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IN-VITRO ANTIMICROBIAL ACTIVITY OF CHLORHEXIDINE POLYMERIC FILMS

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ABSTRACT

The minimum inhibitory concentration (MIC) of chlorhexidine for *Staphylococcus aureus, Bacillus subtilus, Escherichia Coli and Candida albicans* was studied. Chlorhexidine polymeric films composed of ethylcellulose (EC) and hydroxypropyl methylcellulose (HPMC) in a ratio of 8 : 2 were prepared . The inhibition zone diameters of chlorhexidine polymeric film were compared with that of chlorhexidine gauze dressing. Also, the inhibition zone sizes of different concentrations of chlorhexidine (0.5, 1 and 5%) in films plasticized with 20% propylene glycol, in presence of 10% Tween 80 as enhancer, or plasticized with 20% polyethylene glycol 400 were evaluated . Results indicated that the MIC of chlorhexidine was ranged from 1 to 1.8 µg/ml. Also the inhibition zone diameters of the selected polymeric film was higher than that of gauze dressing. There were correlation between drug concentrations and inhibition zone sizes. Also polymeric film containing 20% propylene glycol and 10% Tween 80 showed relatively higher response than polymeric film containing 20% ploeythylene glycol 400. It can be concluded that the tested microorganisms were susceptible to chlorhexidine and the use of polymeric films as drug delivery system enhanced chlorhexidine in-vitro antimicrobial activity. Accordingly, it was recommended to use chlorhexidine polymeric films for topical antisepsis .

INTRODUCTION

Chlorhexidine is an antibacterial agent which has long been used for medical antisepsis[1]. It is a safe antiseptic with low toxicity when used correctly[2] . Its clinical and topically antimicrobial effects were seen among different pateints[3]. Chlorhexidine has wide activity against *Streptococcus mutants*[4]. Also its radiolabeled uptake to *Sacchromyces cervisia and Candida albicans* was very rapid.

The mechanism of action of chlorhexidine includes ribosomal RNA degradation in gram-negative and gram-positive bacteria including *Enterobacteracea, Pseudomonas, Bacillus and Bifidobacterium* [5] . Also chlorhexidine being cationic may interact with bacteria by attraction to negative charge in membrane components[6] .

Different formulations of chlorhexidine as antimicrobial agent available for treating bacterial skin diseases were studied . However, due to their short duration of action, frequent applications are required. On this basis, polymers are used extensively in pharmaceutical formulations. Therefore, formulation of polymeric films which control and deliver drugs over a reasonable time were investigated [7,8,9] .

The present study focus on the in-vitro evaluation of antimicrobial activity of the prepared chlorhexidine polymeric films and gauze dressing (marketing product), each containing 0.5% w/w of chlorhexidine . Also the effect of different concentrations of chlorhexidine on polymeric films in presence of different additives (plasticizer and / or enhancer) was evaluated.

MATERIALS AND METHODS

Materials :

Chlorhexidine gauze dressing 0.5% w/w (Minapharm Egypt). Nutrient agar and Nutrient broth was obtained from Difeco. Chlorhexidine diacetate (Sigma Chemical Co.,St. Louis, Mo.,USA).

Ethylcellulose 14 cp . (BDH chemical ltd poole England). Hydroxypropyl methylcellulose 50 cp. Propylene glycol, polyethylene glycol 400 (El-Nasr Co.Egypt). Tween 80 (Merck, Schuchardt, Munchen, W. Germany). Methanol, methylene chloride (EL-Nasr Co . Egypt). All other reagents were of pharmaceutical grade.

Microorganisms : The following organisms were used :

1- *Staphylococcus aureus*: ATCC 25923

2- *Escherichia Coli* : ATCC 25922

3- *Bacillus subtilus* : ATCC 7972

4- *Candida albicans* : ATCC 753 .

Preparation of polymeric medicated films :

Films formed of EC : HPMC, in a ratio of 8:2, and plasticized with 20% polyethylene glycol 400 or 20% propylene glycol in the presence of 10% Tween 80 (as enhancer), were found [10,11] to be the most suitable films due to its toughness, resiliency and resistant to rapid dissolution in aqueous medium. For there advantageous, films composed of EC : HPMC, in a ratio of 8 : 2, was selected for in-vitro evaluation studies and prepared as follows:

Films were prepared from the polymer solution (EC:HPMC in a ratio of 8:2) in a casting solvent of equal parts (methylene chloride : methanol). The solution contained 6% w/v of the polymer and specific weight of a plasticizer in the mentioned solvent gave a film on a glass substrate that could be easily removed. EC was added gradually, with continuous mixing by magnetic stirrer, to a 100ml beaker containing the solvent, plasticizer or enhancer (if added) and the specific weight of chlorhexidine (0.5, 1.5 and 5% w/w of dry film). HPMC was then added gradualy (EC: HPMC in a ratio of 8:2), with mixing, after all the EC had been dissolved . The beaker was covered with aluminium foil paper to prevent solvent evaportion . The solution was allowed to stand for about 30 minutes to remove entrapped air . Also air was removed from the polymer drug dispersion by ultrasonification for 5 minutes.

The casting solution was transfered to a dust free cleaned and dried petri dish (area = 63.617 cm^2) placed on a flat surface at room temperature . The petri dish was covered with an inverted glass funnel of stem orifice 0.6 cm in diameter . Clearance was provided for the escape of solvent vapours by raising the base of the funnel 2 cm just above the resting surface . The funnel was an aid in controlling the rate of evaporation of the solvent and reducing the blistering of the surface of the deposited film. The solvent was allowed to evaporate for 24 hours, the film was then removed from the petri dish to a desiccator containing anhydrous calcium chloride, where it was stored further 24 hours before use . Rectangular films (13 mg drug content) measuring 3 cm x 4 cm (12 cm^2) were obtained by cutting a selected portion of the cast film with razor blade.

Culture and the in-vitro susceptibility test :

Determination of minimum inhibitory concentration (MIC) was performed by using the tube dilution technique as described by Shadomy and Espinel-Ingroff[12]. On the other hand, the sizes of the zones of inhibition were measured by using the diffusioin method as described by Lorain[13] . *Staphylococcus aureus, E.Coli, Basillus subitilus and Candida albicans* were grown at 37°C for 24 hours on nutrient agar plates. The medium was inoculated with the previous mentioned microorganisms to give approximately 10^6 cells/ml for each microorganisms . Suspensions were prepared and also diluted with sterile saline solution adjusted to pH 6.5 under aseptic conditions. Growth were recorded after 24 hours incubation at 37°C.

The lowest concentration of the drug in µg/ml that prevents in-vitro growth was taken as the MIC. Control was also done in parallel with the test for each sample. The mean of at least 3 readings was determined.

Chlorhexidine gauze dressing (marketing product) and the prepared chlorhexidine polymeric films in different concentrations (0.5, 1 and 5%), in presence of different additives, were tested for antimicrobial activity against the previously mentined microorganisms . Susceptibility testing were done against each individual organisms in an aseptic condition .

The bacterial isolates were used for inoculation of nutrient broth for *Staphylococcus aureus, E.Coli and Bacillus Subtilus* and sabourd's dextrose fluid media for Candida albicans. Cultures were incubated at 37°C over night. A volume of 0.1ml bacterial culture was streaked evenly onto the surface of nutrient agar plate . Discs of chlorhexidine films and gauze dressing were placed and gently pressed down on the surface of agar plate. Plates were incubated for 24-48 hours at 37°C and zone of inhibition were measured .

RESULTS AND DISCUSSION

The in-vitro antimicrobial activity of chlorhexidine acetate against microorganisms was done. Table 1, showed the minimum inhibitory concentration (MIC) of chlorhexidine ranged from 1-1.8 µg/ml. It has been concluded that these microorganisms were susceptible to chlorhexidine. Thus, MIC is a suitable test and critical assessement of antimicrobial activity of chlorhexidine .

In order to compare chlorhexidine film and vaseline gauze dressing the antimicrobial activity was examined . A quantitative comparative study of the antimicrobial activity of chlorhexidine polymeric film and vaseline gauze dressing, each containing 0.5% chlorhexidine, was done. The results were shown in Table 2. It was found that chlorhexidine film showed a higher response in the inhibition zone than gauze dressing.

Table (1): Minimum inhibitory concentration (MIC) of chlorhexidine powder (tube dilution method) .

Microorganism	MIC (µg/ml) ± S.D.
Staphylococcus aureus	1.2 ± 0.2
Bacillus Subtilus	1.5 ± 0.3
E. Coli	1.8 ± 0.2
Candida albicans	1.0 ± 0.1

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Table(3): Antimicrobial effect of chlorohexidine polymeric film plasticized with 20% PG. in presence of Tween 80 .

Drug Conc.	Zones of inhibition (Diameter in mm)			
	Staphylococcus aureus	*Bacillus Subtilus*	*E. Coli*	*Candida albicans*
0.5 %	11	10	9	12
1 %	15	14	12	17
5 %	20	22	21	25

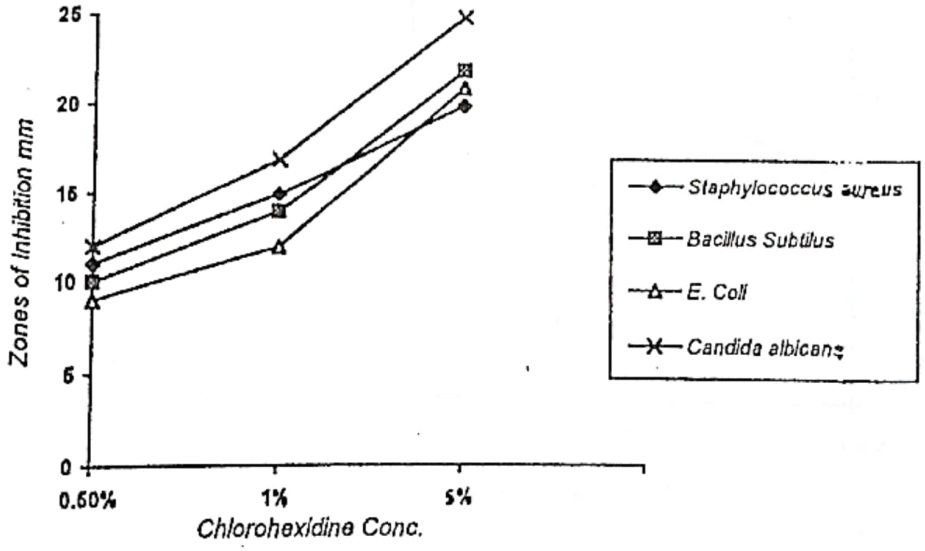

Fig.(1): Antimicrobial effect of chlorhexidine polymeric films plasticized with 20% PG in Presence of 10% Tween 80.

Table (4): Antimicrobial effect of chlorohexidine Polymeric Film plasticized with 20% PEG 400.

Drug Conc.	Zones of inhibition (Diameter in mm)			
	Staphylococcus aureus	*Bacillus Subtilus*	*E. Coli*	*Candida albicans*
0.5 %	10	9	8	11
1 %	13	12	11	15
5 %	18	20	19	23

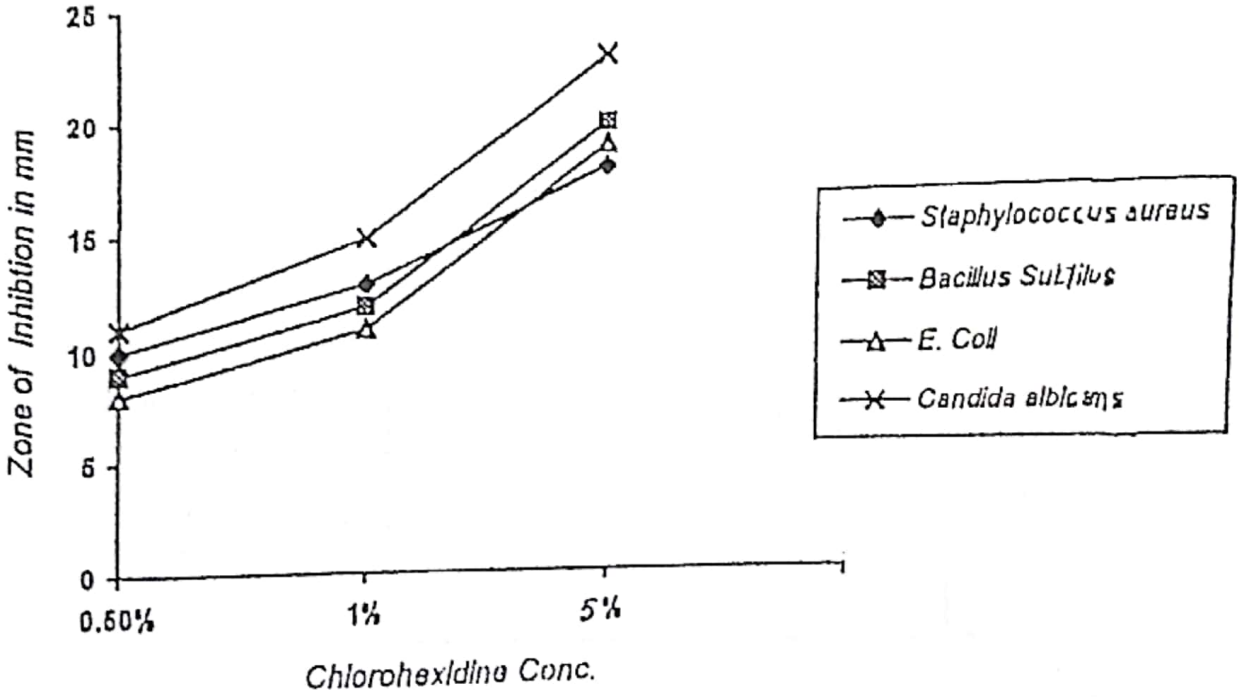

Fig. (2): Antimicrobial effect of chlorhexidine films plasticized with 20% PEG 400.

Table (2): Comparison of inhibition zones of chlorhexidine film with chlorhexidine gauze dressing .

Microorganisms	Zones of inhibition (Diameter in mm)		
	Gauze 0.5% dressing	Film 0.5%	Plain film
Staphylococcus aureus	4 ± 0.5	10 ± 2	0
Bacillus Subtilus	3 ± 0.5	9 ± 1	0
E. Coli	2 ± 0.5	8 ± 1	0
Candida albicans	4 ± 0.5	11 ± 2	0

The sizes of inhibition zones for chlorhexidine polymeric films at 0.5, 1 and 5% concentration showed a dramatic increase in the inhibition zone sizes with increasing chlorhexidine concentrations as seen in tables 3 and 4. However, the use of polymeric films plasticized with 20% propylene glycol and 10% Tween 80 (as enhancer) showed relatively higher response in the inhibition zone sizes than plasticized with 20% polyethylene glycol. The results were graphically illustrated in Figures 1 and 2.

The inhibition zone sizes reflected quantitive concentration gradient established by diffusion of the drug through a given medium and the susceptibility of the tested organisms.

It was speculated that hydrophobic nature of vaseline captured chlorhexidine and retarded its diffusion. While polymeric film exhibited rapid drug delivery due to presence of hydrophilic components in the film such as HPMC, propylene glycol and Tween 80. All of these components promote chlorhexidine diffusion through the medium and thereby enhance its antimicrobial activity .

Also the increased diameter of inhibition zone of chlorhexidine polymeric film may be due to the interaction of chlorhexidine with the bacterial memberane components . This assumption is in agreement with that obtained by Kootongkaew et al. (6), who found that the antimicrobial activity of chlorhexidine was attributed to its attraction to the negative charge in the membrane components of bacteria .

In conclusion : polymeric film is an excellent formulation for topical application. It has many advantages including :

i. It has a defined area,
ii. Easy of application,
iii. Control releasing of drug in a sustained profile over a reasonable time,
iv. Easy to terminate therapy by removing the film which interrupt diffusion of drug .

Accordingly, the use of polymeric films as drug delivery system enhanced chlorhexidine in-vitro antimicrobial activity and it was recommended to use chlorhexidine polymeric films for topical antisepsis .

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دراسة معمليه على الفاعليه الميكروبيولوجية لأغشية الكلورهيكسيدين البوليميريه

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تم قياس أقل تركيز مانع للنمو للكلورهيكسيدين ضد الاستافيلوكوكس أوريس ، باسيلس سابتيلوس ، إيشيرشيا كولاى وكذلك الكانديدا البيكانس . وحضرت الأغشيه البوليميريه للكلورهيكسيدين من مادتى إيثيل سيلبلوز وأيدروكسى بروبيل ميثيل سيليلوز بنسبة ٨ : ٢ . وقورنت أقطار مناطق اللافو للعقار فى الأغشيه البوليمريه مع تلك الخاصه بالشاش وكذلك تم تقييم فاعليه الكلورهيكسدين فى تركيزات مختلفه (٥ر٠ ، ١ ، ٥٪) مع إضافة بعض اللدائن مثل عديد الأيثيلين چليكول ٤٠٠ (٢٠٪) أو البروبيلين چليكول (٢٠٪) فى وجود ١٠٪ توين ٨٠ كمادة مساعدة .

ودلت النتائج على أن أقل تركيزات ممانعه للنمو للكلورهيكسبدين كانت مابين ١ إلى ٨ر١ ميكروجرام/مليمبتر وأن أقطار مناطق اللنمو للغشاء البوليمرى المحضر كانت أعلى من تلك الخاصه بالشاش . وبالإضافة إلى ذلك فإن الأغشية البوليمريه المضاف اليها ٢٠٪ بروبيلين چليكول فى وجود ١٠٪ توين ٨٠ أظهرت فاعلية عالية بالنسبة إلى الأغشية المضاف إليها ٢٠٪ عديد الأيثيلين چليكول ٤٠٠

ويستخلص من ذلك أن إستخدام أغشية الكلورهيكسيدين البوليمريه قد حسنت من فاعلية العقار معمليا ضد الميكروبات المختبره ولذلك فانه يوصى باستعماله كمطهر موضعى .