DEVELOPMENT AND STANDARDIZATION OF SEMISOLID DOSAGE FORM USING PHYTOCONSTITUENT ALONG WITH STANDARD DRUG FOR VAGINAL INFECTIONS

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CHAPTER – 5

SUMMARY AND CONCLUSION

5.1. Summary and Conclusion

This research work was designed with an aim to develop a suitable vaginal formulation that can be effectively used for a wide range of fungal infections. Literature shows that most common vaginal fungal infection is mainly caused due to Candida Spp. It was found that for effective management of such type of infectious condition only oral antifungal therapies are not found sufficient. Again this is also recommended that an alternative topical application should be carried out along with systemic drug therapy for better treatment of vaginal fungal infections.

Therefore, looking towards the demand of the situation and based on a thorough literature study, a therapeutically effective drug combinations were prepared using curcumin and standard synthetic antifungals namely fluconazole and itraconazole. Different drug combinations were prepared and among this most suitable and effective combinations were selected on the basis of *in-vitro* antifungal and pharmacological screening.

Literature shows that uses of bioactive phytoconstituents towards the development of effective drug combinations were found more effective and safe as compared to synthetic drug combinations. Further, this is also a fact that, though fluconazole and itraconazole are considered as the drug of choice for systemic antifungal therapy but at the same time it shows unavoidable side effects like itching, redness and skin sensitivity issues when applied topically at higher concentration.

The rationality behind the use of plant-derived bioactive lies on the fact that these components can enhance the degree of therapeutic effectiveness, reduce the side effects and also reduce the amount of alternative drugs used in combination. Therefore, Use of plant-derived bioactive like curcumin may be considered as a novel approach towards development of effective drug combination.

Mucoadhesive gels were selected as vaginal drug delivery system for safe and effective delivery of the selected combinations to the targeted site. The purposes behind the selection of mucoadhesive gels as a delivery system were to overcome the low retention time inside the vaginal cavity, improved drug absorption and better bioavailability.

This research work is divided into 6 chapters. Chapter 1 starts with an introduction, the background of the research work, aim and objective of the research work, Chapter 2 deals with the review of the literature on brief descriptions about vaginal drug delivery system (VDDS), its importance and present status. This chapter further extended with a discussion on synergistic activities, a brief detail of mucoadhesive vaginal gels and its role and important findings of researchers on the proposed field. Chapter 3 describes the materials and methods used for the experimental work. Chapter 4 summarizes results of the research work. This section also includes discussion of obtained results. Chapter 5 summarizes the whole research outcome and draws a conclusion based on observations and findings. Chapter 6 lists all the references cited in the whole thesis in a format prescribed in CSVTU thesis writing manual.

The objectives of the research work were

- ✓ To develop a safe and effective antifungal combination for the treatment of wide ranges of vaginal fungal infections.
- ✓ To design a suitable vaginal delivery system that can deliver the drug to the infected site easily and safely.
- ✓ To likely improve patients complacence by improving residence time of dosage form in side vaginal cavity

- ✓ To achieve a higher degree of therapeutic assistance with minimum side effects.

Plan of work to carry out the research work was

- Selection and procurement of bioactive plant-derived phytoconstituent and drugs
- Procurement of equipment/instruments, excipients, and chemicals
- Identification and analytical estimation of APIs through determination of solubility, melting point, Assay of APIs and FTIR studies
- Preparation and optimization of suitable and effective phyto-combination based on *in-vitro* antifungal screening
- Preparation, evaluation, and optimization of the suitable dosage form.
  - ✓ Preformulation studies
  - ✓ *In-vitro* evaluation studies like determination of rheological property, pH of formulation, determination of spreadability, estimation of Stability study, compatibility study, mucoadhesion study, estimation of drug content, *in-vitro* vaginal retention test, *in-vitro* drug release study, *in-vitro* vaginal permeability
  - ✓ *In-vivo* evaluation studies like vaginal irritation test, estimation of condition of vaginal surface, antifungal activities and histopathology estimation
- ➢ Statistical analysis, estimation and interpretation of results, compilation, and submission of the research report.

After detail literature survey curcumin as plant-derived bioactive phytochemical and synthetic antifungal drugs such as fluconazole and itraconazole

were selected to develop suitable drug combination for effective treatment vaginal candidiasis or volvo vaginal candidiasis.

Drugs, excipients and essential chemicals were procured from different sources and stored as per the prescribed condition.

First of all the APIs were subjected for essential analytical estimation processes like identification tests (organoleptic evaluation, determination of solubility, determination of melting point and compatibility study), Assay of drugs, preparation of standard calibration curve in a suitable medium.

The received drug samples were first evaluated organoleptically and found that the itraconazole and fluconazole drug powder was odorless, white in colour, crystalline in nature, the texture of powders was smooth and free of any gritty particles. Similarly, received a sample of curcumin was found to be yellows in color, with a characteristic odour, smooth powder without any gritty particle. Therefore, observed identification parameters was found as per the specification of monograph.

The results of solubility study states that curcumin was partially soluble in water and freely soluble in methanol. Similarly, fluconazole was found to be slightly soluble in water and soluble in methanol, acetone. Further, itraconazole was found to be soluble in ethanol, and practically insoluble in water. Again equilibrium solubility of fluconazole, itraconazole, and curcumin in simulated vaginal fluid (SVF) and phosphate buffer pH 4.5 was determined using UV spectroscopic method. The results show that all the drugs are soluble in both the medium. Hence, both the medium are found suitable to carry out the essential *in-vitro* studies.

The melting points of received drug samples were determined using digital melting point apparatus and obtained results were found to be matched the official pharmacopoeia limit. The average melting point of the curcumin and fluconazole

were found 180°C and 138°C respectively and in case of itraconazole, the determined value was 166°C. The recorded values were found as per the limit of standard monograph. Hence, it may be stated that the received drug samples are pure and may be identified as a pure sample of curcumin, itraconazole, and fluconazole.

The assay of fluconazole and itraconazole was performed as per USP, following Potentiometric method and the purity of drug sample was found to be 98.84% and 98.78% respectively. As per the monograph percentage purity of fluconazole should range between 98-102% and similarly in case of itraconazole the percentage purity should range between 98.5 – 101.5%. Therefore, it was found that the purity of received drug sample complies with the standard of USP monograph. Further, the assay of curcumin was performed using UV spectroscopic method using methanol as a solvent system and found the percentage purity 96.28%.

The ƛ max value was determined through UV spectroscopic method. A standard solution of 1mg/ml concentration was prepared for all the received drug samples in SVF and phosphate buffer pH 4.5 respectively and spectrometrically measured. The ƛ max value of curcumin was found at 461nm, similarly ƛ max value of fluconazole was determined at 261nm and itraconazole was found in the range of 226nm. The obtained results were found to be complying with pharmacopoeia standard. Hence, it may be stated that the received sample is pure and suitable for experimental work.

All the received drug samples were subjected to FTIR spectrum analysis. The recorded Spectrum of the tested sample was compared with principal characteristic peaks pure drug as reported in monographs. Principle characteristic peaks of fluconazole (cm^{-1}) as reported in the literature are 1620cm^{-1} appear due to the presence of C=N stretching, 2962cm^{-1} occurs due to CH_2 stretching, 3120cm^{-1}due to

the presence of CH aromatic group, and 3200cm^{-1} due to the presence of OH functional group. Whereas, the received sample of fluconazole show the peaks at1620 cm^{-1}, 2964 cm^{-1}, 3119 cm^{-1} and 3200cm^{-1}. Similarly Principle characteristic peaks of curcumin (cm^{-1}) was reported in literature are 3406.54cm^{-1} due to the presence of OH stretching, 1630.08cm^{-1}, 1615cm^{-1} appears due to C = C and C = O respectively, further, peak reported at 1510 cm^{-1}occurs due to (C = O), while, Peak at 1275 cm^{-1}occurs due to enol C–O bonds. Whereas, the received sample of curcumin shows the peaks at 3404.38 cm^{-1}, 1629.92 cm^{-1}, 1510.24 cm^{-1}, 1274.42 cm^{-1} and1618.92cm^{-1}. Again the principal characteristic IR peak of itraconazole (ITR) as per the literature, at frequency range 400 – 4000 cm^{-1} was reported to occur at 3439cm^{-1}, 3126cm^{-1} and 3069 cm^{-1}due to the absorption of NH2 groups, 2964 cm^{-1} resulted due to CH_2 stretching and a sharp peak occurred at 1698 cm^{-1}due to C=O stretching vibration. Further peaks appeared at 1609 cm^{-1}and 1429 cm^{-1}was due to C=N and C-N bonds, respectively, followed by characteristic peaks occurred at 1510 cm^{-1}due to C-H deformation. The received sample of itraconazole show the peaks at 3128 cm^{-1},3069 cm^{-1}, 2962 cm^{-1}, 1610 cm^{-1}, 1695 cm^{-1}, 1510 cm^{-1} and 1428 cm^{-1}. Therefore, on the basis of comparative peak analysis, it can be stated that the received drug samples were pure and are identified as pure drug sample of fluconazole, curcumin, and itraconazole respectively.

The standard calibration curve of the all the drugs were prepared using a medium like a Phosphate pH 4.5 as well as in a simulated virginal fluid. The linearity values of the standard curve for fluconazole, itraconazole, and curcumin Phosphate buffer pH 4.5medium were found to be 0.987, 0.996 and 0.993 respectively. Similarly, in case of the simulated vaginal fluid medium, the linearity values of

standard calibration curve were found 0.996, 0.995 and 0.994 respectively for fluconazole, itraconazole, and curcumin pure drug samples.

Again, drug excipient compatibility study was also performed with a physical mixture of different combination of drugs with different excipients, as per the formulation design protocol. The compatibility of the different component mixture was analysed by peak overlapping techniques. The changes in peak area, shifting of principle peak and addition of new peak were determined and compared with the parent peak of drug molecules present in the mixture. The results of the study reflects no significant change. Therefore, it may be stated that drug and selected excipients are compatible with each other and hence, found safe and suitable for the development of mucoadhesive gel.

The *in-vitro* antifungal screen study was performed to evaluate the antifungal activity of the individual drug and also well as the designed combination of drugs. The study was performed using fungal strain *candida albicans* MTCC-227. At first the minimum inhibitory concentration (MIC) of individual component namely itraconazole, curcumin and fluconazole were determined by means of disk diffusion method and microdilution technique. The MIC of received drug sample of fluconazole against the pathogenic strain of *Candida albicans* MTCC-227 was found within the range of 32µg/ml to 64µg/ml. Similarly, MIC of itraconazole was found in the range of 8 µg/ml to 32µg/ml and in case of curcumin, the MIC value found in the range of 64 µg/ml to 256 µg/ml.

Further, the antifungal efficacy of different drug combination was determined by means of disk diffusion method. Ketoconazole was used as the reference standard and the result was concluded on the basis of measurement of the radius of the zone of inhibition. The result states that at concentration 10µg/ml all the tested antifungal

agents showed an apparently clear zone of inhibition. The zone of inhibition recorded of curcumin was found to be 14mm, similarly for itraconazole and fluconazole it was found to be 31mm and 21mm respectively, while in case standard drug it was recorded 34 mm.

Further, a different combination of antifungal agents was developed using curcumin along with fluconazole and another set of the combination was developed using curcumin along with itraconazole. However, the overall concentration of every individual combination was restricted to 10µg/ml. The effect of the combination was compared with the effect standard drug as well as the individual component of the combination at the same concentration. The data obtained from the experiment states that at a concentration of 10µg/ml, all most all the combination showed a better zone of inhibition value as compared to the individual drug. Further a few combination shown better effect as compared to the standard drug. Again it was observed that combinations contain a higher concentration of curcumin show comparatively better antifungal effect. Therefore, on the basis of experimental data, it may be clearly interpreted that the combination of natural and synthetic antifungal agents are found to be more therapeutically active as compared to their individual capacity.

Further, for the selection of most effective combination fractional inhibitory concentration index value was determined on the basis of the checker board titer test method. The FIC of each agent is calculated as the MIC of this agent in combination divided by the MIC of this agent alone. Each checkerboard test generates many different combinations and by convention, the FIC value of the most effective combination is used in calculating the FIC index.

Mucoadhesive gels were prepared using hydration followed by continuous agitation method. Different polymer systems at various ratios were tried for the

development of the dosages form. At first 15 different formulation batches were prepared using different polymer like HPMC k100, carbopol P934, carbopol 940, sodium CMC and guar gum at various concentration. Fluconazole was used as a model drug. The purposes behind the preparation of those batches were to evaluate the capacity of different polymer system and to select a few out of them on the basis of their performance to carry out the further study. The polymer systems were mainly selected based on the Parameters like Mucoadhesion capacity, spreadability, vaginal retention capacity and drug release study.

All the prepared formulation were subjected to different *in-vitro* evaluation studies and results of the evaluations are discussed below-

Results of the study suggest that gels prepared using 0.5%, 1% and 1.5% HPMC (F1, F2, and F3) show viscosity within the range of (1080 to 1850cps), mucoadhesion strength ranges from 12.05-15.05 dyne/mm^2 and spreadability within the range of (11.75-12.13 gm/cm^2). Similarly, formulation prepared with Carbopol P934 at a concentration of 0.5%, 1%,1.5% (F4, F5, F6) show viscosity ranges from (1270 cps to 2045 cps), followed by mucoadhesion strength ranges from 14.00-17.25 dyne/mm^2, while spreadability was ranges within (11.56 to 13.25 gm/cm^2). Further , in case of formulation prepared using 0.5%, 1% and 1.5% carbopol 940 (F7, F8,F9) the viscosity of prepared formulations were found within the range of (2225-3440 cps) followed by mucoadhesion strength with the range of (16.05-17.50dyne/mm^2) and spreadability was found within the range of (12.54-13.65 gm/cm^2). The drug content value of the prepared formulations F1 to F9 was ranged between (95.50 - 98.65 %) and released 80% of the drug in 6 hours.

Further, the gels prepared with Sodium CMC (SCMC) using 4%,5%,6% (F10, F11 and F12) and guar gum using 3%, 4%, 5% (F13,F14, F15) as polymer system.

And the result of the viscosity of SCMC gels was found within the range of (1630-2440cps) followed by mucoadhesion strength was found with the range of (4.05-8.85 dyne/mm^2) and spreadability was found within the range of (14.35-18.06 gm/cm^2). Similarly in case of Guar gum gels viscosity was found within the range of (1900-2620cps) followed by mucoadhesion strength was found with the range of (5.5-8.05 dyne/mm^2) and spreadability was found within the range of (14.34-18.30 gm/cm^2). The drug content values of formulation F10 to F15 were found to be ranges within (91.02% -95.20%) and approximately 80% 0f drug releases within 5 hours.

On the basis of resultant data, it may be interpreted that the viscosity of the gel was gradually increased with the concentration of polymer system. Though the viscosity is directly related to the molecular weight of polymer system, but practically is also depends upon the number of other variables related to the preparation of dosage form. Again it was observed that pH of the formulation has an impact on the viscosity of the prepared formulation. Further, the parameters like mucoadhesion force may also be correlated with the viscosity of the formulation. It has been found that viscosity is directly proportional to mucoadhesion force up to a certain extent and inversely proportional to spreadability of the prepared gel. Again on the basis of a literature survey, it may be stated that the mucoadhesion property mainly depends upon the capacity of the polymer matrix to form a reversible bond with the mucous layer. However, form the results of the experimental work it was found that though the formulation F10 to F15 having satisfactory viscosity but they show poor mucoadhesion property and at the same time their consistence was also found poor that reflects in the results of the spreadability study.

Further, the results of *in-vitro* vaginal retention study show that formulation prepared with HPMCk100 (F1-F3) drain out 6.2% to 7.8 % of drug within the

duration of first 60 minutes similarly the resultant value for formulation F4-F6 was found to 4.8% to 5.7% and in case of formulation F7-F9 the value ranges between 5.4% to 5.9%. Further, formulation batches F10-F11 the resultant value range from 25.04%- 28.09% and in case of prepared formulation batches, the drug losses values were ranges between 25.08%-28.07% within first one hour. This study may provide us an approximate idea about the amount of drug retains inside the vaginal cavity at the real-time situation. Based on the result was found that formulation prepared with carbopol P934 and P940 (F4-F9) shown comparatively better amongst all the formulation, followed by formulation prepared with HPMC K 100(F1-F3)

The results of drug release study indicate that, all the prepared formulation release around 75-80% of drug within 6 to 7 hours, which may be considered satisfactory. However the in case of the formulation prepared with SCMC and guar gum an initial burst release was observed. The formulation prepared with carbopol P934 and 940 shows initial slow release.

On the basis of an *in-vitro* evaluation study of prepared gels, three polymer system namely HPMC, carbopol 934, carpool 940 were further selected to design final formulation batches. Unlike previous formulation design, different suitable ratios of these three polymers were prepared using an optimized combination and subjected to different essential *in-vitro* and *in-vivo* parameter to determine their performance.

Total 14 batches of formulations were prepared, among this, formulations (F16 to F22) were prepared by incorporating an optimized combination of itraconazole and curcumin and similarly formulations (F23 to F29) were used for the optimized combination of fluconazole and curcumin.

The prepared formulations were found slightly yellowish in color and translucent in nature. Formulations were found homogeneous, odorless and free from any gritty particle.

Formulations prepared to incorporate optimized combination of curcumin and fluconazole (F16, F17, F18, F19, F20, F21, F22) were evaluated and obtained results state that the viscosity of the prepared formulations was ranges within 1520-4225cps, mucoadhesive capacity was found in the range of 13.70 to 23.50 dyne/$mm^{2,}$ spreadability was found within the range of 11.33- 13.84 gm/$cm^2$and the drug content value was found within the range of (97-98.50 %). The pH of the prepared formulation was ranged between 4.2-4.8. Further, from the result of *in-vitro* release study, it may be concluded that around 75% of drug released from the all the prepared formulation within 4.5 to 5 hours.

Similarly, formulations prepared with optimized combination of curcumin and itraconazole (F23, F24, F25, F26, F27, F28, F29) were evaluated and obtained results state that the viscosity of the prepared formulations was ranges within 1480-4285cps, mucoadhesive strength was found in the range of 13.30 to 24.00 dyne/$mm^{2,}$ spreadability was found within the range of 10.70- 12.80 gm/cm^2 and the drug content value was found within the range of (95.75-98.70%). The pH of prepared formulations was ranged between 4.1- 4.6 Further, from the result of *in-vitro* release study, it may be concluded that around 70% of drug released from the all the prepared formulation within 4.5 to 5 hours.

The results of *in-vitro* vaginal retention study reflect that percentage drug loss during first 60 min for formulation batches F16-F22 was found within the range of (2.10% to 5.8%) for curcumin and (1.70% – 4.1%) for itraconazole. Whereas, for formulation batches F23-F29 the resultant data were found between the range of

(1.02%-2.52%) for curcumin and (1.66%-3.95%) for fluconazole. among all the preparations formulation F21 and F29 show comparably better performance.

On the basis of result analysis, it was found that mucoadhesive gels prepared using a combination of polymers show preferably better performance as compared to the gel prepared off the individual polymeric system. The gels prepared with a combination of polymer significantly improved the mucoadhesion strength of the prepared gel. Further, mark difference in the capacity of drug retention was observed for the formulations prepared with a combination of the polymer system. The drug release rate was also improved for the gels prepared with a combination of the polymer.

The result of the *in-vitro* drug release study indicates that formulation F21 and F29 show significantly superior performance as compared to other prepared formulation. A further evaluation of *ex-vivo* vaginal permeability study, short-term stability, and *in-vivo* animal activity were performed on the selected formulation only.

The *ex-vivo* vaginal permeability study was performed using Franz diffusion cell pig vaginal membrane and simulated vaginal fluid was used as a medium of the study. Result states that approximately 50% of drugs get permeated to the receiver compartment within 180 min. The results of the study indicate that the rate of drug permeation rate was found to be satisfactory.

Six-month stability study was performed on F21 and F29 as per ICH guideline of stability testing of drug Substances and drug Products for topical formulation. This study was performed to estimate the stability condition and shelf life of the prepared formulation. As per the guideline parameters like Appearance, color, odour, clarity, pH homogeneity, viscosity, weight loss, drug content and drug release with respect to time was determined after an interval of 1month, 2 months, 4 months and 6 months.

Formulations are stored in three different environments like 4°C, 25°C, and 40°C at 45% relative humidity. The result of the stability indicates no significant changes in the prescribed parameters. However, a minute change in pH and viscosity was observed after the 4-month interval. Hence on the basis of obtained data, it can be concluded that the prepared formulations were stable.

The experimental protocol of the *In-Vivo* animal study was first approved by the Institutional animal ethical committee (Regd. No. CIP / IAEC / 2015-16/071).The *in-vivo* study was performed to determine the therapeutic efficacy and performance of selected formulations (F21 & F29). At first rabbit, vaginal irritation test was performed. The result of the study shows that both the prepared formulation does not produce any kind significant sign of irritation which is confirmed by visual observation of targeted site. Further, vaginal tissue histopathological analysis was carried out and major haematological parameters like RBC, WBC, platelets count, haemoglobin, and lymphocyte count was also measure to determine any kind of major adverse changes. The results of the study indicates no sign of major abnormal changes in vaginal surface tissue and haematological parameters

- Group I : Normal control group
- Group II : Fungal infected group control group
- Group III : Animal treated with F21test formulation
- Group IV : Animals treated F29 test formulation
- Group V : Animals treated with Marketed standard formulation

Rabbit vaginal area was infected with *Candida albican* suspension and at an incubation period for 7 days. After the 7days incubation period test formulations (F21 & F29) as well as existing marketed formulation (Nizral 2% ketoconazole cream) was applied to the targeted area. The result reveals the fact that both the prepared

formulation having the significant antifungal capacity. It was also observed that formulation prepared with combination take less time to recover the clinical condition indicates better efficiency. The test formulations even show better therapeutic efficacy as compared to the existing marketed formulation.

On the basis of obtained results, it can be concluded that the optimized phyto combinations successfully improve the degree of therapeutic efficacy as compared single drug, even at comparatively low drug concentration.

Mucoadhesive gels prepared using combination of polymers were found suitable as vaginal drug delivery system. Among the different gel preparation F21 and F29 formulation was found to be most suitable, effective and safe as compared to others.

Further, the prepared mucoadhesive gels are found easy to apply and at the same time improved the retention time inside the vaginal cavity, which may improve bioavailability and patients compliance.

Based on the results of stability study, it can be concluded that the prepared formulations were found stable. *In-vivo* results reveal the fact that, optimized formulations F21 and F29 was found efficacious with no sign of allergic reaction and irritation, hence, can be considered safe.

Future scope:

This work creates a vast opportunity for future work. Future work can be carried out for commercial utilization of this concept for the management of infectious diseases.

The work may be extended to find out the effect of same combination against other pathogenic microbes.

Similar kind of research work may be carried out using different plant-derived bioactive to explore more alternative options for the effective management of the different type of clinical situations.

This work was mainly focused on antifungal infections related to the vaginal areas only, however, similar kind of other activities like anticancer, anti-inflammatory, anti-arthritis activities can be conducted exploring the concept to develop effective phyto-combinations.