IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 13, Issue 1 Ver. III (Jan. – Feb. 2018), PP 54-71
www.iosrjournals.org

.Novel Proniosomes as Drug Delivery System for Transdermal Application of Itraconazole: Development, Characterization, Clinical and Bioavailability StudY

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Abstract
Objectives: *The objective of the present study was to incorporateItraconazole (ITC)proniosomes in different gel bases in order to develop ITC proniosomal gel for tansdermalapplication and alsoto achieve high drug plasma levels with enhanced bioavailability.*
Methods: *ITCproniosomes optimized formula was prepared by slurry method and the prepared vesicles were evaluated for their entrapment efficiency (EE%),in-vitroITC release, zeta potential and vesicle size. The prepared optimized formula was then incorporated in different gelling agents as xanthan gum, methyl cellulose (MC), hydroxy propyl methyl cellulose (HPMC) and Carbopol 934. The effect of type and concentration of the employed gel bases on homogeneity, spreadability, extrudability, pH, drug content,the permeation study and viscosity measurement, was studied in all prepared gels. Effect of storage was studied forthe best four formulae at three different temperature for six months. Also,the best formula (G3 containing 5% MC) was used to study the antifungal activity, in vivo therapeutic efficacyand also pharmacokinetic parametersin the ratswhich then compared with commercially available formulations (Sporanox®) capsule and solution.*
Results: *The results showed that the drug permeation and rheological properties of ITC proniosomal gel was affected by polymers type and concentration. G3 containing 5% MC showed the best permeation through rat skin (92.15 % ±1.58), the lowest viscosity, high zone of inhibition (3.2 cm). No change was observed (in drug content, spreadability value, pH, in vitro permeation and viscosity) at room and refrigerated temperature, while there was a change at elevated temperature. In pharmacokinetics study, at all time intervals, it was observed that ITC plasma concentrations in rats treated with proniosomal gel were significantly higher than those treated with marketed products.*
Conclusion: *All the formulated gels were of acceptable physical properties and drug content. They exhibited pseudoplastic flow with thixotropic behavior. Regarding in-vitro permeation and rheological properties G3 (5% MC) formula was the best. The prepared ITC proniosomal gel showed enhanced bioavailability in rats compared with the marketed capsule and solution as it exhibited higher $AUC_{0-\infty}$ for proniosomal gel.*

Keywords: *Itraconazole (ITC); entrapment efficiency (EE%); pharmacokinetics; bioavailability.*

Date of Submission: 15-01-2018 Date of acceptance: 03-02-2018

I. Introduction

ITC is an orally active triazole antifungal agent, which demonstrates broad spectrum activity against a number of fungal species including dermatophytes, Malassesia furfur, Candida species, and Histoplasmacapsulatum [1]. It is also prescribed for systemic infections, such as aspergillosis, candidiasis, and cryptococcosis, where other antifungal drugs are inappropriate or ineffective.The mechanism of action of ITC is impairing the synthesis of ergosterol, an essential component of the fungal cell membrane [2].It is a slightly acidic salt (p*K*a = 3.7), and hence is ionisable only at very low pH values. It is also poorly soluble in water, with an aqueous solubility close to 1 ng/ml at pH 7 and slightly higher than 4 ng/ml at pH 1. So, it can be classifiedaccording to the Biopharmaceutical Classification System, to be a highly lipophilic molecule and belongs to class II, which includes molecules with low solubility and high permeability, for which the dissolution rate is the factor limiting absorption. Like other drugs with poor water solubility, itraconazole is not well-absorbed after oral administration, leading to the highly variable absorption extent and rates and, consequently, to differences in serum levels as well as Area Under the Curve (AUC) values [low and erratic

bioavailability]. The involvement of P-glycoprotein in the oral absorption of itraconazole may be another reason for the high variability observed in the bioavailability of this drug, since this determines thepresystemic first-pass effect.Due to low bioavailability of ITC~55%, maximal if taken with a full meal, an alternative route of administration is preferred as transdermal route.The main obstacle for the delivery of drug through the skin is the stratum corneum; various approaches were put forward to overcoming it [3]. Of these, colloidal carrier is an efficient one as it acts as drug containing reservoirs and can loosen the stratum corneum, thereby modifying the barrier, and can adjust the release rate at the target site [4]. Among the various colloidal carriers, liposome and noisome were the popular ones as they can efficiently encapsulate both hydrophilic and hydrophobic drugs. However, these carriers encountered stability problems, and this led to the discovery of proniosomes [5]. Proniosomes are dry, free flowing, granular product which upon addition of water, disperses or dissolves to form a multilamellar noisome suspension and thereby considered the versatile carrier for transdermal delivery. It also provides additional convenience of storage, transport and dosing.The aim of this study was toformulate and evaluateproniosomal gel of antifungal drug for transdermal delivery to enhance bioavailability through controlled drug release.

II. Materials

ITC, Span 60, Cholesterol and Mannitol were purchased from Sigma Chemical Co., (USA); Soya Lecithin phospholipon 90 H was kindly donated by Lipoid (Lipoid, Germany); Methanol, Chloroform, sodium hydroxide and potassium dihydrogenortho phosphate were purchased from El-Nasr Chemical Co. (Cairo, Egypt); HPMC, (Alpha Chemica, Mumbai, India); MC, Carbopol 934 andXanthan gum Oxford company, Hartlepool, United Kingdom. Methanol, HPLC grade,andAcetonitrile, HPLC grade, Merck (Darmstadt, Germany). All other chemicals used were of analyticalgrade.
Methods

Preparation of ITC proniosomes

The optimized formula of ITC proniosomes was prepared by slurry methodusing span 60 as a surfactantdesigned by box behnken design [6].The required weight of surfactant (Span-60), cholesterol and drug was dissolved in chloroform: methanol (1:1) solution then was poured in a 100 ml round bottom flask containing mannitol as a carrier. The flask was attached to a rotary evaporator(Buchirotavapor (R-3000, Switzerland)), to remove solvent at 60 rpm, a temperature of 45 ºC ± 2, and a reduced pressure of 600 mmHg until the mass in the flask had become a dry, free flowing product. These materials were further dried overnight in a desiccator at room temperature [6].

Evaluation of the optimized formula of ITC proniosomes

Entrapment efficiency of ITC proniosomes

The supernatant of the hydrated ITC proniosomal dispersion was separated, diluted, filtered, and measured using UV spectrophotometer (Shimadzu (2401/PC), Japan) at 262.5 nm.Then the entrapment efficiency was calculated.

In-vitro release of ITC

This study was carried out using a USP dissolution tester (Apparatus I). ITC niosomal dispersion (equivalent to 5mg ITC) was transferred to cylindrical tubes tightly covered with a molecular porous membrane. Five mililiter samples were withdrawn at time intervals of 0, 1, 2, 3, 4, 6, 8, 12, and 24 hr followed by replacement with fresh medium. The samples were analyzed spectrophotometrically at 262.5 nm.

Zeta potential determination and vesicle size analysis

Particle Sizing System(Inc. Santa Barbara) was used in the determination of the zeta potential andvesicle's size of the optimized formula.

Transmission electron microscopy (TEM)

A one dropsamople was diluted and a drop of this diluted dispersion was applied to a collodion-coated 300 mesh, copper grid and left for 5 min. A drop of 2% aqueous solution of uranyl acetate was applied for 1 min. The sample was air dried and examined with TEM[TEM Jeol-200 CX, Japan].

Preparation of ITC proniosomal gels

ITC proniosomal gels (1%) were formulated using four different gelling agents, xanthan gum (1 and 2 %), MC (5 and 10 %), HPMC (2 and 4 %) and Carbopol 934 (1 and 2%), as shown in table (1). The weighed amount of polymer powder was sprinkled gently in a beaker, containing 70 ml boiling distilled water (hot water was used in case of HPMC) and stirred magnetically at a high speed. The aqueous dispersion was allowed to

hydrate for 24 hours in the case of Carbopol. Stirring was continued until a thin hazy dispersion, without lumps, was formed. Tengmglycerin was added as humectant with continuous stirring followed by 0.2 gm methyl paraben and 0.02 gm propyl paraben as preservatives then proniosomes of the optimized formula containing 1 % ITC were added with stirring to get a homogeneous dispersion of pronoisomes in the gel. Neutralization was performed using triethanolamine in the case of Carbopol 934 to attain gelling. Finally, total weight of gel adjusted to 100 gm by distilled water.

Table (1): Proniosomal gel formulations containing Itraconazole

Composition	ITC proniosomal gel formulation							
	G1	G2	G3	G4	G5	G6	G7	G8
Xanthan gum (gm)	1	2						
MC (gm)			5	10				
HPMC (gm)					2	4		
Carbopol 934 (gm)							1	2
Methyl Paraben (gm)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Propyl Paraben (gm)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Glycrine (gm)	10	10	10	10	10	10	10	10
Optimized hydrated proniosomalformualtion (ml)	5	5	5	5	5	5	5	5
Water to (gm)	100	100	100	100	100	100	100	100

N.B. proniosomalpowder equivelant to 1 gm ITC

Evaluation of Gels

Clarity

It was determined by visual inspection under black and white background and it was graded as follows: turbid: +, clear: ++, very clear (glassy): +++ [7].

Homogenity

It was determined by visual inspection for the appearance of gel and the presence of any aggregate [8]. Also,a small quantity of each gel can be pressed between the thumb and index finger and noticethe consistency of the base (weather homogeneous or not) and if there is any coarse particles appeared or detached on fingers.

Spreadability

A spreadability test was conducted by pressing 0.5 g of gel between two glass slides and leaving it for about 5 min. until no more spreading was expected. The diameter of the formed circle was measured and used as comparative values for spreadability [9].

Extrudability

The extrusion of the gel from the tube is an important during its application and in patient acceptance. This study is useful in explaining whether the gel is removing from the collapsible tube during application in proper manner or not. Gels with high consistency may not extrude from the tube whereas, low viscous gels may flow quickly, and hence suitable consistency is required in order to extrude the gel from the tube. Onegm of gel was filled in a clean collapsible tube**;** 0.25 gm weight was placed on the free end of the tube and was just touched for 30 second. Amount of gel extruded was noted [7].

pH

Two grams of gel were dispersed uniformly in 20 ml of distilled water using a magnetic stirrer for 2 hrs. The pH of the dispersion was measured by using a digital pH meter (JENWAY, England) [9].

Drug content

Certain weight of the gel (250mg) was dissolved in 100 ml of phosphate buffer pH 7.4, then was transferred into 100 ml volumetric flask. The volumetric flask containing gel solution was shaken for 2 hours on a mechanical shaker in order to get complete solubility of drug [10]. Finally, samples were analyzed spectrophotometrically at 262.5 nm.

In-vitro permeation of ITC gels

Rat skin samples were mounted in a Franz diffusion cell (A = 2 cm^2)(Hanson research, 50084), with the stratum corneum side facing upwards into the donor compartment and the dermal side facing downwards into the receptor compartment.The receiver phase was 10 ml of phosphate buffer saline pH 7.4. The stirring speed was 100 rpm on a magnetic stirrer, and the temperature was maintained at 37 ± 0.5 °C for 24 hr[11]. One

gram of proniosomal gel was placed in the donor compartment, samples of 1 ml were withdrawn at predetermined time intervals 0, 1, 2, 3, 4, 5, 6, 8, 12, and 24 hrand replaced with fresh medium at appropriate time intervals to maintain a constant volume.The samples were analyzed by UV spectrophotometer at the previously determined λ_{max}(262.5 nm) using phosphate buffer saline (pH 7.4) as a blank.The release experiments were repeated in triplicates.

Permeation data analysis

The average cumulative amount of ITC permeated per unit surface area (μg/cm^2) was plotted as a function of time. The drug flux at steady state (JSS) which is the rate of diffusion or transport of a substance across a permeable membrane. After drug permeation has reached steady state, the steady-state flux (J_{SS}) was calculated from the slope of the straight line [12] using the following equation:
Steady state flux (J_{ss}) = $(dQ/dt)_{ss}$ / A Where dQ is the amount of drug that permeated through a unit cross section area, A, per unit time, t. Permeability coefficient (K_P): The permeability coefficient values (cm hr^{-1}) indicates the penetrating power of a drug through the stratum corneum, which can be calculated using the following equations: $K_P = J_{SS}/C_o$ (where C_o is the initial concentration of the drug). Lag time is the time requested for which the diffusion flow becomes stable. The lag time (hr), was estimated by back extrapolation of the linear portion of the steady-state to abscissa. D (diffusion coefficient, cm^2/min) is thevelocity (cm^2 hr^{-1}) atwhichadrugistransportedbetweenoppositefacesofthe skinwhenthereisunitconcentrationdifferencebetweenthem. Diffusion coefficient was calculated as follows: $D = h^2/6\ L_t$ Where h is the thickness of the skin in cm and L_t the Lag time in minutes [13].
The partition coefficient (K): is the partition of the drug between the skin and the delivery system (skin/vehicle) which calculated according to the following equation: $K = K_p\ h / D$

Kinetic treatment and parameters for the permeation of ITCproniosomal gels

Release kinetics is an integral part for the development of a dosage form to establish in vivo in vitrocorrelation (IVIVC). The data of in vitro permeation from various formulae were tried kinetically using various mathematical models like zero-order, first-order and Higuchi model equations [14].

Rheological properties determination

The rheological evaluation of pharmaceutical semisolids is useful since it provides a method of quality control during and after the manufacturing process, information about the structure of the phases present in a product and the influence of various agents used in its formulation.The viscosity was determined using Brookfield R/S+RHEOMETER(Rotary Viscometer, Brookfield Engineering Laboratories, Inc. (USA)), using spindle CC 14. Measurements were carried out to determine the most suitable gel bases. Samples of the gels were allowed to settle over 30 min at room temperature before the measurements were taken. A defined amount (3 g) of each gel was placed inside the plate which was carefully closed. The measurement was started at 1 rpm; the speed was gradually increased till reaching 200 rpm, the speed was then reduced gradually until reaching the starting rpm. The rheological parameters, including viscosity, shear rate, shear stress were directly obtained from the monitor. A complete rheogram was obtained by plotting the shear rate as a function of the shear stress and plotting the viscosity as a function of shear rate. Viscosity and degree of pseudoplasticity(Farrow's constant) were determined. To study the flow behavior of the gels, Farrow's equation was applied as in the following equation [15]:$\text{Log } G = N \text{ Log } F - \text{Log}\eta$ where: G is the shear rate (sec^{-1}), N is Farrow's constant, F is shear stress (dyne/cm^2) and η is viscosity (c.p.). Log G was plotted against Log F to obtain thevalue of N, which indicates deviation from Newtonian flow.

Microbiological assay of ITC

In vitro antifungal studies were performed with agar-cup diffusion method. These tests were carried out using cultures of Candida albicans (AJ 005123) (0.1%), in Sabouraud dextrose agar. Active culture of Candida albicans strain was inoculated in sterile 0.85% NaCl tube in a ratio of 1 : 9. Further dilution of the culture was prepared in a sterile 0.85% NaCl to get 10^6 CFU/ml. Seeding of culture performed by swabbing method in which sterile swab was dipped into the culture suspension and excess fluid removed by pressing gently against the wall of the test tube. Swab was placed on the edge of the agar plate and move across to the other sides, this was separated to obtain an even spread in sterile conditions. Using borer, wells (cups) were made in the seeded agar plates of 6 mm diameter. Cups were filled with 0.5 ml of the following: (A) niosomal dispersion, (B) selected niosomal gel, (C) ITC suspension and (D) suspension of Sporanox® capsule 100 mg.Theplates were kept in freezer for diffusion for 10–15 min. Then placed in incubator for an incubation period of 24–48 hr at 37°C. Results were seen as the diameter of the zone of inhibition around each cup [16].

Stability study

The stability studies were conducted according to ICH guidelines by storing the formulations at an ambient conditions over a period of six months at varioustemperature conditions: at refrigerator temperature (4 ± 2°C, 60 ± 5% RH), room temperature (25± 2°C, 60 ± 5% RH) and elevated temperature (40± 0.5°C, 75 ± 5% RH.The physical appearance, spreadability,pH,drug content, in vitro permeation and viscosity were determined periodically for any change by procedure stated earlier [17].

In vivo evaluation of therapeutic efficacy

Animals: Male Wister rats weighting 200-250 gmwere obtained from the holding company for biological products and vaccines (VACSERA, Cairo, Egypt). The animals were housed in large polyacrylic cages at temperature 24 ± 2°C. Rats had free access to water and pellet diet. All experimental design in the present study was conducted according to the guidelines approved by the Institutional Animal Care and Ethical committee ofDamanhour University.

Evaluation of therapeutic efficacy:

Twenty four animals were used in this study divided into four groups, six animals each. Group (1) served as normal control, group (2)*Candida albicans* control, group (3)administered MC gel containing standard itraconazole and group (4)administeredthe selected proniosomal gel formula. Group (2) to (4) animals were immunosuppressed with intravenous methylprednisolone (5 mg/kg) for 3 successive days. *Candida albicans* from stock isolates were stored in nutrient agar at 27°C then incubated in nutrient broth at 37°Cfor24 hr. Then, diluted with phosphate buffer saline and injected intradermally in the skin of shaved rats. After 72 hr the animals were treated for a period of one week and visually observed for any changes.

Histopathologic analysis

At the end of the experiment,the rats were killed and the skin was excised. The excised skin was immediately immersed in 10% buffered formalin, dehydrated in graded concentrations of ethanol, immersed in xylene, and then embedded in paraffin block. The 5-µm thick sections of skin were cut using microtome and were mounted on slide using commercial glycerol's mounting fluid. The paraffin wax was removed by warming the slide gently, until the wax melted, and then was washed with xylene followed by washings with absolute alcohol and water. The sections were stained with hematoxylin–eosin to determine gross histopathology and collagen deposition, respectively. The slides were analyzed at 40-fold magnification using optical microscope [18].

In vivo pharmacokinetic study

Male Wister rats weighting 200-250 gm were selected for bioavailability study. Treatment with animals was, as mentioned previously. The rats were divided into 3 groups (n=6) and deprived of food but had free access to water 12 hr before the day of experiment [19]. Then, they were treated as follows:

Group I: treated withthe selected formula G3 (ITC proniosomal gel 0.1 % in 5 % MC gel base) by topical application on the abdominal side of rats after shaving about 15 cm^2 of skin.

Group II: treated with a marketed capsule (Sporanox® capsule).

Group III: treated with amarketed solution (Sporanox® solution).

Blood samples (0.5 ml) were withdrawn through retro-orbital vein of rat at predetermined time intervals (0, 1, 2, 4, 8, 12, 24 and 48 hr) after treatment intoheparinized microcentrifuge tubes. The blood samples were centrifuged using Centrifuge (Biofuge, primo Heraeus, Germany) at 4000 rpm for 20 min for separation of plasma. The separated plasma was stored at -21 ^{o}C until drug analysis. Frozen plasma samples were thawed at room temperature. The plasma samples (100 µl) were separated and 0.9 ml of acetonitrile was added to each of plasma samples to precipitate the protein [2]. The samples were then centrifuged again at 5000 rpm for 5 minutes and the supernatant (20 µl) were filtered and directly injected into the HPLC column and peak area values were recorded. The HPLC instrument used(Hitachi LaChrome Elite, Tokyo, Japan), was equipped with a model series L-2000 organizer box, L-2300 column oven, L-2130 pump with built in degasser, Rheodyne 7725i injector with a 20 µl loop and L-2455 diode array detector (DAD). Separation and quantitation were made on a 250 mm X 4.6 mm (i.d.), 5 µm particle size ODS reverse-phase column kept at room temperature. The mobile phase consisted of a mixture of methanol and water in the ratio of 95:5, and the flow rate was 1.5 ml/min. The column oven was 25 °C. The plasma concentrations vs. time profiles were analyzed using Nonlin® software (Version 1.5, Scientific consulting, Inc., Cary, NC, USA) adopting non-compartmental analysis. Data from the plasma concentration time curve within 48 hr after treatment were employed to estimate the following pharmacokinetic parameters for individual rats in each group, maximum drug concentration (C_{max}), the time to reach C_{max} (t_{max}), area under the plasma concentration vs. time curve from zero to the last sampling time (AUC_{0-48h}) and half life ($t_{1/2}$) for ITC [20].

III. Results And Discussion

Evalation of the optimized formula of ITC proniosomes

Entrapment efficiency, *in-vitro* release, zeta potential and vesicle size

EE% was found to be equal 94.95 ± 0.36, the in-vitro release was 98.13 ± 2.51% at the end of 24 hr, andZeta potential was found to be-30.15 ± 0.41.Figure (1) showed niosomesvesicle sizewhich equal to 340.48 ± 0.58.

Microscopic examination

As shown in figure (2), the examined niosomes appeared as spherical, nano sized, unilamellar vesicles with sharp boundaries and well separated from each other. This could be attributed to the fact that on niosome formation using span, spherical shaped niosomes were obtained in order to minimize the surface free energy.

ITCproniosomal gel formulations

The prepared formulae were described as shown in Table (1). Concentraion of ITC in preparedmarketed gel was 1% w/w [21], so this concentration was used in our formulation.

Evaluation of Gels

As shown in table 2, the appearance of all the prepared gel formulae wassmooth and homogenousand there were no clots or any other particles in the gels, table (2). It was found that spreadability for all preparedformulae ranged from5.2 cm to 8 cm which indicates that the gels can be spread easily on skin surface with a small shear [22].Formulations with higher spreadability values allow ease of application and thereby increased surface area available for drug permeation [23]. The percent of gel extruded was 77.42 to 98.10 %, which was found to be good and compatible. The pH values were found to be in the range of (4.4-6.74) which is within the required physiological range and was considered to besafe, non-irritant and suitable for transdermal application [24]. ITC gel formulae had drug content in the range of 91.52-99.10%, which shows efficient loading and uniform distribution of the drug in the formulations**.**Table (3) showsconclusive rank order concerning the evaluation of ITC proniosomal gels.

Table (2): Evaluation of ITC gel formulae

F. No.	Clarity	Homogeneity	Spreadability (cm)	Extrudability (%)	pH	Drug content (%)
G1	+	Good	6.5±0.19	90.23±1.19	6.71 ±0.17	95.23 ±2.24
G2	+	Good	5.5±0.3	86.46±2.32	6.74 ±0.19	93.44 ±1.67
G3	+	Good	8±0.28	98.10±1.15	6.35 ±0.11	99.10 ±1.48
G4	+	Good	5.4±0.21	83.21±1.28	6.45 ±0.12	92.32 ±2.78
G5	+	Good	5.7±0.23	88.50±1.69	6.50 ±0.18	97.31 ±1.36
G6	+	Good	5.2±0.17	77.42±1.47	6.70 ±0.13	91.52 ±2.71
G7	+	Good	5.8±0.16	91.50±0.87	4.40 ±0.14	98.54 ±2.47
G8	+	Good	5.3±0.24	87.20±1.29	5.00 ±0.16	96.98 ±2.34

+ Satisfactory, ++ Good

*All values are Mean ± SD, (n=3)

Table (3): Conclusive rank order (RO) concerning the evaluation of ITC proniosomal gels

F. No.	Clarity	Homogeneity	Spreadability	Extrudability	pH	Total	RO
G1	1	1	2	3	7	14	**3**
G2	1	1	5	6	8	21	**7**
G3	1	1	1	1	3	7	**1**
G4	1	1	6	7	4	19	**6**
G5	1	1	4	4	5	15	**4**
G6	1	1	8	8	6	24	**8**
G7	1	1	3	2	1	8	**2**
G8	1	1	7	5	2	16	**5**

In vitro skin permeation of ITC gels

The *in vitro* skin permeation of ITC gel formulations was investigated through rat skin. As shown in figure (3), 5% MC gel permeated higher percent of ITC than from other tested gel bases. Figures (4-7) showed the permeation profile of ITC gels. It was found that drug permeation was highly dependent on polymer type and polymer concentration.Proniosomes can act as penetration enhancers, which are useful for increasing the permeation of many drugs. The surfactants in the vesicular form decrease the crystallinity of the intracellular lipid bilayers of the skin and thus enhance drug deposition [25].The permeation of ITCproniosomal gels formulae from different gel bases could be arranged in a descendingmanner as follows: G3 (92.15 %) > G1 (90.25 %) > G7 (89.42 %) > G5 (86.24 %) > G2 (85.23 %) > G4 (83.46 %) > G8 (74.21 %) > G6 (70.48 %).These differences may be attributed to the variation in shape and dimension of the crystallites of the solid fraction and their ordering in the 3-dimensional structure within the resulting network [26]. Also, it was found that xanthan gum had a higher Itraconazole release than Carbopol 934 gels as agglomeration and turbidity of Carbopol 934 gel may be the obvious reason for least drug release [11].It was found that the permeation of ITC from gel base decreased as the concentration of gelling agent increased. The release of ITC from 1 % xanthan gum (G1), 5 % MC (G3), 2 % HPMC (G5) and 1 % Carbopol (G7) gel bases were higher than that from 2 % xanthan gum (G2), 10 % MC (G4), 4 % HPMC (G6) and 2 % Carbopol (G8) gel bases. This may be dueto that at the higher polymer concentrations, the active substance was trapped in polymer chains and it was structured by its close proximity to those polymer molecules thus increasing the diffusion resistance. The mechanism for such enhanced resistance may be due to a reduction in the number and dimension of water channels within the gel structure. Also, the density of chain structure which has been observed in gels microstructure increases at the higher polymer concentration and this limits the active substance's movement area [27]. The higher polymer concentration resulted in lower drug release from the vehicles, this is in agreement with Lauffer's molecular diffusion theory of polymer gels [28], which stated that the diffusion coefficient of a solute was inversely proportional to the volume fraction occupiedby the gel-forming agent.

Permeation data analysis

As shown in table (4), the diffusion flux of 5% MC gel (G3) has the highest diffusion than other gelling agents.Fusion of niosome vesicles to the surface of the skin, results in a higher flux of the drug due to direct transfer of drug from the vesicles to the skin [29]. There were very good correlations were obtained between steady state flux and permeability coefficients [30]. The diffusion coefficient of the ITC gels was in the range of 2.4 E^{-4} Cm^2hr^{-1} for G3 to 4.9 E^{-4} Cm^2hr^{-1} for G4. The partition coefficient of the prepared gels was in the range of 1.283 for G6 to 3.457 for G3. No direct correlation was observed between the lag time and the apparent flux released [31].

Table (4): Permeation parameters of ITC proniosomal gels

F. No.	Steady state flux (J_{ss}) ($\mu g\ cm^{-2}hr^{-1}$)	Permeability coefficient ($Cm\ hr^{-1}$)	Lag time (hr)	Diffusion coefficient ($Cm^2\ hr^{-1}$)	Partition coefficient
G1	162.22± 0.78	0.01622± 0.25	1.46± 0.000151	2.8 E^{-4}± 1.51 E^{-5}	2.84± 0.43
G2	158.83± 0.98	0.01588± 0.18	1.0325± 0.00014	4.03 E^{-4}± 2.24 E^{-5}	1.96± 0.39
G3	172.23± 1.27	0.01722± 0.28	1.67± 0.000136	2.4 E^{-4}± 1.52 E^{-5}	3.45± 0.28
G4	158.28± 0.84	0.01582± 0.24	0.834± 8.93 E^{-5}	4.9 E^{-4}± 2.35 E^{-5}	1.58± 0.37
G5	160.68± 0.94	0.01606± 0.21	1.14± 0.000131	3.6 E^{-4}± 3.23 E^{-5}	2.20± 0.58
G6	125.64± 0.82	0.01256± 0.31	0.851± 8.67 E^{-5}	4.8 E^{-4}± 4.20 E^{-5}	1.28± 0.21
G7	161.15± 0.95	0.01611± 0.19	1.42± 0.000127	2.9 E^{-4}± 3.05 E^{-6}	2.75± 0.34
G8	135.76± 1.05	0.01357± 0.39	1.0324± 0.000121	4.03 E^{-4}± 3.24 E^{-5}	1.68± 0.48

*All values are Mean ± SD, (n=3)

Model dependent kinetics for the in-vitro permeation of ITC gels

The kinetic treatment of the permeation of ITC is critical and has to be investigated to achieve an optimal system with desired release characteristics. As shown in table (5), it was found that the higher correlation coefficient values were for Higuchi diffusion model,suggesting that the permeation release of ITC from all prepared proniosomal gel preparations can be best described by Higuchi's diffusion model [32].

Table (5): The calculated correlation coefficients for the In-Vitro permeation of ITC gels employing different kinetic orders or systems

Formula No.	Correlation coefficient (r)		
	Zero	First	Diffusion
G1	0.8962	0.9317	**0.9564**
G2	0.8965	0.9390	**0.9579**
G3	0.8692	0.9260	**0.9428**
G4	0.8954	0.9342	**0.9550**
G5	0.8804	0.9324	**0.9469**
G6	0.9081	0.9220	**0.9530**
G7	0.8937	0.9364	**0.9574**
G8	0.9034	0.9384	**0.9617**

Rheological properties of gel formulae

Viscosity is an important parameter for characterizing the gels as it affects the extrudability and release of drug [30]. The minimum viscosities ranged from 199.14to 1293.5cp, while the maximum viscosities were in the range 4145.2to 26258.2cp, as shown in table (6). The maximum viscosities of MC gel bases were lower than that of other tested cellulose derivatives. This may be attributed to variation in shape and dimensions of crystallites of different polymers [31]. Itraconazole gels with xanthan gum gave less viscous with a sticky texture and unclear gel while,Carbopol 934 yielded turbid gel.A plot of log G versus log F gives a straight line which allows for the determination of N value (Farrow's constant). Where F is shearing stress, G is rate of shear, η' is viscosity coefficient and N is exponent. N rises as the flow becomes increasingly non-Newtonian and it is a measure of the degree of pseudoplasticity, as shown in figure (8) .All the rheological data of the different gels were fitting to the power's law with (R^2) values ranged from 0.9492 to0.9916.Also, The pseudoplastic behavior was evidenced by that the flow curves approach the origin with no yield values and N value is higher than1, it ranged between 2.2383- 4.7755.In addition, pseudoplastic behavior was evidenced by decreasing viscosity with increasing shear rate (shear thinning) and an increase in the shear stress with increasing the speed. Incorporation of niosomes in semisolid gel forms a colloidal network that aligns itself in the direction of applied shear showing pseudoplastic behavior. This is a desirable property in semisolid preparations, since it should thin during application [33].Thixotropy is a desirable property in gel formulae because these systems retain their high consistency in the container yet can be poured from the containers precisely and spread on the skin easily. Besides, thixotropy improves product stability by decreasing the rate of sedimentation [34].The larger the area of hysteresis loop, the higher the thixotropic behavior [35].

Table (6): Total rank order concerning viscosity, thixotropic behavior and Farrow's constant of ITC proniosomal gels

F. No.	Max. viscosity (cp)	RO	Min. viscosity (cp)	RO	Farrow's constant	RO	Total	RO
G1	4805.4	**2**	199.14	**3**	2.6545	**4**	**9**	**2**
G2	15125.6	**5**	1293.5	**8**	2.2649	**7**	**20**	**8**
G3	4145.2	**1**	136.2	**2**	2.5086	**6**	**9**	**2**
G4	17755.9	**6**	653.7	**6**	2.5878	**5**	**17**	**7**
G5	9251.5	**4**	120.65	**1**	4.7755	**1**	**6**	**1**
G6	26258.2	**8**	645.8	**5**	3.1201	**2**	**15**	**4**
G7	5854	**3**	225.6	**4**	2.2383	**8**	**15**	**4**
G8	21724.9	**7**	653.7	**6**	2.9249	**3**	**16**	**6**

Further study would be completed for the best four formulae regardingspreadability as well as extrudability,drug content, suitable pH,rheological properties, and greatest permeation flux which were (G1, G3, G5 and G7), as shown in table (7).

Table (7): Total rank order concerning the in vitro release, permeation, gel evaluation, drug content and rheological properties of ITC proniosomal gels

F. No.	Gel evaluation	Drug content	% Drug permeated	Rheological properties	Total	RO
G1	3	5	2	2	12	3
G2	7	6	5	8	26	6
G3	1	1	1	2	5	1
G4	6	7	6	7	26	6

G5	4	3	4	1	12	3
G6	8	8	8	4	28	8
G7	2	2	3	4	11	2
G8	5	4	7	6	22	5

Results of stability study

The results of the evaluation tests performed on the selected formulae at different temperatures along the six months showed no marked change in physical appearance in all formulae. Formula G3 was the most stable one as it had slight change in all tests, so was chosen to complete our study on formula G3.Formula G3 stored at (4 ± 2°C, 60 ± 5% RH), after six months exhibited spreadability 7.93 cm, pH 6.36, drug content 96.11%, *in vitro* permeation 92.09% and viscosity 4095.30 cp. All results above showed that there was no marked change in all tests at this temperature, this might be attributed to the rigidization of the vesicles at low temperature that reduced the permeability of the drug through the membrane [36].Formula G3 stored at (25 ± 2°C, 60 ± 5% RH), after six months exhibited spreadability 7.91 cm, pH 6.36, drug content 95.56%, *in vitro* permeation 91.92% and viscosity 4104.60 cp. All results above showed that there was no marked change in all tests at this temperature but, there is a direct relationship between the rate of leakage of drug out of the vesicles and the temperature of storage, the leakage increased with increasing temperature [37], figure (9).

Formula G3 stored at (40 ± 0.5°C, 75 ± 5% RH), after six months exhibited spreadability 8.41 cm, pH 6.42, drug content 91.76%, *in vitro* permeation 91.94% and viscosity 3881.9 cp. Observed changes were found in drug content % and in viscosity at this higher temperature due to high drug leakage from the vesicles. This may be due to the higher fluidity of lipid bilayers at higher temperature, resulting in higher drug leakage. Loss of drug from the vesicles stored at elevated temperature may be attributed to the effect of temperature on the gel to liquid transition of lipid bilayers together with the possible chemical degradation of the phospholipids, leading to defects in membrane packing [38]. So, it is preferred to keep the proniosomal gel in refrigerated conditions.

Microbiological assay of ITC

The microbiological assay was carried out for (G3) proniosomal gel, niosomal dispersion of ITC with marketed product and ITC suspension. The diameter of zone of inhibition (ZOI) obtained with four groups was shown in figure (10). Marketed product (Sporanox® capsule) showed ZOI of 2.8 cm compared to ZOI of 4 cm obtained for niosomal dispersion, 3.2 cm obtained for proniosomal gel and 2.5 cm obtained for ITC suspension after 48 hr. This was explained as the size of ZOI largely depends on the solubility and diffusibility of the sample through the agar media [34]. The obtained results revealed that the developed niosomal dispersion and proniosomal gel were more efficient when compared with marketed product and drug suspension in an antifungal action. These niosomes acts as carriers for drug delivery to the particular site of action. The antifungal activity was created by the drug incorporated in the niosome [39].Smallest zone produced by pure ITC may be due to the minimal solubility of the drug in an aqueous media. Antifungal activity of niosomal dispersion was greater compared to proniosmal gel because the drug release from gel formulation was retarded compared to niosomal dispersion [40].

In vivo evaluation of therapeutic efficacy

Candida albicans,fungal infection was widely used in the evaluation of antifungal activity of the new compounds. After 72 hr the fungal infection was observed in the form of scaling, edema, cracking of the skin, purple patches and inflammation. After treatment with MC gel containing standard drug the main physical changes were disappeared except slight inflammatory area. On the other hand, the selected proniosomal gel formula improved the skin structure with slight cracking [41], as shown in figure (11).

Histopathologic analysis

Histopathological examination of different groups showed the advantages of the selected formula compared to the gel containing standard drug. The uniform dermal and epidermal layers (Black and blue arrows) in normal control group were changed in *Candida albicans* group that showed dermal layer infiltrated (Black arrows, A) with lymphocytes, macrophages and plasma cells, also showed fungal structures at superficial epidermis (Blue arrow, B) (figure 12). The standard group of MC gel with standard ITC showed a uniform dermal layer while the epidermal layer showedhyperkeratosis (Blue arrow) and skin appendages (Black arrow) (Figure 12). On the other hand, the selected formula of proniosomal gel produced more improvement in the skin layers [42].

Results of pharmacokinetic study

To explain the possible improvement in pharmacokinetic behavior of ITC, the plasma concentration-time curve profiles of ITC after topical administration of (G3) proniosomal gel were compared to marketed capsule and marketed solution as depicted in figure (13). As depicted in table (8), The mean C_{max} values for tested proniosomal gel, marketed capsule and marketed solution were (0.657, 0.459 and 0.598 µg/ml), respectively. This result indicated that proniosomes increased the C_{max} of ITC after topical application. This may be attributed to that the vesicular structure of noisome (formed after hydration of proniosomes) which acts as carriers for drugs and helps to overcome the barrier properties of the skin [43, 44]. The mean T_{max} values for tested proniosomal gel, marketed capsule and marketed solution were 8, 4 and 4 hr, respectively; the delayed occurrence of T_{max} with the proniosomal gel may be due to the slow release of the drug from lipid vesicles of niosomes [45]. It was also observed that the increase in the mean$AUC_{o-\infty}$ (17.3312 µg.hr/ml) of the tested ITC proniosomal gel, as compared with the $AUC_{o-\infty}$ (9.12599 and 12.0562 µg.hr/ml) of the marketed capsule and marketed solution, respectively; reflect the sustained release pattern of ITC from the tested proniosomal gel. Therefore, the prepared ITC proniosomal gelhad an efficient, sustained drug release delivery effect in whichniosomes act as a carrier and a slow release vehicle. The percentage of relative bioavailability (RB %) for the tested ITC proniosomal gel in comparison to the marketed capsule and marketed solution was found to be 189.91 and 143.75 %, respectively.The improved bioavailability may be owing to the lipophilic nature of the niosomal formulation and the effect of the nonionic surface-active agent and lecithin on the permeability of the skin. Also, the enhanced bioavailability of Itraconazoleproniosomes was due to avoiding extensive hepatic first pass metabolism, adequately retaining the drug into the skin for longer time duration and systemically deliver the drug to achieve therapeutic blood levels [46].

Table (8): Pharmacokinetic parameters of ITC following administration of the tested ITC proniosomal gel, marketed Spoanox® capsule and marketed Spoanox ® solution to rats (mean ± SD, n=6)

Parameter	G3(proniosomal gel)	Sporanox® capsules	Sporanox® solution
Dose, mg	2.5	2.5	2.5
C_{max}, µg/ml	0.657	0.459	0.598
(T_{max}), hr	8	4	4
(k_{el}), hr^{-1}	0.02769	0.02785	0.02830
$(t_{1/2})_{el}$, hr	25.0255	24.8776	24.4812
AUC_{0-24}, µg.hr/ml	12.5645	6.649	9.1595
$AUC_{24-\infty}$, µg.hr/ml	4.76677	2.4769	2.8967
$AUC_{0-\infty}$, µg.hr/ml	17.3312	9.1259	12.0562
$AUMC_{0-24}$, µg.hr^2/ml	212.054	105.308	148.182
$AUMC_{24-\infty}$, µg.hr^2/ml	228.805	118.8955	139.0448
$AUMC_{0-\infty}$, µg.hr^2/ml	440.859	224.2035	287.2268
MRT hr	25.437	24.567	23.8238
C max / AUC_{0-24}, hr	0.05229	0.06903	0.06528

IV. Conclusion

In this study, ITC proniosomes were formulated and further developed into different gelling agents. It was concluded that the prepared proniosomes were stable with high percent of entrapped drug and in the nanosize. It was also concluded that all the studied gels are of acceptable physical properties and drug content. The results suggest that the studied proniosomal gel may be an appropriate vehicle for the transdermal delivery of lipophilic antifungal drug ITC. The developed system, prolonged drug release up to 24 h, therefore, produce some benefits, such as reduction in total dose, frequency of administration, and dose-related systemic side effects. Formulation of ITC asproniosomal transdermal gel results in high flux of the drug to the skindue to combination of soya lecithin and span. All formulated gels exhibited pseudoplastic flow with thixotropic behavior. Considering drug content, in vitro permeation and rheological properties, G3 (5% MC) formula was the most stable and showed good antimicrobial effect andproduced more improvement in the skin layer's structure. In conclusion, proniosomes represented a promising approach for the formulation of ITC. The results of in vivo pharmacokinetic studies depicted good permeation of Itraconazoleproniosomes to achieve high drug plasma levels with enhanced bioavailability.

References

[1]. Hay R.J., Dupont B. and Graybill J.R., Review Infectious Disease, 9 (1): 1-3, (1987).
[2]. Tang J., Wei H., Liu H., Ji H., DongD., Zhu D., and Wu L., Pharmacokinetics and biodistribution of Itraconazole in rats and mice following intravenous administration in a novel liposome formulation, Drug Deliv., 17 (4): 223-230, (2010).
[3]. Jamakandi V.G., Ghosh B., Desai B.G., and Khanam J., Recent trends in transdermal cardiovascular therapy, Ind. J. Pharm. Sci., 68: 556-561, (2006).
[4]. Schreier H., and Bouwastra J., Liposomes and niosomes as topical drug carriers: dermal and transdermal drug delivery, J. Control Release, 30: 1-15, (1994).
[5]. Thomas L., and Viswanad V., Formulation and optimization of Clotrimazole-loaded proniosomal gel using 32 factorial design, J. Pharm. Sci., 80: 731-748, (2012).
[6]. Samy A.M., Ramadan A.A., Abu El-Enin A.S.M., and Mortagi Y.I.M., Formulation and optimization of itraconazoleproniosomes using box behnken design, Int. J. Appl. Pharm., 10 (2), (2018) in press.
[7]. Sampathi S.,Mankala S.K.,WankarJ.,and Dodoala S.,Nanoemulsion based hydrogels of Itraconazole for transdermal drug delivery, J. Sci. Ind. Res., 74:88-92, (2015).
[8]. Sharma B., Nada DR.S., and Saroha K., Formulation, evaluation and optimization of transdermal gel of Ketorolac tromethamine using face central composite design, Int. J. Pharm Pharm Sci., 6 (4): 133-139, (2014).
[9]. Guleri K.T., and Preet K.L., Formulation and evaluation of topical gel of Aceclofenac, J. Drug Del. Therap., 3 (6): 51-53, (2013).
[10]. Fathy A., Dawaba H., Ahmed M., and Ahmed S., Preparation, characterization and stability studies of piroxicam loaded microemulsions in topical formulations, Drug Discov. &Thera., 4 (4):267-275, (2010).
[11]. Chudasama A., Patel V., Nivsarkar M., Vasu K., and Shishoo C., Investigation of microemulsion system for transdermal delivery of Itraconazole, J. Adv. Pharm. Tech. Res., 2 (1): 30-38, (2011).
[12]. Fathalla D., Abdel-Mageed A., Abdel-Hamid F., and Ahmed M., In-vitro and in-vivo evaluation of niosomal gel containing Aceclofenac for sustained drug delivery, Int. J. Pharm. Sci. Res., 1: 105-115, (2014).
[13]. Sara M., Nevine S., Omaima N., and Abdel Aziz A., Formulation of microemulsion gel systems for transdermal delivery of celecoxib: In vitro permeation, anti-inflammatory activity and skin irritation tests, Drug Discov. &Thera., 4 (6): 459-471, (2010).
[14]. Dash S., Murthy P.M., Nath L., and Chowdhury P., Kinetic modelingon drug release from controlled drug delivery systems,ActaPoloniaePharmaceutica - Drug Res., 67 suppl 3:217-223, (2010).
[15]. Ibrahim H.K., A Novel Liquid Effervescent Floating Delivery System for Sustained Drug Delivery, Drug Discov. Ther., 3: 168-175, (2009).
[16]. Shirsand S.B., Para M.S., Nagendrakumar D., Kanani K.M., and Keerthy D., Formulation and evaluation of Ketoconazole niosomal gel drug delivery system, Int. J. Pharm. Investig., 2 (4): 201-207, (2012).
[17]. Yadav A.V., Murthy M.S., Shete A.S., and Sfurti S., Stability aspects of gel, Ind. J. pharm. edu. & res., 45 (4), 2011.
[18]. Patel K.K., Kumar P., and Thakkan H.P., Formulation of niosomal gel for enhanced transdermal Lopinavir delivery and its comparative evaluation with ethosomal gel, AAPS Pharm Sci Tech., 13 (4): 1502-1510, (2012).
[19]. Park M.J., Ren S., and Lee B.J., In vitro and in vivo comparative study of Itraconazole bioavailability when formulated in highly soluble self emulsifying system and solid dispersion, Biopharm. Drug Dispos., 28 (4): 199-207, (2007).
[20]. Hasbach A.E., Langlois D.K., Rosser E.J.Jr., and Papich M.G., Pharmacokinetics and relative bioavailability of orally administered innovator-formulated Itraconazole capsules and solutions in healthy dogs, J. Vet. Intern. Med., 31 (4): 1163-1169, (2017).
[21]. Sawant A.A. and Mohite S.K., Formulation and evaluation of Itraconazoleemulgel for topical drug delivery, Asian J. Pharm. Tech., 5 (2): 91-96, (2015).
[22]. Singh M.P., Nagori B.P., Shaw N.R., Tiwari M., and Jhanwar B., Formulation, development and evaluation of topical gel formulations using different gelling agents and its comparison with marketed gel formulations, Int. J. Pharm. Erudition, 3 (3): 1-10, (2013).
[23]. Bachhav Y.G. and Patravale V.B., Formulation of meloxicam gel for topical application: In vitro and in vivo evaluation, Acta Pharm., 60: 153–163, (2010).
[24]. Khandre P., Formulation and evaluation studies on Nimesulide topical gel ", M.Sc. thesis, R.R.K. Samithi's College of Pharmacy, I aubad, Bidar, Karnataka, India, (2010).
[25]. Kumbhar D., Wavikar P., and Vavia P., Niosomal gel of Lornoxicam for topical delivery: In vitro assessment and pharmacodynamics activity, AAPS Pharm. Sci. Tech., 14(3): 1072-1082, (2013).
[26]. Gad S., Ahmed A.M.S., Ghourab, M.M., and Queshawy, M.K.E., Design, formulation, and evaluation of Piroxicamniosomal gel, Int. J. PharmTech. Res., 6 (1): 185-195, (2014).
[27]. Fang J.Y., Hong C.T., Chiu W.T., and Wang Y.Y., Effect of liposomesand niosomes on skin permeation of enoxacin, Int. J. Pharm., 219: 61-72, (2001).
[28]. Lauffer M.A., Theory of Diffusion in Gels, Biophys. J., 1 (3): 205-213, (1961).
[29]. Ogiso T., Niinaka N., and Lwaki M., Mechanism for enhancement effect of lipid disperse system on percutaneous absorption, J. Pharm. Sci., 1: 57-64, (1996).
[30]. Lakshmi P.K., RahiHanthal Z., AbdZair M., Kishore C., and Prasanthi D., TransdermalItraconazole for the treatment of basal cell carcinoma; effect of chemical enhancers on skin permeation, Der. Pharma. Chemica., 9 (16): 127-134, (2017).
[31]. Samy A.M., Ghorab M.M., Shadeed S.G., and Mazyed E.A., Design, formulation and evaluation of transdermal Ketoprofen gel, J. Am. Sci., 9 (3): 237-242, (2013).
[32]. Dinu-pirvu C., Ghica M.V., Ivana S., Iordache P.Z., and Szabo F., Formulation and physic-chemical characterization of piroxicam–based carbomer-hydrogel, Advances in Microbiological Hazards and Biotechnologies, 1 (1): 7-15, (2012).
[33]. Pena L.E., Lee B.I. and Sternes J.F., Structural rheology of model ointment. Pharm. Res., 11: 875-881, (1994).
[34]. Mirza M.A., Rahman M.A., Talegaonkar S., and Iqbal Z., In vitro/ in vivo performance of different complexesa of Itraconazole used in the treatment of vaginal candidiasis, Brazilian J. Pharm. Sci., 48 (4): 760-772, (2012).
[35]. El-Bagory I., Bayomi M.A., Mahrous G.M., Alanazi F.K. and Alsarra I.A., Evaluation of mucoadhesive hydrogels loaded with diclofenac sodium–chitosan microspheres for rectal administration, AAPS Pharm. Sci. Tech., 11 (4): 9544-9553, (2010).
[36]. Naggar V.F., El gamal S.S., and Allam A.N., Proniosomes as a stable carrier for oral Acyclovir: formulation and physicochemical characterization, J. Am. Sci., 8 (9): 417-428, (2012).
[37]. Reddy M., Rehana T., Ramakrishna S., Chowdary K., and Prakash V., β- Cyclodextrin Complexes of Celecoxib: Molecular-Modeling, Characterization, and Dissolution Studies, AAPS Pharm. Sci. Tech., 6 (1): 68–76, (2004).
[38]. Benipal G., Design, development and evaluation of proniosomal gel of an antifungal drug- Ketoconazole, Int. J. Pharm. Sci. Rev. Res., 31 (2): 265-272, (2015).
[39]. Pankaj S., Rini T., and Dandagi P.M., Formulation and evaluation of proniosome based drug delivery system of the antifungal drug Cloitrimazole, Int. J. Pharm. Sci. Nanotech., 6 (1): 1945-1951, (2013).

[40]. Desai S., Doke A., Disouza J., and Athawale R., Deveopment and evaluation of antifungal topical niosomal gel formulation, Int. J. Pharm. Pharm. Sci., 3 (5): 224-231, (2011).
[41]. Kumar J.R., Muralidharan S., and Parasuraman S., In vitro and in vivo evalution of microspheres loaded topical gel delivery systems of ketoconazole in male rats against Candida glabbrata, J. pharm. Sci. Res., 6 (11): 376-381, (2014).
[42]. Abdellatif M.M., Khalil I.S., and Khalil M.A.,Sertaconazol nitrate loaded nanovesicularsyystems for targeting skin fungal infection: in-vitro, ex-vivo and in-vivo evaluation, Int. J. pharm., 527 (2): 1-11, (2017).
[43]. Prentice A.G., and Glasmacher A., Making sense of Itraconazole pharmacokinetics, J. Antimicrobial Chemo., 56 Suppl. (1): 17-22, (2005).
[44]. Shaji, and Shah A., Development and characterization of Tenoxicam encapsulated niosomes for enhanced transdermal delivery, Int. J. Pharm. Sci. Res., 7 (1): 307-315, (2016).
[45]. Buchanan C.M., Buchanan N.L., Edgar K.J., Klein S., Little J.L., Ramsey M.G., Ruble K.M., Wacher V.J., and Wempe M.F., Pharmacokinetics of Itraconazole after intravenous and oral dosing of Itraconazole- Cyclodextrin formulations, J. Pharm. Sci., 96 (11): 3100-3116, (2007).
[46]. Yusuf M., Sharma V., Pathak K., Nanovesicles for transdermal delivery of Felodipine: development, characterization, and pharmacokinetics, Int. J. Pharm. Invest., 4 (3): 119-130, (2014).

Tables

Table (1): Proniosomal gel formulations containing Itraconazole

Composition	ITC proniosomal gel formulation							
	G1	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**	**G8**
Xanthan gum (gm)	**1**	**2**						
MC (gm)			**5**	**10**				
HPMC (gm)					**2**	**4**		
Carbopol 934 (gm)							**1**	**2**
Methyl Paraben (gm)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Propyl Paraben (gm)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Glycrine (gm)	10	10	10	10	10	10	10	10
Optimized hydrated proniosomalformualtion (ml)	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**
Water to (gm)	100	100	100	100	100	100	100	100

N.B. proniosomalpowder equivelant to 1 gm ITC

Table (2): Evaluation of ITC gel formulae

F. No.	Clarity	Homogeneity	Spreadability (cm)	Extrudability (%)	pH	Drug content (%)
G1	+	Good	6.5±0.19	90.23±1.19	6.71 ±0.17	95.23 ±2.24
G2	+	Good	5.5±0.3	86.46±2.32	6.74 ±0.19	93.44 ±1.67
G3	+	Good	8±0.28	98.10±1.15	6.35 ±0.11	99.10 ±1.48
G4	+	Good	5.4±0.21	83.21±1.28	6.45 ±0.12	92.32 ±2.78
G5	+	Good	5.7±0.23	88.50±1.69	6.50 ±0.18	97.31 ±1.36
G6	+	Good	5.2±0.17	77.42±1.47	6.70 ±0.13	91.52 ±2.71
G7	+	Good	5.8±0.16	91.50±0.87	4.40 ±0.14	98.54 ±2.47
G8	+	Good	5.3±0.24	87.20±1.29	5.00 ±0.16	96.98 ±2.34

+ Satisfactory, ++ Good
*All values are Mean ± SD, (n=3)

Table (3): Conclusive rank order (RO) concerning the evaluation of ITC proniosomal gels

F. No.	Clarity	Homogeneity	Spreadability	Extrudability	pH	Total	RO
G1	1	1	2	3	7	14	**3**
G2	1	1	5	6	8	21	**7**
G3	1	1	1	1	3	7	**1**
G4	1	1	6	7	4	19	**6**
G5	1	1	4	4	5	15	**4**
G6	1	1	8	8	6	24	**8**
G7	1	1	3	2	1	8	**2**
G8	1	1	7	5	2	16	**5**

Table (4): Permeation parameters of ITC proniosomal gels

F. No.	Steady state flux (J_{ss}) (µg $cm^{-2}hr^{-1}$)	Permeability coefficient (Cm hr^{-1})	Lag time (hr)	Diffusion coefficient ($Cm^2 hr^{-1}$)	Partition coefficient
G1	162.22± 0.78	0.01622± 0.25	1.46± 0.000151	2.8 E^{-4}± 1.51 E^{-5}	2.84± 0.43
G2	158.83± 0.98	0.01588± 0.18	1.0325± 0.00014	4.03 E^{-4}± 2.24 E^{-5}	1.96± 0.39
G3	172.23± 1.27	0.01722± 0.28	1.67± 0.000136	2.4 E^{-4}± 1.52 E^{-5}	3.45± 0.28
G4	158.28± 0.84	0.01582± 0.24	0.834± 8.93 E^{-5}	4.9 E^{-4}± 2.35 E^{-5}	1.58± 0.37
G5	160.68± 0.94	0.01606± 0.21	1.14± 0.000131	3.6 E^{-4}± 3.23 E^{-5}	2.20± 0.58
G6	125.64± 0.82	0.01256± 0.31	0.851± 8.67 E^{-5}	4.8 E^{-4}± 4.20 E^{-5}	1.28± 0.21
G7	161.15± 0.95	0.01611± 0.19	1.42± 0.000127	2.9 E^{-4}± 3.05 E^{-6}	2.75± 0.34
G8	135.76± 1.05	0.01357± 0.39	1.0324± 0.000121	4.03 E^{-4}± 3.24 E^{-5}	1.68± 0.48

*All values are Mean ± SD, (n=3)

Table (5): The calculated correlation coefficients for the In-Vitro permeation of ITC gels employing different kinetic orders or systems

Formula No.	Correlation coefficient (r)		
	Zero	First	Diffusion
G1	0.8962	0.9317	**0.9564**
G2	0.8965	0.9390	**0.9579**
G3	0.8692	0.9260	**0.9428**
G4	0.8954	0.9342	**0.9550**
G5	0.8804	0.9324	**0.9469**
G6	0.9081	0.9220	**0.9530**
G7	0.8937	0.9364	**0.9574**
G8	0.9034	0.9384	**0.9617**

Table (6): Total rank order concerning viscosity, thixotropic behavior and Farrow's constant of ITC proniosomal gels

F. No.	Max. viscosity (cp)	RO	Min. viscosity (cp)	RO	Farrow's constant	RO	Total	RO
G1	4805.4	**2**	199.14	**3**	2.6545	**4**	**9**	**2**
G2	15125.6	**5**	1293.5	**8**	2.2649	**7**	**20**	**8**
G3	4145.2	**1**	136.2	**2**	2.5086	**6**	**9**	**2**
G4	17755.9	**6**	653.7	**6**	2.5878	**5**	**17**	**7**
G5	9251.5	**4**	120.65	**1**	4.7755	**1**	**6**	**1**
G6	26258.2	**8**	645.8	**5**	3.1201	**2**	**15**	**4**
G7	5854	**3**	225.6	**4**	2.2383	**8**	**15**	**4**
G8	21724.9	**7**	653.7	**6**	2.9249	**3**	**16**	**6**

Table (7): Total rank order concerning the in vitro release, permeation, gel evaluation, drug content and rheological properties of ITC proniosomal gels

F. No.	Gel evaluation	Drug content	% Drug permeated	Rheological properties	Total	RO
G1	3	5	2	2	12	3
G2	7	6	5	8	26	6
G3	1	1	1	2	5	1
G4	6	7	6	7	26	6
G5	4	3	4	1	12	3
G6	8	8	8	4	28	8
G7	2	2	3	4	11	2
G8	5	4	7	6	22	5

Table (8): Pharmacokinetic parameters of ITC following administration of the tested ITC proniosomal gel, marketed Spoanox® capsule and marketed Spoanox ® solution to rats (mean ± SD, n=6)

Parameter	G3(proniosomal gel)	Sporanox® capsules	Sporanox® solution
Dose, mg	2.5	2.5	2.5
C_{max}, µg/ml	0.657	0.459	0.598
(T_{max}), hr	8	4	4
(k_{el}), hr^{-1}	0.02769	0.02785	0.02830
$(t_{1/2})_{el}$, hr	25.0255	24.8776	24.4812
$AUC_{0\text{-}24}$, µg.hr/ml	12.5645	6.649	9.1595
$AUC_{24\text{-}\infty}$, µg.hr/ml	4.76677	2.4769	2.8967
$AUC_{0\text{-}\infty}$, µg.hr/ml	17.3312	9.1259	12.0562
$AUMC_{0\text{-}24}$, µg.hr^2/ml	212.054	105.308	148.182
$AUMC_{24\text{-}\infty}$, µg.hr^2/ml	228.805	118.8955	139.0448
$AUMC_{0\text{-}\infty}$, µg.hr^2/ml	440.859	224.2035	287.2268
MRT hr	25.437	24.567	23.8238
C max / $AUC_{0\text{-}24}$, hr	0.05229	0.06903	0.06528

Mean Diameter = 340.48 nm
Stnd. Deviation = 207.7 nm (57.8%)
Norm. Stnd. Dev. = 0.58
(Coeff. of Var'n)
Variance (P.I.) = 0.334
Chi Squared = 20.534
Baseline Adj. = 0.000 %
Z-Avg. Diff. Coeff. = 1.18E-008 cm2/s

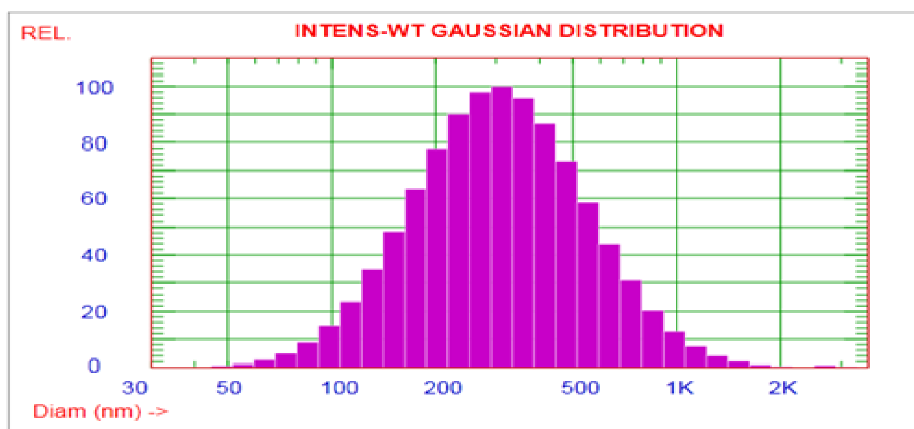

Figure (1): mean vesicle size of ITC proniosomes

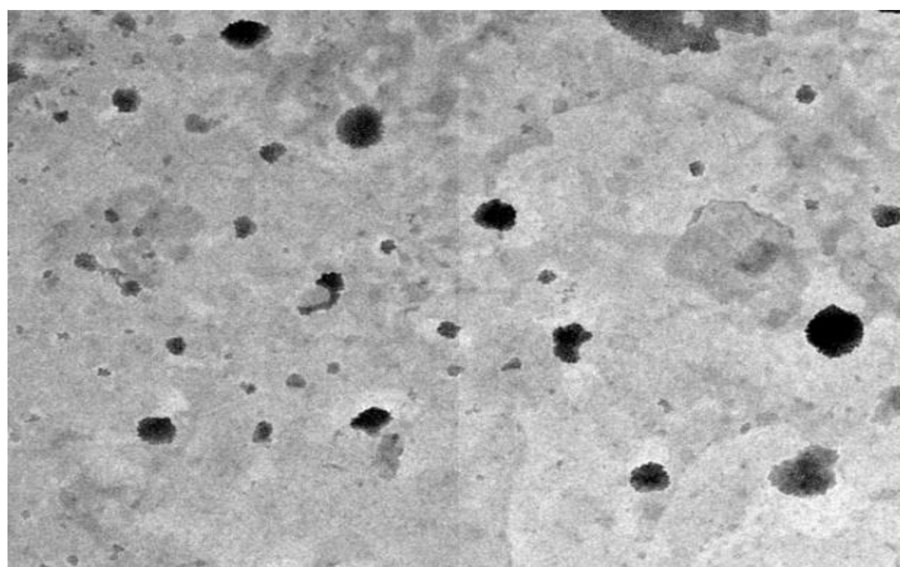

Figure (2): TEM micrograph of the ITC niosomes

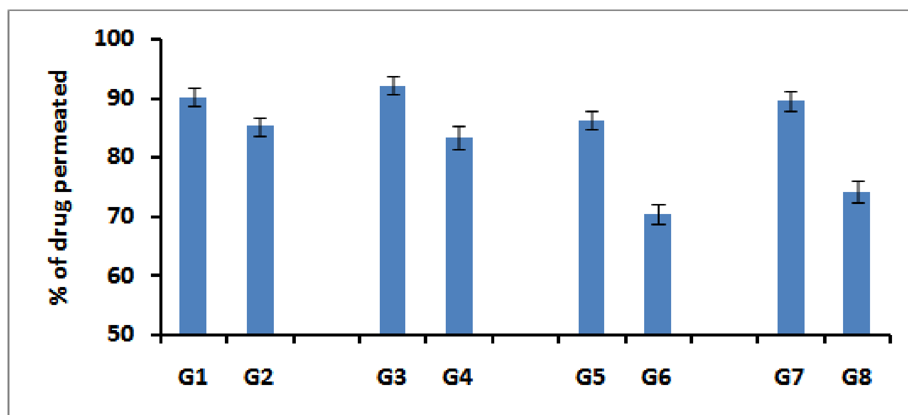

Figure (3): The effect of polymer type and concentration on the in-vitro ITC permeation

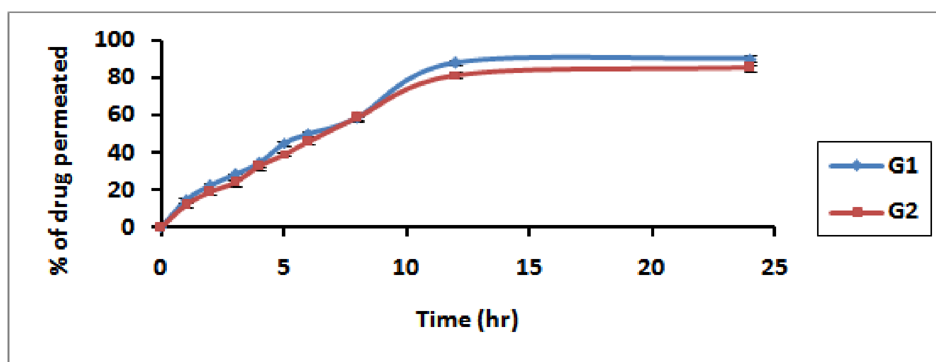

Figure (4): The effect of xanthan concentration on the in-vitro drug permeation of ITC

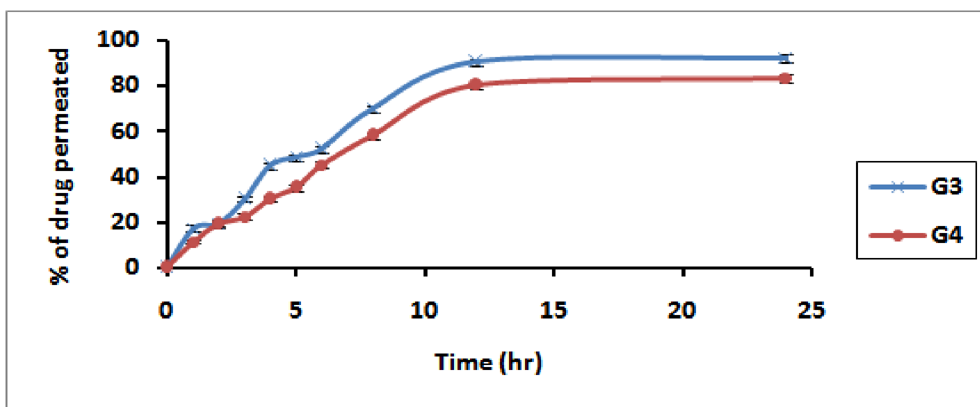

Figure (5): The effect of MC concentration on the in-vitro drug permeation of ITC

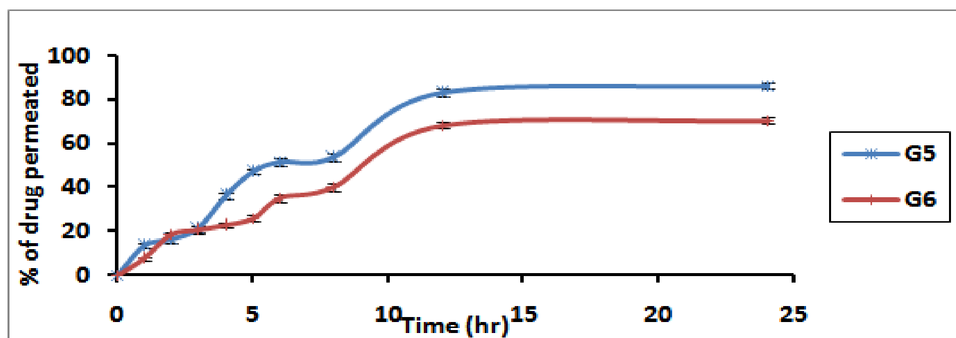

Figure (6): The effect of HPMC concentration on the in-vitro drug permeation of ITC

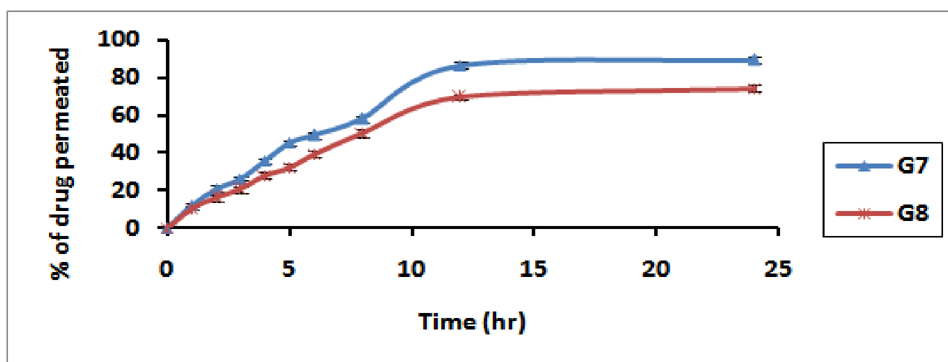

Figure (7): The effect of Carbopol 934 concentration on the in-vitro drug permeation of ITC

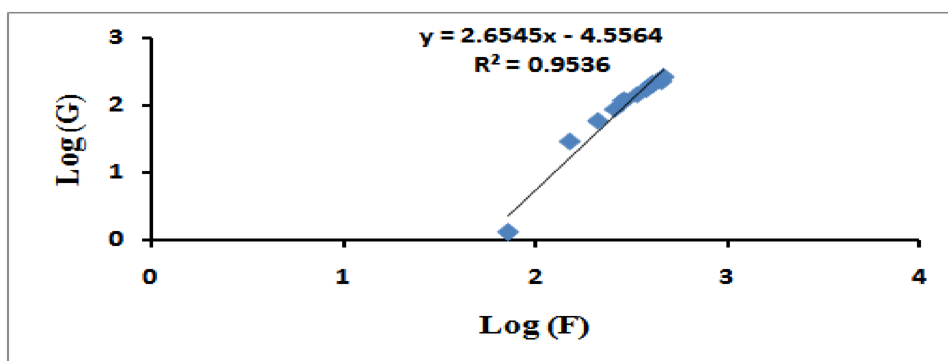

Figure (8): Rheogram of G1

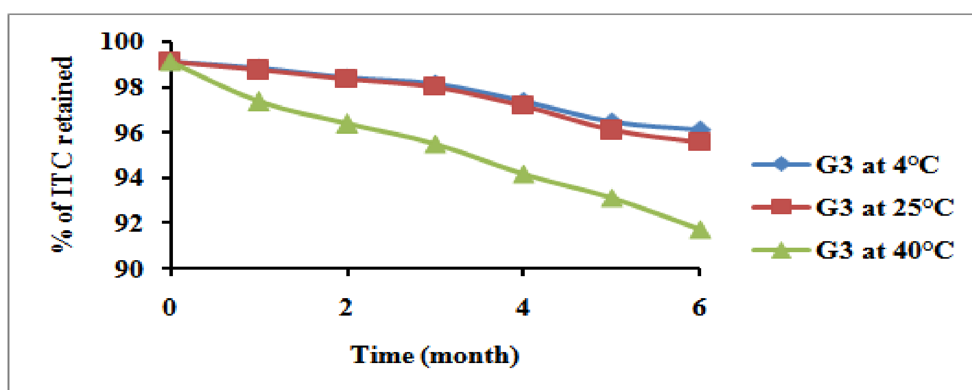

Figure (9): The percent of ITC retained for G3after studying the effect of storage on drug content for 6 months

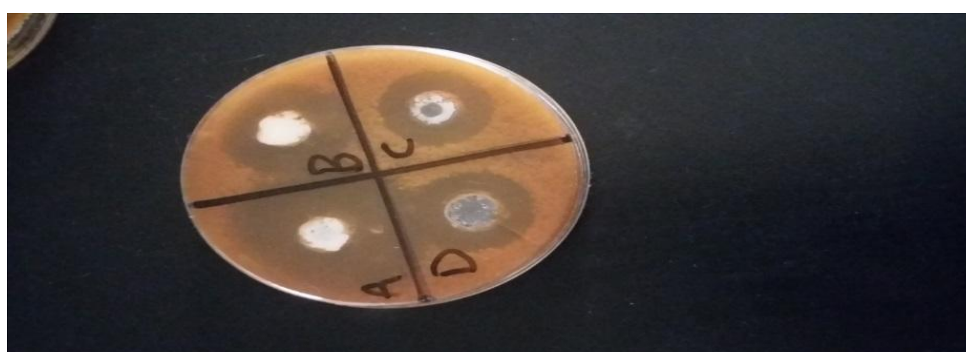

Figure (10): antifungal activity by zone of inhibition of different cups, A: niosomal dispersion, B: proniosomal gel, C: ITC suspension and D: marketed product.

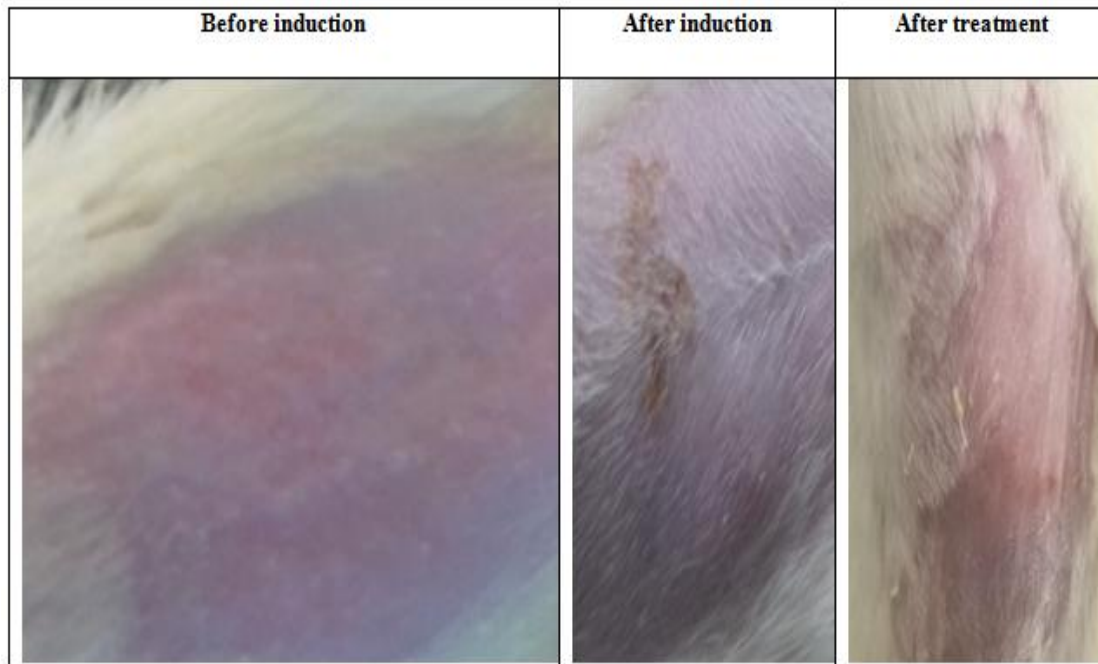

Figure (11): Skin samples before, after fungal infection using candida albicans and after treatment.

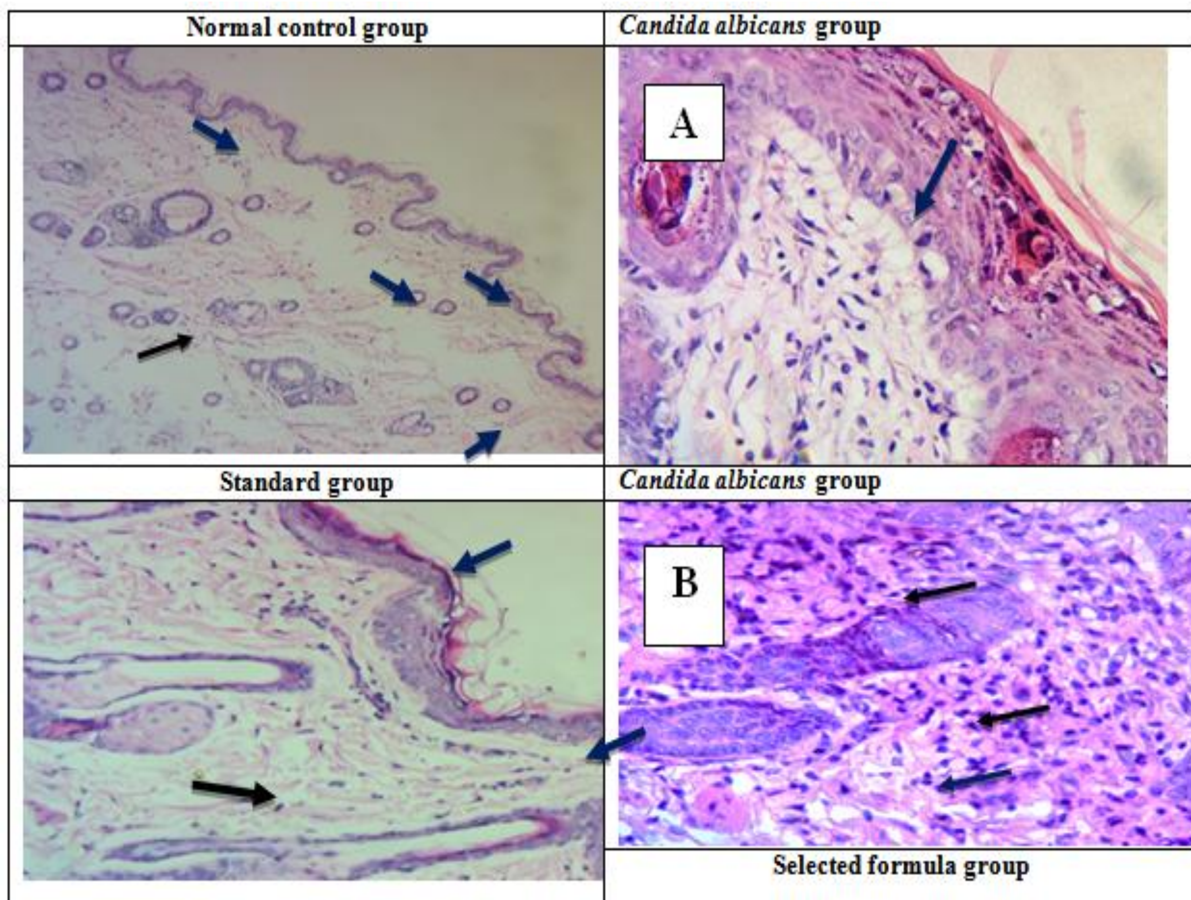

Figure (12): Treatment effect of ITCon the histopathhological changes in dermal fungal infection rats using Candida albicans: clinical postmortem examination histological images (H&E X 20 and 40) for normal control, Candida albicans, standard group and selected formula.

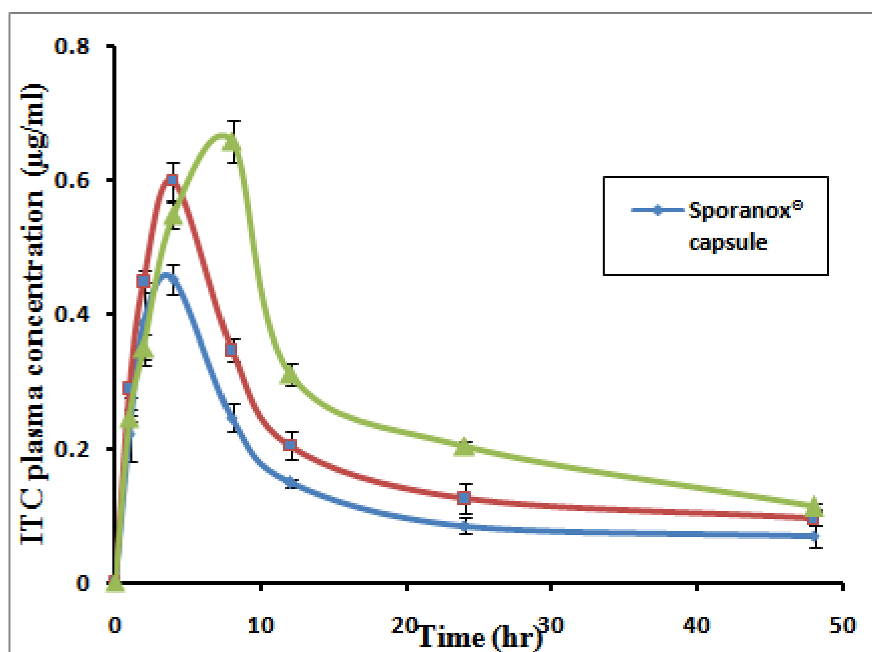

Figure (13): ITC concentration - time profiles in plasma of rats following administration of the tested ITC proniosomal gel, marketed Spoanox® capsule and marketed Spoanox ® solution to rats (mean ± SD, n=6)

IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) is UGC approved Journal with Sl. No. 5012, Journal no. 49063.

Ahmed M. Samy,"Novel Proniosomes As Drug Delivery System For Transdermal Application of Itraconazole: Development, Characterization, Clinical And Bioavailability StudY." IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 13.1 (2018): pp 54-71.