Research article

Rapports De Pharmacie Vol.2 (1), 2016, 192-198
ISSN: 2455-0507

DEVELOPMENT AND *IN VITRO/IN VIVO* CHARACTERISATION OF MODIFIED CALCIUM CARBONATE COMPOSITION OF GUAR GUM AND SODIUM ALGINATE NANO PRECIPITATE OF ECONAZOLE NITRATE

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ABSTRACT

Present study to investigate a method of preparing a modified calcium carbonate guar gum/sodium alginate composition and thereof belong to pharmaceutical field. Polymer is an anion or cation (guar gum/ sodium alginate) in which any one of the polymer react with mixture of sodium bi carbonate and calcium chloride to yield a modified calcium carbonate. The nano precipitates prepared by using polymer as modifier carbonate and calcium chloride and water mixing reaction preparation containing modifier of calcium carbonate. Sustained release of econazole nitrate from modified calcium carbonate nanoprecipitates was obtained by guar gum and sodium alginate as biodegradable polymers. The average particle sizes of nanoprecipitates were in the range of 350 to 512 nm. The loading efficiency of nanoprecipitates was in the range of 80.31 to 90.75%. The nanoprecipitates loaded gel was found to be in the range 45695 to 50659 cps. The maximum gel strength of the EN nanoprecipitates loaded gel was found to be (21 sec.) and maximum mucoadhesion force was found to be (17.51 dynes/cm^2). The formulations exhibited maximum spreadability (13.45 gm.cm/sec), The optimized formulations were able to release the drug up to 540 minutes.

Keywords: Modified calcium carbonate composition, In vivo studies, SEM, nanoprecipitates loaded gel, HPLC

INTRODUCTION

Candidiasis is the fungal infection that can manipulate the private parts. Genital infection is extremely more recurrent in women than men, nevertheless when it does appear in males; thrush affects the head of the penis and the foreskin.

Nanotechnology is found in a wide range of applications in the pharmaceutical industry. Due to new advances in nanotechnology, it is now possible to produce drug nanoparticles that can be utilized in a variety of innovative ways. Nanoparticle introduced in 1991 represent an alternative carrier system to traditional colloidal carriers such as emulsions, liposomes and lipid micro and nanoparticles. Nanoparticles are sub-micron colloidal carriers ranging from 1 to 1000 nm, composed of natural or synthetic polymers and dispersed in aqueous surfactant solution. Nanoparticles offers unique properties such as small size, large surface area, high drug loading and the interaction of phases at the interface and are attractive for their potential to improve performance of pharmaceuticals[1].

Nanoparticles are at the fore-front of the potential applications in drug delivery, clinical medicine and research as well as in other allied sciences. They are manufactured from synthetic/natural polymers and ideally suited to optimize drug delivery and reduce toxicity. In monodisperse systems, nanoparticles are the new generation of nanoparticulated active substance carriers and are attracting major attention as novel drug carriers [2]. Nanoprecipitation is one of the method that is included under second category. It is widely applicable technique that is less energy consuming and less complex method. The principle behind this technique is the interfacial deposition that occurs due to the displacement of a solvent with the non-solvent. The parameters that influence the formation of the nanoparticles in this method are miscibility of the solvents and the presence of the dilute polymer solutions.[3]

This method is considered to be the most sensitive and low energy consuming one as it requires low energy costs and no special equipment requirements. The various polymers involved in this technique are not only Poly(lactide), Poly(lactide-co-glycolide) and Poly caprolactone but also other lactones, cellulose ethers and esters like cellulose butyrate acetate, ethyl cellulose, hydroxyl methylpropylcellulose phthalate, cellulose acetophthalate, naturally occurring polymers(gelatin, Arabic gum), poly(vinyl alcohol acetophthalate), copolymers of acrylate acrylate and methacrylate (Eudragit), poly(vinyl pyrrolidone-vinyl acetate), maleic acid derivatives, etc.

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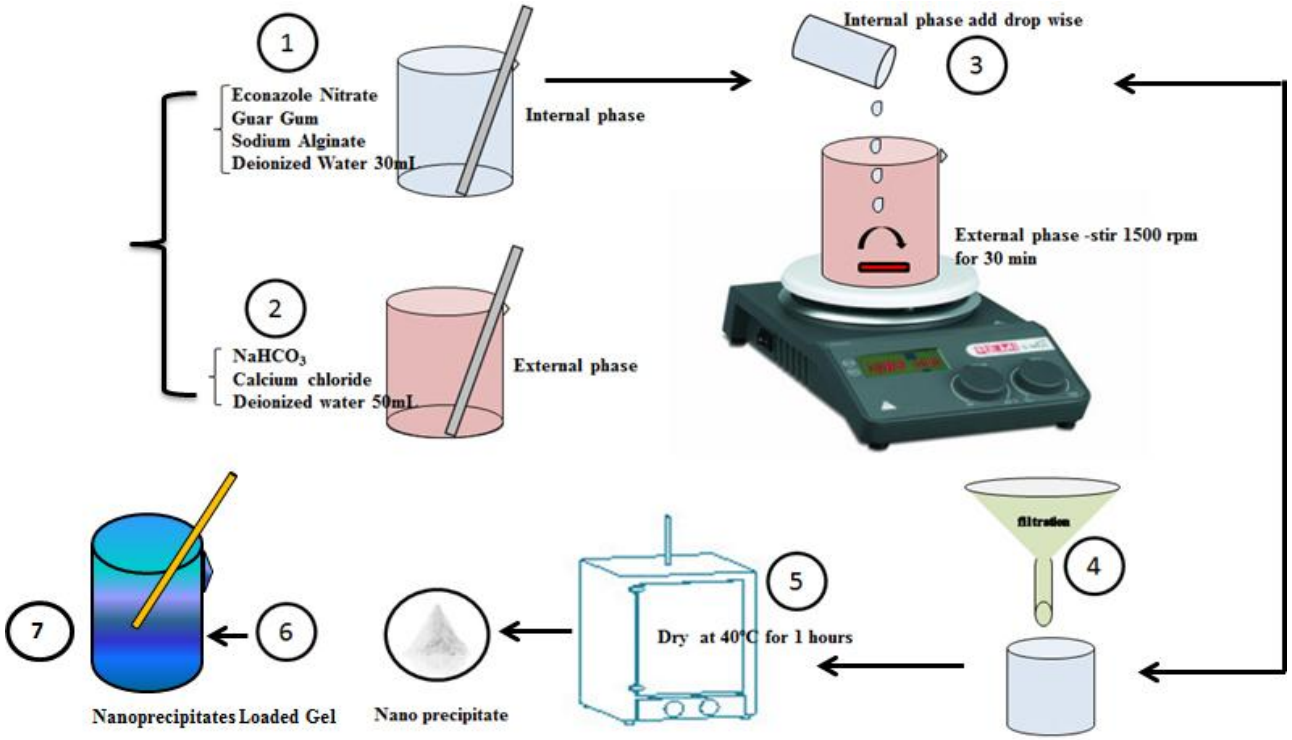
It is most suitable method for hydrophobic drugs and the problem arises when a hydrophilic drug has to be encapsulated in the polymeric matrix by this method. The problem can be minimized by adjusting the pH value or by choosing appropriate solvent/nonsolvent [4,5]. In this study includes formulation of nanoparticles containing econazole nitrate using guar gum/sodium alginate as the retardant polymer which will release the drug for a prolong duration to promote patient compliance**.** Carbopol is one of the most common thickening agent for water phases. It is used after neutralisation and its rheological properties in the aqueous medium are well known. The aim of this work was to investigate the particle size and physical characterization, rheological properties, gel strength, spreadability, mucoadhesive force, diffusion studies, HPLC and scanning electron microscope.

EXPERIMENTAL

Econazole nitrate was gift sample from Kotra Pharma (M) Sdn. Bhd, Malaysia. Guar gum and sodium alginate were purchased from Essex chemicals, UK. Sodium bicarbonate and calcium chloride were purchased from Suka chemicals, Sdn Bhd, Malaysia and all other chemicals used were of analytical grade.

METHOD OF PREPARATION OF ECONAZOLE NITRATE NANO PRECIPITATES:



Preparation of nanoprecipitates loaded gel:

The 10 g gel was prepared by direct dispersion method. In this firstly, propyl paraben was dissolved in water at 80ºC and then accurately weighed quantity of carbopol 940 was dispersed in water at 40ºC with constant stirring for 1 hour. Equivalent to 1% of econazole nitrate nanoprecipitates was incorporated in the above prepared solution of carbopol with continuous stirring. The pH of all gel formulations were adjusted to pH 6, using triethanolamine and stirred slowly until a gel was obtained.

CHARACTERIZATION OF NANO PRECIPITATES

Morphology, particle size:

Morphology of EN nanoprecipitates was snapped by scanning electron microscopy (SEM). Particle size was measured by using zeta sizer 3000 HS (Malvern instruments, UK). Samples were diluted with distilled water and measured at 25ºC. The diameter was calculated from autocorrelation function of the intensity of light scatter.

Determination of loading efficiency:

To determine the loading efficiency, accurately

weighted portions from each batch of econazole EN nanoprecipitates were dissolved in methanol, and this clear solution was analyzed for loading efficiency. The solution was then filtered and determined by RP HPLC method. RP HPLC chromatographic separation was performed on a Shimadzu liquid chromatographic system equipped with a LC-20AD solvent delivery system (pump), SPD-20A photo diode array detector, and SIL-20ACHT injector with 50µL loop volume. The LC solution version 1.25 was used for data collecting and processing (Shimadzu, Japan). The HPLC was carried out at a flow rate of 1.0 ml/min using a mobile that is phase constituted of acetonitrile, 0.5 mm ammonium acetate (pH 3.0) (60:40, v/v), and detection was made at 260 nm. The mobile phase was prepared daily, filtered through a 0.45µm membrane filter (Millipore) and sonicated before use. A Thermo C18 column (25cm × 4.6mm i.d., 5µ) was used for the separation. The drug content was calculated from the calibration curve and expressed as loading efficiency.

Viscosity Studies:

The rheological studies were conducted by using brookfield programmable DVII+ Model pro II type (USA). The viscosity of nanoprecipitates loaded gel was determined at 0.3 rpm and means of two readings were used to estimate the viscosity [7].

Determination of mucoadhesive force:

The mucoadhesive force of all the optimized batches was determined as follows, a section of the chicken mucosa fixed with mucosal side out onto each glass vial using rubber band. The vial with chicken mucosa was connected to the balance in inverted position while first vial was placed on a height adjustable pan. Nanoprecipitates loaded gel was added onto the mucosa of first vial. Then the height of second vial was so adjusted that the mucosal surfaces of both vials come in intimate contact. Two minutes time of contact was given. Then weight was kept rising in the pan until vials get detached. Mucoadhesive force was the minimum weight required to detach two vials. The chicken mucosa was changed for each measurement [8].

Detachment stress (dynes/cm^2) = mg/A

Where m is the weight added to the balance in grams; g is the acceleration due to gravity taken as 980 cm/s^2; and A is the area of tissue exposed.

Measurement of Gel Strength:

A sample of 50g of nanoprecipitates loaded gel was placed in a 100 ml graduated. The apparatus for measuring gel strength (weighing 27g) was allowed to penetrate in gel. The gel 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 [9].

Spreadability:

For the determination of spreadability, excess of sample was applied in between two glass slides and was compressed to uniform thickness by placing 1000g weight for 5 min. weight (50 g) was added to the pan. The time in which the upper glass slide moves over to the lower plate was taken as measure of spreadability [10].

S= ML/T

Where,

M = weight tide to upper slide (g)

L = length moved on the glass slide (cm)

T = time taken (sec)

Diffusion studies:

The in vitro release of EN nanoprecipitates loaded gel was studied using cellophane membrane using modified apparatus. The dissolution medium used was phosphate buffer, freshly prepared (pH 7.4). Cellophane membrane previously soaked overnight in the dissolution medium, was tied to one end of a specifically designed glass cylinder (open at both ends). Equivalent to 1% w/w of EN nanoprecipitates loaded gel was accurately placed into this assembly. The cylinder was attached to stand and suspended in 200 ml of dissolution medium maintained at 37±1°C, the membrane just touching the receptor medium surface. Aliquots, each of 5 ml volume were withdrawn periodically at predetermined time interval of 0.15, 0.30, 1.0, 2.0, 3.0, 4.0, 5.0 up to 9 hours and replaced by an equal volume of the receptor medium. The solution was then filtered and determined by RP HPLC method [11].

In vivo studies:

Animals:

Adult wistar rats (280 ± 10 g) of either gender were obtained from animal house (AIMST university). 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 institute animal ethics committee of the AIMST University, Malaysia and all the animal experiments were carried out according to the committee for the purpose of control and supervision of experiments on animal's 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 a microspheres enriched 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 [12].

Evaluation of therapeutic efficacy:

The male rats were used for the experiment. The rats were divided into the four groups *viz.*, normal control (group I), *Candida glabrata* control (group II), standard treatment group (group III) and EN nanoprecipitates loaded gel treatment group (group IV). Group II to IV animals were changed with intravenous methylprednisolone (5mg/kg) for 3 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, 4^{th} and 7^{th} 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.

Histopathologic analysis:

The liver and pancreas 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, Serial cross sections of the tissues were obtained and stained with hematoxylin and eosin stain for fungal visualization [13].

Statistical analysis:

All the data were expressed as mean ± SEM. Statistical significance between the groups were tested using one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test post-hoc test. A P less than 0.5 were considered significant.

RESULTS AND DISCUSSION

Modified nanoprecipitation method was used for the preparation of nanoparticles [14].This method works best for highly dosed freely water-soluble drugs like econazole nitrate to provide high drug loading and suitable particle size. Nanoprecipitates were prepared with different ratios polymer A&B (0.1-0.3 and 0.1- 0.3 w/w) to investigate the particle size on the physicochemical characteristics of the prepared nanoprecipitates.

The results of the encapsulation efficiency and particle size analysis given in Table 1. The loading efficiency of econazole nitrate into the nanoprecipitates was found in a wide range for all the formulations (80.31 to 90.75%) and were affected by the polymer ratios studied. All of these results indicated that the modified nanoprecipitation method was a very suitable preparation method for encapsulation of hydrophilic drugs.

The numbers characterizing the mean particle size of the EN nanoprecipitates showed that the mean particle diameter was affected by both the polymers ratio for all the formulations as seen in Table 1, the mean particle diameter of the microspheres increased with increasing polymers ratio in the disperse phase based on the viscosity of the all the prepared formulations.

The shape and surface characteristics of the optimized nanoprecipitates are illustrated in Fig. 1. SEM observations showed that all of the nanoprecipitates prepared were cube in shape, and the morphological characteristics of the nanoprecipitates were extensively affected by the polymers ratio investigated.

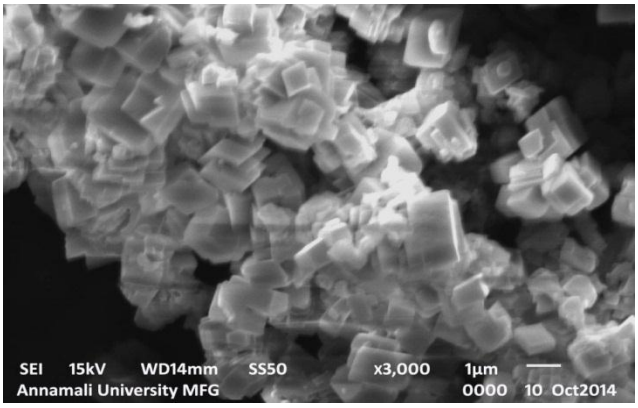


Figure-1: SEM of EN nanoprecipitates

The values of spreadability denote that the gel is easily spreadable by small amount of force. The spreadability of formulation F1, F2 and F3 was found to be 13.45, 12.65 and 11.75 gm.cm/sec respectively; this indicates all the optimized concentration of gel to spread on skin easily.

Gel strength is essential because strong gels will support a much higher pressure than weak gels before they are washed out of the targeted site. The formulations exhibited gel strength as seen in Table 2.

The mucoadhesive force is an important physic-chemical parameter for topical application. The formulations showed maximum mucoadhesive force, these may be due to increase in concentration of carbopol 940 in the formulations as seen in Table 2.

The *in vitro* drug release profile of nanoprecipitates loaded gel is given in Fig 2&3. As the gelling agent concentration increased from 1 to 2%, the drug release rate increased based on the concentration of gelling agent. It is apparent from the drug release profiles that nanoprecipitates loaded gel prepared with 2% of carbopol 940 showed the bursting effect at each time interval.

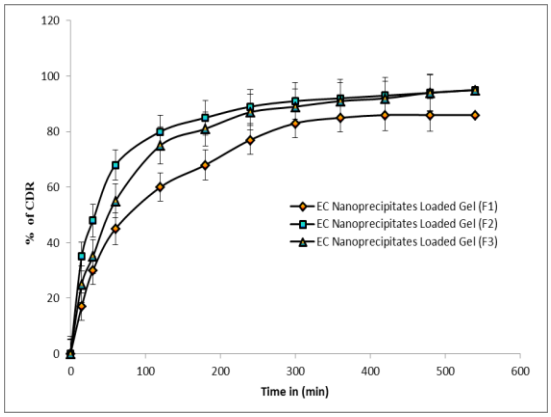


Figure-2: Showing the drug release

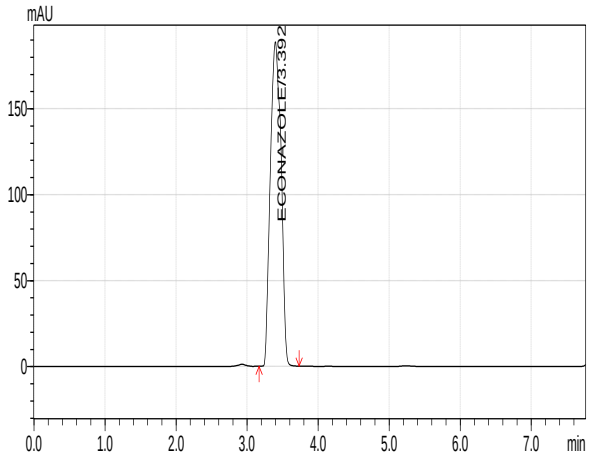


Figure-3: Typical chromatogram of econazole nitrate

The therapeutic efficacy of EN nanoprecipitates loaded gel was compared with *candida glabrata* control by quantitative microbiological analysis and histopathological evaluations. Nanoprecipitates loaded gel and standard marketed formulation treated animals showed significant reduction of CFU count on 4th day of the treatment onwards. The efficacy of the EN nanoprecipitates loaded gel is comparable with standard marketed formulation as seen in (Figure 4 and 5).

Table-1: Composition of econazole nitrate nanoprecipitates

Ingredients (w/v)	A1	A2	A3	A4	A5
Econazole nitrate (g)	0.1	0.1	0.1	0.1	0.1
(A) Guar gum (g)	0.1	0.2	0.3	0.1	0.1
(B) Sodium alginate (g)	0.1	0.1	0.1	0.2	0.3
Deionized water (ml)	30	30	30	30	30
$NaHCo_3$ (g)	2	2	2	2	2
Calcium chloride (g)	3	3	3	3	3
Deionized water (ml)	50	50	50	50	50
Particle Size (nm)	350	423	501	507	512
Drug Loading Efficiency (%)	80.31	85.56	89.11	90.75	90.01

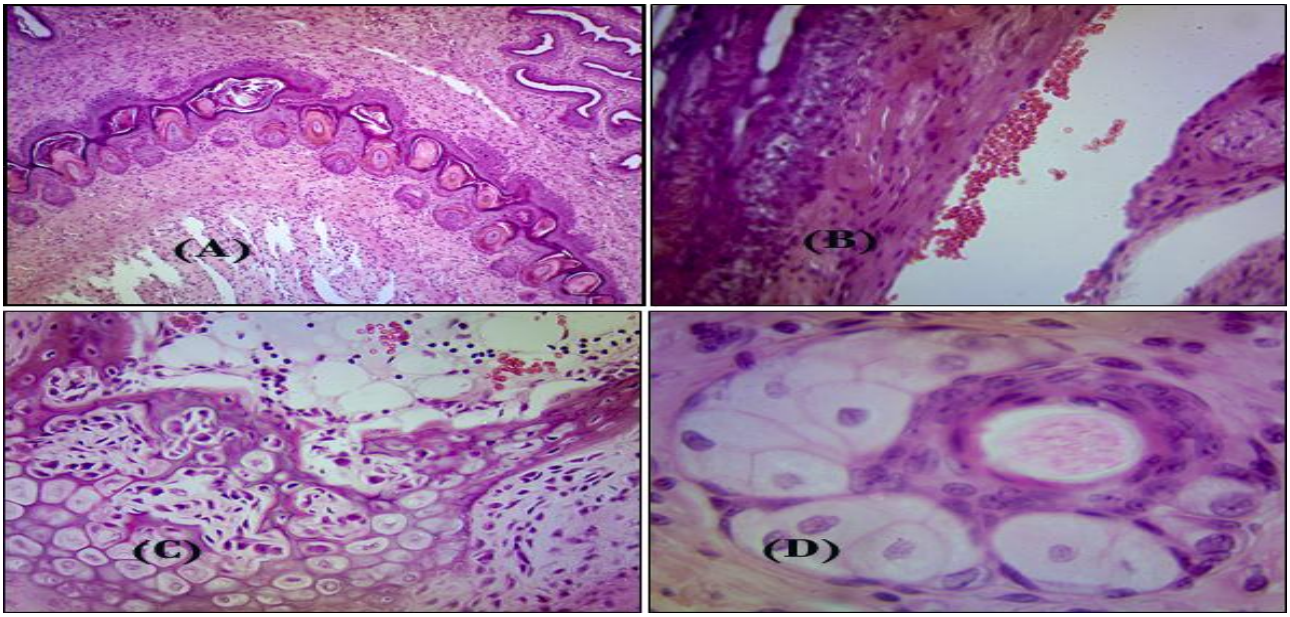


Figure-4: Histological Analysis. Rat penal smooth muscle section from (A) control showed normal articheture and (B) showed Candia microorganism infection in smooth muscle surface (C) and (D) showed reduction in growth of Candia due to antifungal effect of standard and MEG, H&E, 400X.

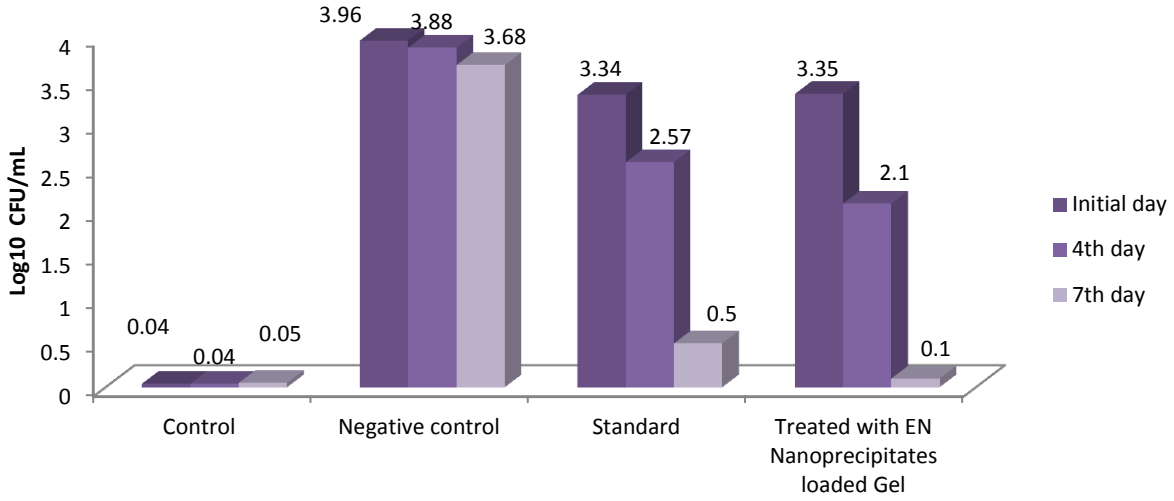


Figure-5: Quantitative microbiological analysis of the Candida glabrata in rat penal smooth muscle surface

Table-2: Characteristics of nanoprecipitates loaded gel

Formulation	Viscosity (cps)	Mucoadhesive force(dynes/cm^2)	Spreadability (gm.cm/sec.)	Gel strength (seconds)
F1 (1% CP 940)	45695	10.23±0.32	13.45 ± 0.34	14
F2 (1.5% CP 940)	47879	14.66±0.25	12.62 ± 0.57	19
F3 (2% CP 940)	50659	17.51±0.17	11.72 ± 0.27	21

CONCLUSION

Econazole nanoprecipitates loaded gel is also used to treat fungal infections of the penis, known as balanitis. Nanoprecipitates loaded gel afforded sustained drug release over 540 (minutes) period. The formulations were therapeutically effective. The in vivo animal studies for penile thrush which was conducted in adult wistar rats revealed that the fungal burden at the end of 7th day was found to be nil for the animals treated with the test formula. The nanoprecipitates loaded gel formulation is a viable alternative to conventional dosage forms such as lotions, creams, powder, ointment by virtue of its ability to enhance bioavailability through its longer residence time and ability to sustain drug release. The nanoprecipitates loaded comfort for administration and decreased frequency of administration succeeding in better patient acceptance.

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