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Comparative Effectiveness of Certain Antimicrobial Agents in Semi Solid Preparations (Pp. 241-251)

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Abstract

*Cosmetics and topical products need not be sterile but may contain low levels of microbial load during use. This study was conducted to determine effectiveness of antimicrobial preservation during storage life of preparations and particularly the suitability of such preservative in terms of safety and broad spectrum activity. In this study, six formulations of both aqueous and oily cream were prepared under laboratory conditions. Four of these preparations were prepared with either methyl parahydroxybenzoate or chlorocresol as preservative. The remaining two formulations, aqueous and oily creams were without preservative serving as control preparations. All the formulations were subjected to microbial qualitative screening, 24 – 48 hours after formulation. Thereafter, the formulations were challenged with bacteria inocula, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (1×10^3 and *Candida albicans* (1×10^2 moulds/g/ml). The result shows that there was initial contamination of the control formulations without preservative (P03 and P04) and oily cream preparation with chlorocresol preservative (P06) with *Proteus* spp, *Aspergillus fumigatus* and *Candida albicans* respectively. Also subjecting the data to statistical inference, ($P < 0.05$) in*

each instance, it was found out that the preservatives used had significant effect on the reduction of bacteria and fungi over a period of 28 days of study. Also there was observed statistically significant difference in the preservative capacity of the antimicrobial agents used. The order of effectiveness is chlorocresol > methylparahydroxylbenzoate. It can be concluded that preservatives used in topical formulations should be thoroughly evaluated to ensure effectiveness. In some instances, reinforcement may be necessary to confer synergy.

Introduction

Semisolid dosage forms are dermatological products of semisolid consistency and applied to the skin for therapeutic or protective action or cosmetic function (Jani, 2003). Semisolid pharmaceutical systems comprise a body of products, which when applied to the skin or accessible mucous membranes tend to alleviate or treat a pathological condition or offer protection against a harmful environment.

They have the property to cling to the skin or mucous membrane for a protracted period of time to exert their therapeutic effect through protection and occlusion. The adhesion is due to their plastic rheologic behaviour which allows semisolid to retain their shape and cling as film until acted upon by an outside force (Swarbrick and Boylan, 1996).

Semisolid dosage forms usually are intended for localized drug delivery. In the past few years, however, those forms also have been explored for the systemic delivery of various drugs. Semisolids constitute a significant proportion of pharmaceutical dosage forms. They can be applied topically to the skin, cornea, rectal tissue, nasal mucosa, vagina, buccal tissue, urethral membrane and external ear lining (Lachman *et. al*, 1991) although they are not required to be sterile, they must be preserved to reduce the level of microbial contaminant to the minimum in order to maintain the integrity of the preparation.

Some workers have been able to report on activities of certain antimicrobial agents in semisolid formulations. Favet *et. al* (2001) were able to conclude that the three zinc gelatin preparations displayed quite different efficacies of antimicrobial preservation. Zinc gelatin preparation without a preservative was effective against bacteria not because of the anti-microbial activity of the zinc but because the low water activity of the preparation inhibits the growth

of organisms. It has some efficiency against *Candida albicans* but fungistatic against *Aspergillus niger*. The zinc gelatin preparation with methyl parahydroxybenzoate has a poor preservation against *Staphylococcus aureus* even though the European pharmacopoeia criteria are met. The preservative efficiency of zinc gelatin semisolid preparation containing phenoxyethanol against bacteria, fungi such as *Candida albicans* and *Aspergillus niger* was optimal.

Hugbo *et. al.* (2003) reported that preservatives employed in some commercially available cosmetic brands did not possess adequate preservative capacity to be able to bring about acceptable low levels of microbial contamination.

Parabens have been the most popular preservative employed in the past. The parabens and their salts are primarily used for bactericidal and fungicidal properties. The low cost and long history of safe use probably explains the common use of parabens (Woedtke *et. al.*, 1999). However, there are some recent studies that have raised doubts as to the safety of parabens. Dabre *et. al.* (2004) reported a controversial finding of presence of parabens in samples of breast tumors suggesting a linkage. Byford *et. al.* (2005) also reported the oestrogenic activity of parabens acting as xenoestrogens. Some oestrogens are known to drive the growth of tumors. Nagel *et. al.* (1977) also reported allergies in sensitive individuals to parabens.

In the present study, activities of parabens and chlorocresol as preservatives are compared in oily and aqueous semisolid preparations.

Materials and Methods

Aqueous and oily cream BP representing both o/w and w/o cream respectively were prepared using chlorocresol and parabens as the indicated preservatives. All samples were maintained at 4°C to minimize growth prior to use. Oxoid media (nutrient and sabouraud) were used in all evaluations.

Evaluation of Microbiological Quality of Preparations

Microbial contamination of the cream samples were determined by thinly spreading a loopful of material withdrawn from the depths of the bulk product on nutrient agar and sabouraud Dextrose Agar and then incubating for 24-28 hours at 37°C and 5 days at room temperature for bacteria and fungi respectively.

In order to assess the degree of contamination, 1g of material was dispersed in 4ml Ringer solution containing 0.25% Tween 80, further dilutions were made by transferring 1ml of the solution into 4ml of sterile Ringer solution (until about 5 dilutions were made), 1ml each from the dilutions was aseptically transferred into 19mls of nutrient agar and then poured into sterile plates. The plates were incubated at 37°C for 24-28 hours. The emerging colonies were counted and recorded as the total viable count. Attempts were made using appropriate biochemical tests to carry out identifications of possible contaminants. All operations were carried out in duplicates.

Challenge Test for Preservative Capacity

Staphylococcus aureus, *Pseudomonias aeruginosa* and *Candida albicans* regarded as common contaminants were used as challenge inocula. An overnight broth culture of the three organisms were prepared using 0.1% peptone water as diluent, compared with 0.5Mc Farland standard containing 1×10^8 organisms. The preparations above were diluted to achieve a population of 1×10^3 for bacteria and 1×10^2 for mould, the population that is regarded as the official specified limits for both bacteria and mould respectively (BP, 1993). 20g of each cream samples were aseptically weighed into 50mls sterile conical flasks, challenged with 1ml suspension of organisms, swirled and stoppered with tight cotton wool plugs.

At days 1, 7, 14 and 28, 1g of each cream sample was weighed into a sterile tube; 4mls of ringer solution containing 0.25%v/v tween 80 was added. 1ml of the resulting mixture was transferred into another sterile tube containing 4mls ringer solution with 0.25% tween 80. Further dilutions were made until 10 dilutions.

1ml of the suspension was transferred each from the last four dilutions and aseptically transferred to 19mls of sterile Sabouraud Dextrose Agar, Nutrient Agar, Centrimide Agar and Mannitol Salt Agar respectively, swirled and poured into sterile plates. The plates were incubated at 37°C for 24 hours (for bacteria), ambient room temperature for fungi. All samples were plated in duplicates. Emerging colonies were counted and recorded.

Results and Discussions

Results are as presented in the tables and figures.

Qualitative tests showed that aqueous cream (P01) without preservative exhibited microbial and fungal contamination whereas the formulations with parabens and chlorocresol preservative (P02 & P03) gave no growth of either bacteria or fungi. On the other hand, oily creams without preservative (P04) and the formulation preserved with chlorocresol (P06) had both bacteria and fungal growth. Table 1 gave quantitative bacterial and fungal counts. Various biochemical identification screening of the contaminants revealed that *Proteus spp.* and *Aspergillus fumigatus* were suspect contaminant in aqueous cream without preservative. The organisms implicated in oily cream preparation without preservative (P04) and the one preserved with chlorocresol (P06) were *Proteus spp.* and *Candida albicans*.

A cream with reliable preservative capacity is one that is capable of inhibiting immediate post-production contaminants as well as subsequent low inocula of in-use contaminants, and thereby maintain acceptable low levels of microorganisms in the preparation. The above revelations indicated that parabens and chlorocresol were able to preserve aqueous cream (P02 and P03) at least at immediate post production. This is not so with oily cream formulated with chlorocresol (P06). This observation tends to be in agreement with a report in British Pharmaceutical Codex, 1979 which states that the effectiveness of chlorocresol is reduced if oils, fats and non-ionic surfactants are present together of which oily cream in this study is a reflection of these agents.

The results of the challenge test on the formulations prepared unsterile is as shown in Table 2 and Figures 1A-1B, 2A-2B and 3A-3B respectively. The cream formulations were stored at ambient temperature for 28 days. Challenge tests that are derived from laboratory prepared-sterile creams cannot closely simulate real situations that are normally encountered by the preparation (Hugbo *et. al*, 2003). This explains the methodology of preparation stated above.

The log survival count of both the aqueous and oily cream formulations without preservative (P01 and P04) initially challenged with 1×10^3 for bacteria and 1×10^2 for fungi regarded as limits allowable in unsterile topical preparations particularly for non-pathogenic organisms were found to be on

the increase as can be seen in Table 2 and Figures 1A-1B, 2A-2B and 3A-3B respectively. This is so for all organisms used in this study.

However, the aqueous cream samples preserved with parabens (P02) showed an initial decline in bacterial counts (*Pseudomonas aeruginosa* and *Stapp. aureus*) for the first 7 days, only to be followed by a resurgence of growth thereafter, though at a much reduced rate. The parabens however, are very active against fungi as it shows continuous reduction in the number of microorganisms throughout the 30 days. Parabens are reliable semi-solid preservatives as they possess most of the attributes desired of an ideal preservative. However, they lack broad spectrum of activity since they are more active against fungi (Lynn and Hugo, 1981).

Aqueous cream sample preserved with chlorocresol (P03) revealed a steady reduction in the number of viable microorganism (for all organisms used in this study) throughout the 28 days as can be seen in Table 2 and Figures 1A-1B, 2A-2B and 3A-3B. This shows that, chlorocresol has a broad spectrum of activity against both bacteria and fungi. Chlorocresol is however a phenol and possesses fairly high dilution co-efficients, it therefore may not exhibit persistent activity in the presence of extraneous matter or upon several dilutions (McDonell and Russell, 1999).

Oily cream formulations preserved with parabens and chlorocresol preservatives respectively (P05 and P06) showed a reduction in both bacteria (*Pseudomonas aeruginosa* and *Staphylococcus aureus*) and fungi (*Candida albicans*) as can be seen in Table 2 and Figures 1A-1B, 2A-2B and 3A-3B. The reduction rate was much lower with chlorocresol as preservative particularly as against *Staphylococcus aureus*. This probably could be due to the fact that efficiency of chlorocresol is reduced particularly in oily medium as explained earlier on.

To be able to draw reliable inference from the data gathered in this study which is quantitative in nature, the data was further subjected to statistical analysis using One-way ANOVA in order to establish if the preservatives used had significant reduction effect on the microbial inocula over a four-week period. Student t-test for independent variables (Unpaired, two-tailed) was used to determine if there was significant difference in the preservative capacity of the two preservatives used for both oily and aqueous creams respectively. The probability of committing type I error 'p' in rejecting null

hypothesis of no significant reduction effect on the microbial inocula over a four-week period was less than 0.05 (i.e. $P < 0.05$). This follows that the data is consistent with alternative hypothesis of significant reduction effect. On the other hand, p-value for student t-test was also less than 0.05 ($P < 0.05$) indicating difference in the reduction rate of the two preservatives studied.

Conclusion

From the foregoing, one is able to infer that both parabens and chlorocresol had significant preservative capacity to be able to bring about acceptable low levels of microbial contamination as demanded by regulatory books (British Pharmacopoeia, 1993). The choice of the preservative to be adopted may be determined by the nature of particular topical formulation. Chlorocresol appear to have reduced capacity as a bacterial preservative in oily cream formulations than the aqueous cream preparation. The overall picture is that chlorocresol tends to have greater preservative effectiveness than parabens in aqueous cream formulation both for bacteria and fungi and for fungi organisms in oily cream formulation. It is however recommended that other alternative antimicrobial agents that may be superior to parabens or chlorocresol in terms of safety and efficiency should be explored.

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Table 1: Microbial Counts (CFU/ml) and Types Found in Cream Preparations

Preparations	Bacteria		Fungi	
	Counts	Types	Counts	Types
P01	6.5×10^{-8}	<i>Proteus spp.</i>	2.5×10^{-8}	<i>Asp. Fumigatus</i>
P02	Negative	-	Negative	-
P03	Negative	-	Negative	-
P04	1.5×10^{-9}	<i>Proteus spp.</i>	2.5×10^{-9}	<i>Candida albicans</i>
P05	Negative	-	Negative	-
P06	1.0×10^{-9}	<i>Proteus spp.</i>	2.0×10^{-9}	<i>Candida albicans</i>

Key:

- P01 = aqueous cream without preservatives
 P02 = aqueous cream with parabens
 P03 = aqueous cream with chlorocresol
 P04 = oily cream without preservative
 P05 = oily cream with parabens
 P06 = oily cream with chlorocresol

Table 2: Challenge Test Preservative Capacity

Samples	<i>Staph aureus</i>	<i>Pseudo aeru</i>	<i>C. albi</i>
<u>1ST DAY</u>			
P01	2.87 x 10 ²	1.82 x 10 ²	5.2 x 10 ¹
P02	7.8 x 10 ¹	8.0 x 10 ¹	3.4 x 10 ¹
P03	6.1 x 10 ¹	5.0 x 10 ¹	3.0 x 10 ¹
P04	2.8 x 10 ¹	1.2 x 10 ¹	4.0 x 10 ¹
P05	1.3 x 10 ¹	0.8 x 10 ¹	1.8 x 10 ¹
P06	2.4 x 10 ¹	1.0 x 10 ¹	3.5 x 10 ¹
<u>7TH DAY</u>			
P01	3.52 x 10 ²	2.20 x 10 ²	6.5 x 10 ¹
P02	4.0 x 10 ¹	5.9 x 10 ¹	2.8 x 10 ¹
P03	6.1 x 10 ¹	2.0 x 10 ¹	2.4 x 10 ¹
P04	3.1 x 10 ¹	1.5 x 10 ¹	4.5 x 10 ¹
P05	1.1 x 10 ¹	0.3 x 10 ¹	1.5 x 10 ¹
P06	2.2 x 10 ¹	0.5 x 10 ¹	3.2 x 10 ¹
<u>14TH DAY</u>			
P01	4.05 x 10 ²	2.50 x 10 ²	7.0 x 10 ¹
P02	4.2 x 10 ¹	6.2 x 10 ¹	0.7 x 10 ¹
P03	1.9 x 10 ¹	0.1 x 10 ¹	0.6 x 10 ¹
P04	4.5 x 10 ¹	1.6 x 10 ¹	7.0 x 10 ¹
P05	0.8 x 10 ¹	Negative	1.0 x 10 ¹
P06	1.9 x 10 ¹	Negative	2.7 x 10 ¹
<u>28TH DAY</u>			
P01	4.57 x 10 ²	2.77 x 10 ²	8.2 x 10 ¹
P02	5.0 x 10 ¹	6.6 x 10 ¹	Negative
P03	0.2 x 10 ¹	Negative	-
P04	5.3 x 10 ¹	1.9 x 10 ¹	9.5 x 10 ¹
P05	0.6 x 10 ¹	Negative	0.8 x 10 ¹
P06	1.3 x 10 ¹	Negative	2.5 x 10 ¹

Fig 1A: Log survival of *Staph aureus* in aqueous cream (PO1-PO3)

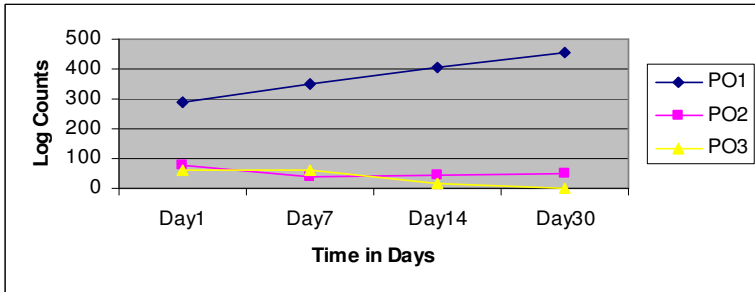


Fig 1B: Log survival of *Staph aureus* in oily cream (PO4-PO6)

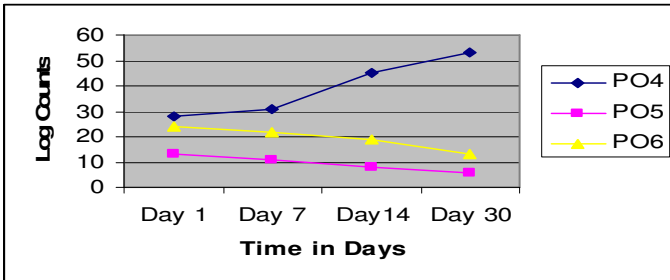


Fig 2A: Log survival of *Pseudo aeruginosa* in aqueous cream (PO1-PO3)

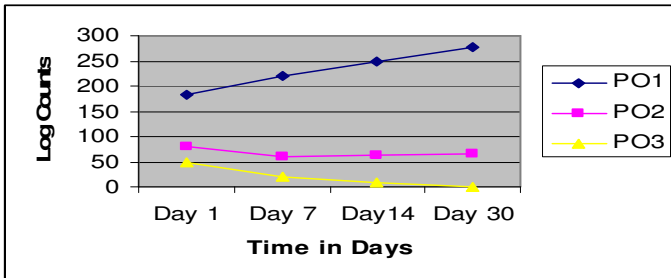


Fig 2B: Log survival of Pseudo aeruginosa in oily cream (PO4-PO6)

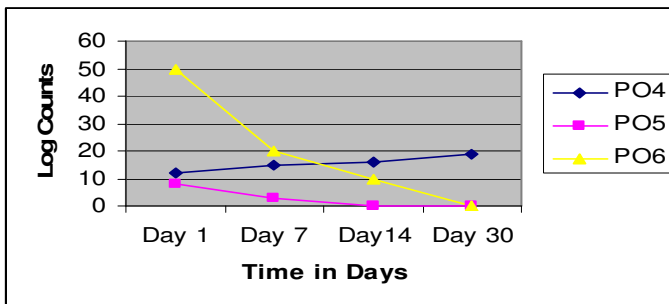


Fig 3A: Log survival of Candida albicans in aqueous cream (PO1-P03)

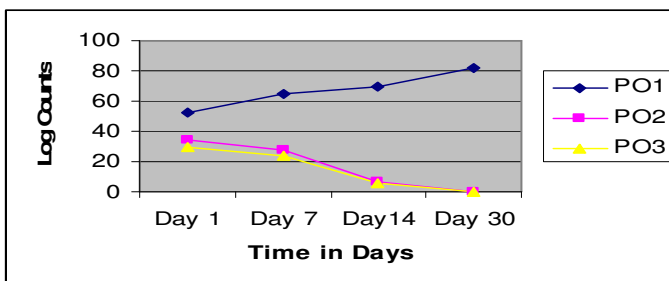


Fig 3B: Log survival of Candida albicans in oily cream (PO4-PO6)

