International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS™ | Volume 8 | Issue 4 | OCT-DEC | 2018 | 623-636
Research Article | Pharmaceutical Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

A COMPARISON STUDY BETWEEN OXICONAZOLE BASED NIOSOMAL AND ETHOSOMAL GEL: NEW APPROACH FOR NOVEL DELIVERY SYSTEMS

Shaik Harun Rasheed[1]*, Rama Kotaiah Mogili[2], Chandra Sekhar Kothapalli Bannoth[3]

***[1]Research Scholar, Jawaharlal Nehru Technological University Anantapur, Anantapuramu - 515 002, Andhra Pradesh, INDIA.**

[2]Department of Pharmaceutics, Malineni Perumallu Educational Society's Group of institutions, Pulladigunta, Guntur - 522017, Andhra Pradesh, INDIA.

[3]Department of Chemistry, JNT University Anantapur, Anantapuramu-515002 Andhra Pradesh, INDIA.

***Corresponding Author Email: shaikharunrasheed@gmail.com**

ABSTRACT

Fungal infection is one of the most common and painful symptoms experienced by both human and veterinary. National Centre for Health Statistics concludes that 80% of patient involved in this problem complained about pain during patient counseling. Pharmaceutical innovation currently has focused on the improvement of novel drug delivery systems (such as liposomes, niosomes, nanosomes, ethosomes, dendrimers, nanoparticles etc) which can increase drug efficacy and potency as compared to other form of drug delivery. The current study focused on to formulate & develop topical antifungal gel in the form of ethosomes and niosomes loaded with oxiconazole as an antifungal drug. Ethosomes and niosomes are well preferred drug delivery system and potential for targeted delivery in anti-cancer, antifungal agents etc. Oxiconazole (OXZ) as an active drug in the form of ethosomes and niosomes could be a novel approach towards skin mediated drug delivery system. Different types of polymers have been introduced to formulate oxiconazole gels. FTIR and DSC techniques used to confirm compatibility study between drug and polymers. Ethosomes and niosomes have been prepared by using injection method and Lipid hydration method respectively. TEM and SEM analysis technique is used to confirm the particle size, shape and morphology of the prepared oxiconazole gel. Particle size played an important role for easy penetration of topical ointment and polyethylene glycol also used as penetrating enhancer in the formulation. All formulations of ethosomes and niosomes based oxiconazole gel subjected for evaluation study. Dissolution study confirms that formulation N15 (niosome) has shown better result in controlling oxiconazole up to 98% in 12h as compare to ethosome. The kinetic release study follows non fickian behavior. Formulation N15 subjected for ex vivo and in vivo diffusion study and shows optimum release rate as compare with commercially available oxiconazole gel.

KEY WORDS

Antifungal; oxiconazole; skin infection; niosomes

INTRODUCTION

Fungal infection mainly caused by microscopic organism such as fungi readily available in the environment which can invade the epithelial tissue. The treatment procedure and surgical care as well as new drug delivery system are more potent against the fungal growth (1). Yeast, molds, rusts and mushroom are belonging to the

family of fungi can grow easily on animals and also obtain nutrients from environment (2, 3). Most of the fungi are useful in biodegradation and helpful for the environment whereas few of them produce infection by entering inside the human body through nasal route, lungs and wounds etc. Fungal infection commonly affects to the hair, nail and skin. In case of systemic infection fungal pneumonia can occur to individuals depending on the favourable climates for the proliferation of fungi (4-6).

The fungal cell wall contains chitin and polysaccharides which make the outer cell very rigid and act as a barrier to prevent the penetration of the drug to the cell. Fungi cell contain ergosterol which control the efficacy of the drug and reduce its potency. Fungal infection is considered as one of the major causes of human disease (7) can leads to systemic infection in a chronic condition. Candidiasis is one of the threatening infections occur by Candida fungi found worldwide showing high risk to the patient life. Candida first adheres to the host cell and releases some virulence factors which damage the host tissue. Similarly, cryptococcal meningitis and invasive aspergillosis are also considered as one of the life-threatening fungi infection. In case of eukaryotic fungi there are very less drugs are available which can kill the pathogen due to the very close evolutionary relationship with the host. Novel antifungal drugs are directly targeted sterol component of cell membrane of fungi either depleting the ergosterol or inhibiting the synthesis. Due to high antifungal resistance of the pathogens a research has been focused on the preparation of novel antifungal compounds.

Medication conveyance frameworks are strategies which are utilized to guarantee that medications get into the body and achieve the region where they are required. These frameworks must consider various requirements, extending from simplicity of conveyance to viability of the medications with exact dosing. Stratum corneum has been viewed as the real boundary to infiltration of substances in to and through the skin. Patients have a tendency to incline toward strategies which are effortless and simple, which is the reason numerous pharmaceuticals come as topical and enteral techniques which can be taken by mouth or connected specifically to the skin (8).

Niosomes are non-phospholipids vesicular contrasting options to liposomes. They are non-ionic surfactant vesicles or surfactant layer vesicles. Niosomes are shaped without anyone else's input - get together of non-ionic amphiphiles with watery media bringing about shut bilayer structures. Niosomes have more infiltrating ability than the past arrangements of emulsions. They are basically like liposomes in having a bilayer, be that as it may, the materials used to plan niosomes make them steadier and in this way niosomes offer numerous more points of interest over liposomes. Ethosomes were created by Touitou et al., 1997, as extra novel lipid bearers made out of ethanol, phospholipids, and water. They are accounted for to enhance the skin conveyance of different drugs (9-11). Ethanol is a productive pervasion enhancer that is accepted to act by influencing the intercellular district of the stratum corneum. Ethosomes are delicate pliable vesicles made predominantly out of phospholipids, ethanol (generally high focus), and water. These delicate vesicles speak to novel vesicles bearers for upgraded conveyance through the skin. The span of the ethosomes vesicles can be balanced from several nanometers to microns.

In this current research work we have formulated ethosomes and niosomes gel which provides a better carrier for OXZ as it builds their dissolvability and offers a controlled discharge due to the polymers such as HPMC, Sodium alginate (SA), chitosan (CH) and carbopol (CP) at different concentrations in the formulations. Different ratios of polymer and OXZ have been taken during the formulation of ethosome and niosome gels to prolong the release rate of active drug. Formulated gels could have the property of greaseless, better spreadability, thermally stable, stable in plasma drug concentration, more stable, compliance and nontoxic in nature (12).

MATERIALS AND METHODS

REAGENTS AND CHEMICALS

Oxiconazole and hydroxy propyl methylcellulose (HPMC K4M) were obtained from Sigma Aldrich chemical laboratory, India. Chitosan and sodium alginate analytical research grade was purchased from SD fine chemicals and used as received. Similarly, carbopol and all other excipients were of analytical research grade and used as received from Cipla, India.

FORMULATION OF ETHOSOME AND NIOSOME GEL

The ethosome has been prepared by using injection method with slight modification of the procedure (13) shown in Table 1. Required amount of oxiconazole as an

antifungal drug is prepared separately with methanol and added with different polymeric solutions prepared (such as HPMC, Sodium alginate, chitosan and carbopol at different concentrations) individually in a water bath at 30oC. Aqueous phase (water) is added in a fine stream to the ethanol with constant stirring at 700 rpm for 15 minutes in a well-sealed container. Obtained solution was left to cool down at room temperature till 1h. In this above-mentioned technique stirring speed, stirring time, rate of injection of aqueous medium are the important factors which played an important role in ethosome formation. Prepared ethosome has been used for entrapment efficiency to find out the percentage of the drug.

Lipid hydration method is used for the preparation of multilamellar vesicles of niosomes (14) shown in Table 2. Weight amount of polymers (such as HPMC, Sodium alginate, chitosan and carbopol) at different concentration has mixed with oxiconazole solution prepared in organic solvent such as methanol in a round bottom flask. Rotary evaporator has been introduced to remove the organic solvent from the prepared sample at room temperature. A thin layer of solid mixture deposited at the bottom of the flask. The thin film was collected and hydrated with aqueous phase with gentle agitation. The obtained product is in the form of niosome and store properly for further evaluation test.

Table 1: Formulation of oxiconazole containing ethosomes gel (%)

Formulation	Drug (%)	CH (%)	HPMC (%)	SA (%)	CP (%)	Soya lecithin (%)	Ethanol (%)	Polyethylene glycol (%)	Distilled water (q.s)
E1	1	1	-	-	-	3	45	2	q.s
E2	1	1.5	-	-	-	3	45	2	q.s
E3	1	2	-	-	-	3	45	2	q.s
E4	1	2.5	-	-	-	3	45	2	q.s
E5	1	-	1	-	-	3	45	2	q.s
E6	1	-	1.5	-	-	3	45	2	q.s
E7	1	-	2	-	-	3	45	2	q.s
E8	1	-	2.5	-	-	3	45	2	q.s
E9	1	-	-	1	-	3	45	2	q.s
E10	1	-	-	1.5	-	3	45	2	q.s
E11	1	-	-	2	-	3	45	2	q.s
E12	1	-	-	2.5	-	3	45	2	q.s
E13	1	-	-	-	1	3	45	2	q.s
E14	1	-	-	-	1.5	3	45	2	q.s
E15	1	-	-	-	2	3	45	2	q.s
E16	1	-	-	-	2.5	3	45	2	q.s

Table 2: Formulation of oxiconazole containing niosomes gel (%)

Formulation	Drug (%)	CH (%)	HPMC (%)	SA (%)	CP (%)	Soya lecithin (%)	Ethanol (%)	Polyethylene glycol (%)	Distilled water (q.s)
N1	1	1	-	-	-	3	45	2	q.s
N2	1	1.5	-	-	-	3	45	2	q.s
N3	1	2	-	-	-	3	45	2	q.s
N4	1	2.5	-	-	-	3	45	2	q.s
N5	1	-	1	-	-	3	45	2	q.s
N6	1	-	1.5	-	-	3	45	2	q.s
N7	1	-	2	-	-	3	45	2	q.s
N8	1	-	2.5	-	-	3	45	2	q.s
N9	1	-	-	1	-	3	45	2	q.s
N10	1	-	-	1.5	-	3	45	2	q.s
N11	1	-	-	2	-	3	45	2	q.s
N12	1	-	-	2.5	-	3	45	2	q.s
N13	1	-	-	-	1	3	45	2	q.s

Formulation	Drug (%)	CH (%)	HPMC (%)	SA (%)	CP (%)	Soya lecithin (%)	Ethanol (%)	Polyethylene glycol (%)	Distilled water (q.s)
N14	1	-	-	-	1.5	3	45	2	q.s
N15	1	-	-	-	2	3	45	2	q.s
N16	1	-	-	-	2.5	3	45	2	q.s

PHYSICOCHEMICAL EVALUATION

ENTRAPMENT EFFICIENCY

The entrapment efficiency of the antifungal drug oxiconazole is determined by UV Visible spectrophotometer. This technique is used to find out the drug content in the formulations. Prepared gel has been diluted into 10 ml of methanol with proper stirring by using magnetic stirrer. A homogeneous solution has been obtained and kept for centrifuge. Centrifuge was carried out at 1200 rpm for 30 min. the supernatant liquid has been analyzed under UV Visible spectrophotometer at 296 nm with suitable dilution. The entrapment efficiency was calculated by formula mention below.

% Entrapment efficiency = (Amount of drug entrapped / Amount of drug added) X 100

PERCENTAGE YIELD

It is calculated to determine whether the drug entrapment in the polymer was efficient could be used to determine the practical yield of the final product. The product results expect close to measuring final output which should compare with the raw materials weight.

Percentage yield = (Practical yield / theoretical yield) X 100

DRUG CONTENT

Prepared gel was weighed up to 1gm and diluted with buffer sample pH 6.8 and make the volume up to 50 ml. From the solution 5ml was pipetted out in 25 ml volumetric flask, and the volume was made up utilizing phosphate buffer pH 6.8. The absorbance was estimated under UV Visible spectrophotometer at 296nm. Medication content was calculated by utilizing standard curve of the oxiconazole.

pH

The pH of various formulations of ethosomes and niosomes were evaluated by using digital pH meter. The pH meter was calibrated before measuring the pH of each formulation and should have done in triplicate to determine the average.

VISCOCITY

Brookfield viscometer is used to measure the viscosity of the prepared gel. The gels were poured in a beaker and rotated at 50 rpm, and the corresponding reading shown on the viscometer was noted. The viscosity of the gel was obtained by Brookfield viscometer. The viscosity was measured in cps. Experiments were carried out in triplicates in case of all the formulations of ethosomes and niosomes.

SPREADABILITY

Prepared ethosomes and niosomes gel was taken for spreadability test. Approximately 350mg of gel was weighed, and then, applied on the glass plate to determine the spreadability of the gel. Another glass plate was dropped at the height of 5 cm to the previously applied glass plate. After one minute the diameter of circle was measured and the test as performed in triplicate, and average values were calculated.

SEM ANALYSIS

Scanning electron microscopy (SEM) was conducted to analyze the surface morphology of ethosomes and niosomes gel. SEM analysis used to determine the shape of the formulations. A drop of formulated gel was mounted on clear glass stub, air dried and visualized under SEM.

***IN VITRO* DRUG RELEASE**

Franz diffusion cell of vertical form is used to determine in vitro drug release study. From each formulation of ethosomes and niosomes 3 mg of freshly prepared gel was spread on the donor side of the cellulose nitrate membrane grade 110 (each sample done in a triplicate manner). The cellulose membrane soaked with isopropyl alcohol to make it more hydrophobic. In receptor vessel 1litre of saline phosphate buffer (pH-7.4) with methanol was used. The study was carried out at 37 ±0.5°C temperature and the speed of agitator maintained as 400rpm for 12h. After a regular interval of time 5ml sample was collected and replaced with the same buffer solution. Collected samples were marked and kept it for analysis under UV Visible spectrophotometer. A suitable dilution has been made for each sample and concentration was measured at 296nm. The obtained result reveals that % of drug release at regular interval of time from the prepared gel.

RELEASE KINETIC STUDIES

The release kinetic study of oxiconazole based ethosomes and niosome has been conducted by using dissolution profile (15). The kinetic study was evaluated by the following equation mentioned below.

- Zero order: $M_t = M_o + K_o t$
- First order: $\ln M_t = \ln M_o + K_1 t$
- Higuchi model: $M_t = K_H \sqrt{t}$
- Korsmeyer–Peppas model: $M_t/M_o = K_k t^n$

Where M_t is the amount of drug dissolved at time t, M_o the initial amount of drug, $K_1$is the first order release constant, K_0 the zero-order release constant, K_H the Higuchi rate constant, K_k the Korsmeyer–Peppas model release constant and n is the diffusional release exponent indicative of the operating release mechanism. The correlation coefficient (R^2) value was used as an indicator of the best fitting, for each of the models considered.

***EX VIVO* DIFFUSION STUDIES FOR BEST FORMULATION**

A male healthy albino rats weighing 150-180 g were sacrificed for abdominal skin. The best formulation in the form of gel was applied on the animal skin. 3mg of gel of oxiconazole drug was applied through donor compartment on the animal skin. Similarly, marketed gel of oxiconazole of 3mg was taken and applied through the donor compartment on other diffusion cell. In both the diffusion cells, reservoir compartment was filled with 10 ml of methanol and 40 ml phosphate buffer solution (pH 7.4) at 37±0.5°C at 400 rpm/min for 12 hrs. Samples were withdrawn from reservoir compartment at regular interval of time and the amount of drug release was determined by UV Visible spectrophotometer at 296nm after suitable dilution. Each time the reservoir compartment was replaced with the same quantity of fresh phosphate buffer solution (pH 7.4). Oxiconazole drug content was estimated prepared in gel form and similarly marketed gel was calculated and reported. All the studies were conducted according to the ethical guidelines of CPCSEA after obtaining necessary clearance from the committee (Approval No: 1688/PO/E/2013/CPCSEA).

***IN VIVO* STUDIES FOR BEST FORMULATION**

PREPARATION OF MICROORGANISM

Sabouraud dextrose agar media is used for the growth of the fungus such as Candida albicans by culture for 48h at 30°C. The cells were collected and hydrated with sterile saline to obtain a final microorganism (concentration of 107 CFU/ml) which is used as a causative organism for skin infection in animal (16).

PREPARATION OF THE ANIMAL

The rats were divided into 4 groups of 6 animals each. The first group was served as negative control without fungal infection. The second group induced by fungal infection for 72h and served as positive control. Groups 3 treated with best formulation of niosome and considered as test group. Group 4 was treated with Marketed drug (Oxistaj cream 1%) and served as standard group.

Group 1: Serves as negative control without fungal infection

Group 2: Serves as positive control induced by fungal infection

Group-3: Serves as test group treated with niosome gel (best formulation)

Group-4: Serves as standard with marketed drug (Oxistaj cream 1%)

All rates were kept under observation during the experiment to make sure any type of clinical features due to fungal infection. Symptoms can be identified as rashes, redness of skin, white discharge, cracking of skin and pimples filled with puss were observed and noted as evidence for skin infection. As time passed the evidence has been recorded in the form of photos and compared between different groups of rats and observed the activity of our best formulation (Niosome based gel) and its control on fungal skin infection. Blood samples were collected in a blood collection tube containing K2EDTA as an anticoagulant, from the tail end of each group of rats. The collected blood samples were subjected for centrifuge at 4°C at 5000 rpm for 5 minutes to separate plasma. The collected plasma stored properly for further investigation of pharmacokinetic parameters such as C_{max}, T_{max}, AUC 0-t K_a, KE, V_d.

RESULTS AND DISCUSSION

PRE-FORMULATION STUDY FOR DRUG AND POLYMERS

BULK DENSITY AND TAPPED DENSITY

Bulk density and tapped density mainly depend on the nature of the compound and its size. These properties of a compound may vary due to the crystallization, milling or in formulation. It also provides the true knowledge of the size of the final dosage form. The density of the solid also affects their flow property after final production. These parameters having significance

towards the preparation of gels which is mostly applied on skin surface. Obtained results of bulk and tapped density have been reported in Table 3 for drug polymer ratios. The bulk density of the drug polymer ratios was found to be 0.299 to 0.542 gm/ml and the tapped density shows 0.211 to 0.41 gm/ml. obtained result shows low interarticular friction between the drug and polymers with better flow property.

FT-IR STUDY

The drug-polymer mixtures were taken, and their agreeableness schedule was performed. This is to set up that other suspenseful therapeutically active cure has not passed through any physicochemical change after it has been subjected to the processing steps during formulation. This may be accustomed on anticipating out the following studies like FTIR. The FTIR spectra of oxiconazole and oxiconazole with HPMC were shown in the Figure 1 and 2.

The FTIR spectral analysis report reveals that oxiconazole has shown its characteristic peaks without any shifting and broadening with the combination of HPMC polymer (similar results obtained with other polymers). From the above results it is concluded that the absorption peaks of oxiconazole remain unchanged in drug-polymer admixture which indicates that there is not any prominent chemical reaction between oxiconazole and the polymers used in the formulations.

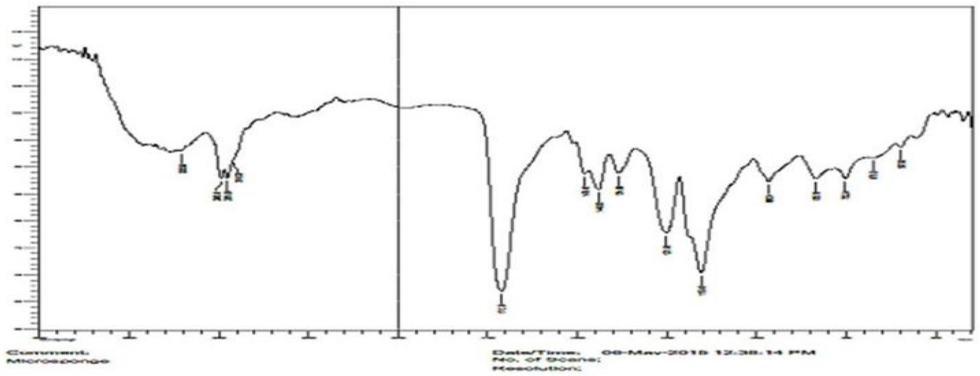

Figure 1: FTIR Spectra of pure Oxiconazole

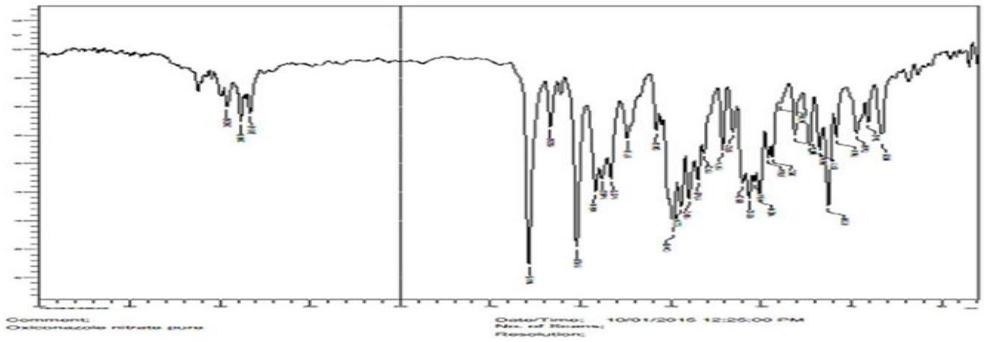

Figure 2: FTIR Spectra of Oxiconazole with HPMC

DSC STUDY

DSC techniques were used to study the compatibility on the active drug such as oxiconazole, different polymers and their compositions. DSC curve of the pure drugs was compared with1:1 ratio of physical mixtures. Thermal sphere of the blends i.e. melting point, the absence of a substantial shift in sudden liquefying point or absence in the display coming from new exothermic/endothermic peak in the blend indicated agreeableness in the middle medicate as well as polymers. Moreover, slight changes in the peak shape, height and width could be the indication of incompatibility. DSC curve of pure oxiconazole, polymers and the complex of drug and polymers has been represented in Figure 3.

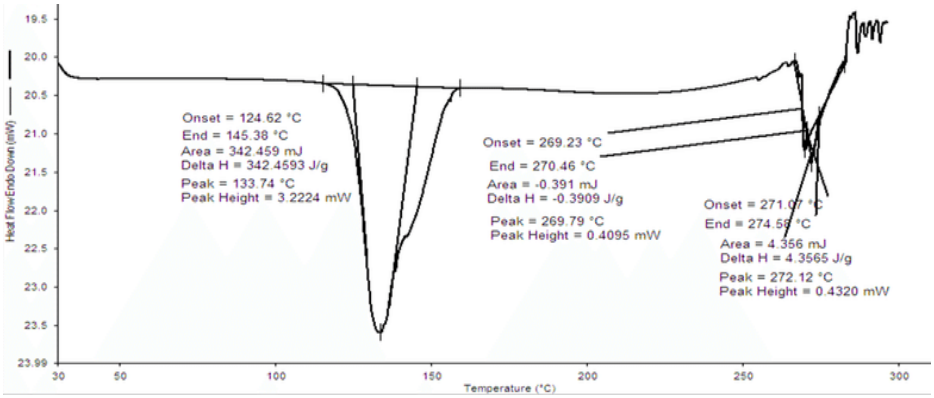

Figure 3: DSC Study of Oxiconazole with HPMC

DSC results reveal that there are no sharp endothermic peaks were detected in furtherance of drug polymers mixture confirms that the polymers used in formulation is compatible with oxiconazole.

EVALUATION STUDY OF NIOSOMES GEL

DRUG ENTRAPMENT EFFICIENCY

Table 4 and 5 shows the drug entrapment efficiency of ethosomes and niosomes containing oxiconazole as an antifungal drug. It provides the data that how much volume of drug entrapped in the prepared formulations. It was observed that 78% to 95% of drugs were entrapped. A larger than involvement was once noticed in spite of formulations upon reducing the particle magnitude tense net appear area of the particles increases. Furthermore thus, a most area appear on the part of medicate entrapment, though in pursuance of longer particles with shorter transpire shows a better result of entrapment rather than adhesive medication. The greater fluidity of ethanol also played an important role to increase the entrapment efficiency. Due to the above reason the amount of ethanol kept constant in each formulation.

Table 4: Evaluation study of oxiconazole containing ethosomes gel

Formulation	Drug entrapment efficiency (%)	Percentage yield (%)	Drug content (%)	pH	Viscosity(cps)	Spreadability (g cm/s)
E1	78.32±1.02	88.73±1.16	86.17±2.72	6.21±0.06	450.35±2.88	4.01±0.06
E2	79.19±1.13	90.64±0.12	88.31±1.73	6.96±0.1	480.74±12.54	4.31±0.1
E3	80.90±1.36	93.97±1.58	91.02±0.69	6.93±0.9	492.09±11.47	4.41±0.9
E4	85.70±1.21	91.32±0.6	90.68±2.92	7.06±0.09	520.46±10.20	4.56±0.09
E5	84.97±1.72	93.52±0.14	87.42±1.61	6.11±0.15	522.73±11.47	4.51±0.15
E6	88.91±2.01	90.37±1.43	91.50±0.93	7.10±0.24	531.46±12.63	4.70±0.04
E7	91.03±1.13	77.28±1.89	94.47±2.56	7.11±0.09	569.72±11.69	4.91±0.09
E8	90.42±2.19	73.12±0.51	94.66±1.82	6.9±0.07	577.82±9.6	4.9±0.07
E9	81.59±1.76	76.26±2.07	89.67±0.78	6.54±0.85	486.87±11.79	4.34±0.05
E10	84.91±3.11	91.97±1.35	91.31±1.71	6.93±0.82	527.39±13.19	4.49±0.02
E11	91.37±2.19	73.24±1.09	94.51±1.71	7.11±0.25	547.63±10.81	4.81±0.15
E12	90.80±1.79	70.73±0.27	93.57±0.58	6.97±0.78	571.97±12.73	4.97±0.08
E13	89.11±1.81	87.26±1.07	86.88±1.78	6.91±0.81	531.86±9.22	4.61±0.11
E14	91.30±2.11	89.26±1.07	92.73±1.77	6.17±0.93	547.95±10.31	4.87±0.09
E15	94.99±1.01	96.32±0.38	95.61±1.07	7.38±0.03	578.95±13.31	5.3±0.03
E16	93.29±2.91	95.37±0.19	94.13±1.32	7.29±0.93	580.05±9.35	5.21±0.33

Table 5: Evaluation study of oxiconazole containing niosomes gel

Formulation	Drug entrapment efficiency (%)	Percentage yield (%)	Drug content (%)	pH	Viscosity(cps)	Spreadability (g cm/s)
N1	78.12±0.92	87.52±1.81	86.81±1.82	6.31±0.03	450.09±1.08	4±0.19
N2	80.19±1.63	88.21±1.28	88.21±1.94	6.51±0.09	469.86±12.24	4.24±0.24
N3	83.90±1.96	90.01±1.38	89.81±1.07	6.68±0.34	487.01±12.27	4.52±0.01
N4	89.70±1.78	92.11±1.17	91.32±1.17	6.91±0.01	518.66±11.09	4.71±0.37
N5	89.97±1.99	92.71±1.03	88.21±1.73	6.8±0.31	528.88±10.39	4.48±0.19
N6	91.91±2.72	89.71±0.73	89.73±0.19	6.09±0.47	542.72±10.16	4.83±0.27
N7	93.03±1.42	78.91±1.01	93.18±1.74	6.73±0.02	571.31±12.06	4.86±0.89
N8	94.02±1.01	72.81±1.28	94.38±1.09	6.82±0.14	576.47±12.03	5.2±0.37
N9	84.32±1.27	94.08±1.15	87.77±2.18	7.1±0.03	489.09±13.83	4.81±0.17
N10	86.73±1.07	92.11±1.03	90.72±1.81	7.16±0.22	530.71±10.23	4.91±0.07
N11	92.91±1.43	88.71±1.73	92.81±1.32	7.21±0.04	547.63±10.81	5.04±0.81
N12	93.80±1.01	70.3±1.91	94.16±1.38	7.34±0.91	574.16±11.19	5.11±0.11
N13	90.51±1.91	77.26±0.38	88.71±1.37	6.75±0.92	529.71±11.81	4.83±0.28
N14	92.92±1.82	87.73±1.82	93.09±1.32	6.92±0.01	569.39±9.91	5.14±0.85
N15	95.01±1.14	95.05±1.79	95.72±0.21	7.31±0.01	580.04±10.18	5.3±0.01
N16	94.81±1.32	73.83±2.71	94.09±0.82	7.17±0.61	578.99±10.07	5.3±0.07

PERCENTAGE YIELD

In the course of the formulation of ethosomes and niosomes the proportion give way executed afterwards the complete deal with used to be one hundred. The percentage of yield is a relation between practical yield and theoretical yield. The percentage yields from the formulations lay in-between 70% to 95%. All these observations values are displayed in Table 4 &5.

DRUG CONTENT

The percentage drug content of ethosomes and niosomeswas found to be in the range of 86% – 96% shown in Table 4 &5. The highest drug content was found in the optimized formulation N15 containing 1:2 ratio of oxiconazole with CP.

pH

Skin compatibility is the major requirement for a good topical formulation. It was found that the pH of all the ethosomes and niosomes gel formulations was in the range of 6.51–7.34 that suits the skin pH indicating the skin compatibility represent in Table 4 &5.

VISCOSITY

The viscosity of ethosomes and niosomes gel formulation ranged between 450 and 580 cps shown in Table 4 &5. Low viscosity was found for the E1 and N1 formulation containing low molecular weight and lower concentration of CH, 1:1 ratio of oxiconazole and CH polymer. Viscosity increases due to the addition of higher concentration of polymer in the formulation. CH is low viscosity grade of polymer as compare to other polymers such as HPMC, CP, SA.

SPREADABILITY

The healing effect of formulation depends on its spreading coefficient. The value of spreadability of ethosomes and niosomes gel ranged from 4 to 5.3 g cm/s shown in Table 4 &5. Spreadability depends on the viscosity and gelling property of the polymers used in the formulation. The formulation N15 having highest viscosity 580.04cps has high spreading coefficient of 5.3 g cm/s, and the formulation E1 and N1 has lesser spreading coefficient of 4 g cm/s as its viscosity is 450.35 cps4 g cm/s as its viscosity is 450.09 cps respectively.

SEM ANALYSIS

The front morphology as well as shape of best formulation of oxiconazole loaded ethosomes and niosome gel have been analysed by using SEM. Formulations such as E15 and N15 shows smooth surface, reveals complete removal of the solvent from the formulation, and it also indicates particles size ranges from 10 µm to 15 µm and 50 nm to 150nm respectively. The SEM image of formulation N15 has found spherical in shape and observe as separated entity whereas formulation E15 has agglomerated in nature represent in Figure 4.

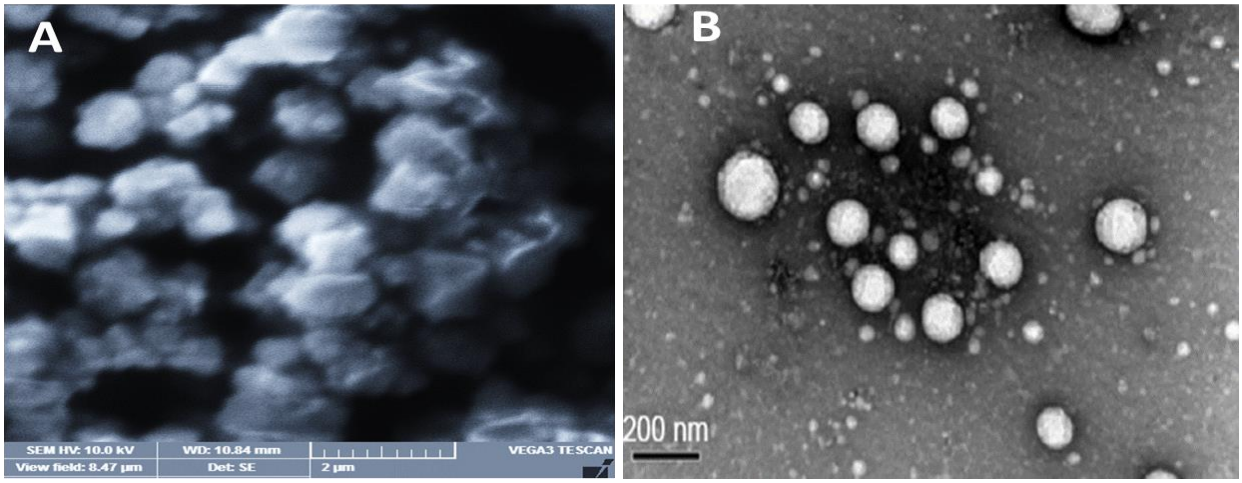

Figure 4: (A) SEM image of Formulation E15 (Oxiconazole as Ethosome gel) and (B) SEM image of Formulation N15 (Oxiconazole as Niosome gel)

***IN VITRO* DRUG RELEASE**

The dissolution investigation was performed in a triplicate way by utilizing the diffusion medium Phosphate buffer with the pH 7.4. The percentage of drug release for all formulations of ethosomes and niosomeprepared gel ranged from 89% to 98% and 90% to 98% at the end of 12 h respectively. Maximum drug release in a sustained manner was observed in the formulation N15 after 12 h. The reason for maximum release may be due to the concentration and the viscosity grade of polymers and their nano form. High viscosity grade of polymers or having gelling nature of polymer could be a useful property for topical formulation to retain the drug molecule for long time and provide a steady plasma drug concentration. HPMC (17) and CP both have gelling property and shown better controlled release as compare to CH polymer. In between HPMC and CP, CP shows better viscosity than HPMC. CP did not disintegrate rapidly due to higher viscosity grade, which could be a barrier for aqueous buffer solution and can easily sustain the release of the active drug. In some cases, the excess viscosity of CP hold the active drug and shows incomplete release. Reports confirm that 1:2 ratios of drug and polymer respectively used for the controlled drug delivery system. CH is a natural and low viscosity grade of polymer (18) which could not able to control the release rate of antifungal drug for optimum time period. Due to this reason CH based formulations having less control on oxiconazole drug release in both ethosome and niosome forms. In few formulations it was observed that if concentration increases a drug release decrease that means drug molecule has been retarded in the formulation and the final percentage of drug release is incomplete. Oxiconazole % of drug release from all the formulations of ethosomes and niosomes shown in Table 6 &7 and in Figure 5.

Table 6: *In vitro* dissolution profile for oxiconazole containing ethosomes gel (Formulations E1-E16)

Formulation	60 min (1h)	120 min (2h)	180 min (3h)	240 min (4h)	300 min (5h)	360 min (6h)	420 min (7h)	480 min (8h)	540 min (9h)	600 min (10h)	660 min (11h)	720 min (12h)
E1	37.09	53.32	79.25	93.31	-	-	-	-	-	-	-	-
E2	35.52	41.39	64.72	77.20	93.11	-	-	-	-	-	-	-
E3	28.81	43.71	61.22	68.81	83.28	90.31	-	-	-	-	-	-
E4	20.22	31.78	47.75	62.81	78.32	88.91	-	-	-	-	-	-
E5	31.67	45.38	69.71	89.27	94.21	-	-	-	-	-	-	-
E6	29.71	41.81	58.32	72.71	84.12	91.98	-	-	-	-	-	-
E7	30.38	41.22	56.39	68.91	81.21	86.72	93.89	-	-	-	-	-
E8	26.33	33.81	49.71	64.71	76.22	85.18	90.33	97.19	-	-	-	-
E9	45.32	57.71	82.71	94.22	-	-	-	-	-	-	-	-
E10	32.87	46.81	62.78	76.32	89.43	94.72	-	-	-	-	-	-
E11	37.82	52.11	65.37	80.23	88.39	95.19	-	-	-	-	-	-
E12	27.31	37.11	48.32	57.68	67.35	78.39	87.12	96.32	-	-	-	-
E13	27.31	32.18	47.31	60.21	72.11	84.22	91.78	-	-	-	-	-
E14	23.56	31.34	42.12	53.77	67.82	75.12	83.17	88.21	92.87	98.22	-	-
E15	19.45	25.61	34.27	47.71	61.87	71.52	78.22	84.32	90.31	94.21	96.73	
E16	17.32	25.34	31.81	43.21	56.32	63.81	70.38	79.31	82.91	86.29	92.22	93.02

Table 7: *In vitro* dissolution profile for oxiconazole containing niosomes gel (Formulations N1-N16)

Formulation	60 min (1h)	120 min (2h)	180 min (3h)	240 min (4h)	300 min (5h)	360 min (6h)	420 min (7h)	480 min (8h)	540 min (9h)	600 min (10h)	660 min (11h)	720 min (12h)
N1	33.59	49.76	76.32	91.32	-	-	-	-	-	-	-	-
N2	29.62	43.90	69.84	81.53	94.81	-	-	-	-	-	-	-
N3	25.96	39.40	57.03	71.13	85.94	91.26	-	-	-	-	-	-
N4	21.85	33.46	49.28	67.96	81.44	90.91	-	-	-	-	-	-
N5	29.58	42..80	67.98	86.54	97.10	-	-	-	-	-	-	-
N6	27.21	38.32	56.33	69.31	82.16	93.27	-	-	-	-	-	-
N7	26.34	35.76	53.26	66.39	78.91	85.21	92.11	-	-	-	-	-
N8	24.72	30.41	47.21	58.99	67.21	78.33	89.23	96.35	-	-	-	-
N9	41.39	56.57	79.33	90.32	-	-	-	-	-	-	-	-
N10	37.33	51.79	69.36	81.55	92.31	-	-	-	-	-	-	-
N11	34.11	49.27	61.71	78.33	89.21	96.32	-	-	-	-	-	-
N12	29.22	41.27	56.26	69.43	82.39	90.28	93.21	-	-	-	-	-
N13	21.72	29.23	42.18	55.32	69.19	77.21	87.35	95.01	-	-	-	-
N14	19.71	26.31	38.32	47.72	61.45	73.26	80.18	87.36	93.32	96.27	-	-
N15	15.32	22.37	32.37	45.78	57.98	65.33	72.32	83.76	89.91	93.32	96.29	98.38
N16	13.45	19.37	27.31	35.87	41.33	49.37	58.36	67.35	75.31	81.38	87.91	90.27

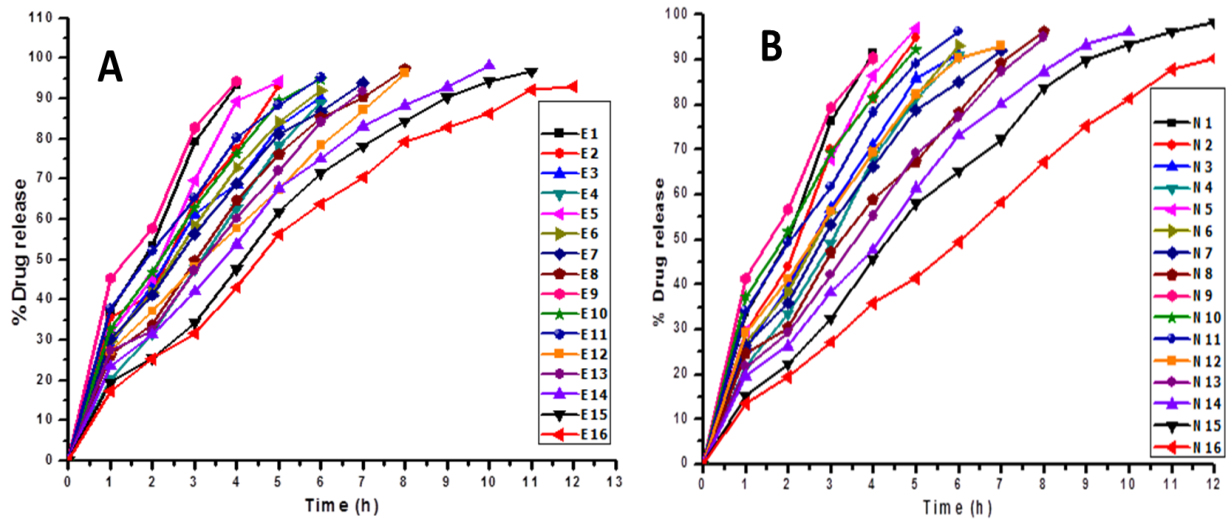

Figure 5: ***In vitro*** **drug release of oxiconazole containing (A) ethosomes gel formulations E1 to E16 and (B) niosomes gel formulations N1 to N16**

KINETIC STUDIES FOR NIOSOMES GEL

Keeping in mind the end goal to decide the correct system of medication discharge from the formulation, the in-vitro dissolution studies was assessed by zero order, first order, Higuchi, and Peppa's equations. The standard of picking the most proper model was in accordance with highest R^2 value as the best fit. The results are shown in Table 8 and 9. The free up illustration data was determined from peppa's plot shown non-fickian release that implies release rates happened by diffusion release of the gels. If n value is less than 0.5, it shows fickian diffusion release, and if n value is between 0.5 and 0.89, it follows nonfickian (anomalous) behavior, i.e., drug release is both diffusion and erosion-controlled mechanism observed in ethosome and niosome formulations.

Table 8: Release kinetics of oxiconazole containing ethosomes gel (Formulations E1-E16)

Formulation	R^2 Values					
	Zero order plots	First order plots	Higuchi plots	Korsmeyer-peppas plots		Order of release
				R^2	Diffusional exponent (n)	
E1	0.998	0.879	0.887	0.999	0.9095	Diffusion & Erosion
E2	0.953	0.901	0.962	0.992	0.37	Diffusion
E3	0.887	0.983	0.991	0.966		Diffusion
E4	0.902	0.863	0.927	0.998	0.979	Diffusion & Erosion
E5	0.911	0.917	0.918	0.997	0.972	Diffusion & Erosion
E6	0.901	0.87	0.978	0.996	0.321	Diffusion
E7	0.884	0.75	0.918	0.969	0.376	Diffusion
E8	0.855	0.975	0.953	0.99	0.421	Diffusion
E9	0.782	0.897	0.988	0.989	0.927	Diffusion & Erosion
E10	0.918	0.898	0.983	0.993	0.87	Diffusion & Erosion
E11	0.952	0.956	0.953	0.985	0.97	Diffusion & Erosion
E12	0.895	0.988	0.991	0.988		Diffusion
E13	0.917	0.954	0.918	0.933	0.988	Diffusion & Erosion
E14	0.9081	0.8663	0.9601	0.9854	0.9728	Diffusion
E15	0.786	0.871	0.987	0.999	0.761	Diffusion & Erosion
E16	0.886	0.899	0.973	0.996	0.821	Diffusion & Erosion

Table 9: Release kinetics of oxiconazole containing niosomes gel (Formulations N1-N16)

Formulation	R^2 Values					
	Zero order plots	First order plots	Higuchi plots	Korsmeyer-peppas plots		Order of release
				R^2	Diffusional exponent (n)	
N1	0.927	0.782	0.899	0.997	0.421	Diffusion
N2	0.933	0.879	0.876	0.988	0.439	Diffusion
N3	0.783	0.989	0.998	0.966		Diffusion
N4	0.911	0.971	0.891	0.993	0.937	Diffusion & Erosion
N5	0.875	0.847	0.908	0.985	0.7.32	Diffusion & Erosion
N6	0.971	0.781	0.971	0.998	0.89	Diffusion & Erosion
N7	0.892	0.878	0.981	0.996	0.78	Diffusion & Erosion
N8	0.871	0.981	0.991	0.998	0.821	Diffusion & Erosion
N9	0.827	0.981	0.979	0.991	0.321	Diffusion
N10	0.874	0.989	0.879	0.998	0.97	Diffusion & Erosion
N11	0.991	0.971	0.893	0.997	0.738	Diffusion & Erosion
N12	0.875	.977	0.879	0.983	0.82	Diffusion & Erosion
N13	0.978	0.997	0.871	0.998	0.728	Diffusion & Erosion
N14	0.891	0.899	0.921	0.999	0.891	Diffusion & Erosion
N15	0.926	0.971	0.998	0.998	0.881	Diffusion & Erosion
N16	0.776	0.877	0.971	0.993	0.731	Diffusion & Erosion

***EX VIVO* DIFFUSION STUDIES FOR BEST FORMULATION**

From in vitro study we observed that niosome N15 is the best formulation as compare to the ethosomes. In ex *vivo* study niosome N15 has been chosen as the best formulation and observed that 94.5% of drug release at 12 h. From the SEM analysis it is concluded that particle size is in nano range. Niosome in nano range as a gel formulation N15 control the release rate for longer period of time as compare to other formulations due to the addition of different type of polymers. The study confirmed significant difference in the drug release and drug content. Figure 6 showed that niosome gel-based formulation N15 when compared with marketed cream (Oxistaj cream 1%). The amount of drug released from marketed cream was 93.9% which is less as compare to niosome gel i.e. 94.5% shown in Table 10. Transdermal flux value for niosome gel found to be 158.91µg/cm^2/hr and marketed cream showed 141.22µg/cm^2/hr. This data confirms that niosome gel N15 reside at targeted site for a longer period of time as compare to marketed product and improved patient compliance was observed. Similarly, the percentage of drug retention in niosome showed better result as compare to the marketed cream. The results obtained for *ex vivo* studies are closely equal to the values of *in vitro* studies. From the study it concluded that oxiconazole gel in the form of niosomes combined with hydrophilic polymer carbopol, exhibit better control in drug release and also forms a good consistency of gel for topical application in fungal infection.

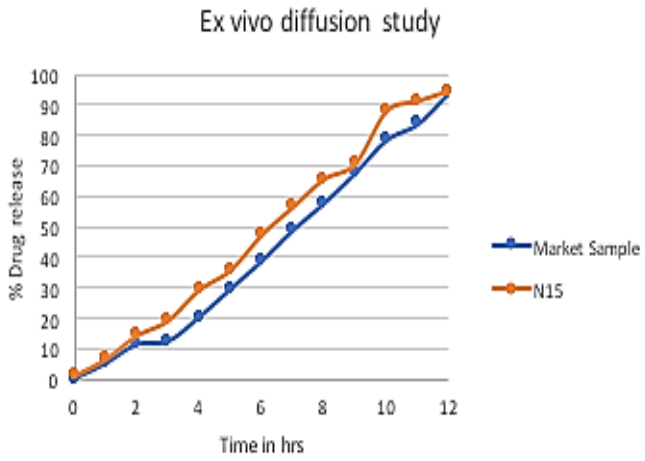

Figure 6: Ex vivo diffusion studies for best formulation N15 and marketed Oxistaj cream

***IN VIVO* STUDIES FOR BEST FORMULATION**

Candida albicans commonly used for the evaluation of antifungal activity (19). Before the application of cutaneous fungal infection, all animals checked thoroughly and showed normal skin without any clinical features of fungal infection such as inflammation, edema, cracking, puss formation or color changes. After the application of fungal infection, the animals showed grayish or purple patches, inflammation, edema, and scaling and cracking of the skin. After treatment with marketed drug Oxistaj cream 1%, the edema and other inflammation disappeared, while the scars were still present with some white spots. The application of oxiconazole based niosome gel showed better results as it transforms the infected part of the skin like a normal skin with slight redness, the study represents in figure 7. The *in vivo* pharmacokinetic parameters like C_{max}, T_{max}, AUC $_{0\text{-}t}$ Ka, K_E, V_d were found to be comparable with marketed Oxistaj cream 1%reported in Table 11.

Table 10: Ex vivo diffusion studies for best formulation N15 and marketed cream

Time in hr	Sqrt time	Log Time	Market Sample		N15	
			% CDR	Log % CDR	% CDR	Log % CDR
1	1	0.1	5.1	0.707	6.5	0.812
2	1.414	0.301	11.3	1.053	14.2	1.152
3	1.732	0.477	12.4	1.093	19.2	1.283
4	2	0.602	20.3	1.307	29.2	1.465
5	2.236	0.698	29.5	1.469	35.7	1.552
6	2.449	0.778	38.7	1.587	47.2	1.673
7	2.645	0.845	48.8	1.688	56.4	1.751
8	2.828	0.903	57.7	1.761	65.5	1.816
9	3	0.954	67.6	1.829	70.6	1.848
10	3.162	1	78.4	1.894	87.8	1.943
11	3.316	1.041	83.7	1.922	91.1	1.959
12	3.475	1.079	93.9	1.972	94.5	1.974

Table 11: In vivo pharmacokinetic parameters for best formulation N15 and Oxistaj cream

Parameters	Marketed formulation (Oxistaj cream 1% w/v)	Test Formulation (N15)
K_E (hr-1)	16.21± 0.19	14.18 ± 0.22
Ka (hr-1)	52.18 ± 0.27	48.21 ± 0.30
Cl_T (lit/hr)	20.38 ± 0.38	17.37 ± 0.32
V_d (lit)	2.41 ± 2.23	2.39 ± 0.19
Cmax (µg/mL)	95.12 ± 2.23	91.00 ± 3.35
Tmax (hr)	4	4
AUC 0-12 (µg.h/mL)	223.18±2.13	234.32 ± 2.10

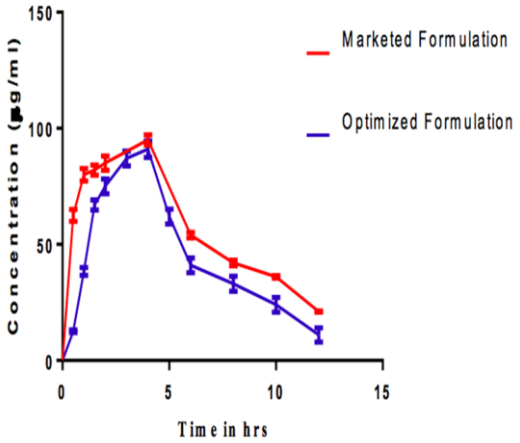

Figure 7: *In vivo* study for best formulation N15 and marketed cream (Oxistaj cream 1%)

CONCLUSION

Oxiconazole drug is prepared in the form of ethosome and niosome gel for the topical skin infection. FTIR, DSC analysis confirms the compatibility between drug and polymers. The *In vitro* Franz's diffusion program of studies conducted for all the formulations for ethosomes and niosomes found that formulations N15 showed optimum drug release in a control manner 98% at 12h and follows non fickian diffusion. Obtained ex vivo results confirms that the niosome gel N15 has shown better drug release as compare to marketed gel Oxistaj cream. *In vivo* study confirms that formulation N15 showed better results as it transforms the infected part of the skin like a normal skin with slight redness as compare to marketed Oxistaj cream. The in vivo pharmacokinetic parameters like Cmax, Tmax, AUC 0-t Ka, KE, Vd were found to be comparable with marketed Oxistaj cream.

REFERENCES

1. Fleming RV, Walsh TJ, Anaissie EJ, Emerging and less common fungal pathogens, Infectious Disease Clinics, 2002; 16(4): 915-933.
2. Lopez-Martinez R, Candidosis, a new challenge. Clinics in Dermatology, 2010; 28(2): 178-184.
3. Heitman J, Microbial pathogens in the fungal kingdom. Fungal Biology Reviews, 2011; 25(1): 48-60.
4. Shapiro RS, Robbins N, Cowen LE, Regulatory circuitry governing fungal development, drug resistance and disease, Microbiology and Molecular Biology Reviews, 2011; 75(2), 213-267.
5. Cannon RD, Lamping E, Holmes AR, Niimi K, Baret, PV, Keniya MV, Monk BC, Efflux-mediated antifungal drug resistance, Clinical Microbiology Reviews, 2009; 22(2): 291-321.
6. Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC, Hidden killers: human fungal infections. Science Translational Medicine, 2012; 4(165): 165rv13-165rv13.
7. White TC, Marr KA, Bowden RA, Clinical, cellular, and molecular factors that contribute to antifungal drug resistance, Clinical microbiology reviews, 1998; 11(2), 382-402.
8. Gisby J, Bryant J, Efficacy of a new cream formulation of mupirocin comparison with oral and topical agents in experimental skin infections, Antimicrobial agents and chemotherapy, 2000; 44(2): 255-260.
9. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M, Ethosomes-novel vesicular carriers for enhanced delivery, characterization and skin penetration properties, Journal of Controlled Release, 2000; 65(3), 403-418.
10. Sinha VR, Bansal K, Kaushik R, Kumria R, Trehan A, Poly-ε-caprolactone microspheres and nanospheres, an overview. International journal of pharmaceutics, 2004; 278(1), 1-23.
11. Pardeike J, Hommoss A, Müller RH, Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. International journal of pharmaceutics, 2009; 366(1-2): 170-184.
12. Cannon RD, Lamping E, Holmes AR, Niimi K, Baret PV, Keniya MV, Monk BC, Efflux-mediated antifungal drug resistance, Clinical Microbiology Reviews, 2009; 22(2): 291-321.
13. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M, Ethosomes-novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. Journal of Controlled Release, 2000; 65(3): 403-418.
14. Azmin MN, Florence AT, Handjani-Vila RM, Stuart JFB, Vanlerberghe G, Whittaker JS, The effect of non-ionic surfactant vesicle (niosome) entrapment on the absorption and distribution of methotrexate in mice. Journal of Pharmacy and Pharmacology, 1985; 37(4): 237-242.
15. Iizhar SA, Ahmed MA, Arief M, Formulation and characterization of mucoadhesive buccal films of trimetazidine dihydrochloride, Latin American Journal of Pharmacy, 2015; 34(8): 1585-1593.
16. Abdellatif MM, Khalil IA, Khalil MA, Sertaconazole nitrate loaded nanovesicular systems for targeting skin fungal infection: In-vitro, ex-vivo and in-vivo evaluation. International journal of pharmaceutics, 2017; 527(1-2): 1-11.
17. Haque SE, Sheela A, Design and in vitro evaluation of interpolymer complex bound metformin sustained release tablet. Journal of Applied Polymer Science, 2014; 131(21).
18. Haque SE, Sheela A, Development of polymer-bound fast-dissolving metformin buccal film with disintegrants. International journal of nanomedicine, 2015; 10: 199-205.
19. Guo F, Wang J, Ma M, Tan F, Li N, Skin targeted lipid vesicles as novel nano-carrier of ketoconazole: characterization, in vitro and in vivo evaluation. Journal of Materials Science: Materials in Medicine, 2015; 26(4): 175.

Received:05.08.18, Accepted: 08.09.18, Published:01.10.2018

***Corresponding Author:**
Shaik Harun Rasheed
Email: shaikharunrasheed@gmail.com