Research Article

Role of Ketoconazole Vaginal Suppository in Management of Vulvovaginal candidiasis

Omar H El-Garhey*Ph.D, Ahmed K Abdelhakem MD and Marwa A. Fouad* B. Pharm.**
* Department of Pharmaceuticals, faculty of pharmacy, Minia University
** Department of Obestetric and gynecolog, Minia University Hospitals

Abstract
Ketoconazole is an imidazole that used as a broad spectrum antifungal, it treats and prevents the fungal infections like thrush, gastrointestinal infections and the skin, nails, and scalp infections.(١) The antimycotic effect of KTZ is to cause a direct damage in membrane of the fungal cell and to inhibit the ١٤-alpha-demethylase, occurring a membrane integrity and fluidity loss, which is an impact of ergosterol biosynthesis insufficiency.(٢) The solubility and dissolution rate of a drug with poor water solubility can be improved by several methods such as the particle size reduction, using the surfactant, complexion inclusion, and solid dispersion which is one of the most efficient methods.(٣&٤) Vaginal drug delivery systems are traditionally used to deliver contraceptive and drugs to treat the vaginal infections. However, vaginal drug delivery is not limited to these drugs as a the vagina has promise as a site to topically deliver drugs which will be absorbed systemically because of the dense network of blood vessels in the vaginal wall(٥). The vaginal route is used for the achievement of local effects and/or for systemic absorption(٦). The vaginal wall is very well suited for the absorption of drugs for systemic use, as it contains a vast network of blood vessels. Antifungals have been important drug candidates for the treatment of gynecological conditions. The vaginal delivery of azole antifungals.(٧)
Keywords: antifungal, Ketoconazole, vulvovaginal candidiasis, vaginal suppositories and drugs

Introduction
The vagina is a fibromuscular sheath-like structure connecting the external genitals with the uterus. It is lined with non-keratinizing squamous epithelium and is ٨–١٢ cm long. The vagina protects the internal genital organs against ascending infections, forms part of the birth canal, and receives the penis in copulation.(٨)

The surface of the vagina is composed of numerous folds, which are often called rugae(٩). The rugae provide dispensability, support and an increased surface area of the vaginal wall.(١٠)

The vagina has an excellent elasticity because of the presence of smooth elastic fibers in the muscular coat.(١١) Vaginal discharge is the second most common gynaecological problem after menstrual disorders. Some women regard almost any secretion from the vagina as abnormal discharge, and the first task for a primary care physician is to ascertain whether it is physiological or pathological (١٢).

Cyclic changes in thickness of the vaginal epithelium, fluid volume and composition, pH and sexual arousal could potentially affect drug release from intravaginal delivery systems(١٣).
Physicochemical properties such as molecular weight, lipophilicity, ionization, surface charge, chemical nature can influence vaginal drug absorption(١٤).

Since vaginal fluid contains a large amount of water, any drug intended for vaginal delivery require a certain degree of solubility in water. In fact, data on the human vaginal permeability of drugs with different physicochemical properties is very limited; much work needs to be done on the effects of physicochemical parameters of drug on vaginal absorption(١٢,١٥).
The vaginal route is used for the achievement of local effects and/or for systemic absorption (١٦). The vaginal wall is

very well suited for the absorption of drugs for systemic use, as it contains a vast network of blood vessels. Vaginal absorption of drugs is dependent upon physicochemical properties of the drug as lipophilicity, ionization, molecular weight, chemical structure and interaction with vaginal secretions and tissues[١٧]. Drugs are transported across the vaginal membrane by the transcellular route, intracellular route or vesicular and receptor mediated transport mechanisms. The physiological factors (e.g. cyclic changes in the thickness and porosity of the epithelium, volume, viscosity and pH of the vaginal fluid) play a role in absorption[١٨].
Absorption is also affected by the thickness of the vaginal wall, the ovarian cycle or by pregnancy[١٩].

What are fungi?
About ٨٠ ٠٠٠ to ١٢٠ ٠٠٠ species of fungi have been described to date, although the total number of species is estimated at around ١.٥ million[٢٠]. This would render fungi one of the least-explored biodiversity resources of our planet. It is notoriously difficult to delimit fungi as a group against other eukaryotes, and debates over the inclusion or exclusion of certain groups have been going on for well over a century[٢١].

Fungal cells are complex organisms that share many biochemical targets with other eukaryotic cells[٢٢]. Therefore, agents that interact with fungal targets not found in eukaryotic cells are needed. The fungal cell wall is a unique organelle that fulfills the criteria for selective toxicity. The fungal cell wall differs greatly from the bacterial cell wall and is not affected by antibacterial cell wall inhibitors such as the β-lactams or vancomycin[٢٣].

<u>Fungal Infections</u>
Fungal infections mean any inflammatory condition caused by a fungus. Most fungal infections are superficial and mild, though persistent and difficult to eradicate. Some, particularly in older, debilitated, or immunosuppressed or immunodeficient people, may become systemic and life threatening[٢٤].

Vulvovaginal Candidiasis:
Candidiasis is mostly due to candida albicans and may be associated with diabetes, pregnancy and prolong use of antibiotics[٢٥]. Patient presents with vaginal discharge and pruritis, discharge appears to be like curdled milk and deep erythema of vulva and vagina is often seen[١٨,٢٠].

Because of lack of specificity of clinical signs and symptoms as many as half of women given this diagnosis may have other conditions[٢٦]. On the other hand, a positive vaginal culture for Candida may reflect colonization in as many as ٥٠٪ of healthy asymptomatic women[٢٧]. So, all the culture positive cases should be correlated clinically and other causes of vaginal discharge should be ruled out if culture reveals mixed or scanty growth of Candida spp[١٨].

Vulvovaginal candidiasis (VVC) risk factors have been identified, including genetic, intermediate age,pregnancy, uses of contraceptive pills, frequent sexual intercourse, uncontroled diabetes mellitus, contraceptive devices, and antibiotics[٢٨].
During pregnancy, the vagina is more susceptible to infections, resulting in a higher incidence of colonization and symptomatic vaginitis[٢٩].

Pregnant women with depressed immune system are also more likely to a higher prevalence of VVC. It occurs in the last trimester of pregnancy, due to the increased amount of glycogen in the vagina and high levels of estrogen hormones[٣٠]. Approximately ٧٥٪ of all pregnant women experience at least one episode of VVC during their lifetime and ٥٠٪ of them suffer recurrent events[٣١]. The mechanisms by which pregnancy encourages Candida colonization are complex[٣٢].

<u>Azole Antimycotics</u>
Azole antimycotics include broad classes imidazoles and triazoles. Both classes share the same antifungal spectrum and mechanism of action. Triazoles are more slowly metabolized and have less effect on human sterol synthesis than do the imidazoles. The currently available imidazoles include clotrimazole,

miconazole, Ketoconazole, econazole, butoconazole and fluconazole[٣٣]

Ketoconazole

Ketoconazole is (±)-cis-١-Accetyl-٤-[p-[[٢-(٢,٤-dichlorophenyl)-٢-(imidazole-١-methyl)-١,٣-dioxolan-٤-y١]methoxy]phenyl]piperazine(١). It has the molecular formula of $C_{٢٦}H_{٢٨}C_{l٢}N_٤O_٤$ and a molecular weight of ٥٣١.٤٤ [٣٤].
Ketoconazole, a synthetic imidazole, is effective in both superficial and deep fungal infections[٣٤, ٣٥].

Although its spectrum of activity is similar to those of other imidazole derivatives, e.g., miconazole, econazole, and clotrimazole, ketoconazole has the advantage of being effective when administered orally[٣٦]. Its therapeutic efficacy was reviewed recently.

Physical properties:

A white or almost white powder with a melting point of ١٤٨-١٥٢ºC, practically insoluble in water, freely soluble in methylene chloride, soluble in methanol and sparingly soluble in alcohol[٣٧].
It displays strongly Ph dependent solubility; it is readily soluble in acidic water (Ph<٣) and has a very low solubility(less than ٤-٦.٧ Mg/Ml) in water at or beyond neutral PH[٣٦].

Pharmacokinetics:

The absorption of ketoconazole from the gastro-intestinal tract is variable and increases with decreasing stomach PH.
The concentration of Ketoconazole in the cerebrospinal fluid (CSF) of patients with fungal meningitis is less than ١٪ of the total drug concentrations of Ketoconazole in urine, saliva and sebum after a single dose of ٢٠٠ mg given orally[٣٨].
Ketoconazole is **metabolized** in liver to be biphasic, with an initial half-life of ٢ hours and a terminal half-life of about ٨ hours. The major **excretory** route is interohepatic. From ٨٥٪ to ٩٠٪ is excreted in bile and faces, ١٠٪ to ١٥٪ in urine and ٢٪ to ٤٪ unchanged[٣٧,٣٨].

Drug interactions:

Administration of drugs that reduce stomach acidity, such as cimetidine, ranitidine or famotidine, may reduce the absorption of Ketoconazole[٣٩].
Absorption of Ketoconazole may also be reduced by sucralfate, however, Concentrations of isoniazid and rifampicin may also be reduced by Ketoconazole[٤٠].

Mechanism of Action:

Ketoconazole interacts with ١٤-α-demethylase, a microsomal cytochrome P٤٥٠ dependant enzyme system, thus impairing biosynthesis of ergosterol for the cytoplasmic membrane leading to accumulation of ١٤-α-methyl sterols[٣٨].

Methodology

Preparation of Ketoconazole Vaginal Pessaries:
Both fatty and water – soluble bases utilized to formulate KTZ vaginalpessaries. Each vaginal pessary was formulate KTZ to contain ٥٠ mg of KTZ. The fatty bases employed were Suppocire NA and Cocoa butter. The investigated water – soluble suppository bases were PEGs alone or mixture combinations; PEG ١٥٠٠ alone, mixture of PEG ١٥٠٠ and PEG ٤٠٠ in weight ratio ٩٠ : ١٠ respectively and a mixture of PEG ١٠٠٠: PEG ٤٠٠٠ in weight ratio ٧٥ : ٢٥ respectively. Equivalent amount to ٥٠ mg of the drug from the prepared KTZ- PVP k٣٠ (١:٤ M) binary system was formulated into vaginal pessaries with water – soluble and fatty bases. Vaginal suppositories of KTZ were prepared by the fusion method (reference) .The suppository bases were melted first in a porcelain dish on water bath at the least suitable temperature and then KTZ powder was added to the melted base but in case of cocoa butter, we were added ١٠٪ bees wax. Gentle stirring of the dish contents was continued after removal from the water bath to ensure complete and uniform distribution of the drug with the base. Just before congealing, the mass was poured into the clean, lubricated mould and allowed to solidify at room temperature.
The prepared KTZ Vaginal pessaries were stored in tightly closed containers and placed in the refrigerator. Before carrying out evaluation tests, the batch was removed from the refrigerator and left for ٢ hours at room temperature.

Evaluation of the prepared Ketoconazole vaginal pessaries:

The prepared Ketoconazole pessaries are subjected to the following evaluation tests:

- Weight variation:

The weight variation of Ketoconazole vaginal pessaries was determined according to the British Pharmacopoeia ١٩٩٨. Twenty pessaries from each formula were weighed individually. The average weight was determined and the percentage deviation of each pessary from the average weight was calculated.

- Uniformity of drug content:

According to the British Pharmacopoeia (١٩٩٨), ten pessaries were taken randomly from each formula and assayed individually. A pre-weighed pessary was melted and dispersed in ٥٠ml methanol, then the obtained suspension is filtered. Samples were taken from the filterate and diluted with phosphate buffer of PH ٥ and assayed spectrophotometrically at λ_{max}٢٣٢nm. The concentration was determined with reference to the previously constructed was calibration curve. A simultaneous blank experiment using plain suppository bases was carried out.

Disintegration of Ketoconazole vaginal pessaries:

The test was performed in distilled water at ٣٧^{o}C using the USP tablet disintegration apparatus. The disintegration times were recorded when pessaries placed in the tubes of the basket either completely melted or dissolved.

Determination of the hardness of Ketoconazole vaginal pessaries:

The hardness of the prepared formulations was evaluated using the Erweka hardness tester at the room temperature. Starting with ٦٠٠mg weight and then ٢٠٠mg weights were added every minute until the pessary was broken or deformed. The weight under which the pessary collapsed was taken as the hardness of the suppository. The hardness apparatus used permitted measurements up to a load of ٥kg.

Melting range determination:

The melting range of Ketoconazole vaginal pessaries was determined using the melting point SMP١ apparatus. Samples of the molten pessaries were taken before congealing in one-end closed capillary tube.

The samples were left to solidify at room temperature and inserted in the apparatus. The melting range was determined from the point of the beginning of the melting until complete liquefaction of the pessary.

In-vitro release of Ketoconazole from the prepared pessaries: The drug release from the prepared Ketoconazole vaginal pessaries was performed using a six –vessel apparatus II of USP XXXV (paddle method). The dissolution medium was ٣٠٠ ml of phosphate buffer of PH ٥. The dissolution medium was stirred at ٥٠ rpm and maintained at a temperature of ٣٧ ± ٠.٥^{o}C. At specific time intervals ٥ml of the dissolution medium was withdrawn and replaced by an equal volume of the same dissolution medium kept at ٣٧ ± ٠.٥^{o}C. The released amount of Ketoconazole was determined spectrophotometrically at λ_{max} ٢٣٢nm after appropriate dilution of the withdrawn samples with the same buffer. The experiments were repeated three times for each sample and the average was considered. The cumulative released percent of Ketoconazole was calculated and plotted versus the time.

Shelf-storage stability testing of Ketoconazole Vaginal pessaries:

Formulation of the prepared vaginal pessaries that gave the highest in-vitro drug release was chosen for this study. The selected formulae were stored in amber colored glass containers, which are tightly closed. The containers were stored for ٦ months on the shelf of refrigerator (٤^{o}C). Other samples of the selected formula were kept at room temperature (٢٥^{o}C) for ٦ months. Samples were subjected to the re-evaluation study to test the drug content remained, physical characteristics as well

Clinical Studies:

Settings: This study was conducted in out patient gynecological clinic of Minya University Gynecological Hospital, in the period of October ٢٠١٤ to December ٢٠١٤.
Patient selection: Women are initially identified by the history of vaginal discharge, acute pruritus (itching) with

presence of typically cottage cheese-like discharge on clinical vaginal examination.

Exclusion criteria included:

١. Women who had any evidence of other types of vulvovaginitis or cervicitis or any suspected mixed infection on examination or after microscopic examination.

٢. Women who were taking any type of antimicrobial treatment (topical or systemic drugs) within one month prior to the first clinical examination.

٣. Pregnant women.

٢-٣) Enrollment and Randomization:

The selected women who met the inclusion criteria were counseled to participate in the study and eligible women who agreed to participate were enrolled after providing informed consent. In a single blinded study, women participate the study were randomly assigned to one of the three studied groups. Group (١), whose patients received ketoconazole vaginal suppo-sitories containing ٢٠٠ mg of the drug alone. Group (٢), whose patients received ketoconazole vaginal suppositories containning ketoconazole with PVP K ٣٠ kneading mixture equivalent to ٢٠٠mg of the drug. The last group was the control group whose patients received miconazole vaginal suppositories contain-ning ٢٠٠mg miconazole (Gynozole™).

Randomization was done by computer generated random numbers, each group contained ٥ patients from an urban residence and another ٥ patients from a rural residence.

Interventions:

Women enrolled after diagnosis of Vulvovaginal Candidiasis (VVC) and the following measures were done for each participating woman:

١. After enrollment of the participating women a vaginal swab was taken from each patient. Aliquots from the swabs were inoculated into Sabouraud Agar plates, which were incubated at ٣٢° C for ٢٤ hours then the colonies of C. albicans were counted.

٢. Each patient assigned to particular group was given the complete dosage of the course of the antifungal treatment (٦ vaginal suppositories, once daily) according to each group.

٣. The patients were instructed to return to the clinic after three days and after seven days. Other swabs were taken from each patient in the two following up visits and similarly treated as the first swab.

The results of clinical examination, microbiological evaluation and the patient's complaints were recorded in an evaluation sheet for each patient.

Results:

Indicated by the number of colonies at different times were observed a significant difference in the response to treatment in each group, although there was no significant difference in the count before the management (Table ١).

Regards the number of colonies after both the ٣rd and ٧th day of treatment in each group, there was a significant difference in group C (mean count was ١٥٦ and ١٤ respectively) and in group B (Mean count was ٤٠ and ٠.٥ respectively), this result compared with the result obtained by Strandberg et al on ٢٠١٠.

The percentage of colonies reduction after ٣ and ٧ days of treatment (Table ٢) shows ٨٦٪ and ٩٩.٨٢٪ of colonies was cleared in Group B, while the Group C has the least clearance percentage (٤٨.٨٢٪ and ٩٥.٥٪ after ٣ and ٧ days of treatment respectively).

Similar results were reported; Antonio Martiniz et al., ٢٠٠١, a significant difference in the percentage of reduction on rats with VVC treated with local topical azasordins for ten days, percent in reduction reached ١٠٠٪ with dose of ١٠ mg/kg[٤١].

After four weeks of the management, the recurrence rate was (٢٠٪, ٣٠٪ and ٤٠٪) in Groups B, Group C and Group A respectively (Table ٣).

As regards to Ventolini et al, ٢٠٠٦ who reported a ٤٠.٢ % of cases during the ١st month after treatment[٤٢].

According to a study conducted by Coric´ et al. (٢٠٠٦), ١٤ days after the treatment of patients with VVC using either a single dose of ١٥٠ mg fluconazole or ٢٠٠ mg clotrimazole as a ٣-day intravaginal regimen, Candida micro-organisms were found in ٢٧٪ and ٣١٪ of the patients respectively[٤٣].

While, only١٠.٣٪ of the patients concomitantly treated with fluconazole and probiotics showed the presence of Candida spp this, in addition to the remission of symptoms and signs of infection, further indicates a successful cure of the infection by probiotic supplementation in most individuals, this study was presented by Martiniz et al on ٢٠٠٩[٤٤].

Once completed the fluconazole + probiotic therapy, during the follow-up period only five women (٩,٨٪) experienced symptom-matologies that could be related to RVC, in particular two after ٢ months and three after ٦ months.[٤٥]

Table (١): Numbers of colonies at different times in the three Groups.

		Group A (n=٢٠)	Group B (n=٢٠)	Group C (n=٢٠)	P value		
					A vs B	A vs c	B vs C
(١)Before Mean±SD		٢٧٢.٤±٣٧.٥٣	٢٨٣.٢±٥٥.٦٥	٢٩٩.٨±٦٠.٦٦	٠.٧٩١	٠.٢٣٠	٠.٥٧٧
(١)After ٣ days Mean±SD		٧٦.٢٥±٢٢.٩١	٤٠.٣٥±٩.٢٣	١٥٥.٩±٧٢.٤٧	٠.٠٣٤*	<٠.٠٠١*	<٠.٠٠١*
(٢)After ٧ days Mean±SD		١٢.٨±١٦.٥١	٠.٥±١.٥٣	١٤±١٤.٨٣	٠.٠٠٣*	٠.٨٦٢	٠.٠٠٣*
P value	(٣)Before vs ٣ days	<٠.٠٠١*	<٠.٠٠١*	<٠.٠٠١*			
	(٣)Before vs ٧ days	<٠.٠٠١*	<٠.٠٠١*	<٠.٠٠١*			

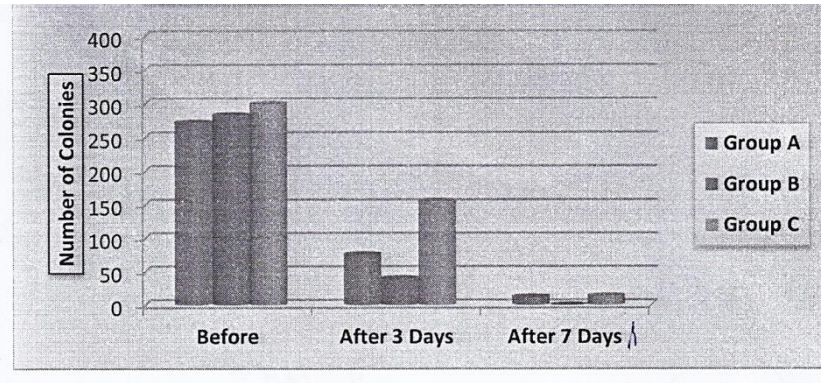


Figure (١): Numbers of colonies before and after treatment in each group

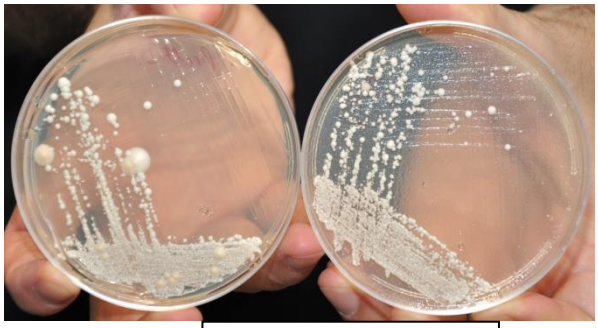
Table (٢): Rate of reduction in numbers of colonies at different times in the three Groups.

	Group A (%) (n=٢٠)	Group B (%) (n=٢٠)	Group C (%) (n=٢٠)	P value		
				A vs B	A vs c	B vs C
(١) Reduction after ٣ days Mean±SD	٧١.٧١±٩.٢٩	٨٥.٦١±٣.٦٤	٤٨.٨٢±١٧.٨٦	٠.٠٠١*	<٠.٠٠١*	<٠.٠٠١*
(١)Reduction after ٧ days Mean±SD	٩٥.٤٩±٦.٠٢	٩٩.٨٢±٠.٥٢	٩٥.٥±٤.٦٩	٠.٠٠٨*	١	٠.٠٠٨*
(٢)P value	<٠.٠٠١*	<٠.٠٠١*	<٠.٠٠١*			

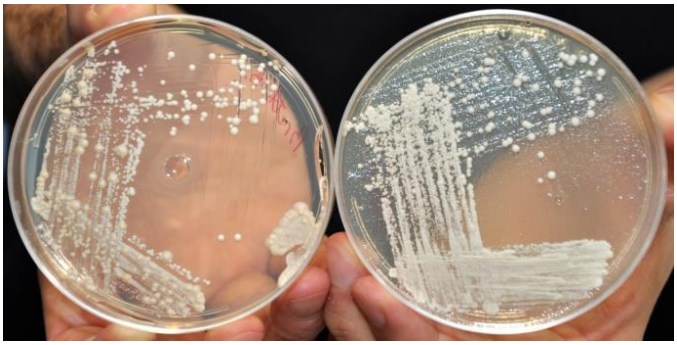
Table (٣): Difference in rate of recurrence after ٢٨ days in the three Groups.

	Group A (n=٢٠)	Group B (n=٢٠)	Group C (n=٢٠)	P value		
				A vs B	A vs c	B vs C
After ٢٨ days No Yes	١٤ (٧٠٪) ٦ (٣٠٪)	١٦ (٨٠٪) ٤ (٢٠٪)	١٢ (٦٠٪) ٨ (٤٠٪)	٠.٤٥٦	٠.٥٠٧	٠.١٦٨

GROUP ١



GROUP ٢



GROUP ٣

Conclusion

Given the therapeutic efficacy and equivalence of the individual antifungal agents as well as route of administration, treatment selection should be driven by the patient's personal preference. Ketoconazole vaginal suppository is a good antifungal drug in management of vulvovaginal candidiasis and specially in immune-compromised women.

References

١. Kumar, Pankaj et al. "Physiochemical Characterization and Release Rate Studies of SolidDispersions of Ketoconazole with Pluronic F١٢٧ and PVP K-٣٠." Iranian Journal of Pharmaceutical Research : IJPR ١٠.٤ (٢٠١١): ٦٨٥–٦٩٤. Print.

٢. Karolewicz, B., Górniak, A., Owczarek, A., Żurawska-Płaksej, E., Piwowar, A. and Pluta, J., ٢٠١٤. Thermal, spectroscopic, and dissolution studies of ketoconazole–Pluronic F١٢٧ system. Journal of Thermal Analysis and Calorimetry, ١١٥(٣), pp.٢٤٨٧-٢٤٩٣.

٣. Alam, M.A., Ali, R., Al-Jenoobi, F.I. and Al-Mohizea, A.M., ٢٠١٢. Solid dispersions: a strategy for poorly

aqueous soluble drugs and technology updates. Expert opinion on drug delivery, ٩(١١), pp.١٤١٩-١٤٤٠.

٤. Górniak, A., Karolewicz, B., Żurawska-Płaksej, E. and Pluta, J., ٢٠١٣. Thermal, spectroscopic, and dissolution studies of the simvastatin–acetylsalicylic acid mixtures. Journal of Thermal Analysis and Calorimetry, ١١١(٣), pp.٢١٢٥-٢١٣٢.

٥. Agata Górniak , Bożena Karolewicz, Ewa Żurawska-Płaksej , Janusz Pluta ١٠.١٠٠٧/s١٠٩٧٣-٠١٢-٢٦٤١-٧.

٦. Kumar, L. and Verma, R., ٢٠١٠. Advantages of intravaginal drug delivery system: an over-view. International Journal of Pharmaceutical Research and Development, ٢(٦), pp.١٥-٢٣.

٧. Constantine, G.D., Bouchard, C., Pickar, J.H., Archer, D.F., Graham, S., Bernick, B. and Mirkin, S., ٢٠١٧. Consistency of Effect with a Low-Dose, Estradiol Vaginal Capsule (TX-٠٠٤HR): Evaluating Improvement in Vaginal Physiology and Moderate-to-Severe Dyspareunia in Subgroups of Postmenopausal Women. Journal of Women's Health.

٨. Johal, H.S., Garg, T., Rath, G. and Goyal, A.K., ٢٠١٦. Advanced topical drug delivery system for the management of vaginal candidiasis. Drug delivery, ٢٣(٢), pp.٥٥٠-٥٦٣.

٩. Lopes, G.P., Brito, A.B., Paim, F.P., Santos, R.R., Queiroz, H.L. and Domingues, S.F.S., ٢٠١٧. Comparative characterization of the external genitalia and reproductive tubular organs of three species of the genus Saimiri Voigt, ١٨٣١ (Primates: Cebidae). Anatomia, histologia, embryologia, ٤٦(٢), pp.١٤٣-١٦١.

١٠. Haylen, B.T., Maher, C.F., Barber, M.D., Camargo, S., Dandolu, V., Digesu, A., Goldman, H.B., Huser, M., Milani, A.L., Moran, P.A. and Schaer, G.N., ٢٠١٦. An International Urogynecological Association (IUGA)/ International Continence Society (ICS) joint report on the terminology for female pelvic organ prolapse (POP). Neurourology and urody-namics, ٣٥(٢), pp.١٣٧-١٦٨.

١١. Koganemaru, M., Nonoshita, M., Iwamoto, R., Kuhara, A., Nabeta, M., Kusumoto, M., Kugiyama, T., Kozuma, Y., Nagata, S. and Abe, T., ٢٠١٦. Endovascular Management of Intractable Postpartum Hemorrhage Caused by Vaginal Laceration. Cardiovascular and interventional radiology, ٣٩(٨), pp.١١٥٩-١١٦٤.

١٢. Kamada, Y., Sakamoto, A., Tabuchi, K., Matsuda, M., Shimizu, K., Nakatsuka, M. and Hiramatsu, Y., ٢٠١٥. Opening the Anterior Vaginal Vault: A Novel Approach to Vaginoplasty with a Modified McIndoe Procedure Using an Artificial Dermis. Journal of pediatric and adolescent gynecology, ٢٨(٥), pp.e١١٩-e١٢٢.

١٣. Tarakaramarao, C., Vijayalekshmi, N.G. and Akhila, S., ٢٠١٣. Applications of Vaginal Drug Delivary: A review. Pharmacie Globale, ٤(١).

١٤. Hussain, A. and Ahsan, F., ٢٠٠٥. The vagina as a route for systemic drug delivery. Journal of controlled release, ١٠٣(٢), pp.٣٠١-٣١٣.

١٥. George, M. and Abraham, T.E., ٢٠٠٦. Polyionic hydrocolloids for the intestinal delivery of protein drugs: alginate and chitosan—a review. Journal of controlled release, ١١٤(١), pp.١-١٤.

١٦. Dutton, P.J. and Rymer, J.M., ٢٠١٥. Physiology of the menstrual cycle and changes in the perimenopause. Managing the Menopause: ٢١st Century Solutions.

١٧. Constantine, G.D., Bouchard, C., Pickar, J.H., Archer, D.F., Graham, S., Bernick, B. and Mirkin, S., ٢٠١٧. Consistency of Effect with a Low-Dose, Estradiol Vaginal Capsule (TX-٠٠٤HR): Evaluating Improvement in Vaginal Physiology and Moderate-to-Severe Dyspareunia in Subgroups of Postmenopausal Women. Journal of Women's Health.

١٨. Kaur, J., Kaur, J., Jaiswal, S. and Gupta, G.D., ٢٠١٦. A Review on Novel Approach of Antifungal Emulgel For Topical Delivery In Fungal Infections. Indo American Journal of Pharmaceutical Research, ٦(٧), pp.٦٣١٢-٦٣٢٤.

١٩. Hussain, A. and Ahsan, F., ٢٠٠٥. The vagina as a route for systemic drug

delivery. Journal of controlled release, ١٠٣(٢), pp.٣٠١-٣١٣.
٢٠. Kale, V.V. and Ubgade, A., ٢٠١٣. Vaginal mucosa-a promising site for drug therapy. British Journal of Pharmaceutical Research, ٣(٤), p.٩٨٣.
٢١. Zaspel, U. and Hamm, B., ٢٠٠٧. Vagina ١٢. MRI and CT of the Female Pelvis, p.٢٧٥.
٢٢. Hawksworth, D.L., ٢٠٠٤. Fungal diversity and its implications for genetic resource collections. Stud Mycol, ٥٠, pp.٩-١٨.
٢٣. Sharma, P., Kumar, D., Mahajan, G. and Mintoo, M.J., ٢٠١٦. COMPLETE UPDATE ON ANTIFUNGAL THERAPY. International Journal of Pharmacology, ٣(٣), pp.١-٩.
٢٤. Sierra, J.M., Fusté, E., Rabanal, F., Vinuesa, T. and Viñas, M., ٢٠١٧. An overview of antimicrobial peptides and the latest advances in their development. Expert Opinion on Biological Therapy, (just-accepted).
٢٥. Pappas, P.G., Kauffman, C.A., Andes, D., Benjamin, D.K., Calandra, T.F., Edwards, J.E., Filler, S.G., Fisher, J.F., Kullberg, B.J., Zeichner, L.O. and Reboli, A.C., ٢٠٠٩. Clinical practice guidelines for the management candidiasis: ٢٠٠٩ update by the Infectious Diseases Society of America. Clinical infectious diseases, ٤٨(٥), pp.٥٠٣-٥٣٥
٢٦. Kent HL. Epidemiology of vaginitis. Am J Obstet Gynecol١٩٩١;١٦٥:١١٦٨–٧٦.
٢٧. Alli, J.A.O., Okonko, I.O., Odu, N.N., Kolade, A.F. and Nwanze, J.C., ٢٠١٧. Detection and prevalence of Candida isolates among patients in Ibadan, Southwestern Nigeria. Journal of Microbiology and Biotechnology Research, ١(٣), pp.١٧٦-١٨٤.
٢٨. Al-Ahmadey, Z.Z. and Mohamed, S.A., ٢٠١٤. Vulvovaginal candidiasis: Agents and its virulence factors. Microbiol. Res. Int, ٢(٣), pp.٢٨-٣٧.
٢٩. Elisabeth, Z.O.M., Lopez, V.C., Soto, S.M. and Fabrice, F.B., ٢٠١٦. Anti-candida biofilm properties of Cameroonian plant extracts. Journal of Medicinal Plants Research, ١٠(٣٥), pp.٦٠٣-٦١١.
٣٠. Sobel, J.D., ١٩٩٩. Vulvovaginitis in healthy women. Comprehensiv therapy, ٢٥(٦), pp.٣٣٥-٣٤٦.
٣١. Ferrer, J., ٢٠٠٠. Vaginal candidosis: epidemiological and etiological factors. International Journal of Gynecology & Obstetrics,٧١,pp.٢١-٢٧.
٣٢. Aslam, M.A.L.E.E.H.A., Hafeez, R.U.B.E.E.N.A., Ijaz, S.A.D.I.A. and Tahir, M., ٢٠٠٨. Vulvovaginal Candidiasis in pregnancy. Biomedica, ٢٤, pp.٥٤-٥٦.
٣٣. Sharma, M. and Solanki, A., ٢٠١٤. Prevalence of Candida infection in pregnant women with and without diabetes. Int. J. Curr. Microbiol. App. Sci, ٣(٤), pp.٦٠٥-٦١٠.
٣٤. Kuriyama, T., Karasawa, T. and Williams, D.W., ٢٠١٤. Antimicrobial Chemotherapy. Biofilms in Infection Prevention and Control: A Healthcare Handbook, p.٢٠٩.
٣٥. Stockley, R.J., Daneshmend, T.K., Bredow, M.T., Warnock, D.W., Richardson, M.D. and Slade, R.R., ١٩٨٦. Ketoconazole pharmacokinetics during chronic dosing in adults with haematological malignancy. European Journal of Clinical Microbiology & Infectious Diseases, ٥(٥), pp.٥١٣-٥١٧.
٣٦. Daneshmend, T.K. and Warnock, D.W., ١٩٨٨. Clinical pharmacokinetics of ketoconazole. Clinical pharmacokinetics, ١٤(١), pp.١٣-٣٤.
٣٧. Hann, I.M., Corringham, R., Keaney, M., Noone, P., Fox, J., Szawatkowski, M., Prentice, H.G., Blacklock, H.A., Shannon, M., Gascoigne, E. and Boesen, E., ١٩٨٢. Ketoconazole versus nystatin plus amphotericin B for fungal prophylaxis in severely immunocompromised patients. The Lancet, ٣١٩(٨٢٧٦), pp.٨٢٦-٨٢٩.
٣٨. Shah, D.K. and Agrawal, A., ٢٠١٦. Formulation and Evaluation of Polymeric Microsponges of Ketoconazole for Topical Delivery.
٣٩. Mutasim, A., ٢٠١٥. Ketoconazole Pharmacokinetics In Sudanese Patients With Mycetoma (Doctoral dissertation, UOFK).
٤٠. Adachi, M., Hinatsu, Y., Kusamori, K., Katsumi, H., Sakane, T., Nakatani,

M., Wada, K. and Yamamoto, A., ٢٠١٥. Improved dissolution and absorption of ketoconazole in the presence of organic acids as pH-modifiers. European Journal of Pharmaceutical Sciences, ٧٦, pp.٢٢٥-٢٣٠.

٤١. Baciewicz, A.M. and Baciewicz, F.A., ١٩٩٣. Ketoconazole and fluconazole drug interactions. Archives of internal medicine, ١٥٣(١٧), pp.١٩٧٠-١٩٧٦.

٤٢. Martinez, A., Ferrer, S., Santos, I., Jimenez, E., Sparrowe, J., Regadera, J., De Las Heras, F.G. and Gargallo-Viola, D., ٢٠٠١. Antifungal activities of two new azasordarins, GW٤٧١٥٥٢ and GW٤٧١٥٥٨, in experimental models of oral and vulvovaginal candidiasis in immunosuppressed rats. Antimicrobial agents and chemotherapy, ٤٥(١٢), pp.٣٣٠٤-٣٣٠٩.

٤٣. Ventolini, G., Baggish, M.S. and Walsh, P.M., ٢٠٠٦. Vulvovaginal candidiasis from non-albicans species: retrospective study of recurrence rate after fluconazole therapy. The Journal of reproductive medicine, ٥١(٦), pp.٤٧٥-٤٧٨.

٤٤. Ćorić, M., Barišić, D. and Lovrić, H., ٢٠٠٦. Fluconazole versus ٣-day clotrimazole in the treatment of sporadic and recurrent vulvovaginal candidiasis. International Journal of Gynecology & Obstetrics, ٩٥(٢), pp.١٧١-١٧٢.

٤٥. Martinez, R.C.R., Franceschini, S.A., Patta, M.C., Quintana, S.M., Candido, R.C., Ferreira, J.C., De Martinis, E.C.P. and Reid, G., ٢٠٠٩. Improved treatment of vulvovaginal candidiasis with fluconazole plus probiotic Lactobacillus rhamnosus GR-١ and Lactobacillus reuteri RC-١٤. Letters in applied microbiology, ٤٨(٣), pp.٢٦٩-٢٧٤.

٤٦. Murina, F., Graziottin, A., Felice, R., Radici, G.L. and Di Francesco, S., ٢٠١١. The recurrent vulvovaginal candidiasis: proposal of a personalized therapeutic protocol. ISRN obstetrics and gynecology, ٢٠١١.