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Evaluation of Preserving Efficacy for Different Cough Syrups Manufactured by Different Pharmaceutical Companies

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Abstract: The aim of the current investigation is to assess the efficacy of different preservatives ingredients of different expectorant cough syrups manufactured by different pharmaceutical companies by comparing the growth of five microorganisms of known quanta of *S. aureus*, *E. coli*, *P. aeruginosa* and *C. albicans*. The microorganisms were inoculated into syrup A (glycerol and propylene glycol), syrup B (propylene glycol and glycerin), syrup C (glycerin, propylene glycol and butyl paraben), syrup D (methyl paraben and probylparaben) and normal saline as a control. All microorganisms were taken from standard stock cultures and incubated for 24 h. Growth of microorganisms into syrup was compared by counting the CFUs from a subculture of inoculated syrup at zero, 3, 6, 12, 24 and 48 h intervals. The data showed that all the combinations of the preservatives in the four studied cough syrups behaved similarly in term of antimicrobial efficiency. The findings suggested that the preservatives mixtures of propylene glycol with glycerol or with glycerin or with butyl paraben preservatives as well as methyl paraben with probylparaben are acceptable clinically and have considerably antimicrobial activity against infectious bacteria during the 48 h studied period.

Key words: Antimicrobial, preservative efficacy, CFU, syrups

INTRODUCTION

Microorganisms are present in the air that we breathe, the food that we eat and the water we drink and medicines we use unless specific measures are adopted to exclude them (Aulton, 2002). Although, some types of pharmaceutical products for example ophthalmic and injectable preparations are sterilized by physical methods (autoclaving for 20 min at 15 pounds pressure and 121°C, dry heat at 180°C or bacterial filtration) many of them also require an antimicrobial preservative to maintain their aseptic condition throughout storage and use. Other types of preparations those are not sterilized during their preparation but are particularly susceptible to microbial growth. Natures of their ingredients are protected by the addition of antimicrobial preservative. Preparations that provide excellent growth media for microbes are most aqueous preparations such as syrups (Allen *et al*., 2005).

Syrups are concentrated solutions of a sugar such as sucrose in water or other aqueous liquids. Flavored and medicated syrups are the preferred dosage form of choice for both children and adults because they contain no or very little alcohol. Patients frequently use multidose syrups and most of the syrups are sold over the counter since the active ingredients are usually not potent, for this reason manufacturers add preservatives to prevent accidental contamination to opened bottles. Preservatives are widely employed in the cosmetics and pharmaceutical industries as well as in a variety of other manufacturing industries (Hugo and Russells, 2005). The preservative system includes both ingredients with known antimicrobial activity and ingredients formula that may contribute directly or indirectly to antimicrobial activity (Spiegeleer *et al*., 2006). Antimicrobial preservatives work by reducing the numbers of organisms and inhibiting the growth of microorganisms that may be introduced during repeated use accidently (Rosenthall *et al*., 2006). The amount of a preservative varies with the proportion of water available for growth, the nature and inherent preservative activity of some formulative materials and the capability of the preservative itself. Among the preservatives commonly used in syrups with the usually effective concentrations are benzoic acid 0.1-0.2%, sodium benzoate 0.1-0.2% and various combinations of methylparabens, propylparabens and butylparabens. Frequently alcohols are used in syrups to dissolve

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alcohol-soluble ingredients but it could act as preservative if is concentration exceeds 15% (Aulton, 2002). The activity of pure preservative must be evaluated using appropriate *in vitro* test, in addition to that other sort of challenge test involving final product is required to be done for determination of the preservative efficacy (Hugo and Russells, 2005). The final product is deliberately inoculated with a suitable microorganisms which may be fungal e.g., *Candida* or bacterial e.g., *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeroginosa.* (Hugo and Russells, 2005). The level of contamination is then monitored at several time intervals (Hugo and Russells, 2005; Spiegeleer *et al*., 2006; Rosenthall *et al*., 2006; Cremieux *et al*., 2005; Souza and Ohara, 2003).

The aim of this study is to evaluate different preservatives (efficacy of preservatives) in different cough and expectorant syrups manufactured by different pharmaceutical companies.

MATERIAL AND METHODS

The methodology of this study was familiar to that followed by Wachowski *et al.* (1999) and Crowther *et al.* (1996). The experimental study has been done during 3 months, April-June 2009. It was conducted at the Department of Biotechnology, Faculty of Science at Philadelphia University. Overnight cultures of *S. aureus* (ATCC 259231), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853) and *C. albicans* (ATCC 14053) (gifted by Dr. A. Shehabi, faculty of medicine, Jordan University) were diluted to a density of 0.5 McFarland units with 0.9% sterile nonbacteriostatic saline using spectrophotometer (Cecil, England). Each organism solution was further diluted 1:50 with sterile 0.9% saline. Aliquots of 0.2 mL of each diluted organism were then added to sterile sealed culture vials containing 20 mL of the following solutions: three vials of syrup (A) A (glycerol and propylene glycol); three vials of syrup (B) (propylene glycol and glycerin); three vials of syrup (C) (glycerin, propylene glycol and butyl paraben); three vials of syrup D (methyl paraben and probylparaben) and three vials of 0.9% nonbacteriostatic saline as a control. Each organism solution was vortexed before addition to the 20 mL vials. After the organisms were added, each vial was vortexed for 1 min and subplated to three plates of Trypticase Soy Agar (TSA). Vials were subplated out at 0, 3, 6, 12, 24 and 48 h intervals for a total of nine plates per solution per sampling period and stored at 20°C between samplings. The plates were then incubated at 37°C for 24 h. Each plated medium was read and numbers of Colony Forming Units (CFUs) were counted and recorded using colony counter (Galaxy 230, USA). For each microorganism, the number of CFUs per plate was averaged for each studied period. Data are presented as the mean of nine replicate assays. A probability of $p = 0.05$ was taken to indicate statistical significance.

RESULTS

As for *S. aureus* (Fig. 1), a significant decline after 3 h in CFUs in 0.9% saline, syrups A and D was observed compared with baseline (zero time). The inoculated syrups B and C showed no significant growth of *S. aureus* during the 48 h study period. Nonetheless, the mean of CFUs of *S. aureus* in syrup D was significantly greater at zero time compared with the saline and the remaining syrups. While propylene glycol with different combination additives in syrups A, B and C reduced the mean of CFUs, while the methyl paraben and probylparaben in syrup D enhanced the formation of *S. aureus* CFUs at zero time.

As for *E. coli* (Fig. 2) in 0.9 % saline, syrups A and C which inoculated with *E. coli* showed significant reduction in the mean CFUs after zero time. On the other hand, *E. coli* inoculated syrups B and D showed no growth during the study time period compared with baseline. The syrups B and D which consist of propylene glycol and glycerin and the methyl paraben and

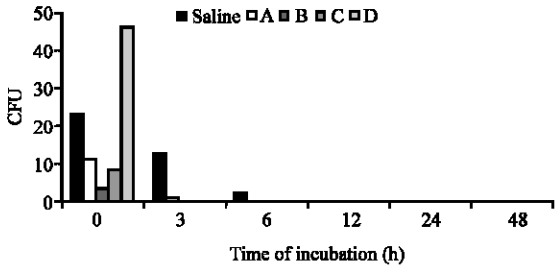

Fig. 1: Number of colony forming units of *S. aureus* counted versus time after inoculation in various cough syrups

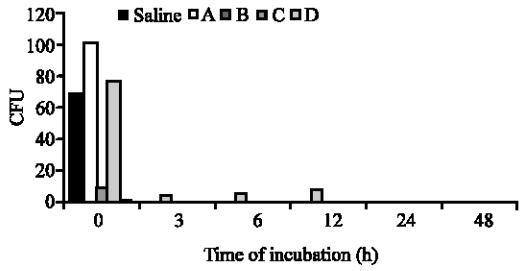

Fig. 2: Number of colony forming units of *E. coli* counted versus time after inoculation in various cough syrups

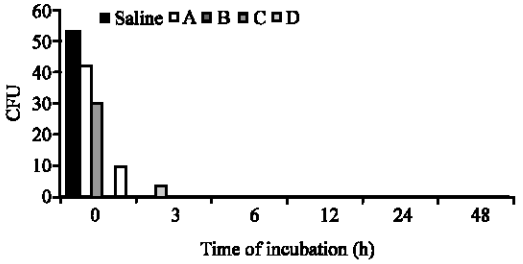

Fig. 3: Number of colony forming units of *P. aeruginosa* counted versus time after inoculation in various cough syrups

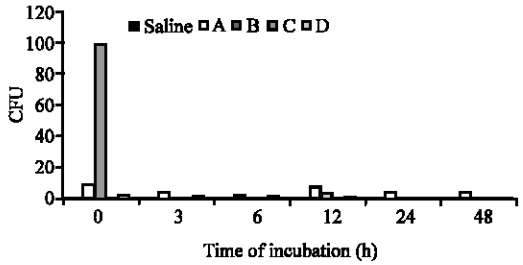

Fig. 4: Number of colony forming units of *C. albicans* counted versus time after inoculation in various cough syrups

probylparaben combination, respectively, suppressed the growth of *E. coli* during the study period. As for *P. aeroginosa* (Fig. 3) in 0.9% saline, syrups A and B, a significant rapid reduction in CFUs was observed at 3, 6, 12, 24 and 48 h. The inoculated syrup D showed no significant changes in the mean of CFUs compared to baseline. *P. aeruginosa*, inoculated syrup C which consists of propylene glycol and butyl paraben combinations, suppressed completely the formation CFUs compared to Syrups A and B. Both did not show inhibition for the CFUs formation by *P. aeruginos* at zero time.

As for *C. albicans* (Fig. 4), static levels were observed in syrups A, B and D during the study period. The 0.9% saline and syrup C which consists of propylene glycol and butyl paraben, however, substantially supressed the formation of CFUs at 0 time and ongoing period time of study). A maximum growth of CFUs at baseline was observed when *C. albicans* inoculated in syrup B which consists of propylene glycol and glycerin combination and which then showed significant reduction at 3, 6, 12, 24 and 48 h. The combination of both propylene glycol, sorbitol and sucrose combination in syrup A therefore, suppressed the growth of *C. albicans* compared to syrup B which consists of propylene glycol and glycerin combination.

DISCUSSION

Development of a pharmaceutical product, those are subjected to microbial contamination, specific efficacy of preservatives are added (EMEA, 1998, 2003). Preservatives efficacy testing is based on a sample inoculation using a microbial suspension with a determined amount of Colony Forming Unit (CFU). The current study is dealing with growth of *S. aureus*, *E. coli*, *P. aeruginosa* and *C. albicans* inoculated into 4 different cough syrups manufactured by local different pharmaceutical companies and which consist of different combinations of preservatives; glycerol with propylene glycol (syrup A), propylene glycol with glycerin (syrup B), glycerin with propelyne glycol and butyl paraben (syrup C), methyl paraben with probylparaben (syrup D). The propylene glycol in combination with glycerol (syrup A) or with glycerin (syrup B) or butyl paraben (syrup C) showed slight growth of *S. aureus* at zero time compared to methyl paraben and probylparaben in syrup D which showed significant growth of *S. aureus* CFUs at 0 time. Moreover, propelyne glycol with butyl paraben enhanced the growth of *E. coli* and suppressed *C. albicans* growing compared to propylene glycol and glycerin which in turn have the opposite action. Enhancement of *C. albicans* and inhibition of *E. coli* growth have recorded. Nevertheless with *P. aeruginosa* growth was the maximum in propelyne glycol in glycerol or glycerin, while *P. aeruginosa* growing was completely suppressed with propylene glycol and butyl paraben. The methyl paraben and probylparaben (syrup D) enhanced the formation of *S. aureus* CFUs at zero time. While suppressed completely the *E. coli* growth, *C. albicans* and slight growing of *P. aeruginosa*.

In conclusion the significant of this study is summarizing the preservatives mixtures of propylene glycol with glycerol (syrup A) or with glycerin (syrup B) or with butyl paraben (syrup C) preservatives and the methyl paraben with probylparaben (syrup D) which they behaved similarly by resisting growth of the microorganisms studied over the 48 h. Therefore, all are acceptable clinically of being have considerably antimicrobial activity against infectious bacteria. Ingredients mixtures of preservatives which offer antimicrobial protection to the patient and therefore used as a means of decreasing the risk of acquired infectious.

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