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*Original Research Article*

# Evaluation of the Protective Effects of Constituents of an Ayurveda Herbal Eye Drop Preparation in Albino Rabbits

**Sadhna Verma[1], Nilkamal Singh[2], Shirley Telles[3*], Acharya Balkrishna[4]**

**[1]Assistant scientist, [2]Scientist C, [3*]Director, [4]General Secretary,**
**Patanjali Research Foundation, Patanjali Yogpeeth,**
**Maharishi Dayanand Gram, Near Bahadarabad, Haridwar, Uttarakhand, India.**

## ABSTRACT

**Background:** Benzalkonium chloride (BAK) is a common preservative used in eye drops and it is known to be corrosive for the eyes. The present study intended to evaluate the possible protective effect of the herbal contents of Ayurvedic eye drops with BAK as the preservative in the eyes of 24 New Zealand, white albino rabbits.

**Method:** The animals were allocated to an experimental (n = 18) and control group (n = 6). The rabbits in the experimental group were given the test substance daily for 28 days while the control group was kept in the same conditions without any treatment. Irritation of the eye, body weight, and mortality rate were observed on Day 7, 14, 21 and 28 after the commencement of the experiment. The animals were sacrificed after 28 days. Hematological and biochemical tests were conducted from the blood samples collected before the sacrifice. Microscopic examination of the tissue and organs were conducted after the sacrifice. Student's t-test was conducted to compare the data of the experimental and control group.

**Result:** There was no evidence of irritation or any pathology in both the experimental and control animals. Ayurveda eye-drops appear to reduce the irritating effects of BAK.

**Conclusion:** The herbal constituents are individually irritating to the eye in combination they did not result in irritation.

**Keywords:** Ayurvedic Eye Drop, Benzalkonium Chloride, Irritation.

***Correspondence to:**

**Shirley Telles Ph.D.,**
Patanjali Research Foundation,
Patanjali Yogpeeth, Maharishi Dayanand Gram,
Near Bahadarabad, Haridwar, Uttarakhand, India.

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## INTRODUCTION

Cataract which is characterized by opacity of the lens of the eye is a leading cause of impaired vision world-wide[1]. The emergence of cataract related to strong UV radiation and growing pollution can be effectively reduced by natural antioxidant biomolecules which may be applied externally or as topical application as eye drops; or taken internally as food supplements or through nanobiotechnology based formulations[2].

Among these options topical applications as eye drops are most commonly used. Most eye drops contain a preservative known as benzalkonium chloride (BAK), a quaternary ammonium salt composed of a mixture of benzododecinium $C_{21}H_{38}N^+$ (BAK $C_{12}$) and myristalkonium $C_{23}H_{42}N^+$ (BAK $C_{14}$) chlorides. BAK is a cationic surfactant and tensioactive compound, acting as a detergent for the lipid layer of the tear film as well as for the lipids of cell plasma membranes, so that it can act as a penetration enhancer for active compounds[3,4]. However it has been reported to induce ocular surface disorders combining irritation, inflammation and cell death processes, especially in long-term treatment. There is a growing body of evidence that BAK induces apoptosis, oxidative stress and inflammation on the ocular surface epithelia[5].

These side effects are believed to be less with herbal preparations[6], though there are very few systematic studies conducted to evaluate this. The present study intended (i) to evaluate the protective effect of a specific preparation of Ayurvedic eye drops which has BAK as a preservative, in reducing irritation and (ii) to assess whether the Ayurvedic eye drops themselves would cause irritation or not, as some of them constituents are considered therapeutically useful, yet possibly irritating to the eye. Tests were carried out on albino rabbits.

## MATERIAL AND METHODS

### Animals and their living conditions

Healthy rabbits without any eye disorder were selected for the study. All rabbits used in this study (n = 24) were New Zealand, white albino rabbits weighing 2.0 – 3.5 kg. They were housed

individually in metal cages fitted with perforated floors. The room was environmentally controlled with a 12-h light/12-h dark cycle, a 30–70% humidity range and 22–24°C temperature range. They were allowed to drink tap water and had access to normal feed *ad libitum*. The research company to which the project was outsourced stated that standard ethical considerations for handling laboratory animals were observed.

**Study Design**

The rabbits were allocated to experimental and control groups. The experimental group had 3 sub-groups (n = 18, 3 males and 3 females in each subgroup) and the control group had 6 rabbits (3 males and 3 females). Each cage was tagged with details such as the study number, study name, dose, group name, animal number, date of initiation and date of termination of the study. The experimental group was given the test substance for 28 days daily. The control group was kept in the same environmental conditions without any treatment.

**Experimental Procedure**

Each 10 ml of the Ayurvedic preparation consisted of (i) juice of *Allium cepa* (1.68 ml), (ii) juice of *Zingiber officinale* (1.66 ml), (iii) juice of *Citrus aurantifolia* (1.66 ml) (iv) honey (5.00 ml) and 0.1% v/v Benalkonium chloride solution as preservative. The pH of the eye drop was found to be 3.09.

The test substance was instilled in the conjunctival sac of the left eye of each animal in the experimental group after pulling the lower lid away from the eyeball. The lids were then gently held together for one second to prevent loss of test material. The experimental groups were divided into three subgroups according to dose of the test substance. The subgroups were (i) the low dose group, consisting of those rabbits which were given 0.1 ml per day for 28 days; (ii) intermediate dose group, which consisted of those rabbits which were given 0.1 ml twice a day for 28 days; and (iii) those rabbits which were given 0.1 thrice a day for 28 days, who were tagged as the high dose group.

**Assessments**

All assessments were blind scored.

Irritation of the eye was assessed on day 7, 14, 21 and 28 using an ocular lesion scale which consist of three parts, part 1 measured opacity (degree of density), part 2 was to check the reaction of the iris (pupil) to light and part 3 was to measure the redness of the palpebral and bulbar conjunctiva, cornea and iris, and to measure chemosis (of the lids and/or nictating membrane).

During the experiment the body weight, signs and symptoms of eye irritation and mortality rate were observed on the day 7, 14, 21 and 28 after the commencement of the experiment. After 28 days of applying the test substance the rabbits were sacrificed. Food was withdrawn overnight prior to the collection of samples and 5-8 ml of blood was withdrawn from an ear vein prior to sacrificing them. The following hematological and serum biochemistry tests were conducted on both the experimental and control group, (i) hematocrit (Hct), (ii) hemoglobin (Hb) (iii) red blood cell count (RBC) (iv) total leucocyte count (WBC) (v) platelet count (PLT) (vi) prothrombin time (PT) (vii) differential leucocyte counts (DLC) (viii) neutrophils (N) (ix) lymphocytes (L) (x) eosinophils (E) (xi) monocyte (M) (xii) basophils (B) (xiii) blood sugar (xiv) blood urea nitrogen (BUN (xv) total protein (TP) (xvi) albumin (ALB) (xvii) serum glutamic oxalo acetate transaminase (SGOT) (xviii) serum alkaline phosphatase (SAP) (xix) serum glutamic pyruvic transaminase (SGPT) (xx) urea (xxi) triglyceride (TG) (xxii) Total bilirubin (T-Bil) (xxiii) direct bilirubin (D-Bil) (xxiv) globulin (GLB) (xxv) creatinine (CRE) and (xxvi) cholesterol (CHO).

**Data analysis**

The data of the experimental group were compared with the data of the control group using Student's t-test.

## RESULTS

**Eye irritation**

There was no evidence of irritation according to the ocular lesion scale.

**Haematological tests**

All parameters were within the accepted limits of normal variations for the animals studied and there was no significant difference between the experimental group and the control group.

**Biochemistry tests**

All parameters were within the accepted limits of normal variations for the species of rabbits studied and there was no significant difference between the experimental group and the control group.

**Organ weight**

There was no significant change in the organ weight of experimental rabbits when compared to its control counterparts.

**Necropsy tests**

There was no sign of pathological changes in the rabbits of both the groups.

**Histopathological tests**

There was no evidence of histopathology in the rabbits.

## DISCUSSION

The results suggest that applying the test substance (Ayurveda eye drops) for 28 days in graded doses does not cause eye irritation in rabbits. This finding may be due to the considerable amount of quantity of honey (50%) present in the test substance, since honey is known to have an anti-inflammatory effect[7] and it is also proven to have wound healing qualities[8]. These properties of honey might have reduced the corrosive effect of BAK.

The finding of the present study is important because the ingredients in the test substance e.g., the juice of *Allium cepa* has been shown to prevent selenite induced experimental cataract in an earlier study[9] and all the ingredients except *Citrus aurantifolia* and Benalkonium chloride are known to contain antioxidants which are useful to prevent the occurrence of a cataract. Hence the test substance can be an effective non pharmacological medicine to prevent and manage cataract without the harmful effect of BAK. Apart from this none of the constituents acted as an irritant.

These results suggest that this Ayurveda preparation may have useful anti-inflammatory properties which need further investigation.

The main limitations of the study were (i) unequal numbers in the two groups, (ii) a small sample size, (iii) carrying out a study on an animal model prevent direct extrapolation to humans. Hence this is an area which is worth studying further.

## SOURCE OF SUPPORT

Patanjali Yogpeeth, Haridwar, Uttarakhand, India.

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**Conflict of Interest:** None Declared.



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