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Development and Antifugal Activity of Itraconazole Loaded Ethosomal Gel in Rat Animal Model

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Abstract

The goal of this study was to create and characterize itraconazole (ITZ) ethosomal gel which may serve as a more efficient agent in local drug deliveryas compared to the current gel preparation available in the market. On the other hand, itraconazole ethosomal gel is fabricated with an aimto solve the limitationsof oral delivery of drugs. The formulations were prepared with varying the quantity of cholesterol (100, 125, 150 and 175 mg), static volume of dichloromethane (DCM), ethanol, phosphate buffer and polaxamer 188.The formulations were evaluated for viscosity, gel strength, spreadability, in-vitro release, in-vivo therapeutic efficacy, microscopic analysis histopathological analysis and Fourier Transformed Infrared (FTIR) studies.

Keywords: Itraconazole ethosomal gel, Topical, Candidiasis, Itraconazole.

INTRODUCTION

The administration of drugs through oral route has been the most conventional route. However, the drugs may undergo degradation when exposed to various environments. On the contrary topical drug delivery system showed promising results. (2) The use of a drug formulation on the skin with the purpose of treating cutaneous disorders (e.g. psoriasis, acne) to confer pharmacological or other effect of the active ingredients to the surface of the skin or within the skin is generally known as topical delivery. Since many years, drug delivery for topical use has been rising as a promising drug delivery system. This is due to the fact that topical delivery of drug has the advantage of high concentration of drugs can be contained at the site of action. At the same time the risk of systemic side effects is much lower relative to parenteral or oral drug administration.In the past twenty years, extensive research that had been conducted lead to the development of the ethanolic liposomes, also known as ethosomes.(1) Ethosomes are the lipid vesicles comprised of phospholipids and alcohol. Both hydrophilic and lipophilic drugs with size rangefrom ten nanometers to microns can be incorporated in ethosomes.(2) The fabricated ethosomes are further integrated into gel formulation to form ethosomal gel. Gel is mainly used for the topical application or to mucosal surface for local action or for percutaneous penetration of drugs. They are preferred due to convenient application on the skin and rapid penetration of the drugs through skin.(3) The present investigation was to design the ethosomal gel containing itraconazole using different concentration of cholesterol and carbopol 940 gel.

MATERIALS

Itraconazole (ITZ), Polaxamer 188 and carbopol 940 were purchased from Sigma-Aldrich Co., dichloromethane (DCM), ethanol, phosphate buffer solution and triethanolamine were procured from ACI Labscan.

METHODS

Preparation of itraconazole ethosomal gel:

Steps involved in Formulation of ethosomes as shown in figure 1.Composition of ethosomes and carbopol gel for topical delivery as given in table 1 and 2.

IN-VITRO EVALUATION

Viscosity:

The viscosity of gel loaded with itraconazole ethosomes were determined using Brookfield programmable DVII +Model pro II type (USA). The viscosity was noted in Centipoise.(4)

Gel Strength:

50gm of the prepared gels was loaded into a 50 ml graduated measuring cylinder. Two points were marked on the measuring cylinder. A spindle was placed at the upper marking point and allowed to drop. The time taken for the spindle to reach the lower marking point was recorded. Three trials were conducted for each run.(5)

Spreadability:

Sample was applied in between two glass slides and was compressed to achieve uniform thickness by placing a weight of 1000g for 5 minutes on top on the slides. Then, the 1000g weight was replace with a weight of 75.87g. The glass slide on top was pulled over a distance of 5cm with a thread tied to it. The time taken for the top glass slide to move over the distance of 5cm was recorded (6). Three trials were repeated for each run. The spreadability can be calculated by using the following formula:

$S = ML/T$

Where, M = weight tide to upper slide (g), L = length moved on the glass slide (cm), T = time taken (sec).

In-vitro drug release:

In-vitro drug release of ethosomal formulation was done by using dialysis membrane (molecular weight cut off 12,000). Phosphate buffer pH (7.4) and ethanol mixture was employed as the release media in the studies. The dialysis bag was soaked in double-distilled water for 12 h before use. Drug loaded ethosomal formulation (2 ml) was poured in dialysis bag. The bag was then suspended in 20

ml of receiving phase i.e. PBS (pH 7.4) and ethanol in a ratio of 3:2v/v, under continuous stirring at 37°C and 200 rpm. Aliquots each of 1 ml were withdrawn at various time points. Sink condition was maintained throughout the experiment. Itraconazole in aliquots was analyzed by UV spectrophotometer at 254 nm (7).

IN VIVO EVALUATION

Animals:

Adult Wistar rats (280 ± 10 g) of either gender were obtained from AIMST University, Malaysia. The animals were housed in large, spacious polyacrylic cages at an ambient room temperature with 12-h light/12-h dark cycle. Rats had free access to water and rodent pellets diet. The study was approved by the AIMST University Human and Animal Ethics Committee he study was conducted according to the Animal Research Review Panel guidelines.

Acute toxicity testing:

The female rats were used for the acute toxicity testing. Hair present in the dorsal surface of the animal (2 X 2 cm) was removed by applying hair remover and cleaned with alcohol. The screening are was marked (1 X 1 cm) and 0.5 g of ethosomal gel was applied to the surface of an animal's skin. During the observation period (14 days), signs such as erythema and edema were assessed (8).

Evaluation of therapeutic efficacy:

Healthy, adult, male Wistar rats were used for the experiment. The rats were divided into the four groups each of six animals *viz.*, normal control (group I), *Candida glabrata* control (group II), standard treatment group (group III) and itraconazole loaded ethosomal gel treatment group (group IV). Group II to IV animals were changed with intravenous methylprednisolone (5mg/kg) for three days for induction and maintenance of cell-mediated immunosuppression (Organisms from stock isolates were stored in nutrient agar at 27°C, streaked onto nutrient broth, and incubated at 37°C for 24 h and included culture was used for further experiment).

Candida glabrata culture was diluted with PBS and swabbed in smooth muscle of rat pennies and allowed to grow for 3 days until the growth of *Candida* was observed on ischiocavernosus smooth muscle. The colony growth was confirmed by counting colony-forming-unit. The animals which as cfu value of more than 3 cfu/ml ware included in the study. The animals were treated for week period and visually observed its physical changes. The swab culture was collected on initial day, 4th and 7th day of the experiment for microscopical evaluation. End of the experiment the animals were sacrificed and ischiocavernosus smooth muscle was collected from all the experimental animals and preserved in 10% formalin.

Microscopical evaluation:

The colony was collected in sterile cotton swab and transferred into 0.5 ml sterile phosphate buffer saline (PBS). The mixture was diluted 10 fold and inculcated in nutrient agar media, incubated for 48 h at 37°C. The yeast count was expressed as log 10 of cfu/ml of PBS.

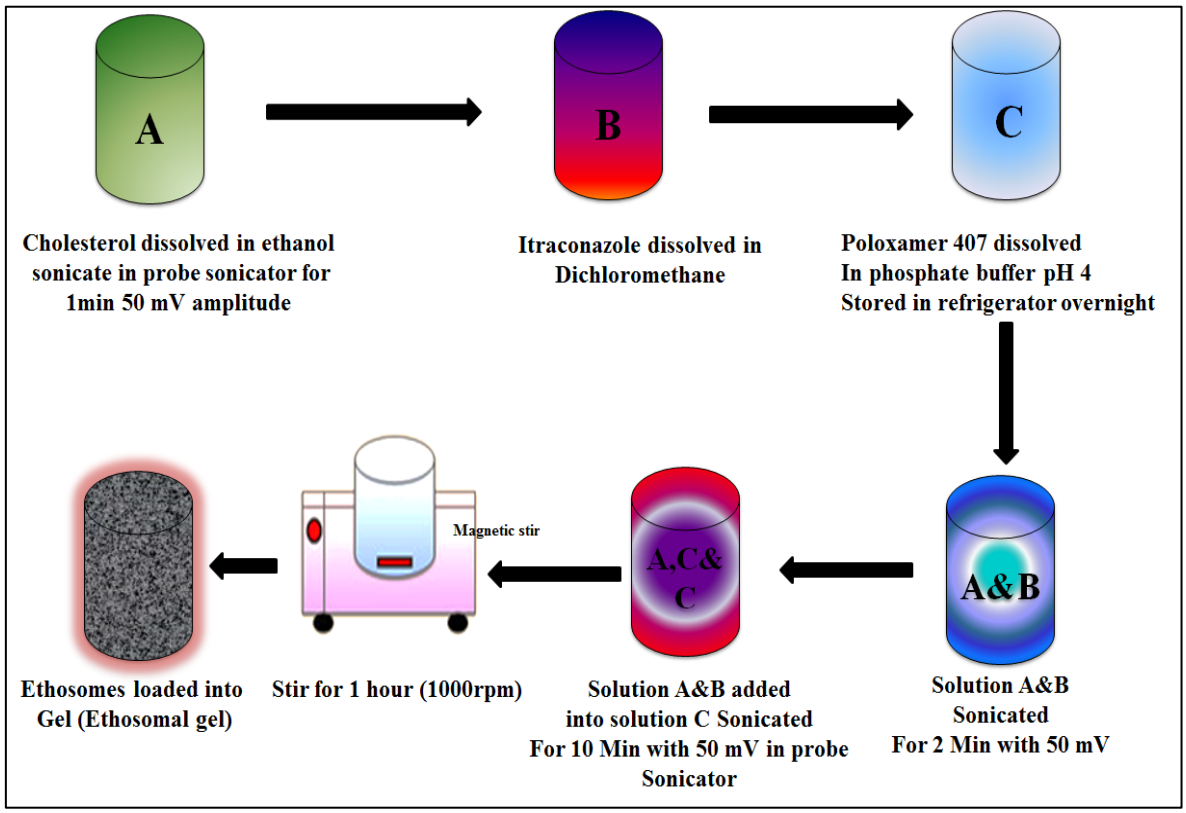

Figure 1: Scheme of (ITZ) ethosomal gel

Table 1: Formulation itraconazole ethosomes

Formulation	Itraconazole (mg)	Dichloromethane (ml)	Cholesterol (mg)	Ethanol (ml)	Phosphate BufferpH 4 (ml)	Polaxamer 188 (mg)
1	100	3	100	7	10	100
2	100	3	125	7	10	100
3	100	3	150	7	10	100
4	100	3	175	7	10	100

Table 2: Composition of carbopol gel

Formulation	Carbopol 940 (g)	Deionized water (ml)	Triethanolamine
F1	1	100	qs
F2	1.2	100	qs
F3	1.4	100	qs
F4	1.6	100	qs

Histopathologic analysis:

The rat penal smooth muscle were dehydrated with alcohol for 12 h each and cleaned with xylene for 15-20 min. The tissue blocks were prepared and the blocks were cut using microtome to get sections of thickness 5 µm. The sections were taken on a microscopic slide on which egg albumin (sticky substance) was applied and allowed for drying. Finally, the sections were stained with eosin (acidic stain) and hemotoxylin (basic stain) (9).

Statistical analysis:

All the data were expressed as mean ± Standard Error of the Mean (SEM). One-way analysis of variance (ANOVA) was employed to test the statistical significance between the groups. Then it was followed by Tukey's *post-hoc* test. A P-value of less than 0.5 were considered significant.

RESULTS AND DISCUSSION

Formulation of ethosomal gel contain itraconazole is prepared by using cholesterol and poloxamer 188.

Physicochemical properties such as viscosity, pH, appearance, clarity, spreadability, and gel strength were performed and the results are recorded in Table 3.

All the formulations of SEM photography were showing as spherical or near to spherical systems as shown in figure 2 and microscopy view of ethosomal gel as shown in figure 3.

The appearance of itraconazole ethosomal gel observed was uniform, the content is homogenized and there was no phase separation as shown in figure 4.

The reological properties of polymer solutions and gels of various formulations were determined at different shear rates. As the rpm increased, the viscosity of gel decreased as shown in Figure 4**.** F1 has the lowest viscosity while F4 has the highest viscosity. F2 and F3 have intermediate viscosity in between F1 and F4. Due to high concentration carbopol 940 in gel increases the viscosity of gel system as shown in figure 5.

The spreadability can relate with viscosity. The spreadabilityis higher with lower viscosity. The values of spreadability indicate tendency of the gel to spread by small amount of shear. The values of spreadability indicate tendency of the gel to spread by small amount of shear. The spreadability of formulation F1 (80.1 gm.cm/sec) was found to be significantly high as compared to other formulations as shown in figure 6.

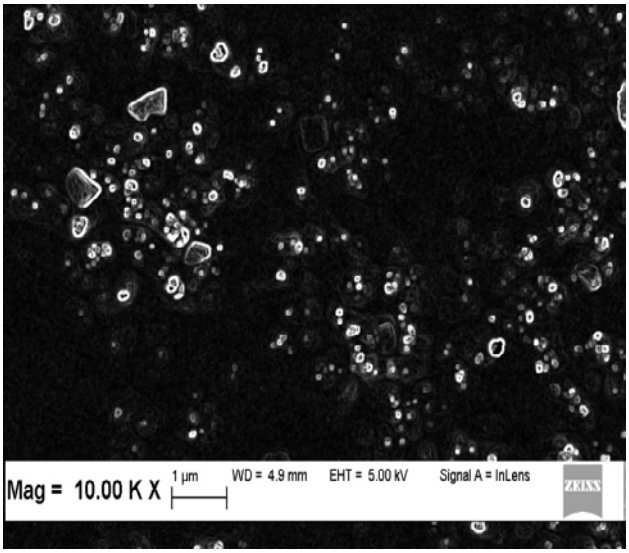

Figure 2: SEM image showing the (ITZ) ethosomal gel

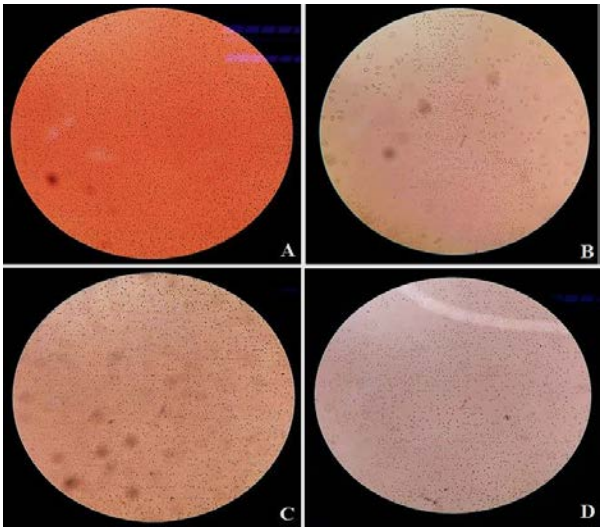

Figure 3: Photography showing the microscopic view of (ITZ) ethosomal gel

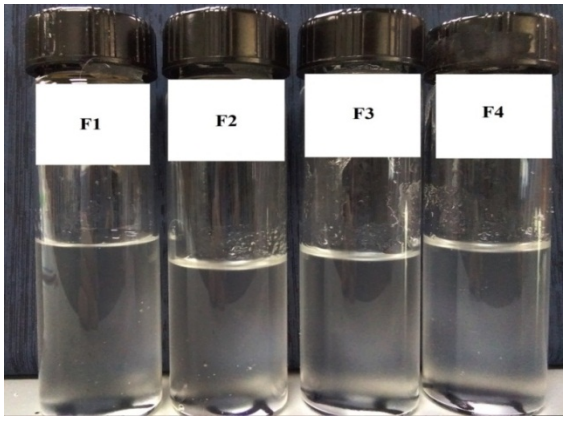

Figure 4: Photography showing the appearance of (ITZ) ethosomal gel

Table 3: Characteristics of various (ITZ) ethosomal gel

Formula code	pH	Viscosity (cps)	Spreadability (gm.cm/sec)	Gel strength (sec)
F1	7.2	2799	80.1	127.3
F2	7.0	9838	64.2	10.2
F3	6.9	16716	40.3	35.16
F4	7.0	14177	46.0	16.7

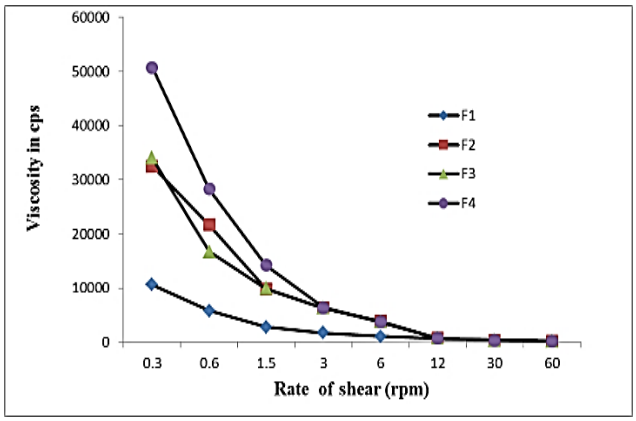

Figure 5: Showing the viscosity of profile of (ITZ) ethosomal gel

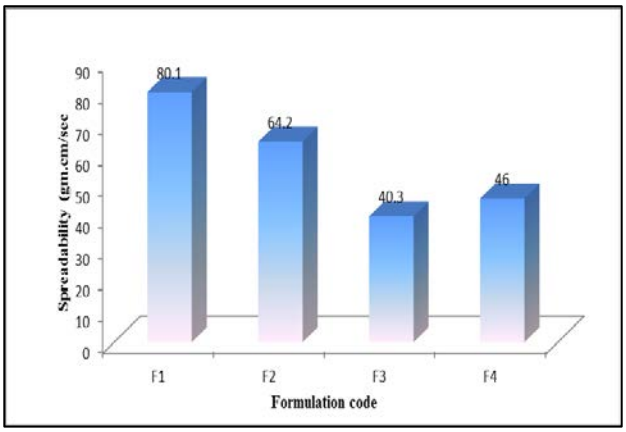

Figure 6: Showing the spreadablity of F code formulation

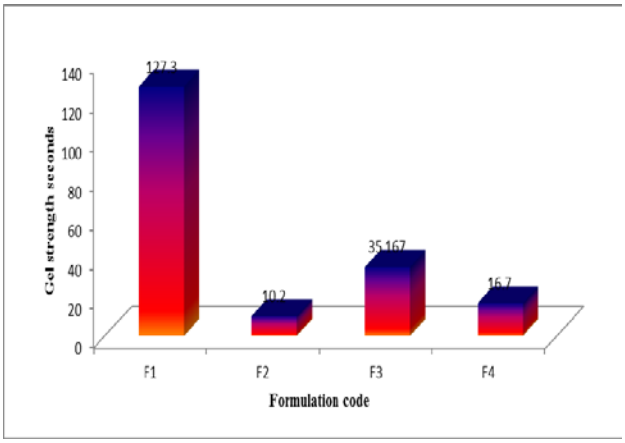

Figure 7: Showing the gel strength of F code formulation

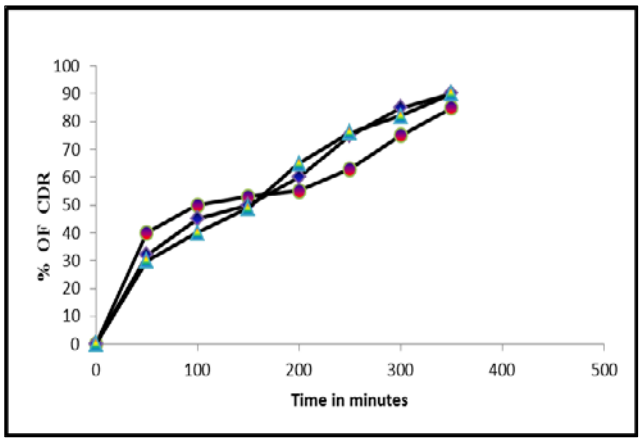

Figure 8: Showing the Diffusion of F Code formulation

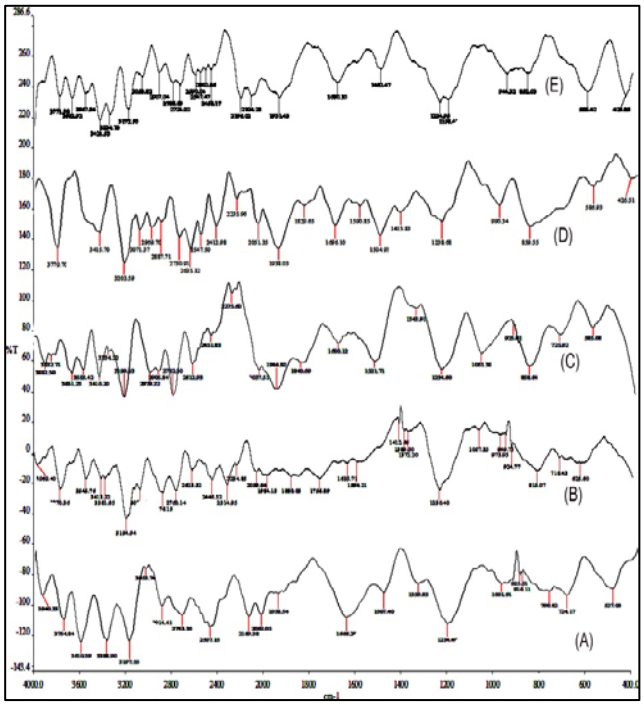

Figure 9: FTIR spectra of (A) Itraconazole, (B) Cholesterol, (C) Poloxamer 188, (D) Cholesterol + Poloxamer 188, (E) Itraconazole + Cholesterol + Poloxamer 188

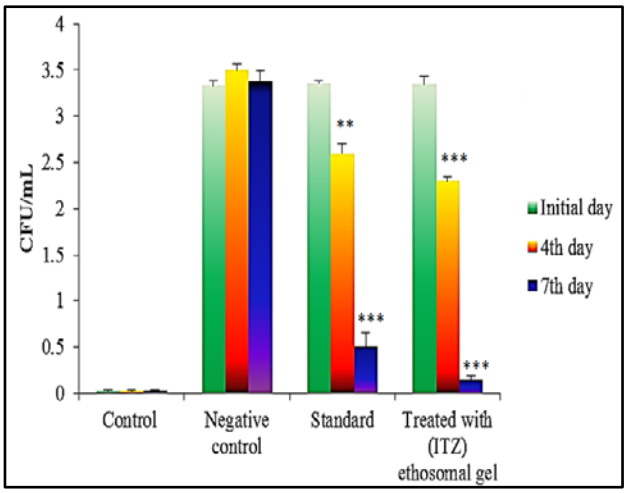

Figure 10: Quantitative microbiological analysis of the Candida glabrata

There is an inconsistent change in the gel strength across different formulations. Apparently, F1 has the highest gel strength which is clearly noticeable as compared to other formulations. The gel strength is important because strong gels will support a much higher pressure than weak gels before they are washed out from the site of administration. The gel strength of formulation F1 (127.3 s) exhibited good gel strength among all the formulations as shown in figure 7.

The *in vitro* diffusion profile of formulation contains itraconazole from ethosomal gel containing various ratio of (carbopol 940) were conducted in diffusion medium pH 7.4. Ethosomal gel containing (F11, F2 and F3) showed 85.02%, 90.01% and 90.05% of drug release 6 hours respectively, as shown in figure 8; from this results it can conclude that it is suitable for extending the drug release in the skin membrane to improve the patient compliance.

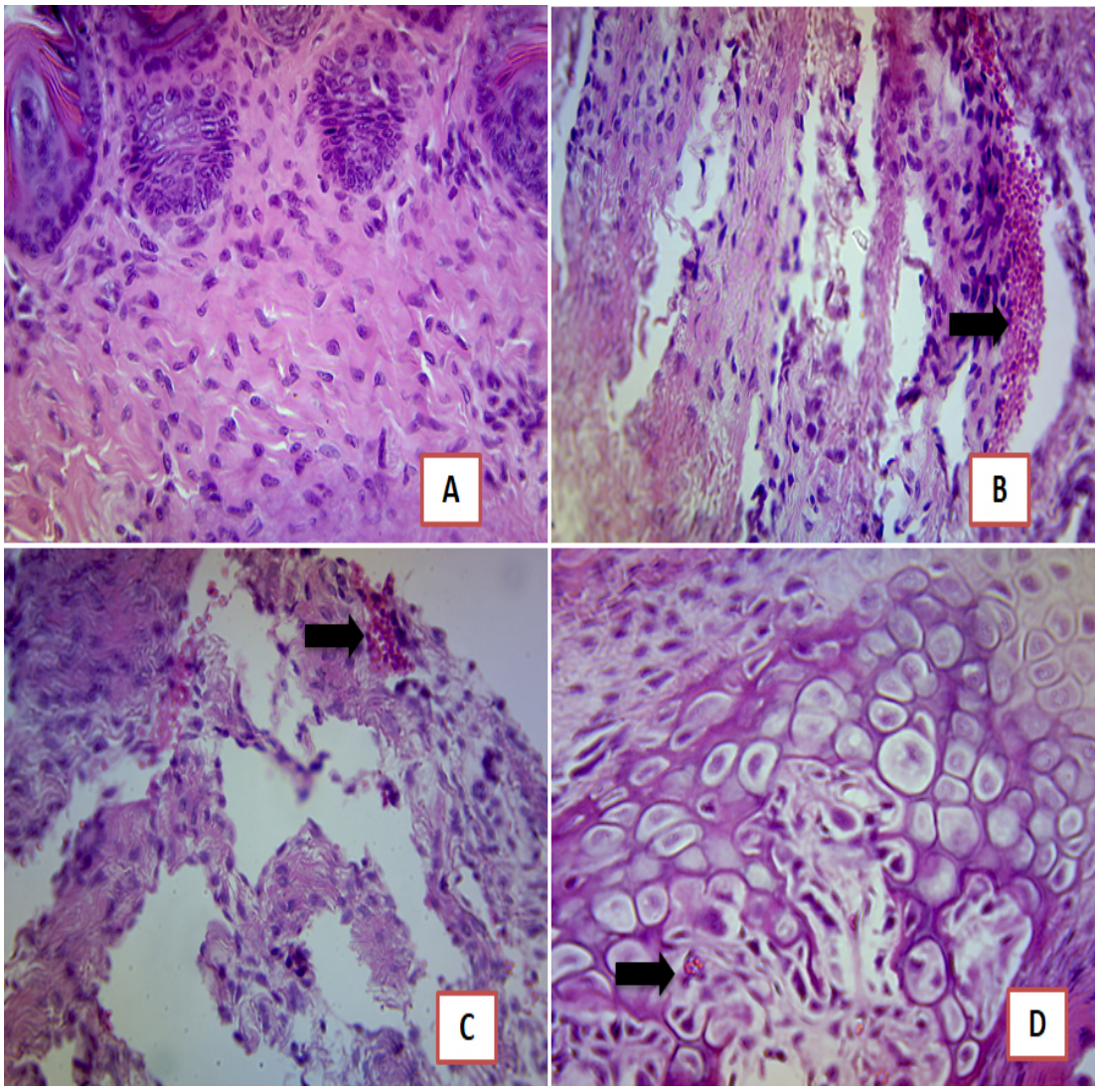

Figure 11: Histopathological analysis of the rat penal smooth muscle section from (A) control showed normal articheture and (B) showed *Candia* microorganism infection in smooth muscle surface (C) and (D) showed reduction in growth of *Candia* due to antifungal effect of standard and Ethosomal gel, H1NI, 400X

It is confirmed that the itraconazole and excipients are compatible with each other by the results of FTIR spectra. Itraconazole formulation formed with no disturbance in the functional group. Hence, the active constituent has no change in effect after polymerization as shown in figure 9.

The therapeutic efficacy of (ITZ) ethosomal gel was compared with *Candida glabrata* control by quantitative microbiological analysis and histopathological evaluations (Figure 10 and 11). Ethosomal gel and standard marketed formulation treated animals showed significant reduction of CFU count on 4^{th} day of the treatment onwards. The efficacy of the Ethosomal gel is comparable with standard marketed formulation.

All the values are mean ± SEM (n= 6). **P < 0.01 and ***P < 0.001 compare to control group (One-way ANOVA followed by Tukey's*post-hoc* test)

CONCLUSION

In summary, an optimized (ITZ) ethosomal gel formulation should have a suitable viscosity, spreadablity and gel strength. In this study, the ethosomal gel formulation was successfully integrated into a carbopol 940 gel matrix and properly characterized. Thus, it was concluded that the prepared formulation is superior than conventional formulations by its nanoscale size and promises better therapeutic efficacy. (ITZ) ethosomal gel can therefore be good substitute for the conventional formulation with the advantages of decreased dose and dosing frequency with better patient compliance.

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