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Anti-inflammatory and Antihistaminic Study of a Unani Eye Drop Formulation

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Abstract: The Unani eye drop is an ophthalmic formulation prepared for its beneficial effects in the inflammatory and allergic conditions of the eyes. In the present study, the Unani eye drop formulation was prepared and investigated for its anti-inflammatory and antihistaminic activity, using *in vivo* and *in vitro* experimental models respectively. The Unani eye drop formulation exhibited significant anti-inflammatory activity in turpentine liniment-induced ocular inflammation in rabbits. The preparation also showed antihistaminic activity in isolated guinea-pig ileum. The anti-inflammatory and antihistaminic activity of eye drop may be due to presence of active ingredients in the formulation. Although there are many drugs in Unani repository which are mentioned in classical books or used in Unani clinical practice effectively in treatment of eye diseases by various Unani physicians. In spite of the availability of vast literature, there is a dearth of commercial Unani ocular preparations. So, keeping this in mind, the eye drop formulation was prepared and its anti-inflammatory and antihistaminic activity was carried out in animal models. Thus, in view of the importance of alternative anti-inflammatory and anti-allergic drugs, it becomes imperative to bring these indigenous drugs to the front foot and evaluate their activities.

Keywords: anti-inflammatory, antihistaminic activity, unani eye drop, turpentine liniment.

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Introduction

Inflammation of the conjunctiva is one of the commonest ocular disorder encountered in the practice of ophthalmology and is most frequently occurring in the developing nations. There are number of medicinal plants which are generally used in ocular ailments from the ancient times. These plants are easily available, rich in chemical constituents and possess biological activity. The Unani eye drop formulation is prepared for beneficial effects in inflammatory and allergic conditions of the eyes. They are cheap, reliable and have little side effects than the costly synthetic drugs, many of which have adverse effects and are beyond the reach of poor patients. The efficacy of many traditional herbal medicines in curing ocular diseases are now being gradually recognized in modern science as well.¹

Unani eye drop is a polyherbal formulation indicated for anti-inflammatory and antihistaminic effect and it contains *Berberis aristata* DC. (stem wood), *Cassia absus* Linn. (seed), *Coptis teeta* Wall. (rhizome), *Symplocos racemosa* Roxb. (bark), *Azadirachta indica* A. Juss (flower), Alum and distillate of *Rosa damascena* Mill. These constituents of Unani eye drop formulation are known to possess anti-inflammatory and antihistaminic properties and are used in traditional medicine for the treatment of a variety of ocular disorders. *Berberis aristata* is documented for its potent anti-inflammatory^{2,3} and antihistaminic effect in isolated guinea pig ileum.⁴ It grows all over the temperate Himalayas at altitude of 6,000–10,000 feet, from Bhutan to Kunawar, Nilgiri Hills and Ceylon.^{5–7} *Azadirachta indica* A. Juss. (Gul-e-neem) is a native of India, and is cultivated in all parts of the country⁸ has been shown to possess anti-inflammatory activity.^{9,10} Its antihistaminic property has also been reported.¹¹ *Cassia absus* Linn. found in wastelands and stony slope upto 1500 m. in India from western Himalaya to Sri Lanka. It is found all over the world in the tropical region.^{12,13} It has anti-inflammatory and astringent properties¹⁴ and was found to be useful in the treatment of Conjunctivitis.^{15–17} Berberine (Fig. 1), an alkaloidal compound isolated from *Berberis aristata*, has anti-inflammatory property¹⁸ may be helpful in reducing the ocular inflammation; congestion and irritation in conjunctivitis. *Coptis teeta* Wall. found more common on the hilly area especially that of Himalaya

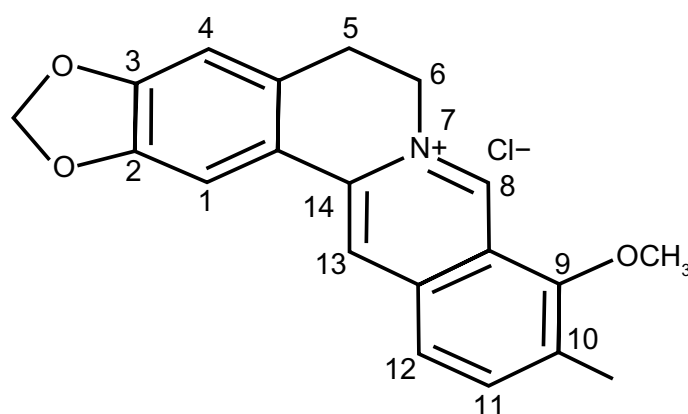


Figure 1. Chemical structure of Berberine chloride.

region and distributed in temperate region of Assam (Mishmi Mountains), Arunachal Pradesh, Sikkim, Bhutan, Nepal and China¹⁹ possess anti-inflammatory property.²⁰ The rose water is used in Conjunctivitis²¹ and has shown analgesic and antiseptic property which helps in curing ocular diseases.²²

Materials and Methods

Plant material

All plant origin drugs and Alum were procured from Dawakhana Tibbiya College, Aligarh Muslim University, Aligarh and rose water was obtained from rose water distillation plant, Hasayan, Aligarh. The drugs were properly identified by the botanical literature available and then confirmed by Prof. S.H. Afaq, a Pharmacognocist in the Department of Ilmul Advia, Aligarh Muslim University, Aligarh. Specimen having voucher Nos. (*Berberis aristata* SC-0111/09, *Coptis teeta* SC-0110/09, *Cassia absus* SC-0108/09, *Symplocos racemosa* SC-0109/09, *Azadirachta indica* SC-0112/09, Alum SC-0113/09 and *Rosa damascene* SC-0107/09) are preserved in the museum at Department of Ilmul Advia, Ajmal Khan Tibbia College, A. M. U., Aligarh, India.

Preparation of eye drop

The herbal eye drop was prepared under aseptic condition as per the method described in Unani Pharmacopeia²³ with slight modification. *Berberis aristata* DC. (stem wood) 140 gm, *Cassia absus* Linn. (seed) 20 gm, *Coptis teeta* Wall. (rhizome) 24 gm, *Symplocos racemosa* Roxb. (bark) 24 gm, *Azadirachta indica* A. Juss (flower) 28 gm were coarsely powdered and soaked in one liter Arq-e-gulab (aqua distillate



of *Rosa damascena* Mill). for over night in round bottom flask. The mixture was refluxed at 60 °C for three hours. The mixture was then cooled, filtered and 4 gm of Alum was dissolved in this solution and again filtered. The resulting filtrate was collected and made up to one liter with Arq-e-gulab. 5 ml Phenyl ethyl alcohol was added as preservative in the solution. Finally the solution was filtered through syringe filter (sterile cellulose acetate 25 mm/0.2 µm) (Axiva Sichem Pvt. Ltd. Delhi-India) and then stored in sterilized vials (sterilized by autoclave).

Chemicals and drugs

Turpentine liniment I.P. (Kapoor Pharmacy, Mumbai, India) was procured from local market Aligarh. The standard drug used was Flurbiprofen Sodium Ophthalmic Solution USP (Nicholas Piramal India Pvt. Ltd, M.P.) and Histamine dihydrochloride (Alfa Aesar).

Anti-inflammatory Activity

Animal's model

The experimental work was carried out on eighteen healthy rabbits (selected at random), having an average weight of 1.5–2.0 Kg. This study was performed as per guidelines and norms of ethics. Permission and approval for animal studies were obtained from Jawaharlal Nehru Medical College and Hospital Animal Ethics Committee, Aligarh Muslim University, Aligarh, India.

Experimental Procedure

Albino rabbits of either sex were used for the study. The rabbits were housed at room temperature 25 ± 2 °C and relative humidity of 60%–70%. The rabbits were given a synthetic diet supplemented and *ad libitum* throughout the experimental period. Ocular inflammation was induced as described by Mitra et al,²⁴ by instilling 100 µl of turpentine liniment I.P. into right eye of all rabbits. Consequently severe discharge into conjunctival sac with chemosis of palpebral and bulbar conjunctiva with slight necrosis occurred and lid margin inflamed within an hour. The cornea, pupil and iris were normal. Eighteen rabbits showing maximum inflammation were divided into three groups of 6 each. Ocular inflammation was scored twelve hours after induction and the score so obtained was considered as the basal value (Day 1). Distilled water was instilled to group I rabbits which serve as a control.

Rabbits in group II and III were instilled with 0.1 ml eye drop (test drug) and Flurbiprofen Sodium Ophthalmic Solution USP respectively for five days. Administration of eye drop in all groups was carried out between 9 am to 17 pm hours at 4 hours intervals. Each symptom was recorded using a four-point scoring system: 0-none; 1-mild; 2-moderate, 3-severe and 4-very severe. Ocular inflammation was scored using student's *t*-test for paired observation comparison on day 2, 3, 4 and 5, after respective assigned treatment as mentioned in Table 1.²⁵

Statistical Analysis

All data were expressed as Mean \pm SE, and the data was statistically analyzed using one-way analysis of variance (ANOVA) method. The minimum level of significant was fixed at $p < 0.01$ and $p < 0.05$.

Antihistaminic Activity

To check the antihistaminic activity, guinea pig ileum was isolated and prepared according to the method described by Ghosh.²⁶ Histamine dihydrochloride (4.0 µg/ml) was added to the bath for spasmogenesis.²⁷ The guinea pig was killed by a blow on the back of the head. The midline incision of the abdomen was made and the ileum was removed. The 10–15 cm portion closest to the ileum was discarded. The rest of the ileum was dissected out of the surrounding mesenteric tissue and was cut into pieces of 2 cm length, which were suspended in a 50 ml organ bath in Tyrode's solution (composition in mM: Sodium chloride-136.89, Potassium chloride-2.68, Calcium chloride-1.80, Glucose-5.5, Sodium bicarbonate-11.9, Sodium hydrogen phosphate-0.4, Magnesium chloride-1.05 were dissolved in 1 liter of distilled water) at 37 °C, aerated with oxygen (95%) and carbon dioxide (5%). The pieces were allowed to stabilize for 30 minutes before any addition of drug. Then to observe the antagonistic effect of the test drug on histamine, the drug was added to the bath one minute before adding histamine.

Results

In this study, we have tested Unani eye drop formulation for its anti-inflammatory activity against turpentine liniment-induced ocular inflammation in rabbit's eye and antihistaminic activity in isolated guinea pig

Table 1. Scoring of ocular inflammation (Homburger¹⁸).

| | | | |
|----|--------------------------------|--|---|
| 1. | Discharge | No discharge | 0 |
| | | Minimal discharge | 1 |
| | | Moderate discharge | 2 |
| | | Sticking of the eyelids with discharge | 3 |
| | | Hair around the eye wetted with discharge and surrounding skin area inflammation | 4 |
| 2. | Lid edema | No lid edema | 0 |
| | | Minimal edema | 1 |
| | | Moderate edema | 2 |
| | | Swelling on both eye lids | 3 |
| | | Puffy swelling of eyelids | 4 |
| 3. | Chemosis | No chemosis | 0 |
| | | Minimal edema of conjunctiva | 1 |
| | | Moderate edema of conjunctiva | 2 |
| | | Obvious conjunctival edema with eversion of eyelids | 3 |
| | | Conjunctival edema clearly visible without eversion of eyelids | 4 |
| 4. | Conjunctival congestion | No congestion | 0 |
| | | Minimal congestion | 1 |
| | | Moderate congestion | 2 |
| | | Bright red conjunctiva | 3 |
| | | Beefy red conjunctiva | 4 |
| 5. | Conjunctival necrosis | No conjunctival necrosis | 0 |
| | | Minimal conjunctival necrosis | 1 |
| | | Moderate conjunctival necrosis | 2 |
| | | Severe conjunctival necrosis | 3 |

ileum. Five days treatment with eye drop formulation reversed the turpentine liniment-induced inflammatory effects in rabbits. The effect was comparable to that of Flurbiprofen ophthalmic solution and was significant ($p < 0.01$) when compared with that of control group (Table 2). The eye drop formulation tested for antihistaminic activity in guinea pig ileum in the dose of 0.5 ml in a 50 ml bath showed relaxant effect on the tissue. The active control 0.1 ml of 4.0 $\mu\text{g/ml}$ histamine dihydrochloride was added in 50 ml organ bath, contraction of the tissue occurred (Fig. 2). After washing, 0.5 ml of the eye drop formulation was put in the organ bath. The test drug caused moderate relaxation of the guinea-pig ileum (base-line went down). After one minute increasing

doses (0.1, 0.2, 0.3 and 0.4 ml) of histamine were added which produced much smaller contractions as compared to active control (Fig. 3). So, the test drug was seen to antagonize the effect of histamine on the tissue. Moreover the magnitude of the contraction of the tissue with addition of 0.1 ml of histamine in organ bath was found to be 48 mm whereas the magnitude of the contraction of the tissue with increasing dose (0.1, 0.2, 0.3 and 0.4 ml of histamine) was found to be 4 mm, 6 mm, 4 mm and 2 mm respectively.

Discussion

Inflammation of the conjunctiva is one of the common ocular disorders which present redness, watering

Table 2. Anti-inflammatory effect of control, test and standard drug in turpentine liniment-induced ocular inflammation in rabbit's eye.

| Group (n = 6) | Ocular lesions score (Mean ± SE) | | | | |
|---------------|----------------------------------|-------------------|--------------------|-------------------|-------------------|
| | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
| Control | 17.3 ± 0.33 | 15.3 ± 0.42 | 14.5 ± 0.43 | 11.2 ± 0.31 | 7.3 ± 0.42 |
| Test drug | 17.0 ± 0.37 | 13.0 ± 0.37 x* | 11.8 ± 0.31 x** | 7.3 ± 0.31x** | 5.5 ± 0.43 x** |
| Standard | 17.0 ± 0.37 | 11.5 ± 0.43 x* y* | 10.7 ± 0.33 x** y* | 5.8 ± 0.31 x** y* | 4.0 ± 0.26 x** y* |

Against control = x, * = $p < 0.05$.
Against test drug = y, ** = $p < 0.01$.

of the eye and conjunctival congestion. Turpentine liniment-induced ocular inflammation also resulted in severe discharge, chemosis and conjunctivitis. The result of the study indicate that the eye drop formulation posses anti-inflammatory activity against the inflammation induced by turpentine liniment in acute phase. The test drug significantly inhibited the ocular inflammation as compared to control. The test drug was also tested for antihistaminic activity in isolated guinea pig ileum. Berberine is the chief alkaloid of *Berberis aristata*, indeed, in the pure form, berberine was clinically tried in patients of chronic trachoma in 1933 by Varma who found its intraconjunctival injections highly effective.²⁸ The chief ingredient of eye drop formulation is *Berberis aristata*. It is an important ingredient of several polyherbal formulations traditionally used for the treatment of eye diseases. Besides this, *Berberis aristata* contains an alkaloid 'Berberine' which has been used for many centuries, for the treatment of eye diseases²⁹ and produced anti-histaminic effect in isolated guinea pig ileum.⁴ Histamine acts on H₁-receptors as an agonist and produces contraction of guinea pig ileum. The test drug was added one minute before histamine in the organ bath,

its action on H₁-receptors was blocked by test drug, in this way the test drug acts as H₁-receptors blocker. So, the test drug was observed to antagonize the effect of histamine on the tissue.

Conclusion

All the above findings reveal the usefulness of the tested Unani eye drop formulation in the treatment of conjunctiva and its significant anti-inflammatory effect was almost comparable to that of flurbiprofen sodium ophthalmic eye drop. The eye drop also exhibited good antihistaminic effect on isolated guinea pig ileum. It was found to antagonize the effect of histamine on the tissue. Thus the eye drop was proved to possess a potent anti-inflammatory and antihistaminic activity.

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Disclosures

This manuscript has been read and approved by all authors. This paper is unique and is not under

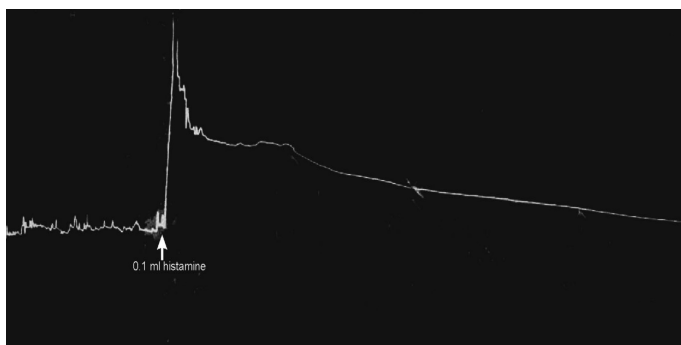


Figure 2. Effect of histamine on isolated guinea pig ileum showing contraction.

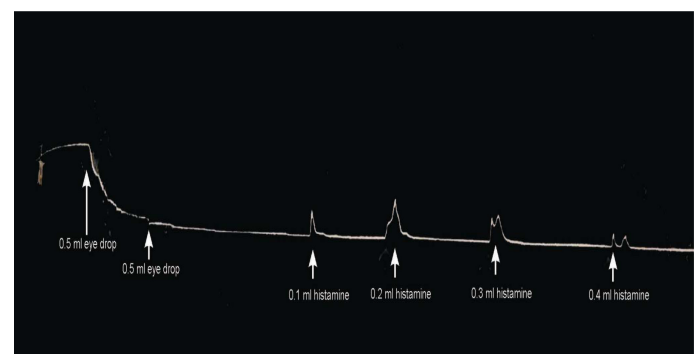


Figure 3. Effect of test drug on isolated guinea pig ileum, showing progressive blockade of histamine-induced contraction with increasing doses.



consideration by any other publication and has not been published elsewhere. The authors report no conflicts of interest.

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