



Contents lists available at CurrentSciDirect Publications

**International Journal of Current Biomedical and Pharmaceutical Research**

Journal homepage: [www.currentscidirect.com](http://www.currentscidirect.com)



## Original article

# Prevention of endotoxin-induced uveitis in rabbits by Triphala, an Ayurvedic formulation

Suresh Kumar Gupta<sup>a\*</sup>, V. Kalaiselvan<sup>b</sup>, Shyam Sunder Agrawal<sup>a</sup>, Sushma Srivastava<sup>a</sup>, Rohit Saxena<sup>c</sup>

<sup>a\*</sup> Department of Pharmacology, Delhi Institute of Pharmaceutical Sciences and Research, Sector 3, Pushp Vihar, New Delhi-110 017.

<sup>b</sup> Indian Pharmacopoeia Commission, Sector 23, Raj Nagar, Ghaziabad, UP 201 002, India

<sup>c</sup> RP Center for Ophthalmic Studies, All India Institute of Medical Sciences, Ansari Nagar, New Delhi, India

## ARTICLE INFO

**Keywords:**  
Triphala  
Uveitis  
Endotoxin  
TNF- $\alpha$ .

## ABSTRACT

**Purpose:** Triphala (TA) is an Ayurvedic formulation used to treat various disorders. The present study was designed to investigate the anti-inflammatory effect of TA aqueous extract on experimental uveitis in the rabbit. **Methods:** Anterior uveitis was induced in rabbits by intravitreal injection of lipopolysaccharide from *Escherichia coli* after pretreatment with TA aqueous extract. Subsequently the anti-inflammatory activity of TA was evaluated by grading the clinical signs and estimating the inflammatory cell count, protein, and TNF- $\alpha$  level in the aqueous humour. **Results:** The anterior segment inflammation in the control group was significantly higher than in TA and prednisolone treated groups, as observed by clinical grading. The inflammatory cell count in the control group was  $31.23 \pm 0.80 \times 10^5$  cells/ml, whereas it was  $3.29 \pm 0.47 \times 10^5$  cells/ml ( $P < 0.0001$  vs. control) and  $1.31 \pm 0.31 \times 10^5$  ( $P < 0.0001$  vs. control) cells/ml in the TA and prednisolone treated groups, respectively. The protein content of the aqueous humour was  $15.43 \pm 0.54$ ,  $3.13 \pm 0.35$  ( $P < 0.0001$  vs. control), and  $1.96 \pm 0.39$  ( $P < 0.0001$  vs. control) mg/ml in the control, TA and prednisolone treated groups respectively. The aqueous TNF- $\alpha$  level in the control group was  $942.20 \pm 6.46$  pg/ml and was  $261.30 \pm 13.60$  ( $P < 0.001$  vs. control) and  $104.00 \pm 4.50$  ( $P < 0.0001$  vs. control) pg/ml in the TA and prednisolone treated groups, respectively. **Conclusions:** Topical administration of aqueous extract of TA prevented uveitis in endotoxin-induced experimental rabbits.

© Copyright 2011. CurrentSciDirect Publications. IJCBPR - All rights reserved.

## 1. Introduction

Uveitis is swelling and irritation of the uvea, the middle layer of the eye. It is a major cause of eye infection in many countries including United States of America [1]. Some of the herbal drug extracts and synthetic drugs have been explored for their preventive effect of uveitis in endotoxin induced experimental models [2-4]. The endotoxin induced animal model of uveitis has been used to represent anterior uveitis on humans. Lipopolysaccharide (LPS), a glycolipid from the outer cell membranes of Gram-negative bacteria is a proinflammatory component and is used to induce uveitis in animals by a systemic or

ocular route.5,6 Experimental studies showed that exposure to LPS induce expression of various mediators of inflammation such as tumour necrosis factor (TNF) -  $\alpha$  and other cytokines.7,8 Increased expression of inflammatory mediators contributes to the development of anterior uveitis, with the breakdown of the blood-ocular barrier leading to infiltration of the aqueous humour with inflammatory cells and the leakage of protein [5]. Experimental uveitis peaks at 24 hours after LPS injection and gradually subsides over the next 24 hours. Triphala is an Ayurvedic formulation consisting of equal parts of the fruit of *Embilca officinalis*, *Terminalia bellirica*, and *Terminalia chebula* [9,10]. The major chemical constituents in Triphala are gallic acid, chebulagic acid, chebulinic acid, Vitamin C and quercetin. Gallic acid showed potent anti-inflammatory activity in experimental studies [11, 12]. Chebulagic acid and quercetin showed potential anti inflammatory effect in lipopolysaccharide

\* Corresponding Author : Prof. Suresh Kumar Gupta  
Department of Pharmacology,  
Delhi Institute of Pharmaceutical Sciences and Research,  
Sector 3, Pushp Vihar, New Delhi-110 017  
Phone: 011 29551490  
Email : [vivekarts@rediffmail.com](mailto:vivekarts@rediffmail.com), [skgup@hotmail.com](mailto:skgup@hotmail.com)

© Copyright 2011. CurrentSciDirect Publications. IJCBPR - All rights reserved.

induced mouse macrophage cell line by inhibition of NF-kappaB activation [13,14]. Triphala formulation (1gm/kg) was also found to have a significant anti inflammatory effects in albino mice with Freund's adjuvant-induced paw oedema [15]. Triphala found to inhibit on PMN-type matrix metalloproteinase expressed in adult periodontitis (inflammatory diseases affecting the periodontium) patients; Triphala showed strong inhibitory activity than doxycycline [16]. It inhibits the growth of Gram positive and Gram negative bacteria [17]. In view of the potent anti-inflammatory and antibacterial activity of Triphala, the present study was designed to evaluate the effect on prevention of uveitis by topical application of its aqueous extract in endotoxin-induced uveitis rabbits.

## 2. Materials and Methods

### 2.1. Animals

New Zealand White rabbits weighing 2.5 to 3 kg were obtained from the animal house facility at Delhi Institute of Pharmaceutical Sciences and Research after getting the approval from Institutional Animal Ethics Committee. The animal experiments were conducted according to the guidelines specified by the committee.

### 2.2. Chemicals

Lipopolysaccharide (LPS) from *Escherichia coli* was purchased from Sigma-Aldrich, St. Louis, MO. The enzyme-linked immunosorbent assay (ELISA) kit was purchased from Diaclone (Besancon, France).

### 2.3. Triphala extract

The aqueous extract of Triphala was provided by Promed Exports Pvt Ltd. (New Delhi, India) and was authenticated by High Performance Thin Layer Chromatography (HPTLC) fingerprinting. The powdered extract was dissolved in 0.25% hydroxy propyl methylcellulose and filtered with 0.25 µm filters (Millipore, Billerica, MA). The filtrate was stored at -4°C in sterile sealed vials until further use.

### 2.4. Study Design

The experimental model of uveitis was carried out as per the method described by Gupta et al [3]. The rabbits were divided into three groups of four rabbits each (eight eyes). The rabbits in groups 1 and 2 were topically instilled with 0.5% and 1% Triphala aqueous extract and Prednisolone acetate respectively, while group 3 (control) received the vehicle. All instillations were performed three times a day for 3 days before induction of experimental uveitis and continued for 3 days after induction. Twenty four hours after the induction of experimental uveitis, aqueous humour sampling was performed for cell count, protein, and Tumour Necrosis Factor (TNF)-α estimation. The rabbits were re examined 72 hours after the endotoxin injection for clinical signs of uveitis.

### 2.5. Rabbit Model of Experimental Uveitis

The endotoxin namely LPS from *Escherichia coli* was used to induce uveitis. The endotoxin was injected intravitreally in both eyes. Topical anaesthesia was done by instilling one drop of

proparacaine HCl (0.5%) solution. After the upper lid was retracted, 20 µL (100 ng) of the endotoxin solution was injected intravitreally at the 12 o' clock position, 3 to 4 mm posterior to the limbus with a 30- gauge needle. Subsequently the eyes were examined for clinical grading, and aqueous humour was tapped for inflammatory cell count, protein and TNF-α estimation.

### 2.6. Clinical Grading of Ocular Inflammation

The clinical signs of ocular inflammation were graded on a scale of 0 to 4 according to the scoring system described by Ruiz-Moreno et al [18]. The grading scale as follows: no inflammatory reaction, 0; discrete inflammatory reaction, 1; moderate dilation of the iris and conjunctival vessels, 2; intense iridial hyperaemia, with flare in the anterior chamber, 3; and the same signs as grade 3 plus the presence of fibrinoid exudation in the papillary area, with intense flare in the anterior chamber, 4.

The grading of clinical signs of uveitis was performed at 24 and 72 hours after intravitreal injection of endotoxin.

### 2.7. Aqueous Humour Sampling

The rabbits were anesthetized with ketamine (20-40 mg/kg) and xylazine (1-2 mg/kg) by intramuscular injection in the hind limb. Proparacaine HCl (0.5%) solution was applied topically to supplement the general anaesthesia. During preliminary studies attempts at aqueous humour (AH) sampling by topical anesthesia was found to be painful and therefore further AH sampling was performed under general anaesthesia. AH (150-200 µL) was aspirated from the anterior chamber with a 30- gauge disposable insulin syringe, and care was taken not to injure the iris or lens during the procedure. Each aqueous humour sample was diluted 50-fold with phosphate buffer.

### 2.8. Total Cell Count

The AH sample was suspended in an equal volume of Turk's stain solution and cell counting was performed on a hemocytometer under light microscope. The cell count per field (each field considered equal to 0.1 mL) was estimated as an average of counts in four fields per sample. Subsequently, the number of cells per millilitre of AH was calculated.

### 2.9. Total Protein Estimation

Protein estimation in AH samples was performed according to the method described by Lowry et al [19]. Briefly, 10 µL of AH sample was diluted with 990 µL of 1 N NaOH and reacted with 4 mL of copper reagent. After 10 minutes, 0.5 mL of Folin's reagent was added and vortexed, and the samples were kept in the dark for 30 minutes. Absorbance was recorded with a spectrophotometer at 620 nm. Bovine serum albumin (BSA) was taken as a protein standard to calculate the protein content of the sample. All estimations were performed in duplicate.

The cell count and protein estimation were performed on the day of AH sampling.

### 3.0. TNF-α Estimation

TNF-α level in AH were estimated by using a commercially available enzyme linked immunosorbent assay (ELISA) kit, per the manufacturer's instructions. All estimations were performed in duplicate.

## 4. Results

### 4.1. Clinical Signs of Uveitis

The rabbits in the control group showed significantly higher degree of ocular inflammation as compared to Prednisolone and Triphala treated groups (Fig.1). After 24 hours of intravitreal injection of endotoxin, the vehicle treated control group had a mean score of  $2.80 \pm 0.20$  (range, 2-4), whereas the Triphala and Prednisolone treated groups showed a mean score of  $1.00 \pm 0.46$  (range, 0-2) and  $0.43 \pm 0.41$  (range, 0-1), respectively, at the same time point ( $P < 0.05$ ; Fig.2).

### 4.2. Total Cell Count

Triphala and Prednisolone treated groups showed a 92.82 % and 96.34 % inhibition of inflammatory cell infiltration, respectively as compared to control group. In the control group, the total inflammatory cell count in the aqueous humour 24 hours after intravitreal injection of endotoxin was  $31.23 \pm 0.80 \times 10^5$  cells/ml (mean  $\pm$  SD,  $n = 8$ ). The rabbits pretreated with Triphala and Prednisolone showed significantly lower inflammatory cell counts ( $3.29 \pm 0.47 \times 10^5$  cells/ml,  $P < 0.0001$  vs. control; and  $1.31 \pm 0.31 \times 10^5$  cells/ml,  $P < 0.0001$  vs. control) in the aqueous humour obtained at the same time point. In addition, an inflammatory cell infiltration was observed in the Triphala treated group was closely related to Prednisolone treated group ( $P < 0.0001$ ; Fig.3)

### 4.3. Total Protein

Total proteins as estimated 24 hours after intravitreal injection of endotoxin showed significantly lower values in the Triphala treated group as compared to control. In the Triphala treated group the protein concentration was  $3.13 \pm 0.35$  mg/ml compared with  $15.43 \pm 0.54$  and  $1.96 \pm 0.39$  mg/ml in the control and prednisolone treated groups, respectively. The protein concentration in the Triphala treated group was significantly lower than in the control group ( $P < 0.0001$ ) and slightly higher than as compared to Prednisolone treated group ( $P < 0.0001$ ; Fig.4).

### 4.4. Tumour Necrosis Factor- $\alpha$

The aqueous humour TNF- $\alpha$  in the control group was  $942.20 \pm 6.46$  pg/ml, whereas it was  $261.30 \pm 13.60$  pg/ml ( $P < 0.0001$  vs. control) and  $104.00 \pm 4.50$  ( $P < 0.0001$  vs. control) pg/ml in the Triphala and prednisolone -treated groups, respectively. The TNF- $\alpha$  level in the Prednisolone treated group was significantly lower than in the Triphala treated group ( $P < 0.0001$ ; Fig.5).

## 5. Discussion

The results of the present investigation demonstrated the protective effect of the topical application of Triphala in endotoxin-induced uveitis. The animals in the treated groups showed not only significant reduction in the severity of clinical signs and also significant reduction in aqueous humour levels of inflammatory cells, protein contents, and TNF- $\alpha$  compared with the control group.

The endotoxin-induced experimental model of uveitis it is not an exact representation of clinical uveitis, but the inflammatory response and cytokine production in response to endotoxin

closely resembles the acute phase of clinical uveitis [20]. The endotoxin-induced inflammation can be observed during antibiotic therapy for ocular infections. The antibiotics, while destroying the bacteria, also release the LPS from their cell walls, thereby leading to inflammatory response. The drugs found effective in endotoxin-induced uveitis may be effective in the treatment of ocular and systemic Gram-negative bacterial infections.

The biological activity of the endotoxin initiates the inflammatory vascular and cellular responses when it is injected through intravitreally. Increased production and release of monocytes, macrophages and other inflammatory cells is associated with the release of potent inflammatory mediators in the aqueous humour. Among those TNF- $\alpha$  is the major one, which appears to be essential in the development of endotoxin-induced inflammation [20, 21]. In our earlier study demonstrated the anti-inflammatory effects of Curcuma longa and Berberis aristata in endotoxin-induced uveitis in rabbits, where the TNF- $\alpha$  level was significantly high in control group as compared to treated [3]. Besides the chemical mediators of inflammation, cell adhesion molecules play a vital role in leukocyte adherence to vascular endothelial cells during the early acute phase of inflammation and contribute to the leakage of protein.

Triphala and its chemical constituents have been demonstrated a significant anti-inflammatory effect in other studies [12-15]. The therapeutic response to topical application of aqueous extract of Triphala has demonstrated for the first time the possible utility as a topical application for the treatment of anterior uveitis. The reduced severity of inflammatory changes observed in clinical manifestations in the inflamed eye was the result of significant inhibition of vascular and cellular inflammatory responses. The release of chemical mediators of inflammation is also suppressed secondary to inhibition of the cellular response. The suppression of vascular and cellular inflammatory responses by herbal extracts was evidenced by significantly low levels of inflammatory cells, proteins, and TNF- $\alpha$  levels in aqueous humour of treated animals. Significantly reduced protein levels in aqueous humour also indicate a possible inhibitory role of extract constituents on leukocyte adherence to the vascular endothelium. Direct binding to LPS of some of the extract constituents may be another mechanism of suppression of endotoxin-triggered uveitis. In earlier study, Chebulagic acid exerts anti-inflammatory effects in LPS-stimulated RAW 264.7 macrophages by inhibition of NF- $\kappa$ B activation and MAP kinase phosphorylation [13]. Inhibition of cyclooxygenase (COX)-2 may also contribute to the anti-inflammatory effect in anterior uveitis, as studies have shown potent COX-2 inhibitory activity of Chebulagic acid [22]. In the present investigation, topical administration of aqueous extract of Triphala showed potential anti-inflammatory activity against endotoxin-induced uveitis in rabbits as comparable to Prednisolone. Further studies of the isolated active principle namely Chebulagic acid are warranted, to explore the full mechanisms of anti-inflammatory activity in endotoxin-induced uveitis.

## Acknowledgements

The authors are thankful to Indian Council of Medical Research for providing financial assistance

## 6. References

- [1] Gritz DC, Wong IG. Incidence and prevalence of uveitis in Northern California; the Northern California Epidemiology of Uveitis Study. *Ophthalmol.*2004; 111: 491-500.
- [2] Umesh CS, Yadav, Subramanyam S, Ramana KV. Prevention of endotoxin-induced uveitis in rats by benotiamine, a lipophilic analogue of vitamin B1. *Invest Ophthalmol Vis Sci.*2009; 50: 2276-2282.
- [3] Gupta SK, Agarwal R, Srivastava S, Agrawal P, Agrawal SS, Saxena R, Galpalli N. The anti-inflammatory effects of *Curcuma longa* and *Berberis aristata* in endotoxin induced uveitis in rabbits. *Invest Ophthalmol Vis Sci.*2008; 49:4036-4040.
- [4] Zhang H, Li X, Li X. Study on the effect and mechanism of qinggan xiehuo decoction in treatment of endotoxin-induced uveitis in rabbits. *Zhongguo Zhong Xi Yi Jie He Za Zhi.* 1196;16:95-98.
- [5] Rosenbaum JT, McDevitt HO, Guss RB, Egbert PR. Endotoxin-induced uveitis in rats as a model for human disease. *Nature.*1980; 286:611–613
- [6] Li Q, Peng B, Whitcup SM, Jang SU, Chan CC. Endotoxin induced uveitis in the mouse: susceptibility and genetic control. *Exp Eye Res.* 1995; 61:629–632.
- [7] Beutler B, Milsark IW, Cerami AC. Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. *Science.* 1985; 229:869–871
- [8] Lehmann V, Freudenberg MA, Galanos C. Lethal toxicity of lipopolysaccharide and tumor necrosis factor in normal and D-galactosamine-treated mice. *J Exp Med.*1987; 165:657–663.
- [9] McIntyre A. Herbal treatment of children: western and Ayurvedic perspectives. Elsevier Health Sciences. 1985,
- [10] Ayurvedic pharmacopoeia committee. The Ayurvedic Formulary of India, Part I, 2nd English ed. New Delhi: Controller of Publications 2003.
- [11] Kroes BH, Van den Berg AJ, Quarles van Ufford HC, Van Dijk H, Labadie RP. Anti-inflammatory activity of gallic acid. *Planta-Med.*1992; 58: 499-504.
- [12] Kim SH, Jun CK, Suk K, Choi BJ, Lim H, Park S, Lee SH, Shin HY, Kim DK, Shin TY. Gallic Acid Inhibits Histamine Release and Pro-inflammatory Cytokine Production in Mast Cells. *Toxicol Sci.* 2006; 91:123-131.
- [13] Reddy DB, Reddanna P. Chebulagic acid (CA) attenuates LPS-induced inflammation by suppressing NF-kappaB and MAPK activation in RAW 264.7 macrophages. *Biochem Biophys Res Commun.* 2009; 381: 112-117.
- [14] Hamalainen M, Nieminen R, Vuorela P, Heinonen M, Moilanen E. Anti-inflammatory effects of flavonoids: genistein, kaempferol, quercetin, and daidzein inhibit STAT-1 and NF-kappaB activations, whereas flavone, isorhamnetin, naringenin, and pelargonidin inhibit only NF-kappaB activation along with their inhibitory effect on iNOS expression and NO production in activated macrophages. *Mediators Inflamm.* 2007;45:673.
- [15] Rasool M, Sabina EP. Anti inflammatory effect of the Indian Ayurvedic herbal formulation Triphala on adjuvant-induced arthritis in mice. *Phytother Res.*2007; 21:889-894.
- [16] Abraham S, Kumar MS, Sehgal PK, Nitish S, Jayakumar ND. Evaluation of the inhibitory effect of triphala on PMN-type matrix metalloproteinase (MMP-9). *J Periodontol.* 2005; 76:497-502.
- [17] Biradar YS, Jagatap S, Khandelwal KR, Singhanian SS. Exploring of Antimicrobial Activity of Triphala Mashi—an Ayurvedic Formulation. *Evid Based Complement Alternat Med.* 2008; 5: 107–113.
- [18] Ruiz-Moreno JM, Thillaye B, de Kozak Y. Retino-choroidal changes in endotoxin-induced uveitis in the rat. *Ophthalmic Res.*1992; 24:162-168.
- [19] Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin-phenol reagent. *J Biol Chem.*1951; 193:265-275.
- [20] Tuailon N, Shen de F, Berger RB, Lu B, Rollins BJ, Chan CC. MCP-1 expression of T lymphocyte subtype in endotoxin induced uveitis. *Invest Ophthalmol Vis Sci.*2002; 43:1493-1498.
- [21] Avunduk AM, Avunduk MC, Oztekin E, Baltaci AK. Characterization of T lymphocyte subtypes in endotoxin-induced uveitis and effect of pentoxifylline treatment. *Curr Eye Res.*2002; 24:92-98.
- [22] Reddy DB, Reddy TCM, Jyotsna G, Sharan S, Priya N, Lakshmi V, Reddanna P. Chebulagic acid, a COX-LOX dual inhibitor isolated from the fruits of *Terminalia chebula* Retz., induces apoptosis in COLO-205 cell line. *J Ethnopharmacol.*2009; 124: 506-512.