic adverse effects and improves adherence due to its localized effects, which is highly desirable. For the treatment of nail infections, a number of traditional formulations such as gels, creams, and oral antifungals are available. There is a need for newer drug delivery strategies to improve the effectiveness of active molecules by achieving the effective concentration of drugs at the right place at the right time [7]. The current review provides a note on nail permeation strategies and recent developments in the TUDD at a glance.

2. HUMAN NAIL

The nail is composed of hard keratinized epidermal cells which are dead and tightly packed and form a transparent and firm coating across the dorsal surfaces of the distal placements of distinct digits. Each nail digit is divided into various sections, such as lunula (the moon), nail bed, nail sinus (*sinus unguis*), nail root (*radix unguis*), nail plate (*corpus unguis*), free margin (*margo liber*), hyponychium, onychodermal band, eponychium, perionyx, nail wall (*vallum unguis*), lateral margin (*margo lateralis*), and paronychium [8-9]. The high resistance of the nail plate is related to the multitude of lateral connections between keratin fibres (disulfide bridges, hydrogen bonds, acid-base bonds, electrostatic bonds). Phospholipids are plentiful in keratin fibres, particularly in the dorsal and intermediate layers, contributing to their flexibility. The nail matrix and nail bed are supplied with an abundant supply of blood by two main artery arches beneath the nail plate. The tissues surrounding the nail have an extensive capillary blood supply; in particular, a capillary loop system delivers blood to the entire nail fold. The nail bed is densely packed with lymphatic veins and glomus bodies, which are considered to assist regulate blood flow to the extremities in cold weather. Despite this extensive vascular network, nail deformities may result from a lack of peripheral circulation [10-11].

3. DISEASES AND DISORDERS OF NAIL

The infected nails are discoloured, thickened, dystrophic with a decreased glow. Various reported nail infections are onychomycosis or nail plate separation (yellow-brown patches caused by *T. rubrum, T. mentagrophytes, Candida albicans*, and *ondermatophyte moulds*), psoriasis (characterized by raw, scaly skin), koilonychia (iron deficiency anaemia) [11], paronychia (Bacteria, fungus, and even viruses can infect the nail fold.), onychogryposis [12], green nail syndrome caused by *pseudomonas* [13], *T. Unguis* (ringworm of the nails), onychatrophia (nail atrophy), onychorrhexis (vertical split in nail plate), beau's lines (trauma, disease, starvation, or any significant metabolic disorder can cause horizontal lines of darker cells and linear depressions) [14], leuconychia (trauma causes white lines or patches on the nail plate), pterygium inversum unguis (the hyponychium grows forward, with living tissue firmly linked to the bottom of the nail plate and contains a blood supply and nerves), yellow nail syndrome is distinguished by cuticle-less yellow nails that develop slowly and are loose or disconnected [15], melanonychia (nail moles in the matrix which are vertical pigmented bands), hematoma or subungual hematoma, and nail bed trauma [16], pterygium (skin moving inward over the nail plate), and nail patella syndrome which is a rare genetic condition characterised by nail and skeletal malformations (among a variety of other associated defects) that is approximately 0.0022%, onychauxis (overgrowth of the nail plate) [17], methyl methacrylate (MMA) damaged nails, although it is banned by the Food and Drug Administration, MMA is a liquid monomer used for acrylic nails by certain unethical establishments, splinter haemorrhages [18] vertical ridges (these are characteristics of ageing, however they are not confined to the old or the elderly). The nail plate develops forward on the nail bed in a 'rail and groove' pattern, similar to how a train moves along its tracks, onychotillomania (patients who pluck and self-mutilate their nails might develop parallel transverse grooves and ridges) [19], pincer nail deformity (the nail plate's transverse over curvature) [20].

4. DRUG DELIVERY ACROSS THE NAIL

Nail problems are rarely fatal, but they can be painful and inconvenient, resulting in major physical and occupational limitations, mental and physiological disturbance, and a drop in quality of life. Deformed nails can cause injury to the surrounding tissue, which can lead to secondary bacterial infection [1]. Treatment of nail infections can be challenging, and

commonly prescribed oral antifungal drugs can cause skin rashes and liver damage. Topical treatment is ideal for treating nail diseases since it has localised effects, resulting in fewer systemic side effects and possibly better adherence. Because of the low drug permeability through the nail plate, topical treatments are restricted. Hence, newer drug delivery systems might be used to more successfully deliver the effective quantity of active molecules to the appropriate location at the appropriate time, maximising the efficacy of the drug [16].

4.1 Drug permeation mechanism

Solvents, film formers, resins to boost adhesion, plasticizers to improve durability and flexibility, suspending agents to maintain viscosity, and colouring agents are all used in topical nail formulations. When the formulation is applied, the solvent evaporates, leaving a water-insoluble film adhering to the nail plate that functions as a drug depot. A drug undergoes release and penetration across the nail for an optimum time period from the depot [21]. The hyper hydration of the nail plate occurs because of the drug depot film, which reduces the moisture loss from the nail plate and enhances drug diffusion. The diffusion can be further increased by various penetration enhancers [22]. The insoluble film functions as a matrix-type controlled release mechanism, dispersing or dissolving the drug evenly. Furthermore, the dispersed drug will be dissolved before release. Fick's law of diffusion governs the release. where increased active concentration will increase the uptake [23].

4.2 Factors affecting drug transport

4.2.1 Size of diffusing molecule

Diffusion of larger molecules is always difficult through the keratin network and leads to less drug permeation. This may be due to the high concentration of keratin fibers which form heavy chain bonds, small pores, or overlapping of pores. According to the reported study, increasing the molecular weight reduces the logarithm of the permeability coefficient. As a result, in order to achieve maximum ungual penetration, active molecules must be small and without a surface charge [17].

4.2.2 Hydrophilicity or lipophilicity of diffusing molecule

Lipophilic actives permeate through the lipidic pathway. Whereas when an aqueous formulation is used, permeation occurs by means of larger pores formed by nail swelling. This swelling is observed due to the expansion of the keratin network by water uptake available in the formulation [24]. A study of the permeation of alcohols (C1-C12) through avulsed human nails reported that the permeability coefficient decreased from C1 to C8, after which increased permeability was observed for > C12. The increasing lipophilicity of a molecule decreases the permeation to a certain extent, after which increased lipophilicity enhances permeation. This concludes the lipidic pathway is very important for lipophilic molecules even though there is a low lipid content in nails (1% of total weight). To test the significance of nail lipid, the nail plate was incubated in a chloroform or methanol combination for 24 h. Even though the permeability of water, methanol, ethanol, and butanol was enhanced via this nail plate, the penetration of decanol and dodecanol was reduced [25].

4.2.3 Nature of the vehicle

In comparison with concentrated alcohols, saline diluted alcohol permeability was five times higher due to swollen pores by hyper hydration of nail plate by vehicle [26]. The nail plate functions as a hydrogel, and when it swells, the distance between keratin fibers increases,

creating wider holes for diffusing molecules, which, in turn, increases the penetration of active ingredients. Additionally, it has been claimed that switching out the water for a non-polar solvent lowers permeability since the mail plate is not as hydrated [27].

4.2.4 The vehicle's pH and solute charge, as well as the degree of ionization

The degree of ionization of mildly acidic and basic drugs is highly influenced by the formulation's pH. This has an impact on the drug's hydrophilicity, lipophilicity, and active drug solubility, as well as how it interacts with the nail plate and keratin fibres. Uncharged molecules penetrate to a larger extent than charged molecules, as has been widely observed.5 However, the pH of the miconazole donor solution was changed from 3.1 (dissociated) to 8.2 (undissociated), demonstrating that neither drug charge nor pH had an impact on the permeability coefficient [25]. It was also reported that the change in the pH of benzoic acid from 2.0 to 8.5, the permeation was decreased by 95.5% with increased lag time. Uncharged molecules have better permeability at pH 2.0 than charged ones did at pH 8.5 [28].

4.2.5 Hydration of nail plates

It was reported that the higher the degree of nail plate hydration, the higher the permeation of active molecules. According to one study, increasing relative humidity from 15% to 100% resulted in enhanced penetration of radio-labeled ketoconazole [5].

4.2.6 The presence of an intact dorsal layer

The tissue with intact or overlapped cells has the highest barrier to molecule permeation. If the layer is altered by means of debridement of any other chemical agent, the permeability increases [29].

4.2.7 Drug binding to keratin and other nail components

The keratin pH was found to be 5. As a result, at pH levels below and above, is both positively and negatively charged. which leads to either binding or repelling molecules based on their charge and ultimately results in less permeability of ionic molecules [26].

5. CONVENTIONAL THERAPIES

The conventional therapies include administration of oral antifungal agents (terbinafine, itraconazole, griseofulvin), administration of topical steroids, vitamin D, analogues, and 5-Flourouracil based on the severity of the infection. In severe conditions, steroidal injections are also reported. However, mono therapy is difficult to cure, but combined therapy may be effective. Avulsion surgery, which is exceedingly severe and unpleasant, is also conducted on a rare basis [30-32].

6. LIMITATIONS OF CONVENTIONAL THERAPY

To reach the site of action in the nail bed, the oral drugs must be delivered systemically. Drug distribution is limited to sub-therapeutic concentrations at the infected location due to inadequate blood circulation to the affected nail bed. This leads to failure of oral therapy. This necessitates the use of higher doses for a longer period of time [30]. High oral dosages have been linked to significant adverse effects, as well as a rise in the number of resistant microorganism strains [31].

7. TRANS UNGUAL DRUG DELIVERY (TUDD)

Topical therapy is an alternative and promising way of delivering active molecules by overcoming the limitations of conventional therapies. This is a non-invasive method and delivery of active molecules can be achieved to the infected tissue. Furthermore, the permeation can be enhanced by various techniques. The pre-absorptive loss occurs due to routine physical activity. The therapeutic concentration of actives at the infected site is reduced due to the binding of absorbed drugs with keratin. The drug must partition into the nail bed to achieve the minimum therapeutic concentration at the infected site. Further, the rate of drug delivery must be sufficient to compensate for the various losses such as metabolism, clearance, and binding of absorbed drugs in the nail bed. If a high concentration of drugs is loaded into the nail bed, ungual treatment can be beneficial [32].

8. CHALLENGES IN TUDD

The major barriers for TUDD are the nail plate thickness and hardness due to disulfide bonds. Both cause a longer diffusional pathway for active molecules. The nail plate acts like a hydrophilic gel membrane, which is different from skin, which is a lipophilic barrier. The differences (physical and chemical) between nail plate and skin help to understand the long duration of treatment and failure of conventional topical preparations [25,33]. As a result, various factors such as active molecule physicochemical properties, formulation characteristics, drug-keratin interactions, and penetration enhancement must be considered when designing an effective topical formulation for improved drug absorption [34].

9. STRATEGIES FOR PENETRATION OF DRUGS ACROSS HUMAN NAIL

9.1 Physical methods

9.1.1 Iontophoresis

It utilizes an electric field (electromotive force) to deliver the drugs across the biological membrane [35]. A study showed that iontophoresis significantly increased salicylic acid compared to passive transport through human nail plates fixed with diffusion cells [36]. Another study reported that increased ungual permeation of glucose and griseofulvin at pH levels above 5 in anodal iontophoresis. factors such as higher drug concentration, higher current density, higher buffer ionic strength, and higher pH [26].

9.1.2 Etching

It is the surface modification of a nail plate by exposing it to phosphoric acid to form microporosities. These microporosities improve the bio adhesion by increasing the wettability and decreasing the contact angle. This promotes additional bonding in the polymeric drug system, resulting in increased diffusion of the drug. The *in-vitro* bio adhesion of hydroxypropyl cellulose films with tartaric acid was found to be 12-fold greater than that of plain films without tartaric acid prepared by hot melt extrusion technology. Further, enhanced permeation was achieved with tartaric acid in etched nails compared with normal nails. Ketoconazole loaded hydroxypropyl cellulose prepared by hot melt extrusion showed 6-fold improved permeation in etched nails (10% phosphoric acid treated) than normal nail plates. Similarly, ketoconazole gel demonstrated 60% more permeability with etched nails compared with normal nails [37-38].

9.1.3 Carbon dioxide (CO_2) laser

It includes avulsion of the afflicted region followed by therapy at a power density of 5000 W/cm^2. As a result, the underlying tissue is immediately exposed to laser treatment. The therapy can also be performed without avulsion where a CO_2 laser beam is directly applied through the nail plate. This causes microporosities on which topical anti-fungal agents can be applied. A study showed that onychomycosis treatment was successful with CO_2 therapy in 21 d with mild or no pain compared with conventional therapy [39].

9.1.4 Hydration and occlusion

As discussed in the early section, hydration makes the increased pore size more elastic, which increases the permeability. According to reported study, raising the relative humidity to 100 percent improved the permeability of [3H]-ketoconazole tenfold [40]. An *in-vivo* study with nail patches of sertaconazole when replaced weekly observed 40–50% enhanced permeability [41]. The success rate was limited to 56% when treatment for onychomycosis was performed with avulsion and topical fungal therapy with and without occlusion. This includes 71% in the occlusion group and 38% from the non-occlusion group [42]. It was also observed that all patients who had avulsion and topical therapy (ketoconazole 2% cream against ciclopirox olamine 1% cream) during occlusion were clear of onychomycosis 1.5 y later; both antifungals were equally efficacious [43].

9.1.5 Micro needle

It is the process of opening holes in the subcutaneous directly to the skin capillaries using arrays of tiny needles; it also has the benefit of being too short to trigger the pain fibers, allowing absorption [11].

9.1.6 Laser therapy and ultraviolet light

In this method, micro holes are made in the nail plate using microsurgical laser apparatus (onycholaser). Topical antifungals can be delivered efficiently through these pores. The keratin in the nail plate would absorb the laser energy, causing vaporization and so the eradication of the nail layers. The use of lasers also causes the creation of craters. The structure, size, smoothness of the wall, presence of fissures, and melted and resolidfied tissue varies amongst laser types [44-47].

9.1.7 Microporation

In this method, the nail plate is drilled to form microconduite holes by using a device (Path Scientific, Carisle, The United States of America, approved by The United States Food and Drug Administration). These microconduites help more penetration of active molecules without effecting the nail bed. It was reported that enhanced permeability of 30% was observed when terbinafine cream and placebo cream were applied to drilled nail plates [48-49].

9.1.8 Low-frequency ultrasound or phonophoresis

The use of ultrasound (low frequency, 20kHz) with a 13 mm ultrasound probe at a distance of 13 mm from the nail enhanced permeability. The possible mechanism would be inertial cavitation or the formation of liquid microjects and further formation of pits. These cavitation's or pits function as conduits or microconduites for ungual drug flux. This method is applied in a pre-treatment procedure for topical therapy. This technique has been utilized to improve percutaneous penetration of joints, muscles, and nerves. Anesthetics, fluocinolone acetonide, and amphotericin B penetrate more effectively [50].

9.1.9 Photodynamic therapy (PDT)

Cells are destroyed using a combination of a sensitising agent and visible light in this therapy. In the field of oncology, PDT based on topical administration of aminolevulinic acid is employed. For complete cure, the nail bed was first treated with a 20% solution of 5-aminolevulinic acid methyl ester, followed by radiation with an excimer laser at 630 nm at 100 J/cm^2 (six to seven sessions). Topical therapy was observed to be ineffective for treating onychomycosis by *T. rubrum*. Additionally, it showed complete recovery in just two weeks when the nail plate was softened by 40% urea (for 7 d), followed by the application of aminolevulinic acid for up to 3 h and broadband red light (630 nm) at 37 J/cm^2 for 7 min and 20 sec to remove dermatophytes. There was no mention of any adverse effects. However, for PDT to work best, all hyperkeratotic debris and diseased nails needed to be completely removed [51].

9.2 Chemical methods

Various chemical agents improve the trans ungual penetration of active molecules. The breaking of chemical and physical linkages that maintain the integrity of the keratin in the nail plate is one potential mechanism. For ungual penetration, disulfide, peptide, hydrogen, and polar bonds are potential targets. Further, the transdermal enhancers are less effective due to the low lipid content in the nail plate. The chemical enhancer may be used either before or simultaneously with the therapeutic formulation to apply to the nail plate [52].

9.2.1 Reducing substances that cleave the disulfide bond on the nail

9.2.1.1 Thiols

These are the sulfhydryl-group-containing substances that weaken the disulfide bonds in the nail's keratin matrix. N-acetylcysteine, mercaptoethanol, N-(2-mercaptopropionyl) glycine, pyrithone, and thioglycolic acid are some of the agents [53]. The cleavage is irreversible in nature. Before applying the medication, the enhancer can be applied to the nail plates (rather than being used in drug formulation) to avoid the drug enhancer compatibility issues. When N-acetylcysteine was investigated, the *in-vivo* improved retention of oxiconazole in upper nail layers was found [54]. Another study found that the antifungal agent tolnaftate was more permeable in the top layers of nail clippings when N-acetylcysteine and 2-mercaptoethanol were present, which was ascribed to swelling and weakening of the nail plate [55]. A thioglycolic acid-mediated redox mechanism involving nail disulfide linkage, which bypasses the nail's barrier integrity, facilitates the drug flow [56].

9.2.1.2 Sulfites

The formation of thiosulfates and thioisulfates occurs when proteins and peptides having disulfide bonds are incubated with sodium sulfite. Sodium sulfite is thought to reduce bonds in the nail plate and increase ungual drug flux [57]. A study showed that enhanced permeation of 5,6-carboxyfluoresceine through nail clippings was observed in the presence of sodium sulfite in healthy volunteers. The enhanced permeation was observed for both pre-treatment and on co-application [52,58].

9.2.2 An oxidizing agent that cleaves the nail's disulfide bond

9.2.2.1 Hydrogen peroxide

It works by breaking the disulfide bonds in the nail plate and oxidizing them. The combination of hydrogen peroxide with urea further improves the ungual permeation. It was reported that hydrogen peroxide (35% in aqueous vehicle) pre-treatment for 20 h improved the permeation of mannitol by 3 fold. The MedNail platform technology revealed an 18-fold improved terbinafine permeability when the nail plate was treated with a reducing agent followed by urea hydrogen peroxide [44,59].

9.2.2.2 Water

Water improves penetration by hydrating and swelling nails when they come into touch with it. Water mostly aids in improving the ungual flow of hydrophilic agents. The enhanced flux of hydrophilic 5-flurouracil, N-acetyl cysteine, and mercaptoethanol from aqueous vehicles [55]. A study showed that increasing ambient relative humidity from 15 to 100% observed a 3 fold *in-vitro* and a 400 fold *in-vivo* flux of [3H]-ketoconazole. This study demonstrates that increased nail plate hydration and relative humidity are responsible for improved permeability [40,60]. It was determined that enhanced drug flow and nail swelling do not coexist. Resorcinol and urea-induced swelling were said to not significantly increase the flow of mannitol. Additionally, it was noted that because the vehicle was lipophilic, increasing tolnaftate flow was not linked to nail swelling [43,55,59,61].

9.2.2.3 Keratolytic enzymes and enhancers

These enzymes enhance drug permeation by hydrolyzing nail keratins, thereby weakening the nail barrier. A study showed enhanced permeation of metformin hydrochloride when bovine hood membranes were treated with keratolytic enzymes. Another study showed that incubation of nail clippings (hoof membranes) with enzymes caused separation of coenocytes, which showed increased flux and enhanced permeability coefficient. The enzymatic damage is irreversible. These enzymes can be formulated into formulations for pre-treatment [22]. The permeability of topical antifungals was increased when nail plates were incubated with papain solution for one day and then treated with salicylic acid for ten days. This may be attributed to aggressive damage to the nail surface, which creates a pathway for drug penetration [44]. For miconazole, ketoconazole, and itraconazole, comparable research has been published. For a 60-day period, no penetration of these antifungals was shown without the presence of keratolytic agents [62]. As penetration enhancers, thioglycolic acid, a reducing sugar, urea, and hydrogen peroxide as oxidizing agents were used to test the permeability of several lipophilic substances including caffeine, methyl paraben, and terbinafine. This combination significantly enhanced the permeation of all the gents by 2-4 fold. The reported mechanism involved breaking down keratin disulphide bonds and creating pores that provide additional drug transport channels that are open [56].

9.2.3 Miscellaneous agents

9.2.3.1 N-acetyl-l-cysteine and mercaptan compounds

It was proven that N-acetyl-l-cysteine and 2-mercaptoethanol together increased tolnaftate permeability [55]. N-acetyl-l-cysteine increased the permeability of oxiconazole, according to an *in-vivo* investigation. Additionally, it improved the retention of oxiconazole in the top layers of the nails [54].

9.2.3.2 2-n-nonyl-1,3-dioxolane

When compared to the control group, the econazole nail lacquer formulation with 18% 2-n-nonyl-1,3-dioxolane demonstrated six times greater penetration into the human nail (without

enhancer). The increased penetration was almost 14000 times more than the minimal inhibitory concentration required to stop the growth of the fungus [4].

N-methyl-2-pyrrolidone, polypropylene glycol 400, dimethyl sulfoxide, labrasol, mercaptoethanol, and transcutol, among other substances, shown improved penetration through the hoof membrane of bovine. Additionally, polyethylene glycols and sodium dodecyl sulphate were recognised as possible trans ungual enhancers. Further, inorganic salts also showed enhanced permeation of terbinafine hydrochloride. This improved permeability was attributable to higher thermodynamic activity and hydration. Gel formulation was used to study the effects of polyethylene glycols on terbinafine hydrochloride through passive and iontophoretic mechanisms. Permeation was better for low molecular weight glycols compared with high molecular weights. This is caused on by the nail plate swelling and increased water absorption [44.63-64].

9.3 Mechanical methods

Dermatologists and podiatrists have utilised invasive, possibly painful mechanical procedures such nail avulsion and nail abrasion.

9.3.1 Nail abrasion

The nail is made up of three layers: the dorsal layer, the intermediate layer, and the ventral layer, which are arranged in the ratios 3:5:2. In this technique, an abrasive is used to fill the nail plate's surface. The dorsal layer of the nail plate, which serves as a main barrier to drug absorption, is removed during this filling. The permeability coefficient was doubled for 5-flurouracil and flubiprofen when abrasive filling was done *in-vitro* [65]. Additionally, the nail plate is sanded on the nail edges using sandpaper with a number 150 or 180. Prior to applying nail lacquer, sanding is done to lessen the crucial fungal mass [2,66].

9.3.2 Nail avulsion

Under local anaesthesia, complete and partial nail avulsions entail the surgical removal of the full or part of the nail plate. The nail plate is made more malleable for avulsion by keratolytic chemicals like urea and salicylic acid [67].

10. PERMEATION STUDY MODELS FOR TRANS UNGUAL DELIVERY

10.1 *In-vitro* models for drug permeation studies

10.1.1 Animal hoof membranes

In terms of keratin, human nail plates and animal hooves are identical. Hence, it is an alternative for human nail permeation studies [68]. Hence, bovine [69], porcine [70], and horse [61] hooves are the best models for *in-vitro* permeation studies. It was revealed that when human nails and horse feet were submerged in control solution, the weight of the hooves increased by 40±9% while the weight of the human nails increased by 27±3%. This demonstrates that due to the less thick keratin in the hoof, an animal's hoof can store more water and is more porous than a human nail [61,71].

10.1.2 Nail clippings

Healthy human volunteers' finger and toe nail clippings are tested for trans ungual formulations. Clippings are hydrated by soaking in suitable media. The hydrated nail

clippings are placed on a nail adapter and sandwiched between the donor and receiver compartments of the diffusion cell to perform permeation [72]. Using these nail clippings, several researchers investigated physical penetration enhancement techniques. However, because of the lack of a nail bed, nail clippings are not regarded as the best model for ungual permeation research [26,36,73].

10.1.3 Cadaver nail plate

These are employed in the evaluation of topical formulations and the investigation of physical techniques [53,74]. In order to study how terbinafine hydrochloride penetrates cadaver nail plates, sodium sulphite was used as a penetration enhancer. A vertical Franz diffusion cell with a nail plate mounted on it was used for the investigation. The addition of salts increased penetration by three to five times [64]. Similar studies were reported for caffeine [12], urea, mannitol, tetraethylammonium [75].

10.1.4 Human keratin film

The film was made from hair after it had been processed and ground into powder. The film was evaluated for permeation of antifungal agents and was compared with the bovine hoof by infecting it with *T. rubrum* prior to the permeation study. According to the study, both models scored equally, and the film model is used to represent an infected nail. It was also mentioned that the thickness is not much greater than 100 µm, offering a flat area for better fungal adhesion and proliferation than human nail clippings or powder [76].

10.2 *Ex-vivo* models for drug permeation studies

TurChub and ChubTur are two platform technologies used to study antifungal efficacy *ex-vivo*. A receptor compartment of TurChub is filled with agar gel, which is used to cultivate fungus. The donor compartment consists of an antifungal agent. In between both, the compartment nail plate (healthy) is fixed for permeation. The study is concluded based on the zone of inhibition, the extraction of drug from the nail plate and further analysis. In ChubTur, the effectiveness is assessed by seeing how antifungal drugs pass through diseased nails as opposed to healthy nails. The formulation is given topically, and viable counts, biomarkers, and enzyme tests are used to track the recovery of microorganisms [77-78]. The research reported antifungal efficacy of bifonazole and sodium pyrithione using an onychomycosis model. The technique comprises of an agar plate containing *T. mentagrophytes* conidia and antifungal agents in various compartments. A healthy nail plate was used as a permeation model. The study's conclusion was based on the zone of inhibition and fungal colony measured by image analysis [79]. Both the above mentioned models were validated by establishing the efficacy of terbinafine hydrochloride, loceryl (Galderma, Lausanne, Switzerland) and penlac (Sanofi-Aventis, Deutschland) permeated through human nail samples. In this study, *T. rubrum* was used as a model fungal agent. Adenosine triphosphate levels in all cells were analysed three weeks following the initial *T. rubrum* inoculation [78].

10.3 *In-vivo* models for drug permeation studies

In a guinea pig *Tinea unguium* model, the pharmacological impact of a triazole antimycotic drug (KP-103) was compared with amorolfine and terbinafine. To create *Tinea unguium* and *Tinea pedis*, paper discs covered with a fungus suspension (*T. mentagrophytes* SM-110) were applied to toes. For 30 d consecutive, 0.1 mL of each agent's 1 % solution was administered once daily to infected feet. The deeper layers of the nail must be penetrated for topical medications to be evaluated for effectiveness; this is not the case with the model [80].

In a different *in-vivo* experiment, rabbits' nails were treated with 0.2 mL of microconidia suspension between the proximal nail fold and the lunula after receiving intramuscular injections of methylprednisolone acetate to suppress their immune systems. Topical ciclopirox (8 % w/v) and amorolfine (5 % w/v) were the model agents. In order to make a nail appropriate for fungal development in 2 weeks, 0.5 mL of sterile water was injected into a nail covered with a finger cot. This condition was then maintained for 0, 2, or 6 weeks without the finger cot and gauze patch. The time period after infection was known as post-infection period (PIP). Following each PIP, the animals were sacrificed, and the nails from the treated paw were removed for histological and microbiological analysis [81]. In an experiment, an excised toe was employed as a model for *in-vivo* trans ungual permeation experiments. However, there is no drug clearance from the nail bed or matrix in this model. Nonetheless, cadaver toes are one of the most ideal models for TUDD experiments since they closely resemble *in-vivo* in many ways [44,74].

11. *IN-VITRO* AND *IN-VIVO* (IVIVC) CORRELATION

It was necessary to compare the *in-vitro* data obtained using radioisotopes and atomic mass spectroscopy with the data obtained *in-vivo* utilising modified diffusion cells, nail clippings, avulsed human cadaver nail plates, and animal hooves as a model for human nail plates. Studies conducted *in-vitro* on people and animals assume that penetration is a passive process without a live component [50,82].

12. FORMULATION ADVANCEMENTS IN TRANS UNGUAL THERAPY

Advanced formulation strategies are needed to deliver a sufficient dose of active to the site of action. Table 1. shows recent advancements in TUDD with various formulations of topical antifungal agents formulated in conventional and novel nano formulations with suitable penetration enhancers.

Table 1. Recent formulation advancements in TUDD system

Formulation	Active	Permeation enhancers	Model used	Method	Treatment	Reference
Lacquer	Ciclopirox	Cyclodextrins	Bovine hooves	*In-vitro*	--	83
Lacquer	Terbinafine	Polyurethane	Human keratinocyte cell line	*In-vitro*	--	84
Gel	Ciclopirox olamine, Terbinafine	Propylene glycol, Polaxomer 407	Keratin film, bovine hooves	*In-vitro*	--	85
Lacquer	Ketoconazole	Thioglycolic acid, Urea, Hydrogen peroxide	Human finger nails	*Ex-vivo*	--	86
Lacquer	Terbinafine	--	Keratin film, Bovine hooves	*In-vitro*	--	87
Lacquer	Terbinafine	Hydroxypropyl-β-cyclodextrin	Human finger nails	*Ex-vivo*	Onychomycosis	88
Lacquer	Naftifine	--	--	--	--	89
Patch	Ciclopirox olamine	Propylene glycol, Thiourea	Cadaver toenails	*In-vitro*	Onychomycosis	90
Patch	Terbinafine	Polypropylene glycol 400, Polyethylene glycol 200, N-methyl-2-pyrrolidone	Porcine hoof	*In-vitro*	--	91
Microspheres injection	Terbinafine	--	Cadaver toe	*Ex-vivo*	Onychomycosis	92
Lacquer	Tolnaftate	Thioglycolic acid	Bovine hoof membrane	*Ex-vivo*	Onychomycosis	93
Microemulgel	Terbinafine	--	Cadaver human skin	*In-vitro*	Onychomycosis	94, 95
Lacquer	Ciclopirox olamine	--	Pig skin, Cow horn keratin slices, Human toenails	*In-vitro*	Onychomycosis	96
Film	Terbinafine	Bio penetrant Beta vulgaris	Keratin film	*In-vitro*	--	97
Gel	Fluconazole	Bio penetrant Pelargonium hortorum	Cadaver human nail	*Ex-vivo*	--	98
Nano vesicles	Terbinafine	--	Cadaver human nail	*Ex-vivo*	Onychomycosis	99

In-situ gel, Lacquer	Terbinafine	--	Cadaver human nail	*Ex-vivo*	Onychomycosis	100
Nanocapsule Suspensions and Nanoemulsion	Tea tree oil (Melaleuca alternifolia)	--	Human nails	*In-vitro*	Onychomycosis	101
Lacquer	Miconazole	Hydroxypropyl-β-cyclodextrin	Cellophane membrane	*In-vitro*	Onychomycosis	102
Cream	Ketoconazole, Oxiconazole	Potassium hydroxide	Human nails	*In-vivo*	Onychomycosis	42
Lacquer	Ketoconazole	--	Cadaver human nail	*In-vitro*	Onychomycosis	103
Solution	Panthenol	--	Cadaver human nail	*In-vitro*	--	104
Patch	Clotrimazole	--	--	--	Onychomycosis	105
Lacquer	Isotretinoin	Glycerol, Polyethylene glycol 400, Thioglycolic acid, Eugenol	Human nails, Bovine hooves	*Ex-vivo*	Nail psoriasis	106
Lacquer	Ciclopirox olamine	Endopeptidase enzyme	Human finger nails	*Ex-vivo*	Onychomycosis	107
Lacquer	Apremilast	Salicylic acid, Dexpanthenol	Human volunteers	*In-vivo*	Nail psoriasis	108
Solution	Amorolfine, Ciclopirox, Efinaconazole, Luliconazole, Terbinafine	Propylene glycol	Healthy human nails	*In-vitro*	Onychomycosis	109
Lacquer	Ciclopirox olamine, Amorolfine	--	Bovine hoof slices	*In-vitro*	Onychomycosis	110
Lacquer	Ciclopirox olamine	--	Healthy males	*In-vivo*	Onychomycosis	111
Gel	Nystatin	Cetylpyridinium chloride, Tween 80	Bovine hoof membrane	*In-vitro*	Onychomycosis	112
Lacquer, Gel	Ciclopirox olamine	Labrasol, Mercaptoethanol Transcutol	Porcine hoof membrane	*In-vitro*	Onychomycosis	70
Powder	Terbinafine	--	Nail plate	*In-vitro*	Onychomycosis	113

Hydrogel colloidal carrier system, Lacquer, Solution	EV-086K	--	Bovine hoof membrane	*Ex-vivo*	Onychomycosis	114
Hydrogel based lacquer	Triamcinolone acetonide, Ciclopirox olamine	Cyclodextrin, Poloxamer polypseudorotaxanes	Bovine hoof	*In-vitro*	Onychomycosis	115
Lacquer	Terbinafine	N-acetyl-L-cysteine, 2-Mercaptoethanol, Thioglycolic acid	Human cadaver toe nail plates	*Ex-vivo*	Onychomycosis	116
Lacquer	Ciclopirox olamine, Clobetasol propionate	Methyl-β-cyclodextrin, Poloxamers	Bovine hooves, Cadaver Finger and toe nail	*In-vitro*	Onychomycosis	117
Nanostructured lipid carriers	Voriconazole	Urea	Porcine hooves	*In-vitro*	Onychomycosis	118
Nano emulgel	Ketoconazole	Thioglycolic acid	Goat hooves	*In-vitro*	Onychomycosis	119
Lacquer	Clobetasol-17-propionate	--	Human patients	*In-vivo*	Nail psoriasis	120
Bilayered lacquer	Terbinafine	Polyethylene glycol 400	Cadaver nails	*In-vitro*	Onychomycosis	121
Lacquer	Ciclopirox olamine	Urea, Potassium hydroxide	Bovine hoof membranes	*In-vitro*	Onychomycosis	122
Microemulgel	Terbinafine	N-acetyl-l-cysteine, Urea	Animal hooves	*In-vitro*	Onychomycosis	123, 124
Lacquer	Tioconazole	Thioglycolic acid, Urea	Cattle hooves	*In-vitro*	Onychomycosis	125
Gel	Terbinafine	Polyethylene glycols	Human cadaver finger nails	*In-vitro*	Onychomycosis	126
Glycerogelatins film	Fluconazole	--	Goat hooves	*In-vitro*	Onychomycosis	127
Film	Fluconazole	Biopenetrant Iresine herbestii	Cadaver human nail	*Ex-vivo*	Onychomycosis	128
Solution	Efinaconazole	--	Guinea pig, Human patients	*In-vivo*	Onychomycosis	129
Microemulgel	Itraconazole	Benzoyl alcohol, Urea, Salicylic acid	Healthy human nail	*In-vitro*	Onychomycosis	130
Gel	Terbinafine	Phosphoric acid, Lactic acid	Human cadaver fingernails	*In-vitro*	Onychomycosis	131

13. USE OF HERBS IN TRANS UNGUAL THERAPY

The methanolic extract of the fruit of *Psidium sartorianum*, the ether extract of the *Nigella sativa* seed (thymoquinone active component), the ethanol extract of seeds and leaves of *Moringa oleifera*, essential oil from *Eugenia caryophyllata* and *Lawsonia inermis* (henna) (lawsone as the principle component) were effective against *candida* and *trichophyton* species. *Ocimum gratissimum* (Ram Tulsi) for its eugenol content, which is used to cure onychomychosis. *Allium sativum* (garlic) is found effective in treating finger nail infections. It has been demonstrated that *Matricaria recuita* (chamomile) and *Camellia sinensis* (green tea) applied topically and consumed as a tea three times daily can stop the development of bacteria and fungus. *Thymus vulgaris* is shown to damage the cell membranes of the fungus due to the presence of thymol. Other agents that have been shown to be effective against fungal infections include *Zingiber officinale* root, *Cayenne pepper*, *Echinacea* (daisy flower family member), tea tree oil extracted from *Melaleuca alternifolia*, and apple cider vinegar. In cases of moderate to severe infection, none of these treatments produce significant results [132-136].

14. CONCLUSION

Nail diseases are difficult to treat since they require long-term therapy and have a high recurrence rate. Topical therapy is an alternative to oral delivery due to its limited systemic effects. The physicochemical factors are significant in the diffusion of molecules. Hydration is important for absorption through the nail because the nail plate functions like a concentrated hydrogel. The highly keratinized barrier has been overcome using a variety of mechanical, physical, and chemical methods. Due to the limited availability of human nails, animal hooves have been used for *in-vitro* permeation studies, despite the fact that hooves have a much greater water absorption capacity than nails, which might cause an overestimation of drug permeability. Keratin films, which are nail substitutes, are the most recent advancement for the permeation model. Researchers have used nail formulations such as nail lacquer, adhesive patches, and gels to improve hydration of the nail plate because of their occlusive qualities. Utilizing nanotechnology also results in reduced degradation of actives, greater patient compliance, and enhanced bioavailability. However, due to limited progress in the development of TUDD, there is a need to establish more data for permeation models, permeation enhancement methods, and novel formulations to improve mathematical modelling of IVIVC correlation. Since, without IVIVC, *in-vitro* and biological interpretations are both suspect.

CONSENT

Not applicable

ETHICAL APPROVAL

Not applicable

REFERENCES

1. Kapil K, Fateh V, Ahmad S, Navin CP, Sweta, P. Drug delivery across human nail: a newer approach. Int J Res Dev Pharm Life Sci. 2014;3(6):1217-22.

2. Di Chiacchio N, Kadunc BV, de Almeida AR, Madeira CL. Nail abrasion. J Cosmet Dermatol. 2003;2(3-4):150-2. DOI: 10.1111/j.1473-2130.2004.00095.x. PMID: 17163921.
3. Verma S, Singh RA. Transungual drug delivery a pivotal remedy in onychomycosis. J Chem Pharm Res. 2016;8(4):370-81.
4. Rajendra VB, Baro A, Kumari A, Dhamecha DL, Lahoti SR, Shelke SD. Transungual Drug Delivery: An Overview. J Appl Pharm Sci. 2012;2(1):203-9.
5. Firoz S, Naga SM, Rajalakshmi R. Transungual drug delivery system – a review. Int J Innov Drug Discov. 2011;1(1):9-14
6. Baden HP, Goldsmith LA, Fleming B. A comparative study of the physicochemical properties of human keratinized tissues. Biochim Biophys Acta. 1973;322(2):269-78. DOI: 10.1016/0005-2795(73)90303-6. PMID: 4765091.
7. Devyani S, Tarun KS, Bhartendu S, Gurpreet S. An overview on transungual drug delivery system. Eur J Biomed Pharm Sci. 2015;2(5):168-178.
8. Gerard JT, Bryan D. Principles of anatomy and physiology. 13th ed. John wiley & sons inc; 2012.
9. Anne W, Allison G. Ross and Wilson anatomy and physiology in health and illness. 9th ed. Churchil livingstone; 2001.
10. Aeena J, Delma D, Joseph M, Sreeja N. Keratinised nail plate: a novel transungual drug delivery platform. Pharmanest Int J Adv Pharm Sci. 2014;5(5):2339-45.
11. Pati NB, Dey BK, Das S, Sahoo S. Nail Drug Delivery System: A Review. J Adv Pharm Edu Res. 2012;2(3):101-9.
12. Vejnovic I, Simmler L, Betz G. Investigation of different formulations for drug delivery through the nail plate. Int J Pharm. 2010;386(1-2):185-94. DOI: 10.1016/j.ijpharm.2009.11.019. PMID: 19941943.
13. Elewski BE, Charif MA, Daniel CR. Onychomycosis in Nails: Therapy, Diagnosis, Surgery. 2nd ed. WB Saunders; 1997.
14. Forslind B, Thyresson N. On the structure of the normal nail. A scanning electron microscope study. Arch Dermatol Forsch. 1975;251(3):199-204. DOI: 10.1007/BF00561761. PMID: 1115523.
15. Suryavanshi KA, Basrur PR, Katedeshmukh RG. Review on Nail (Transungual) Drug Delivery System. Am J PharmTech Res. 2012;2(5):233-44.
16. Vikrant KN, Kiran BK, Vinayak MG, Ramdas TD, Kiran BD Sachin BS, et al. Transungual drug delivery system, a review. Pharmacologyonline. 2011;1(1):126-39.
17. Runne U, Orfanos CE. The human nail: structure, growth and pathological changes. Curr Probl Dermatol. 1981;9:102-49. PMID: 7273840.
18. Effendy I, Lecha M, Feuilhade de Chauvin M, Di Chiacchio N, Baran R, European Onychomycosis Observatory. Epidemiology and clinical classification of onychomycosis. J Eur Acad Dermatol Venereol. 2005;19(1):8-12. DOI: 10.1111/j.1468-3083.2005.01281.x. PMID: 16120199.
19. Philip R. Ciclopirox (Penlac) nail lacquer for onychomycosis. Med Lett Drugs Ther. 2000;42(1080):51–2.
20. Pradeep SP, Sangita VB, Ashwin AT. Nailing the nail trouble by transungal drug delivery. Eur J Pharm Med Res. 2015;2(2):551-71.
21. Murdan S. Drug delivery to the nail following topical application. Int J Pharm. 2002;236(1-2):1-26. DOI: 10.1016/s0378-5173(01)00989-9. PMID: 11891066.
22. Mohorcic M, Torkar A, Friedrich J, Kristl J, Murdan S. An investigation into keratinolytic enzymes to enhance ungual drug delivery. Int J Pharm. 2007;332(1-2):196-201. DOI: 10.1016/j.ijpharm.2006.09.042. PMID: 17097244.
23. Singh DH, Roychowdhury S, Sharma D, Bhandari V, Singh M. Transungual Drug Delivery-A Novel Approach of Unique Features. Indo Am J Pharm Res. 2013;3(6):4460-70. DOI: 10.1044/1980-iajpr.00301.

24. Gupchup GV, Zatz JL. Structural characteristics and permeability properties of the human nail: a review. J Cosmet Sci. 1999;50:363–85.
25. Walters KA, Flynn GL, Marvel JR. Physicochemical characterization of the human nail: solvent effects on the permeation of homologous alcohols. J Pharm Pharmacol. 1985;37(11):771-5. DOI: 10.1111/j.2042-7158.1985.tb04966.x. PMID: 2867157.
26. Murthy SN, Waddell DC, Shivakumar HN, Balaji A, Bowers CP. Iontophoretic permselective property of human nail. J Dermatol Sci. 2007;46(2):150-2. DOI: 10.1016/j.jdermsci.2006.12.010. PMID: 17289349.
27. Murdan S. Drug delivery to the nail following topical application. Int J Pharm. 2002;236(1-2):1-26. DOI: 10.1016/s0378-5173(01)00989-9. PMID: 11891066.
28. Walters KA, Flynn GL, Marvel JR. Penetration of the human nail plate: the effects of vehicle pH on the permeation of miconazole. J Pharm Pharmacol. 1985;37(7):498-9. DOI: 10.1111/j.2042-7158.1985.tb03050.x. PMID: 2863357.
29. Piérard G. Onychomycosis and other superficial fungal infections of the foot in the elderly: a pan-European survey. Dermatology. 2001;202(3):220-4. DOIi: 10.1159/000051640. PMID: 11385227.
30. Nieworth M, Korting HC. Management of onchomycosis. Drugs. 1999;58(2):283-96. DOI: 10.2165/00003495-199958020-00005. PMID: 10473020.
31. Repka MA, O'Haver J, See CH, Gutta K, Munjal M. Nail morphology studies as assessments for onychomycosis treatment modalities. Int J Pharm. 2002;245(1-2):25-36. DOI: 10.1016/s0378-5173(02)00321-6. PMID: 12270239.
32. Scher RK. Onychomycosis: therapeutic update. J Am Acad Dermatol. 1999;40(6):S21-6. DOI: 10.1016/s0190-9622(99)70397-x. PMID: 10367912.
33. Mertin D, Lippold BC. In-vitro permeability of the human nail and of a keratin membrane from bovine hooves: influence of the partition coefficient octanol/water and the water solubility of drugs on their permeability and maximum flux. J Pharm Pharmacol. 1997;49(1):30-4. DOI: 10.1111/j.2042-7158.1997.tb06747.x. PMID: 9120766.
34. Walters KA, Flynn GL, Marvel JR. Physicochemical characterization of the human nail: permeation pattern for water and the homologous alcohols and differences with respect to the stratum corneum. J Pharm Pharmacol. 1983;35(1):28-33. DOI: 10.1111/j.2042-7158.1983.tb04258.x. PMID: 6131961.
35. Kassan DG, Lynch AM, Stiller MJ. Physical enhancement of dermatologic drug delivery: iontophoresis and phonophoresis. J Am Acad Dermatol. 1996;34(4):657-66. DOI: 10.1016/s0190-9622(96)80069-7. PMID: 8601657.
36. Narasimha Murthy S, Wiskirchen DE, Bowers CP. Iontophoretic drug delivery across human nail. J Pharm Sci. 2007;96(2):305-11. DOI: 10.1002/jps.20757. PMID: 17080425.
37. Repka MA, Mididoddi PK, Stodghill SP. Influence of human nail etching for the assessment of topical onychomycosis therapies. Int J Pharm. 2004;;282(1-2):95-106. DOI: 10.1016/j.ijpharm.2004.06.010. PMID: 15336385.
38. Mididoddi PK, Prodduturi S, Repka MA. Influence of tartaric acid on the bioadhesion and mechanical properties of hot-melt extruded hydroxypropyl cellulose films for the human nail. Drug Dev Ind Pharm. 2006;32(9):1059-66. DOI: 10.1080/03639040600683410. PMID: 17012118.
39. Rothermel E, Apfelberg DB. Carbon dioxide laser use for certain diseases of the toenails. Clin Podiatr Med Surg. 1987;4(4):809-21. PMID: 2960438.
40. Gunt HB, Kasting GB. Effect of hydration on the permeation of ketoconazole through human nail plate in vitro. Eur J Pharm Sci. 2007;32(4-5):254-60. DOI: 10.1016/j.ejps.2007.07.009. PMID: 17928205.
41. Susilo R, Korting HC, Greb W, Strauss UP. Nail penetration of sertaconazole with a sertaconazole-containing nail patch formulation. Am J Clin Dermatol. 2006;7(4):259-62. DOI: 10.2165/00128071-200607040-00007. PMID: 16901186.

42. Grover C, Bansal S, Nanda S, Reddy BS, Kumar V. Combination of surgical avulsion and topical therapy for single nail onychomycosis: a randomized controlled trial. Br J Dermatol. 2007;157(2):364-8. DOI: 10.1111/j.1365-2133.2007.08014.x. PMID: 17573877.
43. Baden HP. Treatment of distal onychomycosis with avulsion and topical antifungal agents under occlusion. Arch Dermatol. 1994;130(5):558-9. PMID: 8179339.
44. Shivakumar HN, Juluri A, Desai BG, Murthy SN. Ungual and transungual drug delivery. Drug Dev Ind Pharm. 2012;38(8):901-11. DOI: 10.3109/03639045.2011.637931. PMID: 22149347.
45. Bristow IR. The effectiveness of lasers in the treatment of onychomycosis: a systematic review. J Foot Ankle Res. 2014;7:34. DOI: 10.1186/1757-1146-7-34. PMID: 25104974.
46. Azagury A, Khoury L, Enden G, Kost J. Ultrasound mediated transdermal drug delivery. Adv Drug Del Rev. 2014;72;127–43. DOI: 10.1016/j.addr.2014.01.007.
47. Elkeeb R, AliKhan A, Elkeeb L, Hui X, Maibach HI. Transungual drug delivery: current status. Int J Pharm. 2010;384(1-2):1-8. DOI: 10.1016/j.ijpharm.2009.10.002. PMID: 19819318.
48. Salter SA, Ciocon DH, Gowrishankar TR, Kimball AB. Controlled nail trephination for subungual hematoma. Am J Emerg Med. 2006 Nov;24(7):875-7. DOI: 10.1016/j.ajem.2006.03.029. PMID: 17098113.
49. Boker A, Ciocon D, Kimbell AA. randomized, double-blind, placebo-controlled, pilot study of 1% terbinafine cream applied twice daily and delivered via nail plate microporation for the treatment of subungual toenail onychomycosis. J Am Acad Dermatol. 2007;56 (2):AB114. DOI: 10.1016/j.jaad.2006.10.534.
50. Murdan S. Enhancing the nail permeability of topically applied drugs. Expert Opin Drug Deliv. 2008;5(11):1267-82. DOI 10.1517/17425240802497218. PMID: 18976136.
51. Piraccini BM, Rech G, Tosti A. Photodynamic therapy of onychomycosis caused by Trichophyton rubrum. J Am Acad Dermatol. 2008;59(5):S75-6. DOI: 10.1016/j.jaad.2008.06.015. PMID: 19119130.
52. Saner MV, Kulkarni AD, Pardeshi CV. Insights into drug delivery across the nail plate barrier. J Drug Target. 2014;22(9):769-89. DOI: 10.3109/1061186X.2014.929138. PMID: 24964054.
53. Malhotra GG, Zatz JL. Investigation of nail permeation enhancement by chemical modification using water as a probe. J Pharm Sci. 2002;91(2):312-23. DOI: 10.1002/jps.10058. PMID: 11835191.
54. Hoogdalem EJ, Van den Hoven WE, Terpstra IJ, Zijtveld J, Verschoor JSC, Visser JN. Nail penetration of the antifungal agent oxiconazole after repeated topical application in healthy volunteers, and the effect of acetylcysteine. Eur J Pharm Sci. 1997;5(3):119–27. DOI: 10.1016/S0928-0987(97)00270-4.
55. Kobayashi Y, Miyamoto M, Sugibayashi K, Morimoto Y. Enhancing effect of N-acetyl-l-cysteine or 2-mercaptoethanol on the in vitro permeation of 5-fluorouracil or tolnaftate through the human nail plate. Chem Pharm Bull (Tokyo). 1998;46(11):1797-802. DOI: 10.1248/cpb.46.1797. PMID: 9845958.
56. Brown MB, Khengar RH, Turner RB, Forbes B, Traynor MJ, Evans CR, et al. Overcoming the nail barrier: A systematic investigation of ungual chemical penetration enhancement. Int J Pharm. 2009;370(1-2):61-7. DOI: 10.1016/j.ijpharm.2008.11.009. PMID: 19071202.
57. Stricks W, Kolthoff IM. Equilibrium constants of the reactions of sulfi te with cystine and with dithiodiglycolic acid. J Am Chem Soc. 1951;73(10):4569-74. DOI: 10.1021/ja01154a022.
58. Welsh O, Vera-Cabrera L, Welsh E. Onychomycosis. Clin Dermatol. 2010;28(2):151-9. DOI: 10.1016/j.clindermatol.2009.12.006. PMID: 20347657.

59. Khengar RH, Jones SA, Turner RB, Forbes B, Brown MB. Nail swelling as a pre-formulation screen for the selection and optimisation of ungual penetration enhancers. Pharm Res. 2007;24(12):2207-12. DOI: 10.1007/s11095-007-9368-3. PMID: 17665289.
60. Gunt HB, Miller MA, Kasting GB. Water diffusivity in human nail plate. J Pharm Sci. 2007;96(12):3352-62. DOI: 10.1002/jps.20988. PMID: 17786922.
61. Gradisar H, Friedrich J, Krizaj I, Jerala R. Similarities and specificities offungal keratinolytic proteases: comparison of keratinases of Paecilomyces marquandii and Doratomyces microsporus to some known proteases. Appl Environ Microbiol. 2005;71(7):3420-6. DOI: 10.1128/AEM.71.7.3420-3426.2005. PMID: 16000744.
62. Quintanar-Guerrero D, Ganem-Quintanar A, Tapia-Olguin P, Kalia YN, Buri P. The effect of keratolytic agents on the permeability of three imidazole antimycotic drugs through the human nail. Drug Dev Ind Pharm. 1998;24(7):685-90. DOI: 10.3109/03639049809082373. PMID: 9876516.
63. Murthy SN, Vaka SR, Sammeta SM, Nair AB. TranScreen-N: Method for rapid screening of trans-ungual drug delivery enhancers. J Pharm Sci. 2009;98(11):4264-71. DOI: 10.1002/jps.21743. PMID: 19363796.
64. Nair AB, Sammeta SM, Vaka SR, Narasimha Murthy S. A study on the effect of inorganic salts in transungual drug delivery of terbinafine. J Pharm Pharmacol. 2009;61(4):431-7. DOI: 10.1211/jpp/61.04.0003. PMID: 19298688.
65. Kobayashi Y, Miyamoto M, Sugibayashi K, Morimoto Y. Drug permeation through the three layers of the human nail plate. J Pharm Pharmacol. 1999;51(3):271-8. DOI: 10.1211/0022357991772448. PMID: 10344627.
66. Baran R, Hay RJ, Garduno JI. Review of antifungal therapy and the severity index for assessing onychomycosis: part I. J Dermatolog Treat. 2008;19(2):72-81. DOI: 10.1080/09546630701243418. PMID: 18484426.
67. Hettinger DF, Valinsky MS. Treatment of onychomycosis with nail avulsion and topical ketoconazole. J Am Podiatr Med Assoc. 1991;81(1):28-32. DOI: 10.7547/87507315-81-1-28. PMID: 1825225.
68. Vignardet C, Guillaume YC, Friedrich J, Millet J. A first order experimental design to assess soluble proteins released by a new keratinase from Doratomyces microsporus on human substrates. Int J Pharm. 1999;191(2):95-102. DOI: 10.1016/s0378-5173(99)00283-5. PMID: 10564836.
69. Monti D, Saccomani L, Chetoni P, Burgalassi S, Saettone MF, Mailland F. In vitro transungual permeation of ciclopirox from a hydroxypropyl chitosan-based, water-soluble nail lacquer. Drug Dev Ind Pharm. 2005;31(1):11-7. DOI: 10.1081/ddc-43935. PMID: 15704853.
70. Myoung Y, Choi HK. Permeation of ciclopirox across porcine hoof membrane: effect of pressure sensitive adhesives and vehicles. Eur J Pharm Sci. 2003;20(3):319-25. DOI: 10.1016/j.ejps.2003.07.001. PMID: 14592697.
71. Mertin D, Lippold BC. In-vitro permeability of the human nail and of a keratin membrane from bovine hooves: prediction of the penetration rate of antimycotics through the nail plate and their efficacy. J Pharm Pharmacol. 1997;49(9):866-72. DOI: 10.1111/j.2042-7158.1997.tb06127.x. PMID: 9306253.
72. Kobayashi Y, Komatsu T, Sumi M, Numajiri S, Miyamoto M, Kobayashi D, et al. In vitro permeation of several drugs through the human nail plate: relationship between physicochemical properties and nail permeability of drugs. Eur J Pharm Sci. 2004;21(4):471-7. DOI: 10.1016/j.ejps.2003.11.008. PMID: 14998577.
73. Smith KA, Hao J, Li SK. Effects of ionic strength on passive and iontophoretic transport of cationic permeant across human nail. Pharm Res. 2009;26(6):1446-55. DOI: 10.1007/s11095-009-9854-x. PMID: 19267187.
74. Nair AB, Kim HD, Davis SP, Etheredge R, Barsness M, Friden PM, et al. An ex vivo toe model used to assess applicators for the iontophoretic ungual delivery of terbinafine. Pharm Res. 2009;26(9):2194-201. DOI: 10.1007/s11095-009-9934-y. PMID: 19582550.

75. Hao J, Li SK. Transungual iontophoretic transport of polar neutral and positively charged model permeants: effects of electrophoresis and electroosmosis. J Pharm Sci. 2008;97(2):893-905. DOI: 10.1002/jps.21025. PMID: 17683062.
76. Lusiana, Reichl S, Müller-Goymann CC. Infected nail plate model made of human hair keratin for evaluating the efficacy of different topical antifungal formulations against Trichophyton rubrum in vitro. Eur J Pharm Biopharm. 2013;84(3):599-605. DOI: 10.1016/j.ejpb.2013.01.015. PMID: 23419812.
77. Brown MB, Jones SA, Turner R. Topical ungual formulations. EP 1888029 A2, 2006.
78. Traynor MJ, Turner RB, Evans CR, Khengar RH, Jones SA, Brown MB. Effect of a novel penetration enhancer on the ungual permeation of two antifungal agents. J Pharm Pharmacol. 2010;62(6);730-737. DOI: 10.1211/jpp.62.06.0009. PMID: 20636860.
79. Nakashima T, Nozawa A, Ito T, Majima T. Experimental tinea unguium model to assess topical antifungal agents using the infected human nail with dermatophyte in vitro. J Infect Chemother. 2002;8(4):331-5. DOI: 10.1007/s10156-002-0192-8. PMID: 12525893.
80. Tatsumi Y, Yokoo M, Senda H, Kakehi K. Therapeutic efficacy of topically applied KP-103 against experimental tinea unguium in guinea pigs in comparison with amorolfine and terbinafine. Antimicrob Agents Chemother. 2002;46(12):3797-801. DOI: 10.1128/AAC.46.12.3797-3801.2002. PMID: 12435679.
81. Shimamura T, Kubota N, Nagasaka S, Suzuki T, Mukai H, Shibuya K. Establishment of a novel model of onychomycosis in rabbits for evaluation of antifungal agents. Antimicrob Agents Chemother. 2011;55(7):3150-5. DOI: 10.1128/AAC.00399-11. PMID: 21555762.
82. Murdan S. 1st meeting on topical drug delivery to the nail. Expert Opin Drug Deliv. 2007;4(4):453-5. DOI: 10.1517/17425247.4.4.453. PMID: 17683257.
83. Gómez EC, Anguiano Igea S, Gómez Amoza JL, Otero Espinar FJ. Evaluation of the promoting effect of soluble cyclodextrins in drug nail penetration. Eur J Pharm Sci. 2018;117:270-278. doi: 10.1016/j.ejps.2018.02.028. PMID: 29501459.
84. Gregorí Valdes BS, Serro AP, Gordo PM, Silva A, Gonçalves L, Salgado A, et al. New Polyurethane Nail Lacquers for the Delivery of Terbinafine: Formulation and Antifungal Activity Evaluation. J Pharm Sci. 2017;106(6):1570-7. DOI: 10.1016/j.xphs.2017.02.017. PMID: 28263845.
85. Täuber A, Müller-Goymann CC. Comparison of the antifungal efficacy of terbinafine hydrochloride and ciclopirox olamine containing formulations against the dermatophyte Trichophyton rubrum in an infected nail plate model. Mol Pharm. 2014;11(7):1991-6. DOI: 10.1021/mp400711q. PMID: 24490976.
86. Shireesh KR, Chandra SB, Vishnu P, Prasad MVV. Ungual Drug Delivery System of Ketoconazole Nail Lacquer. Int J Appl Pharm. 2010;2(4):17-9.
87. Sabreen J, Divyakumar B, Kiran B. Preungual drug delivery systems of Terbinafine Hydrochloride Nail Lacquer. Asian J Pharm. 2008;2(1):53-66. DOI: 10.4103/0973-8398.41567.
88. Chouhan P, Saini TR. Hydroxypropyl- β -cyclodextrin: A Novel Transungual Permeation Enhancer for Development of Topical Drug Delivery System for Onychomycosis. J Drug Deliv. 2014;2014:950358. DOI: 10.1155/2014/950358. PMID: 25177500.
89. Indre S, Vitalis B. Effect of Film-Forming Polymers on Release of Naftifine Hydrochloride from Nail Lacquers. Int J Polym Sci. 2017;1-7. DOI: 10.1155/2017/1476270. PMID: 1476270.
90. Biji P, David BL. A Novel Transungual Formulation (Nail Patch) For Delivery Of Ciclopirox Olamine Into The Nail And The Nail Folds. Int J Pharm Med Bio Sci. 2014;3(3):152-64.
91. Ahn TS, Lee JP, Kim J, Oh SY, Chun MK, Choi HK. Effect of pressure sensitive adhesive and vehicles on permeation of terbinafine across porcine hoof membrane. Arch Pharm Res. 2013;36(11):1403-9. DOI: 10.1007/s12272-013-0191-6. PMID: 23812776.

92. Angamuthu M, Nanjappa SH, Raman V, Jo S, Cegu P, Murthy SN. Controlled-release injectable containing terbinafine/PLGA microspheres for onychomycosis treatment. J Pharm Sci. 2014;103(4):1178-83. DOI: 10.1002/jps.23887. PMID: 24497012.
93. Nida A, Soniya S, Kamla P. Antifungal potential of tolnaftate against Candida albicans in the treatment of onychomycosis: development of nail lacquer and ex-vivo characterization. Pharm Biomed Res. 2016;2(3):1-12. DOI: 10.18869/acadpub.pbr.2.3.1.
94. Barot BS, Parejiya PB, Patel HK, Gohel MC, Shelat PK. Microemulsion-based gel of terbinafine for the treatment of onychomycosis: optimization of formulation using D-optimal design. AAPS PharmSciTech. 2012;13(1):184-92. DOI: 10.1208/s12249-011-9742-7. PMID: 22187363.
95. Barot BS, Parejiya PB, Patel HK, Mehta DM, Shelat PK. Microemulsion-based antifungal gel delivery to nail for the treatment of onychomycosis: formulation, optimization, and efficacy studies. Drug Deliv Transl Res. 2012;2(6):463-76. DOI: 10.1007/s13346-012-0109-8. PMID: 25787325.
96. Bohn M, Kraemer KT. Dermatopharmacology of ciclopirox nail lacquer topical solution 8% in the treatment of onychomycosis. J Am Acad Dermatol. 2000;43(4):S57-69. DOI: 10.1067/mjd.2000.109072. PMID: 11051135.
97. Satheesh Madhav NV, Kirti S. Smart ungual biopenetrant from the roots of beta vulgaris. J Appl Pharm Res. 2017;5(2):21-6.
98. Vikram S, Kaushal KC. Inspection of a novel penetration enhancer for transungual drug delivery system: pelargonium hortorum. J Appl Pharm Res. 2015;3(1);22-5.
99. Noha Ibrahim E, Rehab Nabil S, Ghada A. Terbinafine Hydrochloride Trans-ungual Delivery via Nanovesicular Systems: In-Vitro Characterization and Ex-vivo Evaluation. AAPS PharmSciTech. 2017;18(2);551-62. DOI: 10.1208/s12249-016-0528-9. PMID:27138036.
100. Noha Ibrahim E, Rehab NS, Ghada A. In-situ gels and nail lacquers as potential delivery systems for treatment of onychomycosis. A comparative study. J Drug Deliv Sci Technol. 2018;43: 253-61. DOI: 10.1016/j.jddst.2017.10.018. PMID: 140061943.
101. Flores FC, de Lima JA, Ribeiro RF, Alves SH, Rolim CM, Beck RC, et al. Antifungal activity of nanocapsule suspensions containing tea tree oil on the growth of Trichophyton rubrum. Mycopathologia. 2013;175(3-4):281-6. DOI: 10.1007/s11046-013-9622-7. PMID: 23392821.
102. Manavalan R, Barish E, Abraham T, Aswanivm R. Formulation and evaluation of a medicated nail lacquer for the treatment of onychomycosis. Int J Res Pharm Nano Sci. 2016;5(4):201-11.
103. Hafeez F, Hui X, Chiang A, Hornby S, Maibach H. Transungual delivery of ketoconazole using novel lacquer formulation. Int J Pharm. 2013;456(2):357-61. DOI: 10.1016/j.ijpharm.2013.08.082. PMID: 24029171.
104. Hui X, Hornby SB, Wester RC, Barbadillo S, Appa Y, Maibach H. In vitro human nail penetration and kinetics of panthenol. Int J Cosmet Sci. 2007;29(4):277-82. DOI: 10.1111/j.1467-2494.2007.00373.x. PMID: 18489355.
105. Gaddime SB, Nagoba SN, Pattewar SG, Wadulkar RD, Thonte SS. Formulation and Evaluation of Medicated Nail Patches for the Treatment of Onychomycosis. Int J Pharm Sci Invent. 2018;7(4):52-58.
106. Joshi M, Sharma V, Pathak K. Matrix based system of isotretinoin as nail lacquer to enhance transungal delivery across human nail plate. Int J Pharm. 2015;478(1):268-77. DOI: 10.1016/j.ijpharm.2014.11.050. PMID: 25445993.
107. Khattab A, Shalaby S. Optimized Ciclopirox-Based Eudragit RLPO Nail Lacquer: Effect of Endopeptidase Enzyme as Permeation Enhancer on Transungual Drug Delivery and Efficiency Against Onychomycosis. AAPS PharmSciTech. 2018;19(3):1048-60. DOI: 10.1208/s12249-017-0917-8. PMID: 29138987.

108. Kushwaha AS, Repka MA, Narasimha Murthy S. A Novel Apremilast Nail Lacquer Formulation for the Treatment of Nail Psoriasis. AAPS PharmSciTech. 2017;18(8):2949-56. DOI: 10.1208/s12249-017-0776-3. PMID: 28455829.
109. Matsuda Y, Sugiura K, Hashimoto T, Ueda A, Konno Y, Tatsumi Y. Efficacy Coefficients Determined Using Nail Permeability and Antifungal Activity in Keratin-Containing Media Are Useful for Predicting Clinical Efficacies of Topical Drugs for Onychomycosis. PLoS One. 2016;11(7):e0159661. DOI: 10.1371/journal.pone.0159661. PMID: 27441843.
110. Monti D, Saccomani L, Chetoni P, Burgalassi S, Senesi S, Ghelardi E, et al. Hydrosoluble medicated nail lacquers: in vitro drug permeation and corresponding antimycotic activity. Br J Dermatol. 2010;162(2):311-7. DOI: 10.1111/j.1365-2133.2009.09504.x. PMID: 19886884.
111. Monti D, Herranz U, Dal Bo L, Subissi A. Nail penetration and predicted mycological efficacy of an innovative hydrosoluble ciclopirox nail lacquer vs. a standard amorolfine lacquer in healthy subjects. J Eur Acad Dermatol Venereol. 2013;27(2):e153-8. DOI: 10.1111/j.1468-3083.2012.04529.x. PMID: 22449201.
112. Monti D, Egiziano E, Burgalassi S, Tampucci S, Terreni E, Tivegna S. Influence of a Combination of Chemical Enhancers and Iontophoresis on In Vitro Transungual Permeation of Nystatin. AAPS PharmSciTech. 2018;19(4):1574-81. DOI: 10.1208/s12249-018-0977-4. PMID: 29484614.
113. Nair AB, Kim HD, Chakraborty B, Singh J, Zaman M, Gupta A. Ungual and trans-ungual iontophoretic delivery of terbinafine for the treatment of onychomycosis. J Pharm Sci. 2009;98(11):4130-40. DOI: 10.1002/jps.21711. PMID: 19340887.
114. Naumann S, Meyer JP, Kiesow A, Mrestani Y, Wohlrab J, Neubert RH. Controlled nail delivery of a novel lipophilic antifungal agent using various modern drug carrier systems as well as in vitro and ex vivo model systems. J Control Release. 2014;180:60-70. DOI: 10.1016/j.jconrel.2014.02.013. PMID: 24560884.
115. Luis N, Begoña DM, Francisco J. Thermogelling hydrogels of cyclodextrin/poloxamer polypseudorotaxanes as aqueous-based nail lacquers: Application to the delivery of triamcinolone acetonide and ciclopirox olamine. Eur J Pharm Biopharm 2013;83(3):370–7. DOI: 10.1016/j.ejpb.2012.11.004. PMID: 23201053.
116. Patel MM, Vora ZM. Formulation development and optimization of transungual drug delivery system of terbinafine hydrochloride for the treatment of onychomycosis. Drug Deliv Transl Res. 2016;6(3):263-75. DOI: 10.1007/s13346-016-0287-x. PMID: 26926242.
117. Cutrín-Gómez E, Anguiano-Igea S, Delgado-Charro MB, Gómez-Amoza JL, Otero-Espinar FJ. Effect on Nail Structure and Transungual Permeability of the Ethanol and Poloxamer Ratio from Cyclodextrin-Soluble Polypseudorotaxanes Based Nail Lacquer. Pharmaceutics. 2018;10(3):1-15. DOI: 10.3390/pharmaceutics10030156. PMID: 30208659.
118. Rocha KAD, Krawczyk-Santos AP, Andrade LM, Souza LC, Marreto RN, Gratieri T, et al. Voriconazole-loaded nanostructured lipid carriers (NLC) for drug delivery in deeper regions of the nail plate. Int J Pharm. 2017;531(1):292-8. DOI: 10.1016/j.ijpharm.2017.08.115. PMID: 28859937.
119. Mahtab A, Anwar M, Mallick N, Naz Z, Jain GK, Ahmad FJ. Transungual Delivery of Ketoconazole Nanoemulgel for the Effective Management of Onychomycosis. AAPS PharmSciTech. 2016;17(6):1477-90. DOI: 10.1208/s12249-016-0488-0. PMID: 26857516.
120. Sánchez Regaña M, Martín Ezquerra G, Umbert Millet P, Llambí Mateos F. Treatment of nail psoriasis with 8% clobetasol nail lacquer: positive experience in 10 patients. J Eur Acad Dermatol Venereol. 2005;19(5):573-7. DOI: 10.1111/j.1468-3083.2005.01253.x. PMID: 16164711.

121. Shivakumar HN, Vaka SR, Madhav NV, Chandra H, Murthy SN. Bilayered nail lacquer of terbinafine hydrochloride for treatment of onychomycosis. J Pharm Sci. 2010;99(10):4267-76. DOI: 10.1002/jps.22150. PMID: 20737634.
122. Thapa RK, Choi JY, Go TG, Kang MH, Han SD, Jun JH, Son MW, Yong CS, Kim JO. Development of ciclopirox nail lacquer with enhanced permeation and retention. Arch Pharm Res. 2016;39(7):953-9. DOI: 10.1007/s12272-016-0774-0. PMID: 27306785.
123. Thatai P, Sapra B. Transungual Gel of Terbinafine Hydrochloride for the Management of Onychomycosis: Formulation, Optimization, and Evaluation. AAPS PharmSciTech. 2017;18(6):2316-28. DOI: 10.1208/s12249-017-0711-7. PMID: 28116600.
124. Thatai P, Sapra B. Terbinafine hydrochloride nail lacquer for the management of onychomycosis: formulation, characterization and in vitro evaluation. Ther Deliv. 2018;9(2):99-119. DOI: 10.4155/tde-2017-0069. PMID: 29325509.
125. Singh B, Ganesh Kumar B, Preeti K. Formulation and Evaluation of Nail Lacquer Containing Tioconazole for Transungual Drug Delivery System. Indo Am J Pharm Sci. 2015;2(11):1474-85.
126. Nair AB, Chakraborty B, Murthy SN. Effect of polyethylene glycols on the trans-ungual delivery of terbinafine. Curr Drug Deliv. 2010;7(5):407-14. DOI: 10.2174/156720110793566308. PMID: 20955143.
127. Rohit J, Priyanka H, Savita P. Antifungal glycerogelatin: attempt at formulation and evaluation of a potential transungual drug delivery system. Int J Pharm Sci Res. 2014;5(10):4249-53. DOI: 10.13040/IJPSR.0975-8232.5(10).4249-53.
128. Vikram S, Kaushal KC. Formulation and evaluation the transungual drug delivery system of fluconazole with penetration enhancer: iresine herbestii. Indo Am J Pharm Res. 2016;6(2):4521-4. DOI: 10.1044.1980-iajpr.151144.
129. Pollak RA, Siu WJJ, Tatsumi Y, Pillai R. Efinaconazole Topical Solution, 10%: Factors Contributing to Onychomycosis Success. J Fungi (Basel). 2015;1(2):107-14. DOI: 10.3390/jof1020107. PMID: 29376902.
130. Pal P, Thakur RS, Ray S, Mazumder B. Design and development of a safer non-invasive transungual drug delivery system for topical treatment of onychomycosis. Drug Dev Ind Pharm. 2015;41(7):1095-9. DOI: 10.3109/03639045.2014.931966. PMID: 24980914.
131. Vaka SR, Murthy SN, O'Haver JH, Repka MA. A platform for predicting and enhancing model drug delivery across the human nail plate. Drug Dev Ind Pharm. 2011;37(1):72-9. DOI: 10.3109/03639045.2010.491832. PMID: 20545509.
132. Bhaskara-Reddy MV, Anagers P, Gosselin A, Arul J. Characterization and use of essential oil from Thymus vulgaris against Botrytis cinerea and Rhizopus stolonifer in strawberry fruits. Phytochemistry. 1998;47(8):1515-20. DOI: 10.1016/S0031-9422(97)00795-4. PMID: 85944645.
133. Bocchini P, Andalo C, Pozzi R, Galletti GC, Antonelli A. Determination of diallyl thiosulfinate (allicin) in garlic (Allium sativum L.) by high-performance liquid chromatography with a post-column photochemical reactor. Anal Chim Acta 2001;441(1):37-43. DOI: 10.1016/S0003-2670(01)01104-7.
134. Calvey EM, White KD, Matusik JE, Sha D, Block E. Allium chemistry: identification of organosulfur compounds in ramp (Allium tricoccum) homogenates. Phytochemistry. 1998;49(2):359-64. DOI: 10.1016/s0031-9422(98)00191-5. PMID: 9747536.
135. Kharjul M, Kharjul A, Bhairy S, Gupta S, Kale A. Phytochemical and Pharmacological Accounts of Some Reviewed Plants with Antidiabetic Potential. Sch Acad J Pharm. 2014;3(2):162-77.
136. Gupta Shivram, Kharjul Mangesh, Bhairy Srinivas, Maurya Sandeep, Upadhyay Anand. Some Evaluated Potential Therapeutic Agents in Herbalism and their Proposed Dosage Forms. Int J Innov Pharm Sci Res 2014;2(7):1533-54.

DEFINITIONS, ACRONYMS, ABBREVIATIONS

TUDD: Trans ungual drug delivery
MMA: methyl methacrylate
CO_2: Carbon dioxide
PDT: Photodynamic therapy
PIP: Post-infection period
IVIVC: *In-vitro In-vivo* correlation