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Research Article

Nano Formulation Of Itraconazole And Its Synergism Against Candida Albicans When Combined With Silver Nanoparticle

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ABSTRACT

Objective- The study aims to formulate nanoformulation of itraconazole drug and investigate its antifungal response against *Candida albicans* when combined with silver nanoparticle.

Method- The nanoparticle of the itraconazole drug was prepared by using solvent diffusion method. The nanoparticle was incorporated with gel which was formulated using Carbopol 400 and HPMC. SEM was used to investigate the morphology of the nanoparticle. FTIR was used for the study of interaction of drug with polymer. *In-vitro* drug release study was done to see the release of the pure drug and compare it when formulated to nanoparticle. Various physico chemical study like spreadability, viscosity, pH on various formulation of nanogel was done and the suitable one was selected. *In-vivo* antifungal study was performed against *Candida albicans* in *Wistar albino* rats. The antifungal study of itraconazole nanogel and its combination with silver nanoparticle was done which shows a synergistic effect.

Conclusion- The conclusion of the study was that itraconazole nanoformulation shows better antifungal activity when combined with silver nanoparticle than compared to the itraconazole nanformulation alone. Hence, it can be concluded that intraconazole nanoparticles show synergistic effect with silver nanoparticle.

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INTRODUCTION

Fungi are one of those exploitative microorganisms which not only affects the skin and mucous membrane but also produce systemic infections of internal organs(1). They are vegetative organisms but are not considered as plant for the fact that they do not synthesize chlorophyll. If they exist in the form of single cells are commonly called as yeasts while those existing in the form of hyphal threads are called moulds. One of the yeast type pathogenic fungus is candida albicans. These commonly cause cutaneous infection. Candida albicans have the potential to exist as oval blastospores or as pseudohyphae. They are dimorphic in nature(2).One of the yeast type pathogenic fungus is candida albicans. These commonly cause cutaneous infection. Candida albicans have the potential to exist as oval blastospores or as pseudohyphae. They are dimorphic in nature(3). Depending on the level of invasion of the fungi on the skin tissues, fungal infection of skin has been divided into superficial, cutaneous and subcutaneous. When only outermost layer of the skin is infected, it is known as superficial skin infection. The invading site shows elevated skin pH with redness, mild scaling and inflammation. Candida is the yeast that causes cutaneous candidiasis or other form of the candidiasis(4).

For the treatment of Candida infection, a number of drugs of various classes are available. Topical azole or polyenes are used for the treatment of the infection unless the it has a high risk of spreading systemically. In more resistance cases oral itraconazole and fluconazole can be used. Itraconazole is a drug of triazole group of antifungal drugs. These come under class II of BCS class as they have low aqueous solubility(4). It has broad spectrum of activity and generally used as it has high tolerance by the patients(5).

So, in order to overcome drug resistance and further develop beneficial resolution for various skin infections, nanotechnology has become a great aid. Cellular penetration and drug pharmacokinetic properties can be refined by using nanoparticle as drug delivery system. Various complication of drug with the permeability to biological barriers, degradation and low solubility can be resolved by using nanoparticles(6). So, the solubility problem of the itraconazole can be overcome by formulating it as nanoparticles(7).

Amongst the various types of nanoparticles, metal nanoparticles and metal oxide nanoparticles exhibit a broad range of antifungal activity against dermatophyte infections. For the treatment of fungal infection, silver nanoparticle has been a promising antimicrobial agent (6). The concentration of silver nanoparticle required for killing the bacteria is very low which reduces the chances of toxicity in the human cells(8)(9). Unlike other antibiotics, silver nanoparticle do not show bacterial resistance as their site of action is not limited to a single site but involves several levels(10)(9)(11). The use of antibiotic with silver nanoparticles have shown an improvement in the efficacy of the treatment(12). The antimicrobial resistance can be reversed when antibiotic are used in combination with silver nanoparticles(13).

Topical dosage forms exert localized effect by penetrating inside the skin at the site of application(3). For the treatment of cutaneous infections, topical drug delivery system is used. Gel is semi solid dosage form of drug. The preparation of gel involves swelling of cross-linked polymers using a liquid medium. The application of gel involves the less greasiness and easy removal from skin as compared to creams and ointments.

This study aims at overcoming the various pharmacokinetic problems of the itraconazole drug by preparation of the itraconazole nanoparticles. The formulation of the drug in the form of nanoparticles not only improves the solubility and bioavailability of the itraconazole drug but also it improves the efficacy of the therapy. The various side effects caused by the itraconazole oral administration can be resolved by topical application of the drug. The itraconazole gives a synergistic effect with the silver nanoparticles. The antimicrobial property

of silver nanoparticles well known and when it is used in combination with the itraconazole it increases the efficacy of the itraconazole preparation. The fungal resistance which is caused in case of use of itraconazole can be overcome through combination of itraconazole with silver nanoparticle.

MATERIALS AND METHODS

Materials

For the preparation of Itraconazole nanoparticles, itraconazole was procured from Tas Med pvt limited, Chandigarh, India. HPLC grade Carbopol 940 was purchased from High purity laboratory chemicals Pvt. Ltd., Mumbai-02 India. For preparation of silver nanoparticles, silver nitrate (AgNO3, 99.8%) was used as precursor and purchased from thermo Fischer Scientific India pvt. Ltd. HPLC grade tri-sodium citrate dihydrate ($Na_3C_6H_5O_7$) purified was used as reducing agent in the formulation and it was obtained from High purity laboratory chemicals Pvt. Ltd., Mumbai, India. Starch was used as a stabilizer and purchased from Qualigens Fine Chemicals, Mumbai.

Methodology

Preparation of Itraconazole nanoparticles

Itraconazole (pure) synthetic drug was weighed 130 mg, using digital balance. Distilled water of 30ml quantity was taken. For the preparation of itraconazole nanoparticle solvent diffusion method was used. Weighed amount of Itraconazole drug was taken in a beaker. To the above drug a small quantity of the measured amount of purified water was added. The above mixture was then sonicated for small duration of time. The time and speed of the sonicator was adjusted. More water was added to the above mixture to make up the volume. The sonication was again started and the mixture was sonicated for 40 minutes. Sonicating the drug reduces the particle size of the drug. The mixture was then removed from the sonication. The stirring of the above mixture was done using a magnetic stirrer. The stirring was continuous without any break in a low speed. The mixture was stirred for a duration of ten hours at room temperature. The continuous stirring resulted in the conversion of drug into nano form. After the sonication and stirring of the above mixture, the separation of the particle was done. The separation of the particle was done by using ultra-centrifugation method. The suspension was centrifuged for a speed of 15000RPM for 30 minutes.

Synthesis of silver nanoparticles

For the synthesis of silver nanoparticles, all the chemicals were collected and checked for their purity on label. At first 100ml of a 10^{-3} M silver nitrate solution was prepared. This solution was then mixed with 2.0g of starch which contained Ag^+ ions. Next to make solution homogenous, the solution was stirred at a maintained temperature of 60˚C using hot plate magnetic stirrer. When the solution was stable at 60˚C, a solution containing 2×10^{-3}M tri-sodium citrate was added slowly to it. After the solution was once made, it was cooled at room temperature. Dark box was preferred for carrying out the whole reaction to avoid any type of photochemical reaction. The alternative of dark box was created by wrapping the flask with aluminium foil. When tri-sodium citrate was added to starch solution containing Ag^+, the solution turned to yellow colour(15). With time the colour of the solution changed the colour to brown which indicated the formation of silver nanoparticle (fig 1).

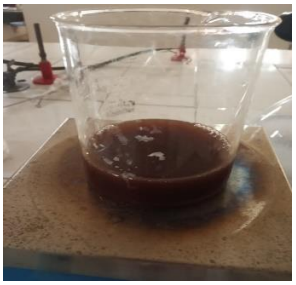


Fig 1: Synthesized silver nanoparticles

Preparation of the gel

Preparation of itraconazole nanoparticle containing gel

For the preparation of gel, Carbopol 940 was used as a gelling agent. Suspension containing itraconazole nanoparticle was measured and taken in required quantity. For preparation of the base required quantity of Carbopol and HPMC were taken and sufficient amount of water was added to it. The mixture was stirred using magnetic stirrer. A small amount of triethanolamine was added to neutralize it. As a moistening agent glycerine was used. Methyl paraben and propyl paraben was used as a preservative. The formulation was continuously stirred until a homogenous gel was obtained. Various concentrations of HPMC and Carbopol 940 was used to prepare the formulations.

Table 1: Formulation of itraconazole nanogel

Formulations	Ingredients							
	Drug (mg)	HPMC (g)	Carbopol (g)	Water (ml)	Propyl paraben (mg)	Methyl paraben (mg)	Glycerine (ml)	Triethanolamine (ml)
F1	25	-	2	80	0.1	0.2	10	5
F2	25	-	2	80	0.1	0.2	10	5
F3	25	1	1	80	0.1	0.2	10	5
F4	25	0.75	0.75	80	0.1	0.2	10	5
F5	25	0.50	0.5	80	0.1	0.2	10	5

On the basis of the above study F5 was selected as best formulation

Characterization of nanoparticles

Scanning electron microscopy (SEM)

Scanning electron microscope was used to study the morphology of the nanoparticle. High energy electron beam scans the sample to create SEM image. To know the shape and size of the nanoparticles, it was subjected to scanning electron microscope. Using EPMA i.e. electron pro- micro analyzer JEOL-J×A 8100 the SEM images of the nanoparticles were taken(16).

FTIR

The possible functional group interaction in the prepared nanoparticles were determined by using FTIR spectra. The structural analysis of organic compounds, polymers, natural products etc. can be easily done by FTIR analysis.This was recorded by FTIR Spectrometer (PerkinElmer Spectrum Version 10.4.00)(17).

***In-vitro* drug release profile**

Dialysis bag diffusion technique was used to determine the *In-vitro* drug diffusion profile. The diffusion of itraconazole drug and itraconazole nanoparticle was performed in phosphate buffer (pH 7.4). The prepared substances in total quantity were placed in cellulose dialysis bag. The bag was tied at both the ends and the bag was kept in the receptor compartment consisting of phosphate buffer (pH 7.4) at 37˚C under magnetic stirring. A small amount of receptor media (1ml) was withdrawn at a determined time upto 500 min and same amount of fluid was replaced with fresh dissolution medium in phosphate buffer having pH 7.4 and then spectrophotometrically analysed at 290nm(18).

Physiochemical characterization of gel

Visual properties

Visual inspection of the formulated gel was done for their physical properties. The observation was made for the colour, transparency and phase separation of the gel. The uniformity of the gel was maintained without presence of any lump in it(19).

pH determination

pH of the above gel was performed by using digital pH meter. The electrodes in pH meter were cleaned properly using distilled water and then wiped and dried. The electrodes were dipped directly in the beaker containing gel. For a while the pH meter was kept and then the reading was noted(16).

Spreadability

For the determination of the spreadability, 2 glass slides were used. 7.5cm each, 2 glass slides were taken and first slide was used to place the gel and the second slide was placed in such a manner sandwiching the gel between them. For the formation of a thin layer of the gel, a weight of 100g was kept above the upper slide compressing the gel. After sometime the weight was removed and a weight of 20g was tied on the upper slide. Now the slides were inclined a little for the upper slide to glide over the first slide. Now the timing for the upper slide to slip over the lower slide is noted. The less the time taken, better will be the spreadability of the formulation(18). The spreadability was then determined by using the formula-

$S = M \times L/T$

Where, S is the spreadability, M is the weight tied on the upper slide, L is the length of the slide and T is the time taken for the upper slide to slip from the lower slide.

Extrudability

The extrudability study was done by filling the gel in an aluminium collapsible tube. To extrude the formulation tube was pressed and the extrudability of the material was checked(18).

Viscosity determination

Viscosity of a liquid refers to the resistance of a fluid to flow. Viscosity of the gel was determined by using DV-E viscometer LV spindle S64. The test was performed and the mean of the formulation was represented in a tabular manner(16).

In vivo pharmacological activity

Anti-fungal activity

Microbial strain

Microbial strain *Candida albicans* (MCCB 0290) was used for the anti-fungal activity. It was obtained from Microbial Culture Collection Bank, Microbiology department, SHIATS, Naini Prayagraj.

Animal

For the purpose of antifungal study, healthy adult male Wistar albino rats were taken. Their age ranged from two to three months and they weighed around 100-150gm. For keeping the rats prescribed atmosphere was provided. The prescribed atmosphere involved 12h light and 12h dark having a temperature range of 25 ± 2^0c and a humidity 30-55%. For daily diet the rats were provided with rat pellets and water. The study was approved by The Institutional Animal Ethical Committee, SIP, Prayagraj, India (SIP/IAEC/February-2020/02).

Procedure

For the in vivo antifungal study of the formulation, *Candida albicans* were used to induce mycosis in Wistar albino rats. The procedure was initiated by cleaning hairs from back surface of area 2×2 of the rats. The hairs were removed using a hair removal cream. Then the cleaned surface was abraded a little with the help of a sandpaper and the skin surface was then inoculated with the inoculum *Candida albicans* using a cotton swab. For the application of the treatment, the animals were divided into four groups containing 4 animals in each group. The groups comprised of one control group, the prepared formulation of itraconazole nanogel, the prepared formulation in combination of silver nanoparticle and the marketed formulation (Itromed gel 1% w/w). All these formulations were topically applied on the cleaned and abraded skin of the rats. The formulations were applied daily one time for an interval of six days. However, no treatment was given to the control group. After the period of six days the response of all the groups were compared and were given treatment scores as 1(not treated), 2(50% treated), 3(75% treated) and 4(100% treated). To check the effect of the treatment given culture study was performed. Ethanol (70%) was used to wipe the treated sites (20).

Result

Itraconazole nanoparticle was formulated by using solvent diffusion technique. The formulation of the drug itraconazole into nanoparticle improves its pharmacokinetic properties. Silver nanoparticle was prepared as per the previously reported data. Then a gel base was prepared using carbopol 940 and HPMC. The itraconazole nanoparticle was

incorporated into the base. Further to show the synergistic effect a combination of itraconazole nanoparticle and silver nanoparticle was used in pharmacological antifungal study in animal. The characterization of itraconazole nanoparticle and nanogel was done and reported.

Characterization of nanoparticle

Scanning electron microscopy (SEM)

The scanning electron microscopy is used to determine the shape and size of the nanomaterial directly. The shape of the nano formulation was found to be spherical. And the size range of 96-130nm was found to be the average size range of the nano formulation. The obtained image of the itraconazole is given below (fig 1).

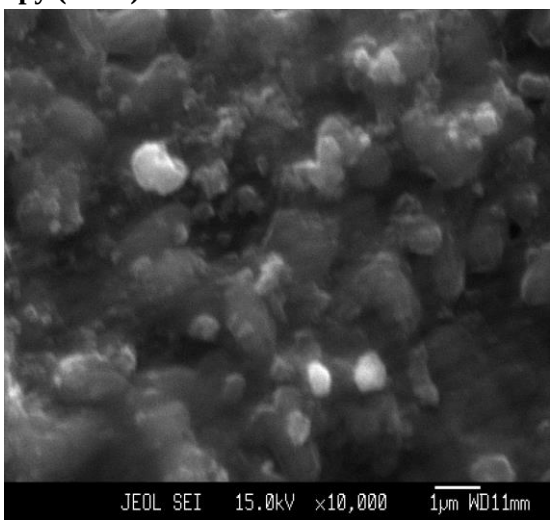


Fig 2: SEM image of itraconazole nanoparticle

FTIR Analysis

For the evaluation of the compatibility of the pure drug with the polymer FTIR is done. FTIR of itraconazole with Carbopol 940 was determined. All the characteristic peaks of the itraconazole and Carbopol 940 was noted. FTIR of itraconazole drug shows the characteristic peaks of O-H stretch at 3600 cm^{-1}, C-H stretch at 3300 cm^{-1}, O-H stretch at 3000 cm^{-1} and C≡C at 2200 cm^{-1}. The FTIR analysis of itraconazole with carbopol 940 shows characteristic peaks of C-H stretch at 3300 cm^{-1} , O-H stretch at 3000 $cm^{-1.}$ The outcome of the study was that there was no interaction between the drug and the Carbopol 940 during the formulation of nanogel.

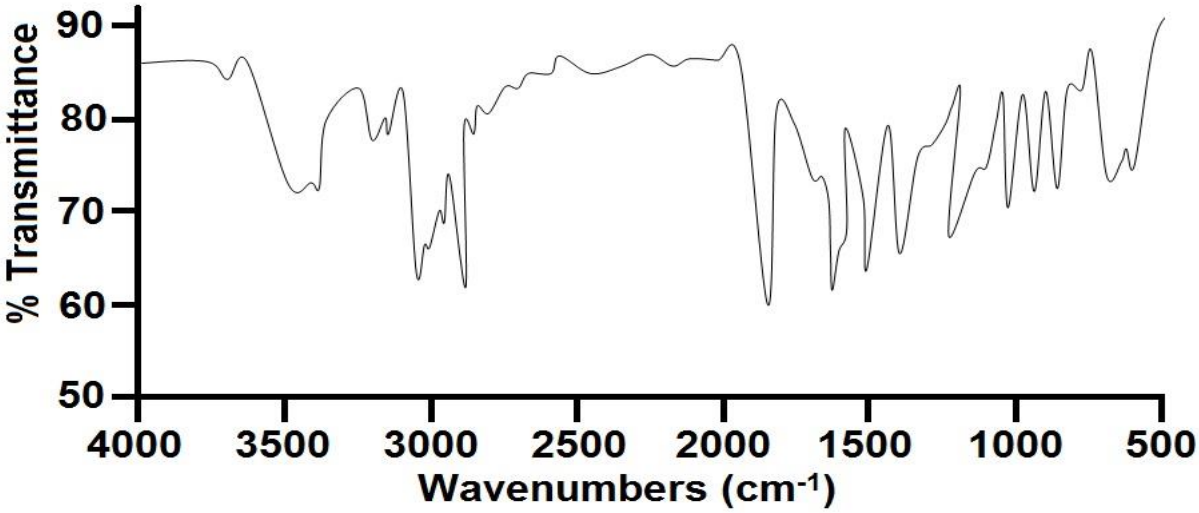


Fig 3: FTIR analysis of itraconazole drug

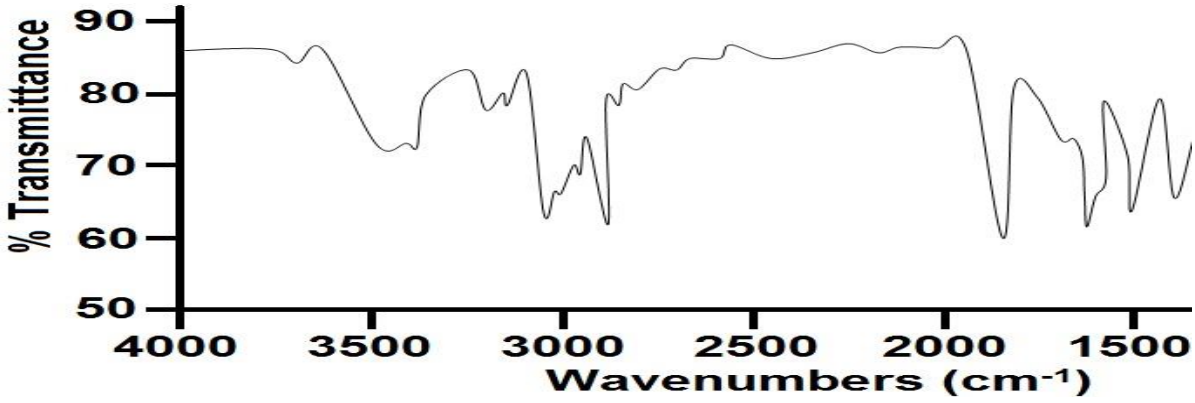


Fig 4: FTIR analysis of drug with Carbopol 940

***In-vitro* drug release profile**

The study was performed and it was observed that the release rate of Itraconazole drug was lesser when compared to itraconazole nanoparticle. The CPR in 100min was found to be 29%, in 300 min released 38% and in 500min CPR was 49% , in case of itraconazole drug. When the CPR of itraconazole nanoparticle was as follows 41% in 100 min, 49% in 200 min and 71% in 500 min.The conclusion was made that the nanoformulation shows more drug release because of its size.

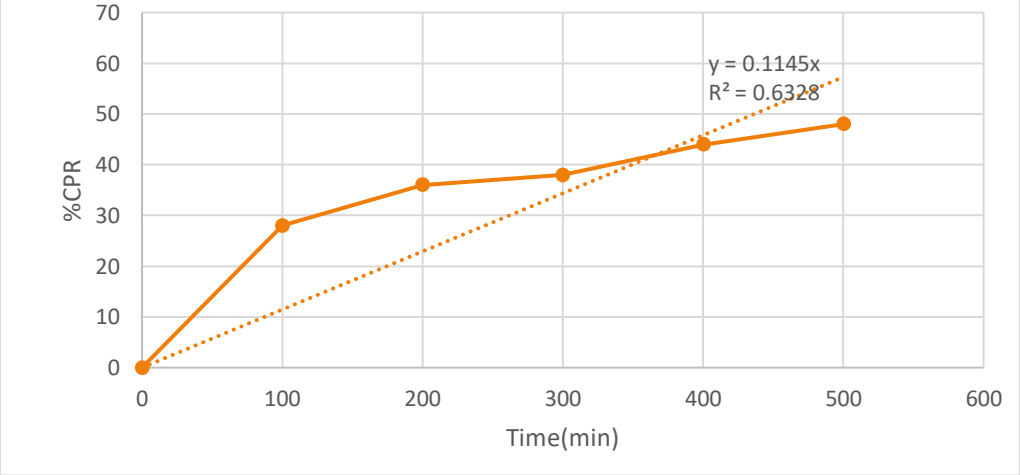


Fig 5: In-vitro drug release of itraconazole drug

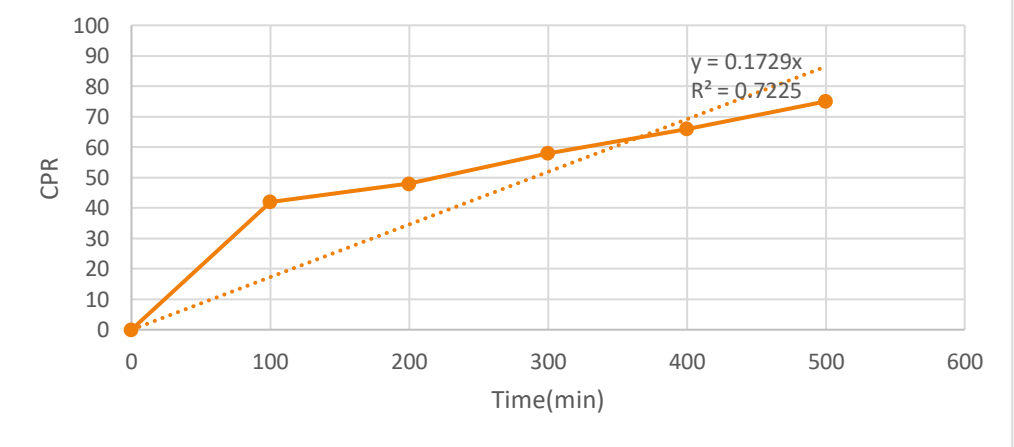


Fig 6: In-vitro drug release of itraconazole nanoparticle

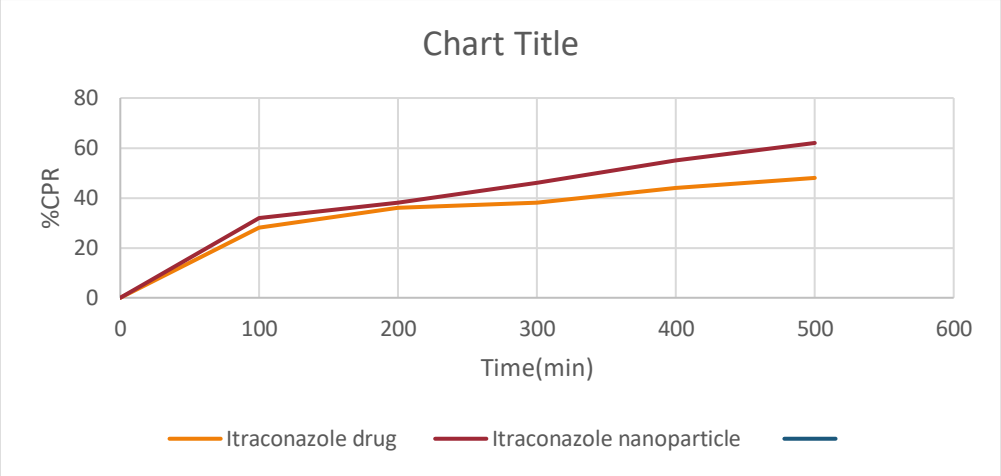


Fig 7: Comparison of In vitro drug release of itraconazole and itraconazole nanoparticle

Physiochemical characterization of gel

Visual inspection

The formulated gel was visually examined for its colour and syneresis. The developed product is homogenous without presence of any lumps in it. The formulation was found to be translucent.

pH determination

The pH of all the formulation was determined and was found in a range of 6.12 - 6.72. So, the pH was determined and was found to be sufficient for the skin without causing any irritation to the skin. The result was represented in table.

Spreadability

Spreadability of the formulation was found out in order to observe the easy application and uniform distribution of the gel. The result of the spreadability was given in the table 2.

Table 2- Physico-chemical characterization

Formulation	pH	Spreadability(g.cm/sec)	Viscosity(cps)	Extrudability
F1	6.71	15.0±0.8	36000	Average
F2	6.23	16.0±0.6	26000	Good
F3	6.12	16.5±0.05	25800	Good
F4	6.68	18.7±0.05	25632	Excellent
F5	6.21	21.4±0.02	25560	Good

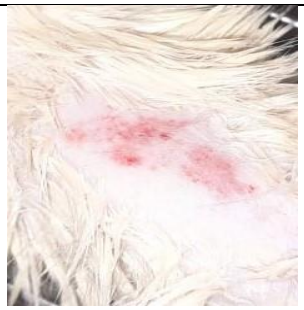
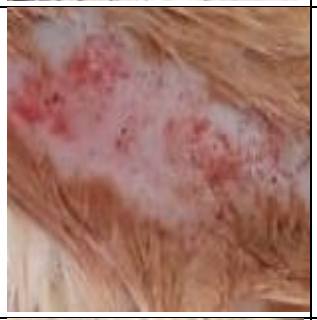
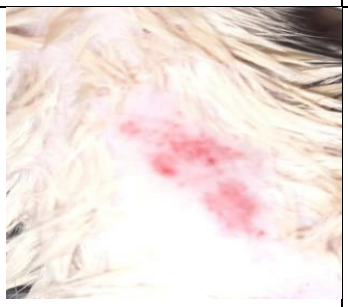
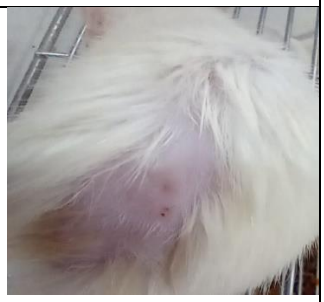
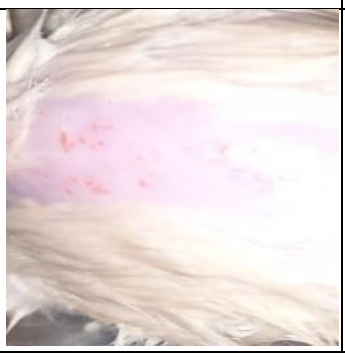
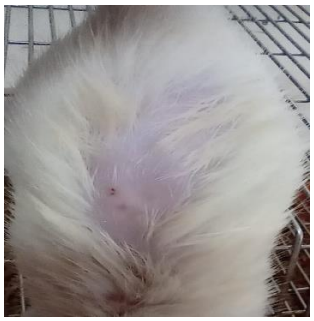
On the basis of the above study F5 was selected as the best formulation.

Pharmacological activity

***In-vivo* antifungal activity**

Determination of the efficacy of the itraconazole nanogel and combination of itraconazole nanoparticle and silver nanoparticle was evaluated in male Wistar albino rat model(100-150g). The candidiasis was induced in albino rats using isolate of *Candida albicans*. Images are given showing rats on different days and showing the cure from the disease because of the treatment given. The efficacy of the itraconazole nanoparticle in combination with silver nanoparticles against cutaneous candidiasis as compared to itraconazole nanoparticle gel and the standard preparation is given in the table. It was observed that rats treated with marketed preparation (Itromed gel 1%w/w) have shown a faster recovery rate as compared to the other formulations. Rats treated with combination formulation of itraconazole nanoparticle and silver nanoparticle have shown a greater efficiency. When observed for the treatment of the itraconazole nanoformulation of the rats, moderate efficacy was observed. Observation was recorded in a tabular form with images (table 3).

Table 3 - Antifungal effect of itraconazole nano formulation and combination with silver nanoparticle on experimental animal

Animal group	Day 1	Day 3	Day 6
Control group (G1)			
Marketed formulation(G 2)			
Itraconazole nanogel(G3)			
Itraconazole nanoparticles +silver nanoparticlesgel (G4)			

DISCUSSION

In the current work the formulation of itraconazole nanoparticle was done by solvent diffusion method. The formulation of itraconazole drug to its nano form improves its overall efficacy. Various pharmacokinetic problem of the itraconazole drug of poor aqueous solubility and low bioavailability can be overcome by formulating it in nanoform (14). The characterization of the nanoparticle was done by SEM for the particle size and shape of the nanoparticle. The average particle size of the itraconazole nanoparticle was found to be 96-130nm. The nanoparticles were found to be spherical in shape which gives a better cellular penetrability in biomedicals. The oral administration of the itraconazole drug causes side effects. So, in order to overcome those side effects topical gel was prepared. Gel was prepared using pure water. No chemical

interaction was observed between the Carbopol and the itraconazole in FTIR analysis. Various physiochemical analysis of the gel was performed, such as pH, viscosity, extrudability and spreadability. The data justified that increase in viscosity results in decreased spreadability. The pH of the gel shows that the pH was compatible with the skin as it was near to the skin pH (16). Silver nanoparticle was synthesized according to the previously existing data (15). The silver nanoparticle was then incorporated with the itraconazole nanogel formulated to see the combinational effect of the two. The gel was then subjected to antifungal activity study against *Candida albicans*. The *in-vivo* antifungal activities show good antifungal activity of the itraconazole nanoformulation but better results were seen when itraconazole and silver nanoparticle were used in combination (21). The antifungal activity of the formulation in combination corresponds activity similar to the marketed preparation. This implies that the combination of itraconazole and silver nanoparticle shows a synergistic effect.

CONCLUSION

This study revealed that the solvent diffusion method can be effectively used for the formulation of itraconazole nanoparticles. The nanoparticles were analysed by SEM which revealed spherical shape and nano size of the formulated nanoparticles. The *In-vitro* release studies show that better drug release was observed in case of itraconazole nanoparticle formulation. Topical nanogel was formulated using carbopol 940. The FTIR analysis was done which confirmed that there was no interaction between the drug and the polymer used. Further, antifungal activity of the itraconazole nanoformulation and itraconazole nanoformulation in combination with silver nanoparticles was done and then it was observed that, the combination shows better antifungal activity then the itraconazole nanoformulation alone. So, the conclusion can be drawn that the itraconazole nanoparticles in combination with silver nanoparticles gives a synergistic effect. However, more study is required to be done further on this synergistic effect of the combination of two.

REFERENCE

1. Verma S, Utreja P. "Vesicular nanocarrier based treatment of skin fungal infections: Potential and emerging trends in nanoscale pharmacotherapy". Asian J Pharm Sci 2019;14(2):117–129.
2. De Pauw BE. "What are fungal infections"? Mediterrnean Journal of Hematology and Infectious Disease. 2011;3(1). 2035-3006.
3. Dowd FJ. "Candida Albicans Infections". Reference Module in Biomedical Sciences. Elsevier Inc.; 2014;1–3 .
4. Zackheim HS. "Cutaneous candidiasis". The Journal of the American Medical Association 1968;204(5):393.
5. Ho K, Cheng T. "Common superficial fungal infections, a short review". Medical Bulletin. 2010;15(11):23–27.
6. Mayer L, Wilson D, Hube B. "Candida albicans pathogenicity". Virulence. 2013;4(2):119–128.
7. Dabas PS. "An approach to etiology, diagnosis and management of different types of candidiasis". Journal of Yeast and Fungal Research. 2013;4(6):63–74.
8. Panáček A, Kvítek L, Prucek R, Kolář M, Večeřová R, Pizúrová N, et al. "Silver colloid nanoparticles: Synthesis, characterization, and their antibacterial activity". Journal of Physical Chemistry B. 2006; 110(33):16248-53.
9. Shrivastava S, Bera T, Roy A, Singh G, Ramachandrarao P, Dash D. "Characterization of enhanced antibacterial effects of novel silver nanoparticles". Nanotechnology. 200;225103
10. Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramírez JT, et al. "The bactericidal effect of silver nanoparticles". Nanotechnology. 2005; 2346
11. Hardes J, Ahrens H, Gebert C, Streitbuerger A, Buerger H, Erren M, et al. "Lack of toxicological side-effects in silver-coated megaprostheses in humans". Biomaterials. 2007; 2869-75.

12. Jain J, Arora S, Rajwade JM, Omray P, Khandelwal S, Paknikar KM. "Silver nanoparticles in therapeutics: Development of an antimicrobial gel formulation for topical use". In: Molecular Pharmaceutics. 2009;1388-1401.
13. Melaiye A, Youngs WJ. "Silver and its application as an antimicrobial agent". Expert Opinion on Therapeutic Patents. 2005; 125-130
14. Ige PP, Sonawane RO."Preparation of itraconazole nanoparticles and its topical nanogel : Physicochemical properties and stability studies". International Journal Of Pharmaceutical Sciences And Developmental Research. 2019;(February). 001-008
15. Vu XH, Thanh T, Duong T, Thu T, Pham H, Trinh DK. "Synthesis and study of silver nanoparticles for antibacterial activity against Escherichia coli and Staphylococcus aureus". Advances in Natural Sciences: Nanoscience and Nanotechnology. 2018; 025019.
16. Dubey NK, Mishra SB, Mukerjee A, Singh A. "Graphene Conjugated Usnic Acid Nano-Formulation for the Treatment of Topical Fungal Infection". Int J Pharm Pharm Sci. 2020;12(5):47–53.
17. Srivastava S, Mishra MK, Dubey NK. "Nano formulation of Itraconazole And Mometasone For The Treatment Of Topical Fungal Infection". Br J Pharm Med Res , 2020;(September). Vol.05, Issue 04, Pg. 2229 - 2237.
18. Kasar PM, Kale K, Phadtare DG. "Formulation And Evaluation Of Topical Antifungal Gel Containing Itraconazole". International Journal of Current Pharmaceutical Research. 2018;10(4):71–4.
19. Pavithra K, Jeganath S, Iqbal A. "Design, development, and characterization of topical gel containing itraconazole-antifungal agent". Asian J Pharm Clin Res.2018;11 153–8.
20. Pershing LK, Corlett J, Jorgensen C. "In-vivo pharmacokinetics and pharmacodynamics of topical ketoconazole and miconazole in human stratum corneum". Antimicrob Agents Chemother. 1994; 38(1):90-5.
21. Sadozai SK, Khan SA, Karim N, Becker D, Steinbrück N, Gier S, et al. "Ketoconazole-loaded PLGA nanoparticles and their synergism against Candida albicans when combined with silver nanoparticles". J Drug Deliv Sci Technol. 2020;56:101574.

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