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Formulation of Microemulsion based vaginal gel-*in vitro* and *in vivo* evaluation

Rahul Nair[1*]. Sevukarajan.M [1], Badivaddin. Mohammed[1] , Jayraj Kumar [2]

[1]*Department of Pharmaceutics, Sree Vidyanikethan College of Pharmacy, Tirupathi, Andhrapradesh, India*
[2]*Rao's College of Pharmacy, Nellore, Andhrapradesh, India*

ABSTRACT

The objective of the present investigation was to develop and evaluate microemulsion based gel for the vaginal delivery of Ciclopiroxolamine (CPO). The solubility of CPO in oils and surfactants was checked to identify components of the microemulsion. The ternary diagrams were plotted to identify the area of microemulsion existence. Various gelling agents were evaluated for their potential to gel the CPO microemulsion without affecting its structure. The prepared formulations of clopirox olamine-Microemulsion based gel(CPO-MBG) was evaluated by checking its Spreadability, Rheological studies, Gel Strength, bioadhesion study, mucoadhesive strength, In-vitro diffusion studies and In-vivo studies The efficacy of the CPO-MBG gel was evaluated in vivo in using rabbits . The CPO-MBG showed good in vitro bioadhesion and anti-fungal activity. The CP-MBG has potential be successfully used for the topical treatment of vaginal candidiasis.

Keywords: Ciclopiroxolamine,-Microemulsion based gel,Anti fungal activity.

INTRODUCTION

Invasive fungal infections are an increasing threat to human health. In the developed world, these infections predominantly occur in the context of increasingly aggressive immunosuppressive therapies. Recently, there have been an increasing number of profound fungal infections caused by fungi such as those belonging to the genus Candida, the genus aspergillus and the genus Cryptococcus. This is a particular complication

encountered in transplant patients, those administered a large quantity of antibiotics, anticancer drugs (cartinostatic) or a steroidal agents over a long period, [1].Most of females have at least suffer with vulvovaginal candidiasis once during their lifetime. There high risk of contracting multiple episodes of vaginitis especially in high risk groups like sexworkers. The episodes of vulvovaginal candidiasis are often painful and very uncomfortable and can include itching, irritation, continous vaginal discharge and dysuria.[2,3,4]. The most commonly prescribed treatment for vulvovaginal candidiasis in recent years has been the imidazole antifungals. Imidazole antifungal agents which are available in various dosage forms such as vaginal creams and pessaries and oral tablets. Ciclopiroxolamine (CPO) is an exciting microbicidal candidate with a long history of proven safety in clinical use as a vaginal and skin product with anti-infective properties. It is already formulated, manufactured, and marketed worldwide in vaginal products. (5,6) . CPO is highly hydrophobic nature and has poor topical permeation posses certain challenge in for vaginal drug delivery. Hence, for solubilization of CPO microemulsion appeared to be a suitable approach. Microemulsion is defined as a dispersion consisting of oil, surfactant, cosurfactant and aqueous phase, which is a single optically isotropic and thermodynamically stable liquid solution with a droplet diameter usually within the range of 10–100 nm [7]. Microemulsions have several advantages such as enhanced drug solubility, good thermodynamic tability, ease of manufacturing and enhancement effect on transdermal ability over conventional formulations [8,9]. The solubilization of CPO in microemulsions would improve its vaginal availability. However, it is also essential to have a dosage form which adheres to the vaginal mucosa and increases the residence time of CPO in vagina. This functionality can be imparted by gelling of the CPO using bioadhesive agent. Thus, in the present investigation, the potential of microemulsion based bioadhesive gel MBG of CPo was investigated for vaginal delivery. The developed microemulsion based bioadhesive gel of CPO MBG was evaluated for in vitro antifungal activity, vaginal irritation in rabbits and its in vivo anti fungal efficacy was evaluated by rabbit models.

MATERIALS AND METHODS

2.1. Materials

CPO was kindly gifted by Fourrts India ltd chennai., Mumbai, India. Olive oil, linseed oil, and Castor oil (Yarrow chemicals ltd., Mumbai, India). Tween 80, Propylene glycol, sodium alginate , Carbopol and triethanolamine (All AR grade)were purchased from s.d. Fine Chemical Ltd., (Mumbai, India). Sabaraud dextrose agar was purchased from HiMedia Ltd, (Mumbai, India). Double distilled water was used whenever required.

2.2. Construction of pseudo-ternary phase diagrams:

In order to find out the concentration range of components for the existing range of micro emulsions, pseudo-ternary phase diagrams were constructed using water titration method at ambient temperature (25°C). (10) Olive oil, linseed oil, and Castor oil were screened as the oil phase. Tween 80 and Propylene glycol were selected as surfactant and cosurfactant, respectively. Distilled water was used as an aqueous phase. Three phase diagrams were prepared with the 1:1, 2:1 and 3:1 weight ratios of Tween 80 to propylene glycol, respectively. For each phase diagram at a specific surfactant/cosurfactant weight ratio, the ratios of oil to the mixture of surfactant and cosurfactant were varied as 0.5:9.5, 1:9, 1.5:8.5, 2:8, 2.5:7.5, 3:7, 3.5:6.5, 4:6, 4.5: 5.5, 5:5, 5.5:4.5, 6:4, 6.5:3.5, 7:3, 7.5:2.5, 8:2, 8.5:1.5, 9:1, 9.5:0.5. The mixtures of oil, surfactant and cosurfactant at certain weight ratios were diluted with water drop wise, under magnetic

stirring.The point at which the mixture became signs of phase separation was considered as the end point of the titration. The area of microemulsion existence was denoted as ME. After being equilibrated, the mixtures were assessed visually and determined as being microemulsions, crude emulsions or gels. No attempt was made to distinguish between oil-in-water, water-in-oil or bicontinuous type microemulsions.

2.3 Preparation of microemulsions

From the phase diagrams, suitable composition was chosen for the further studies. The various microemulsion formulated are described in Table no 1. In order to prepare the drug loaded microemulsions of CPO was dissolved in a mixture of oleic acid and propylene glycol. Tween 80 was taken and solubilized in the distilled water. Then water was added to the clear oily phase drop by drop. The o/w microemulsions containing CPO were obtained under a magnetic stirring at ambient temperature.

2.4. Formulation of microemulsion based gel (MBG) of CP

Various gelling agents namely, sodium alginate and Carbopol were evaluated for their ability to gel CPO microemulsion. To the prepared microemulsion gelling agent was dispersed slowly with the help of mechanical stirrer. (Table no-1)

Table 1: Different formulations of Drug loaded Microemulsion Based Gels

	Formulation-A	**Formulation-B**	**Formulation-C**	**Formulation-D**	**Formulation-E**
Drug-ciclopirox olamine	100 mg	100 mg	100 mg	100 mg	100 mg
Oil phase	Linseed oil [LO]	Linseed oil [LO]	Oleic Acid [OL]	Oleic Acid [OL]	Oleic Acid [OL]
Surfactant/cosurfact-ant weight ratio. [S : CoS Ratio]	2:1	3:1	1:1	2:1	3:1
Surfactant/cosurfactant Used.	Tween 80/propylene glycol	Tween 80/propylene glycol	Tween 80/propylene glycol	Tween 80/propylene glycol	Tween 80/propylene glycol
Gelling Agent (Sodium alginate or carbopol)	0.2 gm	0.3 gm	0.3 gm	0.2 gm	0.2 gm
Aqueous phase	q.s	q.s	q.s	q.s	q.s

3. Characterization of the CP -MG

3.1. Determination of pH:

The pH of the 10% (w/w) gel was determined using digital pH meter (Elico make) calibrated using suitable buffer solutions.

3.2. Spreadability:

The spreadability of the gel was determined using the following technique: 0.5g gel was placed within a circle of 1 cm diameter premarked on a glass plate over which a second glass plate was placed. A weight of 500 g was allowed to rest on the upper glass plate for 5min. The increase in the diameter due to spreading of the gels was noted.

3.3 Determination of Drug Content

For determination of drug content, about 1 g of the gel was weighed in a 100-ml volumetric flask and dissolved in methanol the samples were appropropriately diluted and the absorbance was measured at 298 nm using UV-VIS spectrophotometer

3.4. Rheological studies on the MBG:
Brookefield Viscometer was used for rheological studies. The sample was placed in a beaker and was allowed to equilibrate for 5min before measuring the dial reading using a T-C spindle at 0.5, 1, 2.5, and 5 rpm. At each speed, the corresponding dial reading on the viscometer was noted. The spindle speed was successively lowered and the corresponding dial reading was noted.

3.4. Measurement of Gel Strength:
A sample of 50 gms of gel was placed in a 100ml graduated cylinder and gelled in a thermostat at 37^{O}C. The apparatus for measuring gel strength (weighing 10 gms) was allowed to penetrate in CPO -MBG gel. The gels strength, which means the viscosity of the gels was determined by the time (seconds), the apparatus took to sink 5cm down through the prepared gel.

3.5. In vitro bioadhesion study:
The bioadhesive potential of the CPO MBG was evaluated using a method reported by Nakamura et al.(11) . Briefly, an agar plate (1%, w/w) was prepared in pH 4.5 citrate phosphate buffer. Test sample, 50mg was placed at the center of plate. After 5min, the agar plate was attached to a USP disintegration test apparatus as shown in Fig. 12 and moved up and down in pH 4.5 citrate phosphate buffer at 37±1 ◦C. The sample on the plate was immersed into the solution at the lowest point and was out of the solution at the highest point. The residence time of the test samples on the plate was noted visually.

3.6. In vitro antifungal activity:
Antifungal activity of CP-MG, and CP standard dissolved in DMSO) was evaluated against *Candida albicans* ATCC 10231 by using a petri plate method. The mean zone of inhibition was recorded for the formulations. Table no 3.

3.7. In-Vivo Studies:
Rabbit model:
Female rabbits weighing 1.5–2.5 kg were used for the study . Animals were acclimatized before the beginning of the study. Animals were divided into three groups (n = 3) as follows:
Group 1: No application (Control).
Group 2: Marketed formulation (Clotrimazole gel).
Group 3:CP-MG.

Treatment:
Before intravaginal treatment, animals were anesthethetized with intraperitoneal injection of 100 mg/kg ketamine hydrochloride. For fungal challenge, the animals were infected by intravaginal inoculation of *C. albicans* suspended in sterile saline containing 10 c.f.u. / ml. Various formulations were applied into the vagina of rats at a CT using a stomach sondle needle. At each time vaginal lavage samples were collected with 100 ml saline by washing the for the fluid three times up and down in the vagina fluid was then plated onto sabouraud dextrose agar modulus (and incubated for 48 h at 37 8C and c.f.u. values were recorded. A vaginal smear was taken 2 days after the challenge to was performed using a confirm the establishment of infection observation for 2 days. After 48 hours, the viable counts and inflammation were visually assed.

3.10.In-vitro permeation study:

The in-vitro permeation rate of F-MG was determined by using Keshary- Chien diffusion cells fitted with 0.45µ cellulose acetate membrane (Sartorius) at 37 ± 0.1°C using a thermostatic water pump. The effective diffusion area was 2.54 cm2 (18mm orifice diameter), and the receptor compartment was filled with 13.5 ml of phosphate buffer pH 7.4. The receptor fluid was constantly stirred by externally driven teflon coated star head magnetic bars. . Donor compartment contains of 1g of test microemulsion gel equivalent to 8 mg of CP. The receptor compartment was filled with fluid. 1ml of sample was withdrawn at a time intervals of 30 minutes from sampling port of receptor compartment and same volume was the replaced with receptor fluid solution in order to maintain sink condition. The samples were appropropriately diluted and the absorbance was measured at 298 nm using UV-VIS spectrophotometer.

RESULTS AND DISCUSSION

4.1Pseudo ternary phase diagrams of microemulsions

The microemulsion existence region was determined by constructing phase diagrams. Fig.1 describes the pseudo ternary phase diagram with various weight ratios of Tween 80 to Propylene glycol . The translucent region presented in phase diagram reveals the microemulsion existence region. No distinct conversion from water-in-oil (w/o) to oil-in-water (o/w) microemulsion was observed. The rest of region on the phase diagram represents the turbid and conventional emulsions based on visual inspection.

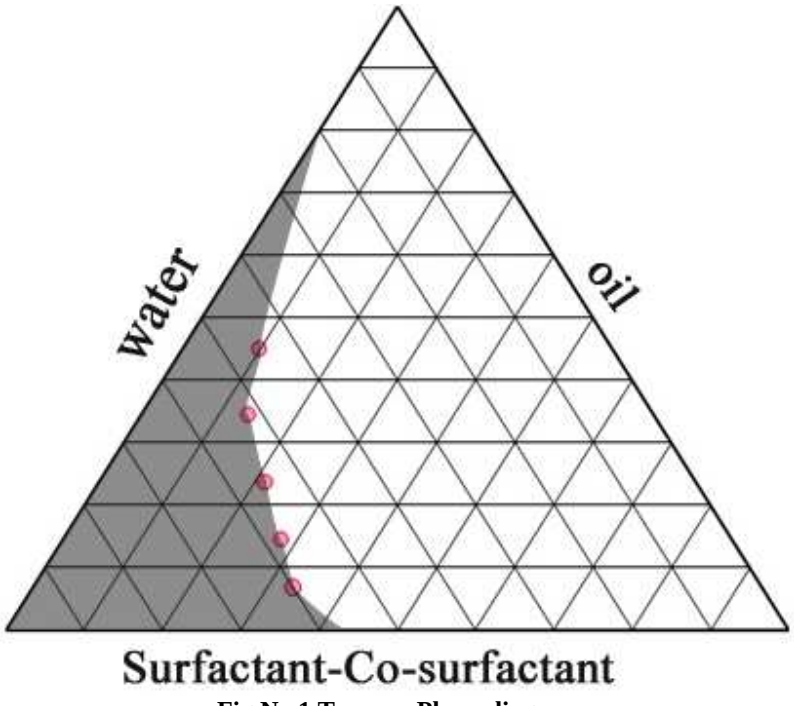

Fig No 1.Ternary Phase diagram

4.2 pH studies

The ph of the prepared formulations of CPO-MBG is given in.(Table-2) which were found equivalent to the vaginal pH.

4.3 Spreadability

It is an important property of vaginal formulation from patient compliance point of view. The Spreadability diameter for different formulations of CPO-MBG are summarized in.(Table-2) Which is indicaties of good spreadability.

4.4 Rheological studies

The viscosity for different formulatons- CPO-MBG are tabulated in(Table-2) . Among this formulations the formulation-B having more viscosity which indicates concentration of gelling agent is more in formulation-B.

4.5 Gel Strength

The gel strength of different formulations CPO-MBG are shown in.(Table-2) All the formulation was found to have good gel Strength.

4.6 Bioadhesieve Force

The bioadhesive potential of ciclopiroxolamine-MBG was evaluated by in vitro method. The retention time by different formulation of ciclopiroxolamine-MBG was determined. (Table-2).All the formulations shows good retention time.

4.7 Mucoadhesive Force

The mucoadhesive force is an important physicochemical parameters for prolonging vaginal retention time and there by better therapeutic effects. Detachment stress of different formulations of ciclopiroxolamine-MBG was determined (table-2). The formulations shows adequate mucoadhesion.

Table2: Different parameters of CPO Microemulsion Based Gels

S. No.	Formulations	pH	Spreadability in cm	Viscosity (cps)	Gel Strength in sec.	Bioadhesive Retention Time(mins:sec)	Mucoadhesive Force (dynes/cm^2)
1	A	4.25	2.8	6400	90	11.00	15138
2	B	4.33	2.9	7000	87	10.30	14314
3	C	4.23	2.7	6250	101	17.00	15449
4	D	4.28	2.9	6300	109	18.10	15844
5	E	4.40	3.0	6250	113	19.50	15988

4.8 In vitro antifungal activity

The values of zone of inhibition produced byCPO standard, CPMBG and Candid-V gelwere 3.0±0.16, 3.5±0.12 and 2.8±0.15mm,respectively (n = 3). It is clearly evident that CPMBG showed higher anti-fungal activity as compared to the marketed Candid-V gel ($P < 0.05$) and CP standard. The anti-fungal activity was signifi-cantly higher than Candid-V gel ($P < 0.05$) whereas it was slightly higher thanCP standard ($P > 0.05$).

4.9 In-Vivo Studies

Studies performed in rabbits showed less inflammation in animals treated with ciclopirox olamine MBG and culture showed lower viable count, when compared with animal treated with standard drug. This finding indicates that Ciclopirox olamine microemulsion based gel shows potential anti fungal activity against C.albicans strain.(fig no2)

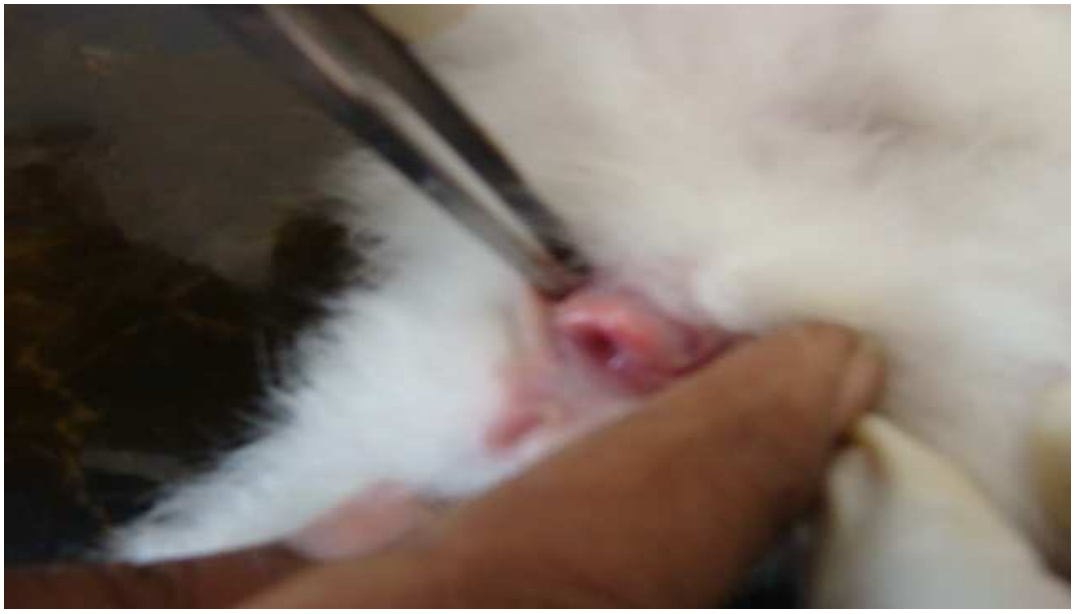

Figure No.2: Visual observation of vagina after administration of ciclopirox olamine-MBG

4.10 Invitro Diffusion studies

Fig. 3 illustrates the permeation rates of CPO thhrough the different MBG formulations. E showed the highest perfusion rate (54 ± 1.15) among the formulations tested. The surfactant mixture content in the formulation affected the permeation rate significantly.

CONCLUSION

The CPO-MBG can be successfully formulated for the topical treatment of vaginal candidiasis. The developed CPO-MBG showed good antifungal activity in rabbits when compared with standard. The studies indicated that CPO-MBG could be a viable alternative to the current topical formulations available for the treatment of vaginal candidiasis.

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