Topically applied standardized aqueous extract of *Curcuma longa* Linn. suppresses endotoxin-induced uveal inflammation in rats

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Aqueous extract of *C. longa* when administered 4 h after induction of *E. coli* lipopolysaccharide-induced uveitis in rats showed significantly suppressed inflammation with a significantly lower mean clinical grade, histopathological grade and aqueous humor (AH) protein level compared to vehicle treated group. Although, prednisolone group showed significantly lower clinical grade, histopathological grades and AH protein levels compared to *C. longa* group, TNF-α levels did not differ significantly. Moreover, when the aqueous extract was administered starting from 3 days before induction of uveitis, the mean clinical and histopathological grade as well as AH protein and TNF-α levels were comparable to *C. longa* group when treatment was administered 4 h after induction of uveitis. It is concluded that topically applied standardized aqueous extract of *C. longa* suppresses endotoxin-induced uveitis in rats by reducing TNF-α activity.

**Keywords**: *Curcuma longa*, Endotoxin, TNF-α, Uveitis

Uveitis, defined as intraocular inflammation of uncertain etiology, is a commonly encountered clinical condition and depending upon the amount and location of scarring can lead to multiple complications and even blindness. The management largely involves topical or systemic use of steroids that is often associated with local adverse effects like cataract and glaucoma besides numerous systemic adverse effects. It is, therefore, imperative to look for safer and more effective treatment options. One such option of interest is to investigate bioactivity of plant-derived drug molecules. *Curcuma longa* Linn. has been investigated for its anti-inflammatory properties. Among the bioactive substance isolated from *C. longa*, curcumin is the principal and most active curcuminoid. Besides being a potent antioxidant, it shows significant anti-inflammatory properties owing to its ability to suppress Tumor Necrosis Factor (TNF)-α. However, its benefits in ocular inflammatory diseases have not been investigated widely. One of the studies has shown its anti-inflammatory effects in patients with uveitis after oral administration of curcumin. The anti-inflammatory effects of topically applied aqueous extract of *C. longa* were observed in endotoxin-induced uveitis in rabbits when the crude extract was instilled as pre-treatment. However, the possible therapeutic benefits can be utilized effectively if the anti-inflammatory properties of *C. longa* are significant when used after the onset of inflammation rather than when given as pre-treatment.

Therefore, the purpose of the present study is to evaluate the effects of the aqueous extract of *C. longa* following its administration after the induction of uveitis. Anti-inflammatory effects were compared with those observed following its use as pre-treatment. Further, the standardized extract of *C. longa* is compared for its anti-inflammatory effects with that of prednisolone. Since, aqueous humor levels of cytokines, in particular TNF-α are elevated in endotoxin-induced uveal inflammation and *C. longa* has demonstrated significant TNF-α suppressing property, the study also measured the effects of topical *C. longa* on aqueous humor TNF-α levels.

**Materials and Methods**

The animal handling and all procedures undertaken in this study were in accordance to the ARVO...
statement for the Use of Animals in Ophthalmic and Vision Research. The study was approved by Institutional Animal Ethics committee. Wistar rats weighing 180-200 g were maintained under standard laboratory condition with 12:12 h cycle of day-night and ad libitum access to food and water. The rats that were healthy on general clinical and ophthalmic examination were included in the study.

Plant Extract—The aqueous extract of *C. longa* was provided by Promed Exports Pvt Ltd, New Delhi, India, and the extract was standardized to 1% curcumin.

Experiment 1

Animals were randomly divided in 3 groups of 24 animals each. The experimental uveitis was induced in all rats and bilateral topical treatment was started 4 h after induction. Group 1 was topically instilled with vehicle consisting of 0.25% hydroxypropyl methylcellulose (HPMC), Gr. 2 with the extract of *C. longa* and Gr. 3 with 1% prednisolone. The topical instillation of respective eye drops (20 µL) was done every 30 min for 2 h, every 2 h for 4 h and then every 6 h till 24 h. At 24 h post-induction, anterior segment and fundus photography were done. Rats of all groups were then sacrificed by overdose of pentobarbital and in each group, pooled samples of aqueous humor from 4 eyes (n=6 from 24 eyes) were used for estimation of protein and TNF-α. Ten randomly chosen eyes from each group were subjected to histopathological examination.

Experiment 2

Animals were randomly divided in two groups of 24 animals each. Group 1 was the control group treated with vehicle while the Gr. 2 rats received bilateral treatment with *C. longa* extract, topically. All instillations (20 µL) were done thrice daily for three days before induction of experimental uveitis and continued for 24 h post-induction. After 24 h of induction of experimental uveitis, anterior segment and fundus photography was done and rats were sacrificed as in experiment 1. In each group, estimation of aqueous humor protein and TNF-α estimation and histopathological examination was done as in study 1.

Induction of uveitis in rats—Experimental uveitis was induced as described previously.4,5 Briefly, each animal was injected with *Escherichia coli* 055: B55 lipopolysaccharide (LPS), 100 µg, (Sigma Chemical Company, St Louis, MO, USA) dissolved in 100 µl, subcutaneously in each of the hind footpad.

Anterior chamber and fundus photography and clinical grading—Anterior chamber photography was done using slit lamp biomicroscope at 16X magnification. Posterior chamber and fundus photography was done using slit lamp with same magnification plus a 90D lens placed in front of the rat eye. Clinical grading for severity of inflammation was done by a masked observer before and after dilatation of pupil with tropicamide 1% eye drops. With undilated pupil the severity of uveal inflammation was graded based on the extent of iris hyperemia and the alteration in the shape of pupil. With dilated pupil the grading was based on the extent of distortion of the shape of pupil, presence or absence of exudates and the extent of haziness in the posterior chamber (Table 1). Total score for each rat was calculated and mean score among groups was compared for statistically significant differences.

Aqueous humor protein and TNF-α estimation—Rats were anesthetized by light ether anaesthesia. The eye was thoroughly washed with normal saline and dried completely with a cotton bud. Cornea was now gently pricked using a 30 gauze needle and the aqueous that escaped was collected using a micropipette. Pooled samples of aqueous humor from 4 eyes from 4 different rats (within a group) were used for estimation of proteins and TNF-α. Thus, from 24 animals in each group, 6 aqueous humor samples were obtained. Protein estimation in aqueous humor was done according to the method described by Lowry et al.6. TNF-α levels in aqueous humor were estimated using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Diaclone, France) as per manufacturer’s instructions. All estimations were done in duplicate.

Histopathological examination—The enucleated rat eyes, 10 from each group, were fixed in 10% formaldehyde. The sections (6-8 µm thick) were stained with hematoxylin and eosin for light microscopy. The inflammatory changes observed in anterior chamber, ciliary body and posterior chamber were graded by a masked observer on a scale of 0-5: 0 = none, 1 = minimal, 2 = discrete, 3 = moderate, 4 = severe, 5 = intense.

Statistical analysis—All values were expressed as mean ± SD. Statistical comparison was done using
two-way ANOVA with Bonferroni’s test. \(P<0.05\) was considered significant.

**Results**

**Clinical signs of uveitis**—After 24 hours of induction of uveitis, anterior chamber photographs in control groups, in both the experiments, showed signs of severe ocular inflammation. Intense conjunctival and circumcorneal inflammation was observed in all rats of control group. Pupil was irregular on dilatation and exudates were clearly visible. Fundus photography showed hazy appearance of the fundus. The mean score in control groups on clinical grading in experiments 1 and 2 is shown in Table 1 and Figs 1 and 2. In experiment 1, *C. longa* treated group showed mild conjunctival and circumcorneal congestion. Pupil was round and fundus was clearly visualized. In prednisolone treated group, the conjunctival and circumcorneal congestion were minimal, pupil was round and details of fundus architecture were clearly seen. The mean scores on clinical grading in *C. longa* and prednisolone treated groups were significantly lower than the control group. Further, clinical grading showed significantly lower degree of inflammation in prednisolone treated group as compared to *C. longa* treated group (Table 2, Fig 1). In experiment 2, *C. longa* treated group showed significantly reduced conjunctival and circumcorneal congestion as compared to control group. Pupil was round on dilatation. No haziness was observed in posterior chamber and details of fundus were clearly visualized on fundus photography. The mean score on clinical grading in this group was significantly lower than the corresponding control group (Table 2, Fig. 2), however, no difference was observed from that of *C. longa* treated group in study 1.

**Total aqueous humor protein**—Aqueous humor protein levels were found to be significantly lower as compared to corresponding control when topical treatment with *C. longa* started after induction of uveitis (experiment 1). Prednisolone lowered the mean aqueous protein and this reduction was significantly greater than both the control and *C. longa* treated groups.). Pre-treatment with *C. longa* (experiment 2) also lowered the aqueous humor protein levels (Fig. 3) and this effect of pre-treatment did not differ significantly from that observed in experiment 1.

**TNF-alpha**—Topical treatment with *C. longa* significantly reduced aqueous humor TNF-\(\alpha\) levels in rats receiving treatment after induction of uveitis. The mean TNF-\(\alpha\) level in *C. longa* treated rats (experiment 1) was significantly lower than control group and was comparable to prednisolone treated group. *C. longa* pre-treated rats in experiment 2 also showed significantly lower TNF-\(\alpha\) levels as compared to control (Fig. 4).

**Histopathological signs of uveitis**—Histopathological examination of rat eyes in control groups revealed signs of severe ocular

<table>
<thead>
<tr>
<th>Table 1—Clinical grading of the severity of uveal inflammation</th>
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<td><strong>Undilated pupil</strong></td>
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<tr>
<td>A. Iris hyperaemia</td>
</tr>
<tr>
<td>1. No iris hyperaemia - 0</td>
</tr>
<tr>
<td>2. Mild iris hyperaemia – 1</td>
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<tr>
<td>3. Moderate iris hyperaemia – 2</td>
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<tr>
<td>4. Severe hyperemia – 3</td>
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<tr>
<td>5. Severe hyperaemia with hemorrhage - 4</td>
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<tr>
<td></td>
</tr>
<tr>
<td>B. Shape of pupil</td>
</tr>
<tr>
<td>1. Round and regular – 0</td>
</tr>
<tr>
<td>2. Irregular pupil – 1</td>
</tr>
<tr>
<td>3. Irregular pupil with exudates - 2</td>
</tr>
<tr>
<td>C. Haziness in posterior chamber</td>
</tr>
<tr>
<td>1. Posterior chamber clear of haziness; fundus architecture clearly visible – 0</td>
</tr>
<tr>
<td>2. Haziness in posterior chamber; fundus architecture not seen clearly – 1</td>
</tr>
<tr>
<td>3. Hazy posterior chamber with fundus architecture not visible – 2</td>
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inflammation with vasodilatation, interstitial edema and extensive inflammatory cell infiltration in ciliary body, anterior chamber and posterior chamber. The histopathological features in rats in *C. longa* treated group, when treatment was started after induction of experimental uveitis (experiment 1), showed significantly lower grade of inflammation compared to corresponding control group. Prednisolone treated group showed significantly lower histopathological grade compared to corresponding control and *C. longa* treated group. The histopathological grade in *C. longa* treated group of experiment 1 did not show significant difference from the mean grade of the *C. longa* pre-treated group of experiment 2. (Table 2, Fig. 5).

**Discussion**

Acute anterior uveitis can be produced experimentally in rats by a single injection of endotoxin or LPS at a peripheral site remote from the eye. The LPS component of gram-negative bacteria, endotoxin, when injected in susceptible animals at a site remote from eye can induce ocular inflammation and this phenomenon is known as endotoxin-induced uveitis. Strong biological activity of endotoxin initiates vascular and cellular inflammatory responses.

Fig. 1—Endotoxin-induced uveitis in rats (experiment 1). Control group (A) intense inflammation and exudates seen through pupil, (B) dilated and irregular pupil with exudates in the pupillary area, (C) haziness in posterior chamber and fundus not seen clearly. *C. longa* treated group (A) mild inflammation indicated by slightly congested vessels, (B) completely dilated pupil with regular margin and no exudates, (C) fundus clearly visualized. Prednisolone treated group (A) no signs of inflammation, (B) completely dilated pupil with no exudates, (C) clearly visible fundus [16X; C: 90 D lens was added to 16X magnifications].

Fig. 2—Endotoxin-induced uveitis in rats (experiment 2). Control group (A) intense inflammation and exudates seen through pupil, (B) dilated pupil with irregular shape and broken synechiae, (C) haziness in posterior chamber and fundus not seen clearly. *C. longa* treated group (A) mild inflammation as indicated by slightly congested vessels, (B) completely dilated pupil with regular margins and no exudates, (C) fundus clearly visualized [16X; C: 90 D lens was added to 16X magnifications].
As a result there is breakdown of the blood-aqueous barrier and subsequently, there is accumulation of inflammatory cells in the anterior and posterior segments. Infiltration starts after 4 h and reaches maximum at 24 h post-induction. Extensive inflammatory cell infiltration is associated with release of potent inflammatory mediators and several studies have suggested that TNF-α has an important role in the development of uveitis.

**Table 2**—Effect of topical treatment with C. longa and prednisolone on clinical signs of uveitis and histopathological grade

<table>
<thead>
<tr>
<th>Clinical grading</th>
<th>Histopathological grading</th>
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<tr>
<td></td>
<td>Experiment 1 (n=48)</td>
</tr>
<tr>
<td>Control group</td>
<td>6.25 ± 1.04</td>
</tr>
<tr>
<td>C. longa group</td>
<td>1.75 ± 0.27*</td>
</tr>
<tr>
<td>Prednisolone group</td>
<td>1.08 ± 0.49*#</td>
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P values: *<0.0001 versus corresponding control, #<0.05 versus corresponding C. longa treated group, **<0.01 versus corresponding control, $<0.01 versus corresponding C. longa treated group.

Fig. 3—Effect of topical treatment of C. longa and prednisolone in endotoxin-induced uveitis in rats on aqueous humor protein levels. The extract of C. longa significantly lowered the aqueous humor protein level when given after induction of uveitis as well as when given as pre-treatment. [Values are mean±SD; P values: *<0.0001 versus corresponding control, #<0.001 versus corresponding C. longa treated group.]

Fig. 4—Effect of topical treatment of C. longa and prednisolone in endotoxin-induced uveitis in rats on aqueous humor TNF-α levels. C. longa significantly lowered the aqueous humor protein level when given after induction of uveitis as well as when given as pretreatment. [Values are mean±SD; P value: *<0.0001 versus corresponding control.]

Fig. 5—Micrographs of hematoxylin and eosin stained sections of rat eyes 24 h after induction of experimental uveitis. A. Control group: the arrow indicating extensive inflammatory cell infiltration in posterior chamber (PC). Extensive infiltration also seen in anterior chamber (AC) and ciliary body (CB). Blood vessels in ciliary body are also dilated. B. C. longa pre-treatment group: very few inflammatory cells seen in anterior chamber, posterior chamber and ciliary body. C. C. longa: treatment group: very few inflammatory cells seen in anterior chamber, posterior chamber and ciliary body. D. Prednisolone treatment group: minimal inflammatory cell infiltration seen in anterior chamber, posterior chamber and ciliary body. As the histopathological features in control group of both the treatment (experiment 1) and pre-treatment (experiment 2) groups were similar, only one control group photograph is shown here [20X].

As a result there is breakdown of the blood-aqueous barrier and subsequently, there is accumulation of inflammatory cells in the anterior and posterior segments. Infiltration starts after 4 h and reaches maximum at 24 h post-induction. Extensive inflammatory cell infiltration is associated with release of potent inflammatory mediators and several studies have suggested that TNF-α has an important role in the development of uveitis.
role to play in the development of endotoxin-induced uveitis. As the inflammatory response and cytokine production in response to endotoxin injection closely resembles the acute phase of uveitis in human, this model of uveitis in rats has successfully been used for efficacy evaluation of experimental drugs.

The present results clearly demonstrate the efficacy of topical application of C. longa aqueous extract in the treatment of endotoxin-induced uveitis in rats. When treatment was initiated after induction of uveitis, there was significant protection against development of ocular inflammation in response to endotoxin injection as indicated by significantly low clinical and histopathological grades in treated group as compared to control group. These observations were further supported by significantly low aqueous humor protein and TNF-α levels in treated group as compared to control group. Comparison with prednisolone, although, showed significantly higher clinical and histopathological grades and aqueous humor protein levels in C. longa group, the TNF-α levels between the two treatment groups did not differ significantly from each other. The efficacy of C. longa when used as pre-treatment as an anti-inflammatory agent was in accordance with Gupta et al. where topical application of the crude extract of C. longa aqueous extract was found to protect significantly against the development of experimental uveitis in rabbits. Importantly, comparison of all parameters between experiment 1 and 2 did not show significant differences indicating that the efficacy of C. longa aqueous extract as an anti-inflammatory agent in the treatment of endotoxin-induced uveitis is equivalent to that observed when it was used as prophylaxis.

Multiple mechanisms and molecular targets for anti-inflammatory effects of C. longa and its biologically active component curcumin have been evaluated by many researchers. For the first time, the present study demonstrated that the suppression of TNF-α caused by C. longa is comparable to that caused by prednisolone. Higher efficacy of prednisolone as compared to C. longa as observed in clinical and histopathological evaluation may probably be attributed to multiple molecular targets for anti-inflammatory effects of steroids. Although, in addition to its antioxidant effects, curcumin suppresses TNF-α and NF-κB and downregulates the expression of proinflammatory enzymes such as cyclooxygenase-2 and 5-lipooxygenase and expression of various cell surface adhesion molecules, whether these molecular targets play a role in the anti-inflammatory effect of topically applied C. longa remains to be determined. Further studies are in progress to evaluate the role of these molecular targets in the anti-inflammatory activity of C. longa in ocular tissue on topical application.

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References


