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An *In Vitro* Study on the Effect Of Human Tear Fractions Against the Human Pathogens

KEYWORDS: Tears, Ion- exchange chromatography, Disc diffusion method, Microorganisms

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ABSTRACT *The present study was designed to evaluate the antimicrobial activity of human tears against the human pathogens using disc diffusion method. The tears were effective with maximum and minimum zone of inhibition of 3 mm and 1 mm against Bacillus subtilis and Proteus mirabilis respectively. E. coli, P. aeruginosa and Candida albicans were found to be resistant to human tears. Commercially available lysozyme was also found to be effective against Bacillus subtilis and Proteus mirabilis only with maximum and minimum zone of inhibition of 5 mm and 2 mm respectively. This investigation clearly indicates about the use of enzymes present in tears for the treatment of infectious diseases especially caused by Gram (+) bacteria.*

INTRODUCTION

Tears provide a mechanical defense via flushing of foreign particles from the surface of the eye and transporting antimicrobial agents to the surface as defensive measures (1). Lacrimation is the process of tears secretion, which serves to clean and lubricate the eyes. Tear composition varies from tear types. Mainly, tears are composed of water, salts, antibodies and lysozymes (antibacterial enzymes). The composition of tears is proteins (lysozyme, lipocalin and lactoferrin), enzymes, lipids, metabolites and electrolytes. The protein concentration differs between emotional tears and the tears produced by irritants. The complex composition of tears, though, differs throughout the daily cycle of human existence. Tear fluid contains water, mucin, lipids, lysozyme, lactoferrin, lipocalin, lacritin, immunoglobulins, glucose, urea, sodium, and potassium. Some of the substances in lacrimal fluid fight against bacterial infection as a part of the immune system. Human tears contain a variety of proteins involved in the defense against invading micro-organisms. One of the proteins in nonspecific defense, lactoferrin, was first described in human tears by Masson *et al* (2). *Pseudomonas aeruginosa* is a gram-negative opportunistic bacterium capable of causing severe corneal infection. There are at least two types of *P. aeruginosa* isolated from infected corneas; those that invade corneal epithelial cells (3). *Escherichia coli* is a gram-negative rod that is found as a normal commensal in the GI tract, which can produce ocular infection including corneal ulcer and endophthalmitis, which can result in a devastating outcome. Early recognition and appropriate treatment is crucial. *Proteus mirabilis causes* Post-keratoplasty endophthalmitis (4). *Candida spp.* is the most common cause of endogenous endophthalmitis, although initial infection with the dimorphic fungi may lead to infection and scarring of the chorioretina (5). The present study was investigated to determine the antimicrobial activity of human tears fractions, obtained from Ion- exchange chromatography, against human pathogens. The inhibitory action of tears was also compared with the effect of commercially available lysozyme against the microbes tested.

MATERIALS AND METHODS

Tear collection

Reflex tears were collected from four female volunteers at the age between 18-20 years. The selection of the female donors was based upon the following criteria like they are not using spectacles, they have not ever gone for any eye treatments and they are not using any eye cosmetics. The donors face was washed with water and the area below the eyelid was wiped with 95% ethanol before collecting the sample. Around 500 µl of tears were collected from each volunteer using microcapillary tubes by exposing their eyes to onion vapours over approximately 30 minutes. Tears were stored at -20°C for further use.

Collection of tear fractions using Ion- exchange chromatography technique

The empty column was washed with hot water (90°C). Top cap of the already packed column with CM-Cellulose was removed. The bottom cap was removed and the column was equilibrated with 50ml of 1 X Equilibration buffer. Tear sample and sterile distilled water were added in equal proportion. It was mixed in the tube provided for 10 minutes to get a homogenous solution. The pH of the tear was adjusted to 7.0 by slowly adding neutralizing solution. Now the tear sample was loaded to the column for equilibration. Top and bottom caps of the column were replaced and incubated for an hour at room temperature with intermittent mixing. After an hour the column material was allowed to settle. Slowly the supernatant of the tube was pipette out without disturbing the column. Now the column was washed with 50 ml of 1 X Wash buffer. The elutes were collected in test tube as 2 ml fractions**.** Total five fractions were collected**.**

Optical density determination of tear fractions

The optical density of each fraction was noted at A_{280} nm using UV- Spectrophotometer.

Preparation of commercially available lysozyme powder solution

Commercially available lysozyme powder was mixed vigorously in sterile distilled water as a concentration of 0.1 gm/ml.

Microorganism of interest

E.coli, Bacillus subtilis, Pseudomonas aeruginosa, Proteus mirabilis and *Candida albicans* were obtained from Department of Biotechnology, Faculty of Science and Humanities, SRM University, Chennai. The microbes were sub-cultured and were preserved at 4°C for further use.

Antimicrobial susceptibility test of tear fractions and commercially available lysozyme

The collected different fractions of tears were subjected to antimicrobial assay by Agar disc diffusion method using Mueller Hinton Agar (MHA) plates. Potato Dextrose Agar (PDA) plates were used for *Candida albicans.* 25 µl of fractions were

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loaded in sterile agar plates. The plates were incubated at 37°C for 24 hours. The inhibitory activity was observed by measuring zone of inhibition developed on the plates. The susceptibility test of tear fractions was also compared to the inhibitory activity of commercially available lysozyme against the same microbes. The diameter of zone of inhibition was measured in millimeter.

RESULT AND DISCUSSION

Optical density (OD) of tear fractions was measured at 280 nm using UV- Spectrophotometer. Maximum value (1.97) was obtained in fraction number 2 (Table-2). The antimicrobial activity of tear fractions was determined using disc diffusion method. *Bacillus subtilis* was found to be more susceptible to tear fractions with maximum zone of inhibition of 3 mm. The tear fractions were less effective against *Proteus mirabilis* with 1 mm of zone of inhibition. Other microbes were found to be resistant to the tears. Maximum inhibitory activity of tear fractions against *Bacillus subtilis* and *Proteus mirabilis* was obtained from fraction number 2. Commercially available lysozyme powder was also found to be effective against *Bacillus subtilis* and *Proteus mirabilis* only. But the activity of lysozyme powder was more compared to the tear fractions against the same microbes. Commercially available lysozyme powder was showing maximum zone of inhibition of 5 mm against *Bacillus subtilis*. *E.coli*, *Psuedomonas aeruginosa* and *Candida albicans* were also resistant to commercially available lysozyme powder (Table- 1 and Fig.- a). Exposure of the eye directly to the environment renders it vulnerable to a number of uncommon infectious diseases caused by bacteria, fungi and parasites. Host defenses directed against these microorganisms, once anatomical barriers are breached, are often insufficient to prevent loss of vision. Therefore, the timely identification and treatment of the involved microorganisms are paramount. Xiao-Dan Qu *et al* (6) reported that enzymes present in tears were responsible for inhibiting the growth of Gram (+) bacteria but the enzymes lacked bactericidal activity against Gram (-) bacteria. In our investigation the tear fractions were also found to be effective against *Bacillus subtilis* and ineffective against Gram (-) bacteria. The bactericidal activity of human tears against the Gram (+) bacteria may be due to the composition of bacterial cell wall. Our finding favours the work of Fleming (7) and Selsted *et al* (8) who demonstrated that human tear lysozyme hydrolyzes the glycosidic linkages in the polysaccharide components of the cell wall and kills *Micrococcus leisodeikticus* and *Bacillus subtilis*. Less activity of the tear fraction may be due to the reason that the inhibitory activity of tears depends upon the age and sex of the person.

CONCLUSION

The data collected in this study suggest that effect of tear on the microbes may be complex mechanism. Studies aimed at inhibitory effects of tears on human pathogens are likely to improve our understanding about the infection and what circumstances predispose to infectious disease. Furthermore, the identification of contributing tear factors based on age and sex of the individual could eventually lead to new approaches to the prevention and treatment of infections.

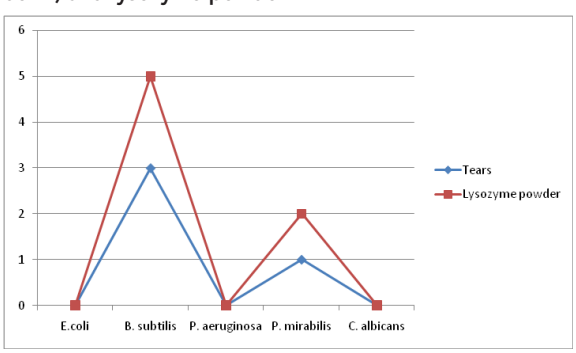
TABLE: 1- Antimicrobial activity of tears (fraction number 2) and commercially available lysozyme (in mm)

Microorganisms	Tears (Fraction number 2)	Lysozyme powder
E. coli	-	-
Bacillus subtilis	3 mm	5 mm
Pseudomonas aeruginosa	-	-
Proteus mirabilis	1 mm	2 mm
Candida albicans	-	-

TABLE: 2- Determination of optical density of tear fractions

Tear fractions	Optical density at 280 nm
Fraction 1	1.934
Fraction 2	1.977
Fraction 3	1.933
Fraction 4	1.933
Fraction 5	1.941

Fig: (a)- Antimicrobial activity of human tear (fraction number 2) and lysozyme powder



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