Formulation And Evaluation Of Itraconazole Nano-Ointment For The Topical Fungal Infection

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ABSTRACT

In recent year, there is many research performed in the development of novel drug delivery systems using nanoparticles. Nanoparticles shows significant advantages over the conventional drug delivery and easy in administration with different route of administration. The current study is aimed to formulate and evaluate the nanoformulation of Itraconazole for treatment of topical fungal infection. Itraconazole shows triazole structure based antifungal agent that is active against a broad spectrum of fungal species and used for the treatment of local and systemic fungal infection. Nanoformulation formulation of ointment containing Itraconazole was formulated from the polymers like polyethylene glycol 400 and polyethylene glycol 4000, Sodium Lauryl Sulphate, glycerin. Raw drug was converted into nanoparticles and then incorporated into ointment base. After that preformulation studies were done for the Itraconazole(API) for the identification and characterization of drugs. In this procedure various chemical tests were performed like pH, Melting point, solubility of drug, extrudability, spreadability, viscosity, tests of physical characteristics like appearance, odor, and taste for knowing its potency. Itraconazole ointment nano-formulations also showed sustained release of drug. Scanning Electron Microscope showed the formulation is in nano-range and found spherical in shape. By the study of FTIR it shows no interaction between the drug and the carrier. Then antifungal study was carried out on the wistar albino rat with 8 days protocol. The results comes that formulation having better stability, good viscocity and better compatibility. These results suggested that decided aim of work to improving pharmacokinetic profile of drug has been improved by the formulation of nano-formulation. In summary, the nanoformulation of Itraconazole ointment drug delivery systems has various advantages like topical application on to skin, easy way of route of administration, least side effects and nano sized. It can postulate that nanoformulation may be a best approach to treat the fungal skin disease.

Keywords: Itraconazole, Nano-ointment, fungal infection, topical

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INTRODUCTION
Fungal infection of the skin is now a day’s becomes one of the most common dermatological diseases in the world. The drug delivery onto the skin is found effective and easiest way for local therapy in the treatment of many dermatological diseases example fungal infection. Topical drug delivery is the most common conventional administration route for the treatment of cutaneous disorder such as acne, aging, or some cutaneous diseases such as skin inflammation.
Most commonly drugs used to treat fungal infections like itraconazole, Miconazole and Ketoconazole have been administered orally but they shows side effects, hepatic, effects, hepatic, systemic side effect in longer duration of treatment. So because of that there is need to bypass the drugs to avoid hepatic, systemic side effect and gastric disorders. Ointment formulations become one of the most suitable dosage forms for topical use to treat cutaneous infection. Itraconazole shows triazole structure based antifungal agent that is commonly used to treat cutaneous mycoses owing to its high activity against a broad spectrum of pathogenic fungi causing the disease such as dermatophytes and yeasts. Itraconazole, comes under a triazole antifungal medication was approved by the United States Food & Drug Administration in 1992. Itraconazole is BCS class2 drug with half-life 21 hours, protein binding (99.8%) and absolute bioavailability (55%) due to its low aqueous solubility. Its mechanism of action is like that, triazole contains the azole ring in their structure, which have free nitrogen atom. This nitrogen atom compete oxygen atom that cause cytochrome P450 enzyme inhibition which is essential for ergosterol in the fungal cell wall. Then the cytochrome P-450 is important for Cl4 demethylation of lanosterol which helpful for synthesis of ergosterol. Then the prevention of synthesis of the ergosterol loss membrane fluidity of cell. The accumulation of phospholipids and the unsaturated fatty acid is there in the fungal cell, cause cell death. The gastric acidity affected the bioavailability of itraconazole. Itraconazole reflects a great diversity of non-fungal activity that works by slowing the growth of fungi cause infection and used for the treatment of fungal infections due to its good tolerance for patients. Ointment route of drug delivery has suitable way because it avoids first-pass effects, gastrointestinal irritation, and metabolic degradation associated with oral administration. Nanoparticles offer new opportunities for the treatment of skin diseases. The present study is aimed to formulate and evaluate the nanoformulation of itraconazole for treatment of topical fungal infection. The preferred method of preparation of nanoparticles is depends on the nature and physiochemical parameters of drug and polymer. Some methods are available for preparation of nanoparticles, emulsion solvent evaporation method, double emulsion and solvent evaporation method, salting out method, emulsions diffusion method, solvent displacement or precipitation method, dialysis method, etc. Nano-formulation of ointment containing itraconazole has been prepared from the polymers like polyethylene glycol-4000, polyethylene glycol 400, sodium lauryl sulphate, glycerin and nanocrystals of itraconazole. Nanoparticles explore the controlled release profiles for many drugs in topical route and exhibiting low toxicity which is produced in the drug. Nanoparticles are used in topical products because they have ability to target the skin, to cure skin related diseases. The some examples of the drugs delivered through topical route are antifungal, antiviral, antibiotics, antiseptics, local anesthetics and corticosteroids. Drug loaded nanoparticles were characterized through particle size, zeta potential, polydispersity index (PDI), in vitro drug release, IR spectroscopy, X-ray powder diffraction (XRPD) and surface topography. The prepared nanoparticles were dispersed into ointment and it was characterized through pH, homogeneity, spreadability, viscosity, drug content, in vitro drug release and in vitro antifungal activity.
Itraconazole is (+)-1-[(RS)-sec-butyl]-4-[p-[4-[(2R,4S)-rel-2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-Δ²-1,2,4-triazolin-5-one in IUPAC and having formula C₃₅H₃₈Cl₂N₈O₄ and its molecular weight is 705.64g/mol. The bioavailability of itraconazole is 56% and it metabolized in liver excreted from renal and faecal. It binds with protein 99.8%. Itraconazole consists of antifungal class of medication used for the treatment of fungal infections due to its good tolerance for patients. Itraconazole consists of highly lipophilic, poorly soluble at physiological PH and extensively protein-bound in circulation. Itraconazole is commonly used for the treatment of fungal infections because of its good tolerance for patients. Topical drug delivery is the conventional administration route for the treatment of cutaneous disorders such as acne, aging or some cutaneous disease such as skin inflammation. Itraconazole is mostly administered to the skin by passive diffusion from the plasma to the keratinocytes, with strong drug adherence to keratin. Itraconazole decreases synthesis of ergosterol by inhibiting fungal enzymes cytochrome p450. Chronic and acute both fungal infections can be treated by this agent as it safe for prolonged uses.

**MATERIALS AND METHODS**

Chemicals and reagents:
- Methanol H.P.L.C. grade
- Water H.P.L.C. grade
- Polyethylene glycol-400
- Polyethylene glycol-4000
- Glycerin
- Sodium Lauryl Sulphate

**Instrumentation:**
- Electronic weighing balance
- Ultrasonic bath sonicator
- Magnetic stirrer
- Centrifuge
- Brookfield Viscometer
- pH meter
- FTIR
- SEM

**Formulation method of Itraconazole**

Solvent diffusion method was used for preparation of formulation. 6.5 mg of Itraconazole were measured through weighing balance and taken into a 20 ml volumetric flask. Then little amount of water was added to this and sonicate for few minutes. After that volume make up to 20 ml and sonicate for 40 minute then the solution was stir at low speed through magnetic stirrer for 10 hrs after that it get converted into nano from. Nanoparticle were separated by centrifuge it at 15000 rpm this process was done for 30 minute. Lastly the separated particles were dried into hot air oven until it dried. Dried nanoparticles were collected into eppendorf.

**Preparation of topical nanoformulation ointment:** Ointment base was used for
Itraconazole and Mometasone Nanoformulation. This base was prepared by dissolving polyethylene glycol 400 into polyethylene glycol 4000 on hot plate and adding pinch of sodium lauryl sulphate and little bit of glycerin. Let it cool and when it reaches to room temperature it gets ointment like texture. Nanoparticles of Itraconazole and Mometasone were added to the ointment base by geometrical method by continuous folding the drug and base on the slab. Now it stored into air tight container. Six different composition of formulation were prepared for evaluating best one. Quantities of different compositions are as follows

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**Solvent diffusion method** was used for preparation of formulation. 44.2 mg of Itraconazole and Mometasone were measured through weighing balance and taken into a 20 ml volumetric flask. Little amount of water was added to this and sonicate for few minutes. After that volume make up to 20 ml and sonicate for 40 minute. This solution was stir at low speed through magnetic stirrer for 10 hrs after that it get converted into nano form. Nanoparticle were separated by centrifuge it at 15000 rpm this process was done for 30 minute. Separated particles were dried into hot air oven until it dried. Dried nanoparticles were collected into eppendorf.

**Table 1: Formulation development for nanoformulation ointment**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Ingredients</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Itraconazole</td>
<td>1.1</td>
<td>1.2</td>
<td>1.5</td>
<td>1.3</td>
<td>1.4</td>
</tr>
<tr>
<td>2</td>
<td>Polyethylene Glycol-400(ml)</td>
<td>50</td>
<td>40</td>
<td>60</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>3</td>
<td>Polyethylene Glycol-4000 (ml)</td>
<td>50</td>
<td>60</td>
<td>40</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>Glycerine(ml)</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>5</td>
<td>Sodium lauryl sulphate(mg)</td>
<td>0.2</td>
<td>0.15</td>
<td>0.10</td>
<td>0.5</td>
<td>0.15</td>
</tr>
</tbody>
</table>

**RESULTS AND DISCUSSION**

A) Identification of Drug

**Appearance:** By testing the physical parameters of itraconazole it was noticed with naked eye and looks like white powder and it complies with the guidelines.

**Odor:** Itraconazole was checked odourless.

**Chemical Test:** Chemical test for identification of itraconazole was performed and found positive.

**Melting Point:** For the testing of melting point of Itraconazole the melting apparatus was used and outcomes were 167 degree Celsius.

**Solubility of Drug:** Different solvent were used for the analysis solubility. Itraconazole was tested form its solubility when dissolving these drugs into different solvent and take result that Itraconazole is soluble in methanol and it is insoluble in water and 0.1 HCL.

B) **pH**
The obtained results in pH testing shown in following tables:

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>7.2</td>
</tr>
<tr>
<td>F2</td>
<td>7.5</td>
</tr>
<tr>
<td>F3</td>
<td>7.4</td>
</tr>
<tr>
<td>F4</td>
<td>7.8</td>
</tr>
<tr>
<td>F5</td>
<td>7.9</td>
</tr>
</tbody>
</table>

C) Viscosity
The obtained results in viscosity testing shown in following tables:

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Viscosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>1300cp</td>
</tr>
<tr>
<td>F2</td>
<td>1500cp</td>
</tr>
<tr>
<td>F3</td>
<td>1600cp</td>
</tr>
<tr>
<td>F4</td>
<td>1100cp</td>
</tr>
<tr>
<td>F5</td>
<td>1000cp</td>
</tr>
</tbody>
</table>

D) Spread ability:
The obtained results in spread ability testing shown in following tables:

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Spreadability</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>1.7</td>
</tr>
<tr>
<td>F2</td>
<td>1.9</td>
</tr>
<tr>
<td>F3</td>
<td>1.2</td>
</tr>
<tr>
<td>F4</td>
<td>1.5</td>
</tr>
<tr>
<td>F5</td>
<td>1.6</td>
</tr>
</tbody>
</table>

E) Extrudability
The obtained results in extrudability testing shown in following tables:

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Extrudability</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>Average</td>
</tr>
<tr>
<td>F2</td>
<td>Excellent</td>
</tr>
<tr>
<td>F3</td>
<td>Good</td>
</tr>
<tr>
<td>F4</td>
<td>Excellent</td>
</tr>
<tr>
<td>F5</td>
<td>Average</td>
</tr>
</tbody>
</table>
F) U.V Analysis of Itraconazole
Methanol was used for preparing dilution of Itraconazole and standard curve was taken on UV spectrophotometer. The spectra were observed at \( \lambda \) max 262 nm. And the regression value was 0.993.

\[
y = 0.0199x + 0.0181 \\
R^2 = 0.9935
\]

G) Fourier transform infrared analysis:
Nanoparticle were tested for their particle size by X-Ray Powder Diffractometer and after that graph obtained from analysis.
The FTIR test was carried out to detect the chemical interaction between the compounds. It was found that the pure drug that is Itraconazole showed the peaks at Itraconazole nanoparticles tested for their morphology through scanning electron microscope and then results were obtained. 3414.49 cm\(^{-1}\) N-H stretch, amine group, 2931.82 cm\(^{-1}\) O-H stretch, Phenols group, 1451.20 cm\(^{-1}\) C-H stretch, Alkanes group, 1232.13 cm\(^{-1}\), C-O Stretch, alcohol groups. After the nanoformulation the itraconazole shows peaks at 3423.46 cm\(^{-1}\), 2929.56 cm\(^{-1}\), 1451.57 cm\(^{-1}\), 1229.33 cm\(^{-1}\) which depicts that there is no chemical interaction happen during the nano-formulation.

H) Scanning Electron Microscope
H) Spreadability

The spreadability testing was performed and following results was obtained:

<table>
<thead>
<tr>
<th>Formulation code</th>
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<tbody>
<tr>
<td>F1</td>
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<td>1.5</td>
</tr>
<tr>
<td>F5</td>
<td>1.6</td>
</tr>
</tbody>
</table>

I) Entrapment efficiency:

Formulation-1 65.95%
Formulation-2 94.59%
Formulation-3 81.86%
Formulation-4 89.49%
Formulation-5 85.45%

Test for activity of candida albicans:

Firstly, skin irritation studies was carry out by using Itraconazole topical ointment formulation by using wistar rats as animal model. The immune efficacy of topical ointment itraconazole was assessed in male albino rat model (wistar; 100-150gm.). The antifungal activity of Itraconazole topical ointment formulation was determined by cup and plate method as the test fungi. In this study sabouraud dextrose agar used as a culture media for culturing candida albicans. Then after further study placed little amount of our nanoformulation and kept in incubator for 48 hours. Then after that zone inhibition was recorded it was 4mm due to which we can pretend that nanoformulation of Itraconazole is capable for inhibiting the fungal growth and cause cell death of fungi by inhibiting synthesis of ergosterol in fungal cell.

DISCUSSION:

Topical therapy allows directly delivery of drug to the skin having least risk of systemic side effects with easy administration. In this study the prepared ointment of Itraconazole was stable and could be used with promising potential for fungal infection of skin through penetrates different barrier layer of skin like stratum corneum. Topical itraconazole shows high local tissue level, more rapid drug delivery. Topical therapy allows Itraconazole ointment with loading capacity and small particle size ensured a sustained release profile and shows a good permeation through mouse skin with retained drug content in skin. Itraconazole shows low solubility in water and consists of low bioavailability at about 55%. This type of antifungal drug when orally administered produce hepatic and gastrointestinal side effects. When fungal infection is treating for longer duration, then it shows the adverse effect of itraconazole which may further increase to chronic. So to overcome above problems, the itraconazole was formulated into topical nano ointment. In this preparation the drug nanoparticle were prepared by using solvent diffusion method. Then base was prepared from polymer like polyethylene glycol 400, polyethylene glycol 4000, glycerine and sodium lauryl sulphate. After that nanoparticles were incorporated into ointment base. Raw drug was converted into nanoparticles and then incorporated into ointment base. After that preformulation studies were done for the Itraconazole (API) for the identification and characterization of drugs. In this procedure various chemical tests were performed like pH, Melting point, solubility of drug, extrudability, spreadability, viscosity, tests of physical characteristics like appearance, odor, and taste for knowing its potency. FTIR were performed for functional group identification. Nanoparticle size determination
was done by Scanning Electron Microscopy. Finally nanoformulation prepared which shows better antifungal activity towards the site of application and treat the local site which is infected by fungal disease.

CONCLUSION:
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REFERENCES:
Nan and Microemulsions for Skin Delivery, Pharmaceutics 2017;9(40):37.


