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Anti-inflammatory, Antioxidant and Antimicrobial Activity of Ophthacare Brand, an Herbal Eye Drops

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ABSTRACT

In the present study, the herbal preparation of Ophthacare brand eye drops, was investigated for its anti-inflammatory, antioxidant and antimicrobial activity, using in vivo and in vitro experimental models. Ophthacare brand eye drops exhibited significant anti-inflammatory activity in turpentine liniment-induced ocular inflammation in rabbits. The preparation dose-dependently inhibited ferric chloride-induced lipid peroxidation in vitro and also showed significant antibacterial activity against Escherichia coli and Staphylococcus aureus and antifungal activity against Candida albicans. All these findings suggest that Ophthacare brand eye drops can be used in the treatment of various ophthalmic disorders.

Keywords: Ophthacare brand, herbal eye drops, antioxidant, antimicrobial, anti-inflammatory

INTRODUCTION

Ophthalmic problems afflict a substantial segment of the population. These problems are generally managed with antibiotics and steroids, but prolonged use of these drugs may have potential side effects. The Ayurvedic system of medicine has described a number of medicinal plants useful in the treatment of ocular diseases and disorders. Inspite of the availability of vast literature, there is a dearth of commercial herbal ocular preparations. Thus, considering the potential side effects of corticosteroidal therapy, it is worthwhile to explore the benefits of Ayurvedic drugs.

Even though there is enough literature available regarding the efficacy of various medicinal plants in the treatment of ophthalmic disorders, no herbal preparation is available for clinical use. Moreover, the plants used in the traditional system of medicine have not been studied extensively using experimental models (Srinivas and Prabhakaran, 1989; Barde, 1994).

In the present study, Ophthacare brand eye drops (The Himalaya Drug Co., Bangalore), a preparation containing *Carum copticum* (seeds) 0.60% w/v, *Terminalia belerica* (fruits) 0.65% w/v, *Emblica officinalis* (fruits) 1.3% w/v, *Curcuma longa* (rhizome) 1.3% w/v, *Ocimum sanctum* (leaves) 1.3% w/v, *Rosa damascena* (petals) 1.1% w/v, *Cinnamomum camphora* 0.05% w/v and honey 3.7% w/v, was evaluated for its anti-inflammatory, antioxidant and antimicrobial activity using *in vivo* and *in vitro* experimental models.

The constituents of Ophthacare brand eye drops are known to possess antimicrobial and anti-inflammatory properties and are used in traditional medicine for the treatment of a variety of ocular disorders. *Carum copticum* has been shown to possess antibacterial activity against *Salmonella typhosa, Micrococcus pyogenes* and *Escherichia coli* (Krishnan and Badhwar, 1953). It is also recommended as a potential source of natural antioxidant (Mehta *et al*. 1994). Fruits of *Terminalia belerica* were found to be useful in the treatment of many ocular diseases. Fully ripe or dried fruit, mixed with honey is used as an external application in ocular diseases (Nadkarni, 1976a). It also possesses significant antimicrobial activity against both Gram-positive and Gram-negative organisms (Valsaraj *et al*., 1994). The exudate obtained from the incisions of *Emblica officinalis* fruits is used as an external application for ocular inflammation (Nadkarni, 1976). Aqueous extract of *Emblica officinalis* was found to be a potent inhibitor of lipid peroxide formation and as a scavenger of hydroxyl and superoxide radicals *in vitro* (Jose and Kuttan, 1995). Extracts of *Curcuma longa* exhibit anti-inflammatory, antioxidant and antimicrobial properties (Ammon and Wahl, 1991; Bone, 1991). Volatile and fixed oils of *Ocimum sanctum* are known to possess anti-inflammatory activity (Singh and Agrawal, 1991). Essential oil of *Ocimum sanctum* possesses bactericidal activity against Gram-positive and Gram-negative bacteria (Prasad and Rao, 1987). Rose water obtained from petals of *Rosa damascena* is known for its soothing effect and is also found to be beneficial in ophthalmopathy (Kiritikar and Basu, 1987). The extract of *Cinnamomum camphora* showed antibacterial activity against Gram-positive and Gram-negative organisms (Naqvi *et al.* 1985). Honey is generally recommended for sore eyes. It is also reported to prevent infection and promote healing, as it has ingredients similar to antibiotics (Mitra, 1985).

MATERIALS AND METHODS

Preparation and Standardization of Ophthacare Brand Eye Drops:

Carum copticum (seeds) 6 g, *Terminalia belerica* (fruits) 6.5 g, *Emblica officinalis* (fruits) 13 g, *Curcuma longa* (rhizome) 13 g, *Ocimum sanctum* (leaves) 13 g, *Rosa damascena* (petals) 11 g and *Cinnamomum camphora* 0.5 g were coarsely powdered and 37 g of honey was added and the volume made up to 1 liter with distilled water. The total mixture was refluxed at 95^{o}C for two hours. The mixture was then cooled and filtered. The filtrate was subjected to charcoal treatment (2%) to remove coloring matter and was then refiltered. The resulting filtrate was collected and made up to 1 liter with distilled water. The solution was made isotonic with NaCl and the pH adjusted to 7.2 using disodium phosphate. Finally, the solution was filtered through 0.2-µ filters. Two or more batches of such preparations from raw materials of different origin were standardized using fingerprint analysis for characterize the solution by Gas Chromotography according to the following method.

Gas chromatographic analysis

A 10 µl quantity of Ophthacare brand eye drops was injected into a 20 M stainless steel column filled with 10% Carbowax. Nitrogen was used as the carrier gas at 30 ml per minute and a flame ionization detector was used. The temperatures of the oven, injector and detector were 85^{o}C, 110^{o}C and 150^{o}C, respectively. The chromatogram was recorded with the help of an Aimil chromatography data station.

Anti-inflammatory activity

New Zealand white rabbits of either sex weighing between 2 - 2.5 kg were used for the study. The rabbits were housed at a temperature of 22 ± 2^{o}C and relative humidity of 60-70%. The rabbits were fed a synthetic diet supplemented with lucerne and had free access to water throughout the experimental period. Ocular inflammation was induced by instilling 200 µl of turpentine liniment I.P. in the left eye (Bhalla *et al.,* 1994). Instillation of turpentine liniment resulted in severe discharge, chemosis, conjunctival congestion and flare within 10-12 hours. Ocular inflammation was scored 12 hours after induction and the score so obtained was considered as the basal value (Day 0). Twenty-four rabbits showing maximum inflammation were randomized into three groups of 8 each. Normal saline was instilled to Group I rabbits to serve as a control. Rabbits in Groups II and III were instilled with 0.1 ml of Ophthacare brand eye drops containing 10% v/v of herbal extracts and Betamethasone eye drops (0.1% w/v) respectively for 2 days.

Administration of eye drops in all the groups was carried out between 9 to 17 hours at 2-hour intervals. Ocular inflammation was scored on day 1 and day 2 after respective assigned treatment as mentioned in Table 1 (Homburger, 1983).

Table 1: Scoring of Ocular Inflammation

1.	**Discharge**	No discharge	0
		Minimal discharge	1
		Sticking of the eyelids with discharge	2
		Hair around the eye wetted with discharge and surrounding skin area inflammation	3
2.	**Chemosis**	No chemosis	0
		Minimal edema of the conjunctiva	1
		Obvious conjunctival edema with eversion of eyelids	2
		Conjunctival edema clearly visible even without eversion of eyelids	3
3.	**Conjunctival congestion**	No congestion	0
		Minimal congestion	1
		Bright red conjunctiva	2
		Beefy red conjunctiva	3
4.	**Iris**	Structure of the iris seen clearly	0
		Structure of the iris seen with difficulty through the hazy cornea	1
		Structure of the iris not seen	2
5.	**Flare**	No flare	0
		Minimum flare details of iris visible	1
		Increased flare and details of iris visible with difficulty	2

Antioxidant activity

Antioxidant activity was evaluated by inhibition of *in vitro* lipid peroxidation induced by ferric chloride, a well known pro-oxidant. Ten percent of normal rat liver homogenate was prepared in 0.15 M of potassium chloride and centrifuged at 2000 rpm for 10 min. Different

concentrations of Ophthacare brand eye drops (60-120 µl/ml) and the standard reference drug α-tocopherol (10-30 µg/ml) were taken in a test tube, to which 1 ml of liver homogenate and 0.2 ml of 250 mM ferric chloride were added. For blank, 0.2 ml of water was used. All samples were incubated for 20 minutes at 37°C after which the reaction was terminated by the addition of 50 µl 10% butylated hydroxy toluene. The samples were boiled for 30 minutes at 90°C after the addition of 2.5 ml of 1% phosphoric acid and 2.0 ml of 0.8% thiobarbituric acid. The samples were cooled and 5 ml of *n*-butanol was added and vortexed. The *n*-butanol layer was separated and its optical density (O.D.) was read spectrophotometrically at 532 nm (Trush *et al*., 1985). All the tests were conducted in triplicate.

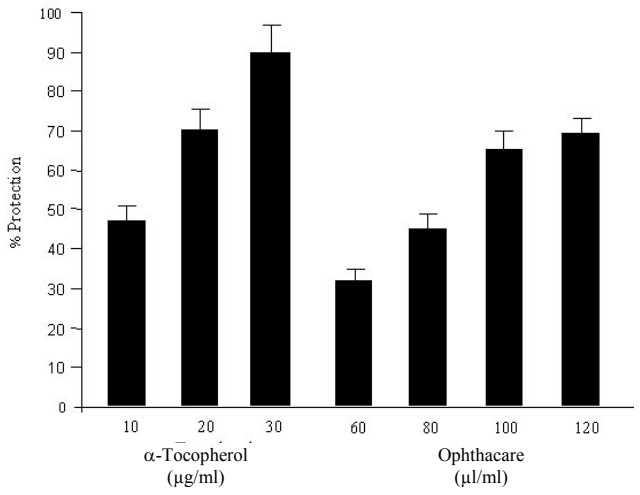
Percentage protection for the test samples was calculated as follows:

$$\frac{\text{O.D. of positive control} - \text{O.D. of test sample}}{\text{O.D. of positive control}} \times 100$$

Antimicrobial activity

Inoculum preparation: Cultures were maintained in 10% glycerine saline at –20°C. These were sub-cultured on soyabean casein digest agar and sabouraud dextrose agar and incubated at 37°C for 18 hour. After 18 hour a single colony of bacteria was sub-cultured on soyabean casein digest media and a colony fungus was sub-cultured on sabouraud dextrose agar and incubated for 18-24 hours. Micro-organisms were recovered by centrifugation at 4000 RPM for 10 minutes, washed twice in distilled water, and finally resuspended in sterile distilled water in order to obtain an inoculum concentration of about 10^9 CFU/ml (Leak *et al.,* 1996).

Antimicrobial activity was determined using the hole-plate method. The method involved diffusion of the drug from a vertical hole through the solidified agar layer of a petri plate to such an extent that growth of the added micro-organisms was prevented entirely in a circular area or zone around the hole containing a solution of the drug sample. The agar medium is homogeneously inoculated with a culture of the test organism. Holes (6-8 mm diameter) were dug using a flamed cork borer and aseptically filled with Ophthacare brand eye drops (100 µl/well). The plates were incubated at 37°C for 18-48 h, after which zone of inhibition was measured. All the tests were performed in triplicate of the following micro-organisms: *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella, Proteus, Candida albicans, Shigella flexneri* (Chand *et al.,* 1994).



ig. 1: Antioxidant activity of Ophthacare brand herbal eye drops *in vitro* lipid peroxidation

Statistical analysis

The data was analysed statistically using the unpaired Students test to discover the difference. The minimum level of significance was fixed at $p<0.05$.

Standardisation of the Ophthacare eye drops

Fingerprints of Gas Chromatography was presented in Figure 2. The fingerprints of different samples were identical and reproducible. Two-days' treatment with Ophthacare brand eye drops reversed the turpentine liniment-induced inflammatory effects in rabbits. The effect was comparable to that of Betamethasone and was significant when compared with that of the control group (Table 2). The drug also showed significant antioxidant potential against ferric chloride-induced lipid peroxidation (Figure 1). The antioxidant activity of 120 µl/ml of Ophthacare brand eye drops was comparable with 20 µg/ml of α-tocopherol. The preparation also showed marked antibacterial (against *E. coli, S. aureus, P. aeruginosa, Klebsiella, Proteus* and *Shigella*) and antifungal (against *Candida albicans*) activity (Table 3).

Table 2: Anti-inflammatory effect of Ophthacare brand eye drops in turpentine liniment-induced ocular lesions in rabbits

Group	Ocular lesion score		
	Day 0	Day 1	Day 2
Control	13.00 ± 0.00	11.83 ± 0.31	11.00 ± 0.51
Ophthacare brand eye drops	13.00 ± 0.00	6.83 ± 0.40*	4.50 ± 0.43*
Betamethasone	13.00 ± 0.00	6.00 ± 0.86*	5.00 ± 0.66*

Values are Mean ± SEM (n=8), *$p<0.001$ as compared to the control group.

Table 3: Antimicrobial activity of Ophthacare brand eye drops

Organism	Zone of inhibition (mm)
S. aureus	27.60 ± 1.45
E. coli	19.00 ± 0.58
P. aeruginosa	10.33 ± 0.40
Klebsiella	13.66 ± 0.40
Proteus	14.33 ± 0.67
C. albicans	37.66 ± 0.40
Shigella	21.33 ± 0.70

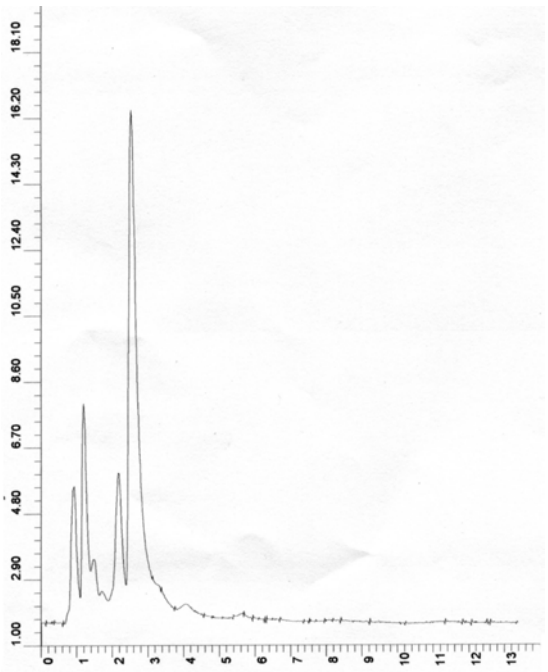


Fig. 2: Gas chromatographic fingerprints of Ophthacare brand eye drops

DISCUSSION

Inflammation of the conjunctiva is one of the commonest ocular disorders encountered in the practice of ophthalmology. Discomfort, burning, dryness, sensation of a foreign particle under the eyelids and irritation are common complaints. Watering of the eye, with eyelids sticking together and chemosis, is also observed. Turpentine liniment-induced ocular inflammation also resulted in severe

discharge, chemosis and conjunctivitis. Ophthacare brand eye drops offered marked protection in this model and the anti-inflammatory effect was almost comparable to that of betamethasone. The observed anti-inflammatory effect may be due to the inhibition of inflammatory mediators by the ingredients present in the formulation.

Free radical-induced oxidative stress has been implicated in the pathogenesis of a variety of human diseases. The natural antioxidant defense system has been found to be defective in many of these diseases. In neonatology, premature retinopathy is a common sequel, when premature infants with an incompletely vascularized retina are exposed to a high concentration of oxygen. In severe cases it may lead to blindness. This problem appears to be limited with α-tocopherol (Howard, 1990). Recent theories also suggest that oxidative stress due to the accumulation of free radicals is involved in the pathogenesis of cataracts (Bhuyan and Bhuyan, 1984; Varma *et al*., 1984) and senile macular degeneration (Varma, 1991). There is also evidence of linking senile macular degeneration to free radical oxidative activity and reduced antioxidant defense in the ageing retina (Richer, 1993). Thus, the antioxidant effect exhibited by Ophthacare brand eye drops might prove beneficial in the treatment of ocular disorders involving free-radicals damage.

Acute conjunctivitis is one of the most frequently occurring ocular diseases among infants and children. Therapy of acute conjunctivitis includes agents effective against Gram-positive and Gram-negative bacteria. While many antibacterial agents have similar spectra of activity, they are not equally potent against several organisms and vary in their pharmacokinetic properties (Neu, 1991). Ophthacare brand eye drops revealed marked antibacterial and antifungal activity. The effects of Ophthacare brand eye drops against both Gram-positive and Gram-negative bacteria make it a promising drug in the treatment of ocular diseases due to infections.

All the above findings reveal the usefulness of Ophthacare brand eye drops in the treatment of various ophthalmic disorders such as acute and chronic conjunctivitis, eye strain, dacryocystitis and pterygium.

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