

In Vitro Assessment of Different Concentration of Allopathic and Ayurvedic Medicinal Preparation on Microbial Inhabitants of Eye

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Abstract: Present study is an *in vitro* assessment of the effect of both allopathic and ayurvedic medicinal preparations on the commonly inhabiting bacteria in eyes. The selected ayurvedic medicines were Elaneer Kuzhambu and Himalaya Ophthacare eye drops; allopathic medicines were Ciplox and Catina. For concentration study four different eye drops were taken in three ratios pure form, 1:1 and 1:2. In pure form the eye drops are used directly. For each ocular medicine their 3 different concentrations with three replicas were obtained. By disc diffusion method these eye drops produced inhibition zones on the swab culture of ocular micro flora after 24 hours of incubation. The inhibition zone for each eye drops varied in diameter. The inhibition zone indicates the destruction of microbial population in the culture by the activity of the eye drops. As per the results from the present investigation, among allopathic eye drops, Ciplox produced maximum zone of inhibition than Catina. Among ayurvedic eye drops, Himalaya produced maximum zone of inhibition than Elaneer Kuzhambu.

Key words: Allopathic and ayurvedic ear drops, Catina, Ciplox, Himalaya, Elaneer Kuzhambu

1. INTRODUCTION

The human micro biota is the aggregate of microorganisms that reside on the surface and in deep layers of skin, in the saliva and oral mucosa, in the conjunctiva, and in the gastrointestinal tracts (Lee *et al.*, 1989). Eye is one of the sense organ which is important throughout our life. It has been said that, the eyes are window to the world. The external ocular surface acquires a microbial flora at birth and some of the commensals may become resident flora in the conjunctiva and lids and have a potential to turn into pathogens. Apart from the resident flora any microorganism from the environment can form a transient flora in the eye and given an opportunity invades the ocular tissues. Under normal circumstances the conjunctiva and eyelids support a population of bacteria (Stephen *et al.*, 2008).

Normal ocular flora is diverse. They are similar to those found in the upper respiratory tract and on the skin (Osato, 1996). Individual microorganisms within the ocular flora interact with each other as well as with defense mechanisms of the eye and immune system. Tears function as one such antimicrobial defense—they contain the antimicrobial enzyme lysozyme, and also act together with the mechanical action of the eyelids in washing away pathogens. Under normal conditions, this results in a balance preventing the overgrowth of a particular microorganism and therefore infection. Bacteria are the most common agents causing external ocular infections, including conjunctivitis, blepharitis, keratitis, dacryocystitis and orbital cellulitis. They are responsible for 70 – 80% of conjunctival morbidity which poses a huge socio-economic burden to the general public (Epling *et al.*, 2012).

Ciprofloxacin is in a class of antibiotics called fluoroquinolones. It is used to treat bacterial infections of the eye including conjunctivitis and corneal ulcers. Ophthacare is a herbal eye drop preparation containing basic principles of different herbs viz *Carum copticum*, *Terminalia bellerica*, *Curcuma longa*, *Ocimum sanctum*, *Cinnamomum camphora*, *Rosa damascene*, and *Melde spumapum*. In most cases improvement was observed with the treatment of the herbal eye drop (Burkil, 1966). Honey is an anti-inflammatory agent which soothes the eyes and heals wound quickly.

2. MATERIALS AND METHODS

The present study aimed to analyze the *in vitro* assessment of different concentration of natural and artificial medicines on microbial inhabitants of eye. The selected ayurvedic medicines are Elaneer Kuzhambu, Himalaya Ophthacare eye drops. The allopathic medicines are Ciplox and Catina.

CONTENTS OF MATERIALS USED

1. Soyabean Casein Digest Medium (Tryptone soya Broth)

INGREDIENTS

Pancreatic digest of casein	17.00 gm/l
Papaic digest of soyabean meal	3.00 gm/l
Sodium chloride	5.00 gm/l
Dipotassium hydrogen phosphate	2.50 gm/l
Dextrose (Glucose)	2.50 gm/l

2. Nutrient HiVeg Agar

INGREDIENTS

HiVeg peptone	10.00 gm/l
HiVeg extract	10.00 gm/l
Sodium chloride	5.00 gm/l
Agar	15.00 gm/l

3. Ciplox Eye Drops

CONTENTS

Ciprofloxacin Hydrochloride IP	0.3% w/v
Benzalkonium chloride IP	0.01% w/v
Aqueous vehicle	q.s

4. Catina Eye Drops

CONTENTS

Potassium Iodide I.P.	3.3% w/v
Calcium Chloride Anhydrous I.P.	1.05% w/v
Sodium Chloride I.P.	0.83% w/v
Methyl Hydroxy Benzoate I.P.	0.023% w/v
Propyl Hydroxy Benzoate I.P.	0.011% w/v
Sterile aqueous base	q.s.

5. Himalaya Ophthacare Eye Drop

CONTENTS

Madhu (Purified Honey)	37.00% w/v
Amalaki (<i>Embllica officinalis</i>)	13.00% w/v
Haridra (<i>Curcuma longa</i>)	13.00% w/v
Vishnupriya (<i>Ocimum sanctum</i>)	13.00% w/v
Satapatri (<i>Rosa damascena</i>)	11.00% w/v
Vibhitaki (<i>Terminalia bellerica</i>)	6.50% w/v
Yawani (<i>Carcum copitum</i>)	6.00% w/v
Karpoora (<i>Cinnamomum camphora</i>)	0.50% w/v

6. Elaneer Kuzhambu

CONTENTS

Amalaki (<i>Emblica officinalis</i>)	96 g
Darvi (<i>Berberis aristata</i>)	96 g
Haritaki (<i>Terminalia chebula</i>)	96 g
Vibhitaki (<i>Terminalia bellirica</i>)	96 g
Madhuka (<i>Glycyrrhiza glabra</i>)	144 g
Coconut water	8.172 liters

CONCENTRATION STUDY

For concentration study four different eye drops were taken in three ratios pure form, 1:1 and 1:2. In pure form the eye drops are used directly. In 1:1 ratio 1 ml of eye drop and 1 ml of distilled water was taken. For 1:2 ratio 1 ml of eye drop and mixed with 4 ml of distilled water. The four different eye drops: Ciplox, Catina, Himalaya Ophthacare and Elaneer Kuzhambu are made to the above mentioned concentration.

ANTIBACTERIAL SUSCEPTIBILITY TEST DISC

Test discs of 7 mm diameter were made with Whatman No.1 filter paper and were sterilized in hot air oven at 160°C for one hour. For preparation of test discs with medicines, the different concentration of allopathic and ayurvedic medicines are taken in a sterilized containers and the pre-sterilized test discs were soaked in the medicines for 10-20 minutes and allowed to dry for preventing unwanted spreading of the content on bacterial lawn.

PRE ENRICHMENT OF TEST MICROBE

Human ocular micro flora were obtained through swab collection from the ocular surfaces and enriched in Tryptone Soya Bean Agar with incubation at 37°C for 24 hours.

CULTURE MEDIA PREPARATION

The culture media used was 3.8 g of Nutrient HiVeg Agar was dissolved in 100 ml distilled water, boiled on a hot plate, to mix evenly, cotton plugged and sterilized in an autoclave at 121°C and 15 lb/in² pressure for 15 minutes.

ANTIBACTERIAL SUSCEPTIBLY TEST

The antibacterial assay was carried out by using disc diffusion method, also known as Kirby- Bauer method performed in laminar air flow. Using a sterilized cotton swab, heterogeneous carpet culture of ocular bacteria was made on the Nutrient HiVeg Agar plate with pre enriched inoculums. Antibacterial test discs were placed at sufficiently distances from each other in order to avoid the mixing of inhibition zone. Three replicas were also made with each test disc to minimize sampling error. Culture plates were then kept in incubator at 37°C for 24 hours. After 24 hours of incubation, the culture plates were observed to check antibacterial activity of selected eye drops against the bacterial species. Inhibition zone diameters (IZDs) were measures using standard scale in nearest mm.

STATISTICAL ANALYSIS

Descriptive statistics: Mean (M) and Standard deviation (SD) for the values of IZDs produced for different treatments were calculated using MS Excel software. Two way ANOVA was conducted with IZD obtained through four treatments using MS Excel software.

3. RESULT

The present study aimed to analyze the *in-vitro* assessment of different concentration of allopathic and ayurvedic medicinal preparation on microbial inhabitants of eye with an incubation period of 24 hours at 37°C. The inhibition zone diameter were measured and recorded.

The inhibition zone diameter produced by the effect of four selected medicines, both ayurvedic and allopathic, on ocular micro flora from three different treatments were analyzed separately. Two ayurvedic and two allopathic ocular medicines with different concentrations (Pure form, 1:1 and 1:2) were used in this study. For each ocular medicine their 3 different concentrations with three replicas were obtained.

Table 1: Inhibition zone produced by Ciplox eye drop against ocular bacteria in three treatments

	Pure Form (mm)	1:1 Dilution (mm)	1:2 Dilution (mm)
Treatment 1	13	1	9
Treatment 2	13	9	8
Treatment 3	12	9	8
Mean ± SD	12.66 ± 0.5773	9.33 ± 0.5773	8.33 ± 0.5773

Zone of inhibition produced by Ciplox, in pure concentration at first and second treatment showed the maximum zone of inhibition, that is 13 mm. The third treatment showed the inhibition zone of 12 mm. The mean and standard deviation for the pure concentration were calculated as 12.66 mm and 0.5773 respectively. In 1:1 dilution, the first treatment showed the maximum zone of inhibition, 10 mm. The second and third treatment showed the same inhibition zone of 9 mm. While in 1:2 dilution the second and third treatment, zone of inhibition value is same and is 8 mm. (Table 1)

Table 2: Inhibition zone produced by Catina eye drop against ocular bacteria in three treatments

	Pure Form (mm)	1:1 Dilution (mm)	1:2 Dilution (mm)
Treatment 1	9	7	6
Treatment 2	8	6	5
Treatment 3	9	7	6
Mean ± SD	8.66 ± 0.5773	6.66 ± 0.5773	5.66 ± 0.5773

In pure concentration, the first and third treatment showed maximum zone of inhibition that is 9 mm. In 1:2 dilutions the first and third treatments showed the maximum zone of inhibition, that is 6 mm. (Table 2)

Table 3: Inhibition zone produced by Himalaya Ophthacare eye drop against ocular bacteria in three treatments

	Pure Form (mm)	1:1 Dilution (mm)	1:2 Dilution (mm)
Treatment 1	8	6	6
Treatment 2	7	6	5
Treatment 3	8	7	5
Mean ± SD	7.66 ± 0.5773	6.33 ± 0.5773	5.33 ± 0.5773

In its pure concentration, the first and third treatment shows the maximum inhibition zone, 8 mm and the second have 7 mm in diameter. The mean and standard deviation of the pure concentration is 7.66 mm and 0.5773 respectively. In 1:1 dilution, the first and second treatments produce 6 mm and the third produce 7 mm. In 1:2 dilutions, the inhibition zone produced by first treatment is 6 mm in diameter. The second and third treatment produces 5 mm. (Table 3)

Table 4: Inhibition zone produced by Elaneer Kuzhambu against ocular bacteria in three treatments

	Pure Form (mm)	1:1 Dilution (mm)	1:2 Dilution (mm)
Treatment 1	7	6	5
Treatment 2	8	6	5
Treatment 3	7	5	4
Mean ± SD	7.33 ± 0.5773	5.66 ± 0.5773	4.66 ± 0.5773

From their second treatment produces the maximum zone of inhibition 8 mm. The first and second treatment of 1:1 dilution produces the inhibition zone of 6 mm and the third treatment produce 5 mm. In 1:2 dilution, the first and second treatment produces 5 mm of inhibition zone and the third treatment produces 4 mm in diameter. (Table 4). Two way ANOVA showed there was significant variation of IZD of different Allopathic and Ayurvedic eye drops in different concentration (F= 15.65322835; P<0.05) and also between each eye drops (F=17.38492261; P<0.05) (Table 5).

Table 5: Results showing analysis of variation between eye drops and between concentrations

Source of Variation	SS	df	MS	F	P-value	F crit
Concentration	19.00815	2	9.504075	15.65322835	0.00416	5.143253
Between Eye drops	31.66649167	3	10.55549722	17.38492261	0.002307	4.757063
Error	3.642983333	6	0.607163889			
Total	54.317625	11				

4. DISCUSSION

The human body is an ecosystem. It is the home to trillions of bacteria, viruses, fungi and other tiny organisms. These organisms are known as microbes. Together they form communities that make up the human micro biome. Human need microbes to stay healthy and many microbes need the environments provided by the human body to survive. Different species live in different places in and on our bodies, and they are adapted to the conditions in these places. The surface of human body inside and out, for example the skin, mouth, eyes, intestines etc are covered in millions of individual microorganisms that don't do us any harm, they are known as the normal body flora. Changes in the composition of the normal flora can occur by the application of antiseptics or antibiotics to the eyelids or conjunctiva (Osato, 1996).

The analysis on *in vitro* susceptibility shows the comparison between the effect of various eye drops including both ayurvedic and allopathic with their different concentrations on the heterogeneous micro flora of eye including both resident flora and invaders. The selected four eye drops which treated against the ocular bacteria culture produce clear and discrete zone of inhibition. The zone of inhibition produced by selected eye drops slightly varied in diameter among each other. The bacterial growth among the diffusion disc of eye drops disappears and thus be inhibition zones where developed. Hence it was found that each eye drop had varied in their effects on ocular micro biota.

Among four eye drops tested, Ciplox showed highest antibacterial effect. Ciplox is an allopathic eye drop and used to treat bacterial infections of the eye including conjunctivitis and corneal ulcers. The active content of Ciplox is Ciprofloxacin, is in a class of antibiotics called fluoroquinolones. Ciplox produced an average IZD of 12.6 mm for pure concentration, 9.3 mm for 1:1 concentration and 8.3 mm for 1:2 concentrations. Ciprofloxacin is effective in the reduction of bacterial ocular flora including both gram negative and gram positive ocular pathogens (Ermis *et al.*, 2004).

Catina is an allopathic eye drop. The major contents include potassium iodide which have an anti cataract property. The observed variation in inhibitions on diameters of Catina was 8.6 mm in pure concentration, 6.6 mm in 1:1 concentration and 5.6 mm in 1:2 concentrations. It has broad spectrum activity against ocular bacteria and has been used in the management of childhood bacterial conjunctivitis. Himalaya Ophthacare eye drop is a brand of eye drop is advertised as natural ayurvedic eye drop and the ingredients are extracts of natural ayurvedic medicines. The inhibition zone diameter produced by Himalaya Ophthacare eye drop is 7.6 mm for pure concentration, 6.3 mm for 1:1 concentration and 5.6 mm for 1:2 concentrations. The effects of Ophthacare eye drops against both Gram-positive and Gram-negative bacteria make it a promising drug in the treatment of ocular diseases originating from infections (Neu, 1991).

Elaneer Kuzhambu is an ocular preparation referred in Sahasrayoga. It is also an ayurvedic eye drop with natural ingredients. It is characterized as fine homogenous thick liquid which was sticky and slow falling as drop, brownish black in colour, sweet smelling aromatic in odour, bitter and astringent in taste. The zone of inhibition produced by Elaneer Kuzhambu was 7.3 mm, 5.6 mm and 4.6 mm for pure concentration, 1:1 dilution and 1:2 dilutions respectively. The eyelids and conjunctiva contain normal flora comprised of bacteria and fungi. These micro organisms play an important beneficial and protective role, but they can also cause infection when any component of the natural control mechanisms is insufficient. Antibiotics are generally regarded as anti-bacterial medications that are expected to prevent further infection and thus improve health. Both antibiotics and antiseptics alter the normal ocular flora.

5. CONCLUSION

The human ocular surface consisting of the cornea and conjunctiva, is colonized by an expansive, diverse microbial community. The analysis on *in vitro* susceptibility shows the comparison between the effect of various eye drops including both ayurvedic and allopathic with their concentrations on the heterogeneous micro flora of eye including both resident flora and invaders. The allopathic medicines used in this study were Catina and Ciplox which contains chemical ingredients. The ayurvedic medicines include Himalaya Ophthacare eye drop and Elaneer Kuzhambu and the ingredients of both of them are extracts of natural ayurvedic medicines. The inhibition zone for each eye drops varied in diameter. The inhibition zone indicates the destruction of microbial population in the culture by the activity of the eye drops. Among four eye drops tested, Ciplox showed highest effects on ocular micro biota and produces maximum zone of inhibition and Elaneer Kuzhambu shows the least inhibition zone diameter. Microorganisms play an important beneficial and protective role and it cause infection when the environmental conditions alter. Application of the antibiotics and antiseptics to the eyelids or conjunctiva changes the composition of the normal ocular flora.

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