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FORMULATION AND EVALUATION OF LIPOSOMAL FLUCONAZOLE AND PROPOLIS AS TOPICAL ANTIFUNGAL AGENTS IN *CANDIDA KERATITIS*

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ABSTRACT

The aim of this study was to formulate each of fluconazole and propolis in liposomal system and to evaluate their antifungal activities in solution or liposomal formulation, in both *in vitro* and *in vivo* (rabbits cornea) against *Candida albicans*. Each of fluconazole (2 mg/ml) and propolis (5%) were prepared under sterile condition, in solution form, as well as were entrapped in multilamellar liposomes, composed of egg yolk phosphatidylcholine and cholesterol (up to 50 mole% of cholesterol). The effect of cholesterol incorporation on the encapsulation efficiency of drug was most pronounced with liposomes prepared from 33% of cholesterol .In vitro, the diameters of the growth inhibition zones, which indicate the antifungal activity of fluconazole and propolis in solution and liposomal formulation, were measured using cup diffusion method. They were compared with that of polyvinyl alcohol (solution) and liposomes alone as control groups.In vivo, the study was carried out in sixteen Newzland rabbits. *Candida keratitis* was induced by inoculating *Candida albicans* 15 µl into the central corneal stroma. One drop of the previous preparations were applied into the conjunctival sac every hour for 14 hours daily until complete healing of the *Candida keratitis* or appearance of sever complications (Resistant corneal ulcer or atrophia bulbi). The antifungal activity of all preparations were evaluated by observation of the rate of corneal healing using sterile fluorescein solution daily. The results revealed that, all control groups exhibited no antifungal activity either *in vitro* or *in vivo*.In vitro, fluconazole and propolis in solution or liposomal formulation exhibited antifungal activity which could be arranged in the following descending rank order : liposomal fluconazole > liposomal propolis > fluconazole in solution > propolis in solution.In vivo, there were a highly significant differences between each of fluconazole and propolis in liposomal formulation, in comparison with both drugs in solution form ($P = 0.001$ and 0.002 respectively) . There were no significant differences between liposomal fluconazole and liposomal propolis ($P = 0.45$). There were no significant differences between fluconazole and propolis in solution ($P = 0.46$) .Accordingly, it could be concluded that liposomal encapsulation seems to offer a simple and potent means of enhancing and improving the antifungal activity of the drugs.

INTRODUCTION

Liposomes (microscopic membrane-like structures) have been widely used in the recent years as a drug delivery system for many drugs including antimicrobial agents[1,2]. In this regard, liposomes have been used as carriers and as a potentially valuable system for the controlled delivery of therapeutic agents. Encapsulation of pharmaceutically active drugs within liposomes seems to offer a simple and potent means of modifying and controlling the pharmacology of a variety of drugs[3].

Liposomal structures can encapsulate lipophilic and hydrophilic drugs, so they have the ability to control the rate of release of the encapsulated drugs, to protect the drugs from metabolic enzymes present at tear/corneal epithelium interface. Moreover they prolong contact with corneal surface. Thereby improving the bioavailability and therapeutic efficacy of the drug through the cornea[2,4].

Fungal infections of the cornea especially *Candida* has considered a major problem in ophthalmology because of diagnostic and therapeutic difficulties[5,6]. Fungal *keratitis* can impair vision or lead to total blindness. So various specific antifungal agents have been used[7].

Fluconazole is a synthetic, antifungal agent belonging to the group of triazoles. It is effective against *Blastmyces dermatitidis*, *Coccidiodes immitis*, *Cryptococcus neoformans*, *histoplasma capsulatum*, *Microsporum* spp, *Trichophyton* spp, and *Candida* spp[8]. It acts as a fungistatic agent .It is less lipophilic and more hydrophilic when compared to other azole antifungal agents[9]. The high penetration into the aqueous humour and low toxicity of fluconazole, makes it a good candidate for consideration as a topical ocular antifungal agent[10,11].

Propolis (bee glue), a resinous hive product, is collected by honey bees from various plant sources. It has been reported to exhibit a wide spectrum of activities including antibacterial, antifungal, anti-viral and antitumour properties[12,13]. Propolis had no toxic effect on albino mice, guinea pigs, and rabbits as experimental animals[14]. Propolis is widely used for apitherapeutical purpose in different forms such as granules, powder, soft and dry extracts as well as some other preparations, honey with propolis, suppositories, syrups, and ointments[15].

Ophthalmic antifungal agents are not commercially available and the physicians depend in the treatment of fungal ocular infections on the preparation of topical antifungal agents prepared from systemic antifungal drugs[16,17].

Fluconazole and propolis have been previously reported to be effective antifungal agents. There was no previous reports about the effects of liposomal fluconazole or liposomal propolis[6,10,18,19].

The aim of this study was to formulate and evaluate the *in vitro* and *in vivo* antifungal activities of topical fluconazole and propolis in a solution or a liposomal formulations, using the cup diffusion method and experimental *Candida albicans keratitis* in rabbits eyes respectively.

MATERIAL AND METHODS

1. Materials:

Fluconazole powder, was kindly supplied by EPICO, Cairo, Egypt. Egg yolk phosphatidylcholine was prepared and purified in our laboratory according to the method of Faure[20]. Cholesterol was purchased from Sigma chemical company, St. Louis, Mo., USA. Propolis was obtained as a gift from department of insects, faculty of agriculture, Ain Shams University, Cairo, Egypt. Polycarbonate membrane and membrane holders were obtained from Nucleopore corp. All other chemicals and solvents were of the analar grade and were gifted by El-Nasr company for pharmaceutical chemicals, Cairo, Egypt. Sabouraud dextrose agar, Oxoid Co., England. *Candida albicans* was kindly provided by Microbiology Department, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt.

2. Experimental animals:

Sixteen healthy Newzland rabbits, weighing 1.5 - 2 kg were used in this study. Animals were obtained from the Animal Breeding Center, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt. The animals were kept in restraining boxes during the treatment.

3. Methods:

A. Preparation of fluconazole solution:

Stock solutions of fluconazole were prepared by solubilizing the drug in polyvinyl alcohol to get a final concentration of 2 mg/ml. These solutions were protected from light and stored in dark at 4°C for not more than one week.

B. Preparation of propolis ethanolic extract solution (PEE):

Propolis ethanolic extract was prepared according to the method of Miyataka[21]. The method in brief with some modifications is as follows: Propolis slices (1-2 mm) were macerated with 50ml of ethanol (95%) for 2 days. The non dissolved residues were separated by filtration through fine filter (Whatmman. No.1) under vaccum. The solvent was evaporated under vaccum at 30°C until a constant weight. The obtained extract has a characteristic odour, insoluble in water but soluble in alcohol. The PEE was weighed and redissolved in polyvinyl alcohol to produce a concentration of 5% (W/V).

C. Preparation of liposomes

1. Preparation of multilamellar liposomes:

Multilamellar liposomes were prepared according to the method of Bangham et al., (1965)[22]. The lipid used for preparation composed of egg phosphatidylcholine and cholesterol which was dissolved in a minimal amount of chloroform. A thin film was formed on the surface of the flask by removal of the organic solvent using a rotatory evaporator. The dried lipid film was then hydrated with appropriate amount of polyvinyl alcohol solution to give lipid concentration of 60 mmole lipid/ml aqueous phase. The flask was shaken gently by hand for one hour at 25°C, followed by ten times of vigorous shaking and standing each of 30 seconds.

2. Preparation of liposomal fluconazole:

Fluconazole was dissolved in polyvinyl alcohol and incorporated into the aqueous compartment of liposomes to get 20 mg/ml concentration of the drug. The non-encapsulated drug was removed by centrifugation at 10.000 rpm for 30 minutes at 4°C. To ensure the complete removal of the free drug, the processes of centrifugation and resuspending were repeated three times. The final supernatents of liposomal suspension was collected and assayed spectrophotometrically for the presence of free drug at 261 nm[9]. The liposomal suspension was resuspended in adequate amount of fresh saline to get 2 mg/ml final concentration of the drug.

3. Preparation of liposomal propolis:

Propolis ethanolic extract with a concentration of 5 mg/ml was prepared and added to the solution of cholesterol/phospholipid 33% in chloroform and mixed with gentle shaking. The solvents were then completely removed by a rotary evaporator under vaccum at 25°C to obtain a thin film on the inner wall of the flask. The preparation of liposome suspension was completed as mentioned previously.

4. Sequential extrusion of the liposomal preparation:

A homogenous liposomal preparations with controlled size distribution was obtained by sequential extrusion method. The total lipid concentration was 60 µmol/ml and the liposomes were diluted to 12 µmol/ml in saline prior to extrusion. The dispersion was passed through polycarbonate membrane of 3.0 µm pore size so as to remove large aggregates of liposomes. These liposomes were sequentially extruded through 2.0, 1.0 and 0.8 µm pore size polycarbenate membrane filters. The extrusion process was accomplished at a relatively low pressure (10 pounds/square inch) in 25 mm membrane holder.

5. Determination of encapsulation efficiency of fluconazole within liposomes:

The encapsulation efficiency was calculated as the ratio of amount of the drug remaining within liposomes, after separation of liposomes from un-encapsulated drug, to the total amount of drug. The calculations were conducted according to the following equation:

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Amount of drug encapsulated}}{\text{Total amount of drug}} \times 100$$

D. Culture and *in vitro* susceptibility test:

Diameters of the growth inhibition zones (mm) produced by different formulations of fluconazole, propolis and control groups (polyvinyl alcohol solution and liposomes) were measured using the cup diffusion method[23]. *Candida albicans* was grown at 37°C for 24 hours on sabouraud's agar plates. The medium was

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inoculated with *Candida albicans* to give approximately 10^6 cells/ml. The results were recorded after 24 hours. The mean values of three readings for each sample were calculated.

E. *In vivo* study:

1. Inoculum procedure:

The rabbits were sedated by the intramuscular injection of a mixture of 0.5 ml ketamin (Ketalar 50 mg/ml) and 1ml of 2% xylocaine. The corneas were anesthetized by topical application of Benoxinate Hydrochloride (0.4 %).

Intrastromal Injection of 15 µl of *Candida albicans* suspension was done under an operating microscope, by inserting a sterile 27- gauge needle into the center of the cornea to a depth of one half of the corneal thickness.

The animals were excluded from the study if there was penetration of the inoculum into the anterior chamber or reflux of the inoculum was observed.

2.Treatment procedure:

This study included sixteen rabbits, divided into two equal groups. In the first group (8 rabbits), the right eyes of 4 rabbits received fluconazole solution (fluconazole group) and the left eyes received polyvinyl alcohol solution (control group). The right eyes of the other 4 rabbits received liposomal fluconazole drops (liposomal fluconazole group) and the left eyes received liposome drops only (control group).

In the second group (8 rabbits), the right eyes of 4 rabbits received propolis solution (propolis group) and the left eyes received polyvinyl alcohol solution (control group). The right eyes of the other 4 rabbits received liposomal propolis drops (liposomal propolis group) and the left eyes received liposomes drops only (control group).

3. Application of eye drops and the follow up:

The previously mentioned formulations were prepared and sterilized by filtration[24] as eye drops and were applied topically in the eye as 50 µl/application .

Eye drops were instilled into the conjunctival sac of rabbits after 48 hours of the inoculum procedure . The instillation continues every hour for 14 hours until complete healing of the corneal ulcer or appearance of severe complications (resistant corneal ulcer, atrophia bulbi), then, 5 times daily for 5 days.

The area of corneal epithelial defect was stained with sterile fluorescein solution, photographed and then measured every day. The mean epithelial defects were compared between all groups.

The surface area of corneal lesion was calculated using πab formula[25]

where $\pi = 22/7$

a = ½ radius of smallest axis.

b = ½ radius of biggest axis.

Occurrence of complications such as desmatocele, corneal perforation, resistant corneal ulcer, atrophia bulbi and hypopyon were noted.

F. Statistical analysis:

Results were expressed as mean value ± SE of the mean . Number of experiments were three in case of *in vitro* study and four in case of the *in vivo* study . Statistical analysis was carried out using the unpaired "t" and ANOVA tests within and between groups . The total area under the surface area (mm^2) of corneal lesions and time (day) was calculated using trapezoidal method[26] .

RESULTS AND DISCUSSION

1. The effect of cholesterol on the encapsulation efficiency of liposomes:

The results in Table (1) showed the influence of cholesterol incorporation into the phospholipid bilayers on the encapsulation efficiency of fluconazole in liposomes.

It is obvious that as the concentration of cholesterol was increased , the encapsulation efficiency increased. The maximum encapsulation of the drug (61.4%) was attained at 33 mole % of cholesterol and decreased to 36.4% as the concentration of cholesterol was increased to 50 mole %. The presented data clearly indicated that the incorporation of cholesterol was very important for obtaining liposomal formulations. Multilamellar liposomes composed of phosphatidylcholine and cholesterol have been used for the *in vitro* and *in vivo* studies, to provide liposomes with maximum stability and maximum encapsulation of the drug[27].

Table (1): Effect of cholesterol content of liposomal membranes on the encapsulation of fluconazole.

Cholesterol content mole (%)	Encapsulation (%)
00	20.4
10	30.5
20	34.5
30	56.9
33	61.4
36	56.6
40	40.2
50	36.4

2. *In vitro* antifungal activity study:

The *in vitro* antifungal activity of fluconazole and propolis in solution or liposomal formulations as determined by the growth inhibition zones in mm was shown in Table (2) and Fig. 1(A & B) .

Table (2): The Mean* diameters of the growth inhibition zones and the percentage effect of fluconazole and propolis in solution or liposomal formulations

Treatment	solution		Liposomes		Relative potency
	Mean diameter of growth inhibition zone (mm)	% effect	Mean diameter of growth inhibition zone (mm)	% effect	
Control	3±0.2	--	2±0.1	--	--
Fluconazole	15±0.5	100	28±0.6	186.7	1.86
Propolis	10±0.3	100	18±0.3	180	1.8

*Mean of three readings

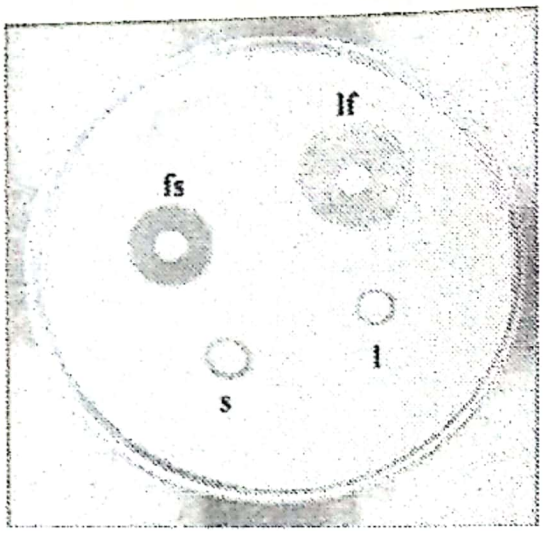

Fig. 1(A): The growth inhibition zone with liposomal fluconazole (lf), fluconazole in solution (fs), solution (s) and liposomes (l).

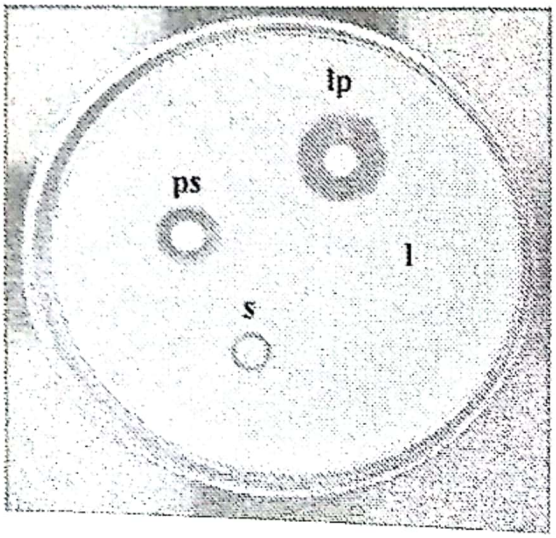

Fig. 1(B): The growth inhibition zone with liposomal propolis (lp), propolis in solution (ps), solution (s) and liposomes (l).

Results of the present study showed that diameters of the growth inhibition zones for the liposomal fluconazole and propolis were significantly higher than that of fluconazole and propolis in solution (P = 0.001 & 0.004 respectively). Polyvinyl alcohol and liposomes had no significant antifungal activity.

From the above results, one can arrange the antifungal activity for all tested preparations, as described by growth inhibition zone, in the following descending order: liposomal fluconazole (30 mm) > liposomal propolis (20 mm) > fluconazole in solution (18 mm) > propolis in solution (13 mm) > polyvinyl alcohol (solution) (3 mm) > liposomes alone (2 mm).

Accordingly, liposomes encapsulated drugs were more effective than the drugs in solution, where an increase of inhibition zone of 86.7 % and 80% for fluconazole and propolis was demonstrated respectively. The relative potency of the liposomal preparations to the drugs alone were 1.86 and 1.80 for fluconazole and propolis respectively.

3. *In vivo* antifungal activity study:

All eyes inoculated with *Candida albicans* developed corneal ulcer on day 2 with an average surface area of 58.3 mm^2 (Figs. 2 and 3).

Signs of corneal healing were observed only in groups treated with fluconazole and propolis in solution or liposomal formulaion (Fig. 4-7). No corneal healing occurred in control groups (Fig. 8).

Corneal perforation and atrophia bulbi occurred in control groups and not observed in fluconazole or propolis groups solutions or liposomal formulations (Fig. 9).

The mean time of complete healing of *Candida keratitis* was 11 days in liposomal fluconazole group (range 9-13), 20 days in fluconazole group (range 19 - 22 days), 12 days in liposomal propolis group (range 10 - 13 days), and 21 days in propolis group (range 18-23). (Fig. 10).

There were no significant differences between topical fluconazole and propolis in solution as regard to antifungal activity and rate of corneal healing (p = 0.45) . Addition of liposomal encapsulation to both drugs increase the rate of corneal healing significantly (p = 0.001 and 0.002 respectively), (Fig. 11 - 12).

There were a highly significant differences between fluconazole and propolis in solution or liposomal formulation and control group, as regard to antifungal activity , and the rate of corneal healing.

In addition to the above observations, the results in Table (3), showed that there were significant reductions in the total area under the curve of the surface area of corneal ulcers .

Both fluconazole and propolis in liposomal system reduced the area under the curve of the surface area of corneal ulcers by 52.2 & 51.3 % of the total area under the curve, recorded by fluconazole and propolis in solution respectively .

Table (3): Total area under the curve (mm^2. day) of the surface area of the *Candida keratitis* treated with solution, liposomes (as a control), fluconazole and propolis in solution or liposomal preparations.

Treatment	Solution	Liposomes	% Reduction of the area under the curve
Control	1548 ± 75	1619 ± 63	--
Fluconazole	[a] 510 ± 33.11	[a,b] 248.72 ± 33.83	52.2
Propolis	[a] 565.44 ± 61.78	[a,b] 270.78 ± 20.06	51.3

(a) Significant difference from the control groups ($p < 0.05$).
(b) Significant difference from the fluconaz-ole and propolis in solution ($p < 0.05$)

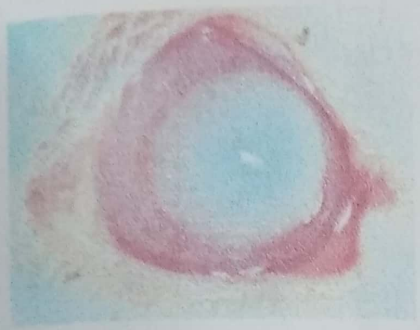

Fig. (2) : Corneal haze following intrastromal injection of 15ul candida albicans suspension.

Fig. (3) : Candida keratitis 48 hours following inoculation with candida albicans suspension....

Fig. 4 (A) : Candida keratitis 4 days after treatment with fluconazole solution.

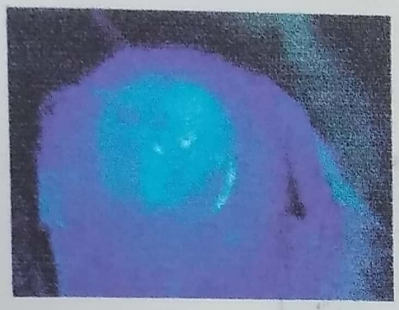

Fig. 4 (B) : Candida keratitis 8 days after treatment with fluconazole solution.

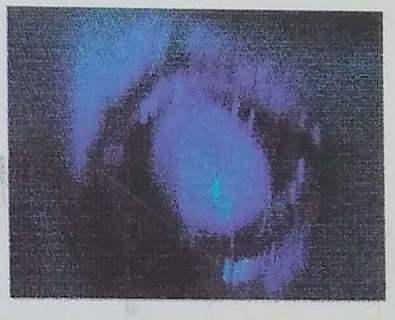

Fig. 4 (C) : complete healing with corneal opacity 19-22 days after treatment with fluconazole solution.

Fig. 5 (A) : Candida keratitis 4 days after treatment with liposomal fluconazole.

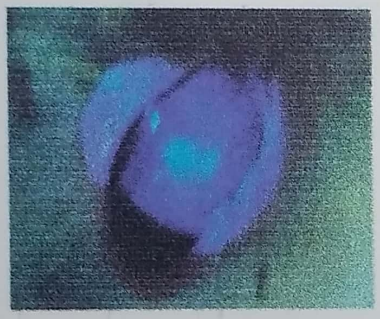

Fig. 5 (B) : Candida keratitis 8 days after treatment with liposomal fluconazole.

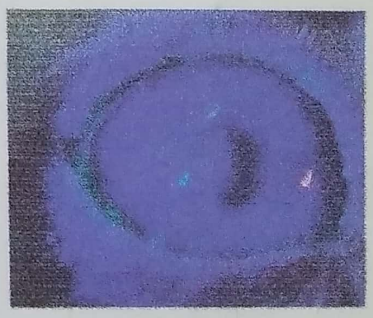

Fig. 5 (C) : complete healing with corneal opacity 9-13 days after treatment with liposomal fluconazole.

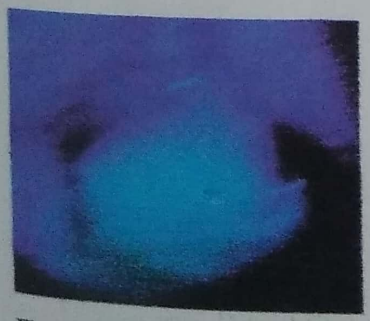

Fig. 6 (A) : Candida keratitis 4 days after treatment with propolis solution.

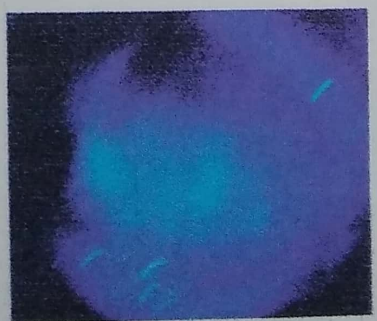

Fig. 6 (B) : Candida keratitis 8 days after treatment with propolis solution.

Fig. 6 (C) : complete healing with corneal opacity 18- 23 days after treatment with propolis solution.

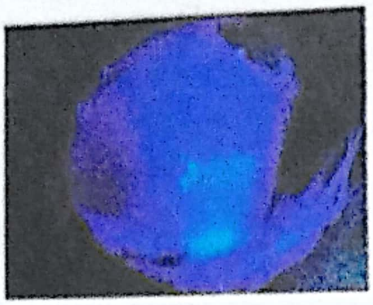

Fig. 7 (A) : Candida keratitis 4 days after treatment with liposomal propolis.

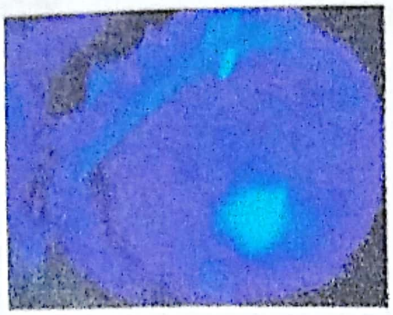

Fig. 7 (B) : Candida keratitis 8 days after treatment with liposomal propolis.

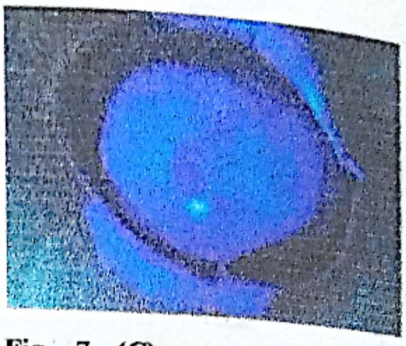

Fig. 7 (C) : complete healing with corneal opacity 10- 13 days after treatment with liposomal propolis.

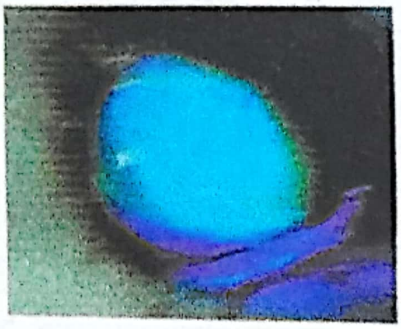

Fig. (8) : No signs of corneal healing in control groups.

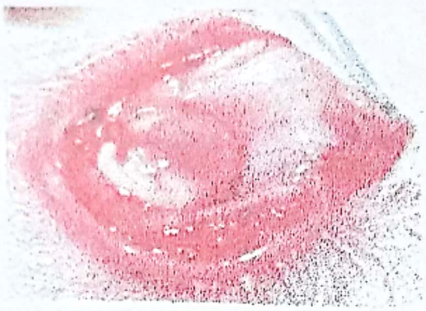

Fig. (9) : Corneal perforation and atrophia bulbi (control group).

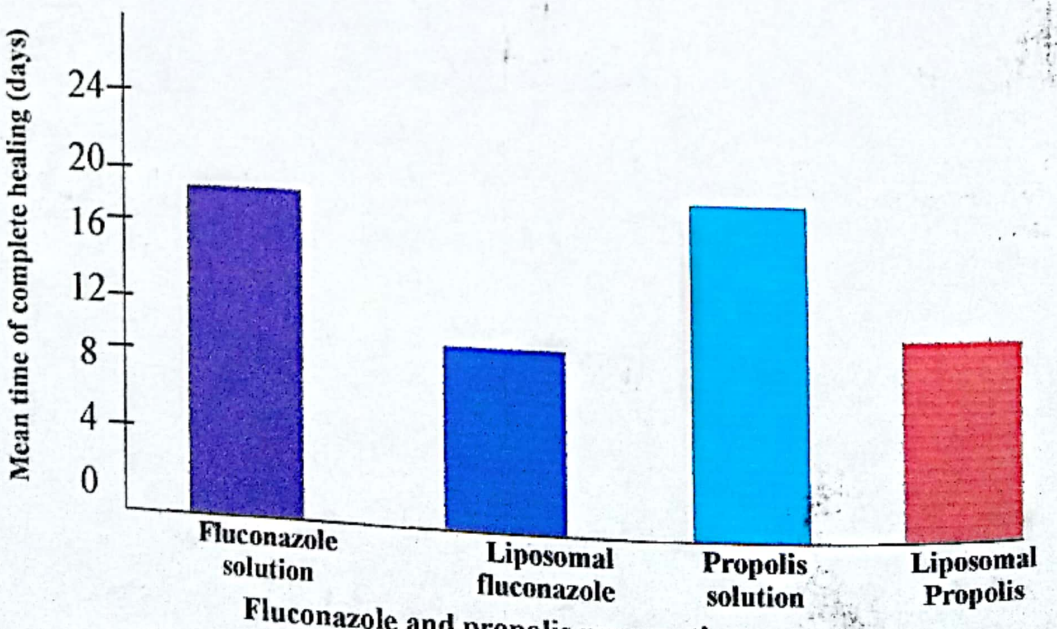

Fig. (10) : Mean time of complete corneal healing in different preparations of fluconazole and propolis.

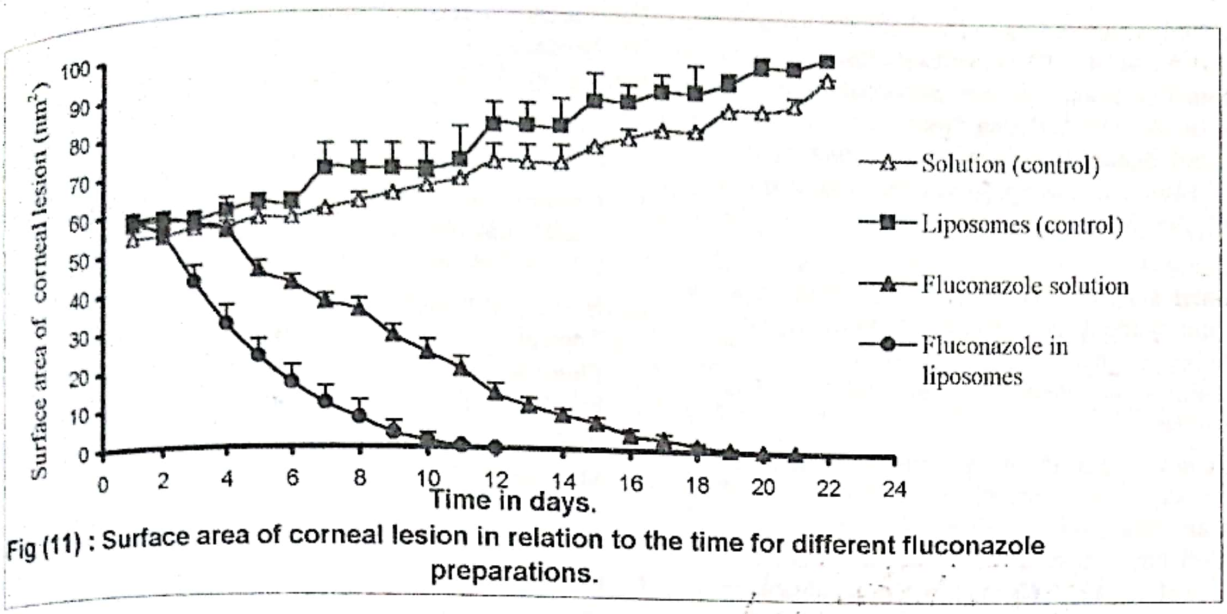

Fig (11) : Surface area of corneal lesion in relation to the time for different fluconazole preparations.

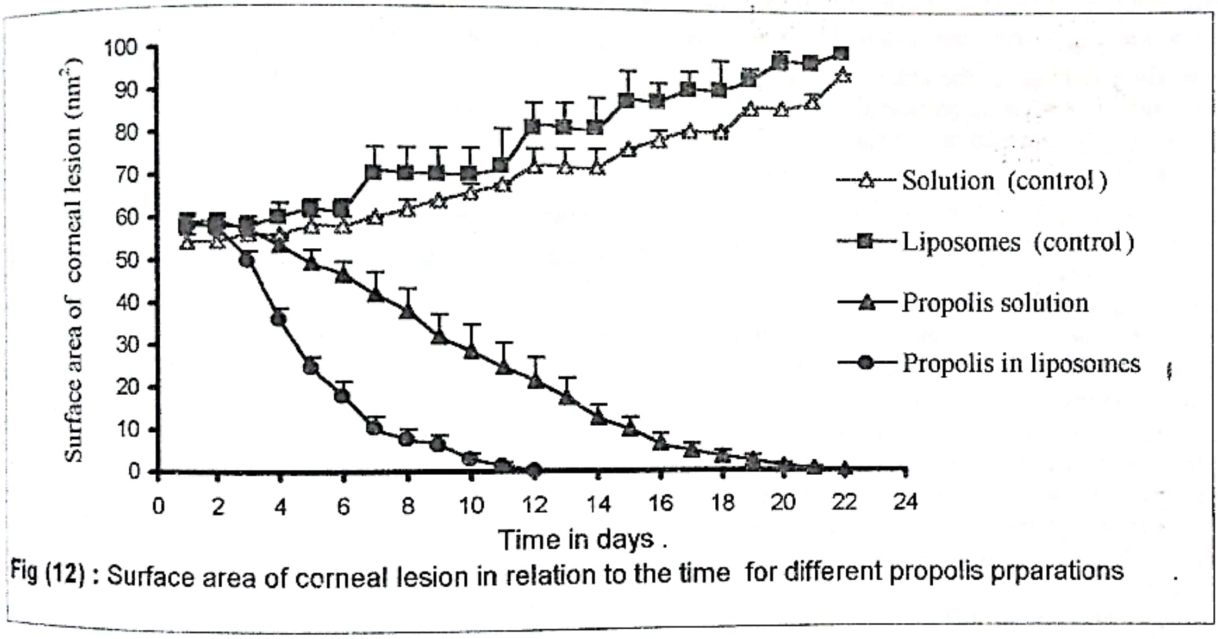

Fig (12) : Surface area of corneal lesion in relation to the time for different propolis prparations .

Candida albicans has been chosen in this study as a test organism as it is the most common isolated fungal corneal pathogen[17,28].

Rabbit's cornea has been chosen as an experimental model because of the similarity to that of the human cornea[29]. The choice of topical fluconazole as antifungal agent was based on its availability, low toxicity, and penetration into the aqueous humour[10].

Our results are in accordance by that reported by Baumann et al., (1990)[30], who found that fluconazole applied topically produced significant antifungal effect compared with the control group. The antifungal activity of fluconazole may be in part due to the inhibition of cytochrome p 450-dependent enzymes[8]. This may result in impairment of ergosterol synthesis in fungal cell membrane. Ergosterol was reported to have the ability to control the permeability of cell membrane. The net result is leakage of potasium and magnesium ions from the fungal cell and death[9].

Propolis as a natural honey bees product has been used in this study for comparing its antifungal activity with that of fluconazole. Propolis was reported to facilitate corneal epithelial wound healing. The mechanisms of this effect and its clinical use still remain to be undetermined[31].

Tikonov et al., (1987)[15] reported that propolis has antioxidant and membrane stabilizing activity. These effects of propolis not only reduce but also stabilize the liposomal membranes against lipid peroxidation[32].

It was previously reported that the ethanolic extract of propolis has a large number of polyphenols. The flavonoid components are responsible for the antifungal activity of propolis[32].

The antifungal activity of fluconazole and propolis in liposomal formulation is better than these agents in non liposomal solution . It was previously reported that, liposomal encapsulation increases the lipid solubility and hence their permeability through cell membrane. Moreover the liposomes may enhance the binding of the drug to the active sites inside the cells[33].

Liposomal system may increase the intracellular concentration of the drug. It has ability to circumvent cell membrane permeability barriers by cell membrane-liposome interactions (adsorption, fusion, and endocytosis)[34].

In our study, one of the most important benefits of preparation of these antifungals in the form of liposomes are reduction of both onset and duration of recovery and healing from candidiasis (Figs. 10-12). This may lead to the reduction of the incidence of other infections and complications that may occur due to the prolonged time required for the antifungal to start their effects and to induce complete recovery.

Moreover, the reduction of the recovery time may increase the patient compliance, reduces the chance of the incidence of microbial resistance, reduces the drug doses and concentration and may reduces their side effects .

In our study, there was a similarity between the liposomal preparation of both fluconazole and propolis *in vivo*, despite the difference in their effects in the *in vitro*. This may be due to the absence of the immune system defense mechanism. Since in rabbits there is an immune system stimulation actually occurs during candidiasis. Liposomes could protect the delivered drug from metabolic and immune attacks, reduce drug toxicity, and enhance the therapeutic effects[35]. On the other hand, the differences between the activity of the liposomal preparations between fluconazole and propolis *in vitro* may be due to the difference in the molar ratio of the effective molecules of both agents. Since most propolis components are not antifungal[32].

All the above results showed that the use of liposomes as a vehicle could contribute to the enhancement of the bioavailability of the drug .

It could be concluded that the findings obtained from *in vitro* and *in vivo* studies demonstrate the efficacy of liposomes as ocular drug delivery system. Therapy with topical liposomal fluconazole 2 mg/ml or liposomal propolis 5% were successful in eliminating experimental *Candida albicans* infection of the rabbit cornea, in comparison with topical fluconazole (2 mg/ml) or propolis (5%) in solution . Moreover, propolis as a natural product and in the given concentration, is as potent antifungal as fluconazole against *Candida keratitis*.

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REFERENCES

1- Crommelin, D.J.A. and Schreirer, H.; Liposomes. In: Colloidal Drug Delivery Systems, J. Kreuter Ed., M. Dekker, New York., pp. 73-190 (1994) .

2- Barber, R.F. and Shek, P.N.; Liposomes as a Topical Ocular Drug Delivery System In: Pharmaceutical particulate carriers, A. Rolland Ed., M. Dekker, New York.,pp. 1-20 (1993).

3- Kirby, L.J,; Gregoriadis, G.; Liposomes. In: Mathiowitz E , editor. Encyclopedia of Controlled Drug Delivery , Vol 1. New York . john Wiley and Sons, Inc., p 461-492 (1996).

4- Pirce, C.; Horton, J. and baxter, C.; **Surgery**, 115 (4), 480-48, (1994).

5- Lund, O.E.; Mino, D.H. and Klauss, V.; **Klin-Monatsbl-Augenheilkd**, 202(3): 188-94 (1993).

6- Espinel-Ingroff-A.; **Rev Esp Quimioter.**; 13(2) :161-60 (2000).

7- Martin, M.J.; Rahman, M.R.; Johnson, G.J.; Srinivasan M. and Clayton Y.M.; **International Ophthalmology**, 19:299-302 (1996).

8- Price, M.F.; **Antimicrob. Agents Chemother.**, 38, 1422-4 (1994).

9- Dash, A.K. and Elmquist, W.F.; Fluconazole In : Brittain H.G., Ed. Analytical profiles of drug substances and excipients, Vol 27. Academic Press., p. 67-113 (2001).

10- Yee, R.w.; Cheng, C.J.; Meenakshin, S.; Ludden, T.M.; Wallace, J.E. and Rinaldi, MG.; **Cornea**, 16(1) :64-71 (1997).

11- Abbasoglu, O.E.; Hasal, B.M.; Senser, B.; Erdemoglu N. and Gursel E. **Experimental Eye Research**, 72(2): 147-51 (2001).

12- Banskota, A.H.; Tezuka, Y.; Adnyana, I.K.; Midori, Kawa, K.; Matsushige, K.; Message, D.; Hue-rtas A.A, and Kedota S.; **Journal of Ethnopharmacology**, 72(1-2):239-46 (2000).

13- Burdick, G.A.; **Food Chem. Toxicol.**, 36(4) :347-63 (1998).

14- Okonenko, L.B.; Sallmonella infections and propolis, **Zdravookhr Kaz**, 1, 55-57 (1988).

15- Tikonov, AI.; Rogozin, B.A.; Porokh, L.A.; nyak, N.S.; Mamontova, T.G.; Yarnykh, T.M.; Budnikova, and Yavtushenko, VSV.; **Fasmatsevtichnii–zhurnal**., 6, 65-66 (1987).

16- O`Day, D.M.; **Cornea**, 6: 234- 450 (1997).

17- Abu-hussain, Hi.; EIN-aggar, A.B.; EL-Bedeiwy, M.Y. and Abu-Elsoud, S.; **Bull Ophthalmol Soc. Egypt.**, 91 (6):965-971 (1998).

18- Drago, L.; Mombelli, B.; De Vecchi, E.; Fassina, M.C.; Tocalli, L. and Gismondo, M.R.; **Journal of**

Chemotherapy, 12(5): 390-5 (2000).

19- Ota, C.; Unterkircher, C.; fantinato, V. and Shimizu, M.T.; **Mycoses**, 44 (9-10) :372-8 (2001).

20- Faure, M.; **Bull. Soc. Chem. Biol.**, 32,503-512 (1950).

21- Miyataka, H.; Nishiki, M.; Matsumoto, H.; Fujimoto, T., Matsuka, M. and Satoh, T.; **Biol Pharm. Bull.**, 20(5), 494-501 (1997).

22- Bangham, A.D.; Standish, M.M. and Watkins, J.C.; **J. Mol. Biol.**, 13, .238-252 (1965).

23- Shadomy, S. and Espinel-Ingroff, A.; Manual of Clinical Microbiology, 3rd Ed American Society for Microbiology, Washington, D.C., p. 647-653 (1980).

24- Delaat Adrian, N.C.; Microbiology for the Allied Health Professions, 3rd, 45-46, (1984).

25- Mizrah, I.A. and Sullivan, M.; Calculus and Analytic geometry, Wadsworth publishing company, Balmont, California, 488-504 (1982).

26- Gibaldi, M.; Biopharmaceutics and Clinical Pharmacokinetics. In: Delivery of Drugs Dosage Form and Their Evaluation, Lead and Febiger Eds. 4th Ed. Philadelphia, London, pp. 41-73 (1991).

27- Ezz El-Din, M.M; Abu-Zaid, S.S. and Ghanem, E.H.; **Afr. J. Myco. and Biotech.**, 3(2) :89-99 (1995).

28- Ayoub, M.I.; Abdel Hakim, A.S., Katamish, T.A.; Sobhi, F.S, and Ehsan, A.A.; **Cataract and Cornea**, 3:4-16 (1997).

29- AL-Hussains, A.K., EL-Shanawany, A.; Daef, E.A. and Abd EL–latif, M.M.; **Bull. Ophthalmol Soc. Egypt.**, 90(5):809-812 (1997).

30- Baumann, W.; Klinge, B. and Ruchel, R.; **Brit. J. Ophthalmol**, 74 : 40-42 (1990).

31- Ozturk, F.; kurl, E.; Inan, U.V.; Emiraglu, L. and Ilkei, S.S.; **Cornea**, 18(4) 466-71 (1999).

32- Franco, T.T.; kurebayashi, AK.; **Revista do instituto adolfo Lutz.**, 46: 81-86 (1986).

33- Oh, Y.K.; Nix, D.E.; Straubinger, R.M.; **Antimicrob. Agents Chemother.**, 9, 2104-2111 (1995).

34- Pagano, R.E.; Weinstem, J.N.; **Annu. Rev. Biopsy Bioeng.**, 7, 435-468 (1978).

35- Bar-Ilan, A. and Neumann, R.; Basic Considerations of Ocular Drug - Delivery Systems In: Zimmer-man, T.J., Editor. Textbook of Ocular Pharmacology. Lippincott, Raven Philadelphia, p. 139-150 (1997).

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صياغة وتقييم الفلوكونازول والبروبولیز الليبوزومى كعقار موضعى مضاد للفطريات فى الهاب الفرجى المبايضى

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كان الهدف من هذه الدراسة هو صياغة كل من الفلوكونازول والبروبوليز فى الليبوزوم ، وتقييم نشاطهم المعملى والحيوى ضد فطور المبيضة البيضاء (*Candida albicans*). لذلك تم تحضير كل من الفلوكونازول والبروبوليز فى صيغة محلول وتغليفهم فى الليبوزوم المتعدد الطبقات ، والمكون من الفوسفاتيديل كولين (المستخلص من صفار البيض) مع الكوليسترول (حتى نسبة ٥٠ مول % كوليسترول). وكان للكوليسترول المضاف تأثيراً واضحاً على فاعلية التغليف عند نسبة ٣٣ مول% كوليسترول. ولقد تم الاختبار المعملى لكل من الفلوكونازول (٢ مج/مل) والبروبوليز (٥%) فى صيغة محلول وكذلك على هيئة ليبوزوم ، وذلك بقياس أقطار مناطق اللانمو ضد فطور المبيضة البيضاء ، ومقارنتها بكل من المحلول والليبوزوم الخالى من العقار . بالاضافة الى ذلك تم التقييم الحيوى على ستة عشر أرنب وذلك بحقن منتصف القرنية فطور المبيضة البيضاء (١٠ ميكروليتر). وقد تم تقطير المستحضرات السابقة للأرانب كل ساعة لمدة ١٤ ساعة يوميا مع ملاحظة إلتئام القرنية باستخدام محلول الفلورسين المعقم يومياً .

وقد أوضحت النتائج أن المحلول أو الليبوزوم الخاليين من العقار ليس لهم أى نشاط مضاد لفطر الكانديدا وذلك من خلال الاختبارات المعملية والحيوية. كما أظهر الاختبار المعملى أن الفلوكونازول والبروبوليز سواء فى المحلول أو مغلفاً بالليبوزوم لهم نشاط مضاد للنمو الفطرى وكان ترتيبهم كالاتى: الفلوكونازول الليبوزومى > البروبوليز الليبوزومى > محلول الفلوكونازول > محلول البروبوليز ومن ثم فقد أظهر الاختبار الحيوى وجود فروق ذوات دلالة احصائية بين الفلوكونازول الليبوزومى أو البروبوليز الليبوزومى بالمقارنة بمحلول الفلوكونازول أو البروبوليز. كما أنه لا توجد فروق ذوات دلالة احصائية بين الفلوكونازول الليبوزومى والبروبوليز الليبوزومى ، وكذلك بين محلول الفلوكونازول ومحلول البروبوليز . وبالتالى يمكن استنتاج أن التحوصل الليبوزومى يعتبر طريقة بسيطة وذات فاعلية لتحسين النشاط المضاد للفطريات لكل من الفلوكونازول والبروبوليز.