J. Pharm. Pharmacol. 1995, 47: 997–1001
Received March 13, 1995
Accepted September 15, 1995

Comparative Evaluation of *Butea frondosa* and Flurbiprofen for Ocular Anti-inflammatory Activity in Rabbits

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Abstract

The roots and leaves of the plant *Butea frondosa* were evaluated for ocular anti-inflammatory activity on the subacute model of ocular inflammation in rabbits.

The arkas (liquid preparations obtained by distillation of certain liquids or drugs soaked in water, using the Arka-Yantra or any other convenient modern distillation apparatus) were prepared using the roots and leaves of the plant. The arkas were formulated as gels using Pluronic F-127 (PF-127) 30% w/w as the polymer. The anti-inflammatory activity of the preparations were assessed by determining their effects on elevated intraocular pressure consequent to breakdown of blood/aqueous humour barrier. A commercial eyedrop of flurbiprofen 0·03% w/w was used to compare the ocular anti-inflammatory activity of the arkas of the plant. A marketed root arka was included in the study for comparison. The anti-inflammatory activity of the arkas formulated as gels were compared with flurbiprofen gel prepared using the same polymer. The changes in intraocular pressure were monitored at various time intervals after a single dose administration of the aqueous as well as gel formulations. In multiple dose studies the aqueous preparations were administered three times a day, while the gels were administered once a day up to day 30 and the intraocular pressure was monitored on different days post-administration.

The findings reveal statistically significant differences ($P < 0·05$) between the arkas of the plant and the commercial eyedrop of flurbiprofen. The arkas of the plant proved to be better than the eyedrop of flurbiprofen, while with respect to gels, the intraocular pressure monitored at various time intervals revealed no statistically significant difference ($P > 0·05$) between the gel formulations. However, the changes in intraocular pressure monitored on different days post-administration until day 30, demonstrated that the gel produced from *B. frondosa* leaves arka was superior to all the other gels with respect to the extent of reduction of elevated intraocular pressure elicited experimentally.

In recent years inhibitors of prostaglandin synthesis have been introduced in clinical ophthalmology. Flurbiprofen sodium, a non-steroidal anti-inflammatory drug has gained importance and is recommended for inhibition of intra-operative miosis (Keates 1984), treatment of cystoid macular oedema (Drews 1990) and treatment of ocular inflammation following cataract surgery (Diestelhorst et al 1991) and argon laser trabeculoplasty (Blumenthal et al 1987). The non-steroidal anti-inflammatory drugs have gained importance due to the side-effects of corticosteroid therapy (Timothy et al 1989). Thus on the one hand with the existing potential side-effects of corticosteroid therapy and the limitations of non-steroidal therapy, the potential of indigenous drugs can be exploited. The Ayurvedic system of medicine describes the medicinal properties of a number of plants. *Butea frondosa* (Koen ex Roxb) (synonym *Butea monosperma*) is a plant of the family Leguminosae native to India, Burma and Sri Lanka and is popularly known as dhak, palas or flame of the forest. The roots and leaves of *B. frondosa* are indicated to be useful in the treatment of eye diseases and disorders (Shastri 1916; Nadkarni 1976). However, the claims of Ayurveda need to be validated by a suitable experimental and clinical model. There also exists a great need for an inter-disciplinary approach (Sane & Kuber 1992). There is a paucity of data pertaining to the pharmacological and phytochemical investigation of the roots and leaves of the plant. No report so far exists in the literature pertaining to the examination of ocular anti-inflammatory activity of any part of the plant. However, several reports are available with respect to screening of flowers and seeds for antifertility activity (Razdan et al 1970) and anthelminitic activity of seeds only (Shaw & Tripathi 1982). Except for mycotoxic activity (Mishra et al 1991) and antimicrobial activity (Zafar et al 1989) of the leaves, no other activity of the roots and leaves of the plant has been reported in the literature. The literature reveals a preliminary phytochemical investigation of the roots of the plant by Tandon et al (1969). The present study deals with the investigation of roots and leaves for their ocular anti-inflammatory activity. An arka, as defined by Ayurvedic texts, is a liquid preparation obtained by distillation of certain liquids or drugs soaked in water, using the Arka-Yantra or any other convenient modern distillation apparatus. The modern version of the Arka-Yantra is the Soxhlet extractor (Narayana 1993). The arka can be used as an eyedrop. In the present study the arkas were prepared using the roots and leaves. These arkas along with marketed arka were studied and compared with commercial eyedrops of flurbiprofen for their effects on the elevated intraocular pressure induced experimentally by the procedure described earlier (Mengi & Deshpande 1992).

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Although the conventional eyedrop formulations are ubiquitously used in ocular disease management, the bioavailability achieved ranges from 1–7% (Lee & Robinson 1986). The poor ocular bioavailability of drugs has been attributed to the precorneal loss processes that quickly flush the drug out of the precorneal area. Consequently bioavailability of ophthalmic formulations needs to be improved. One of the ways is by prolonging the retention of the drug at the precorneal site (Zaki et al 1986). There has been an increasing use of polymers to increase the viscosity of the formulations and consequently localize the drug in the precorneal area (Saettone et al 1980). Poloxamers or pluronics, a class of gel-forming polymers, have been used in ophthalmic dosage forms. They possess several properties such as low toxicity, mucomimetic properties and optical clarity which make them suitable for use in opthalmic formulations. Pluronic F-127 (PF-127) has been found to be least toxic and hence this polymer was chosen for our experimental study. In our present investigation flurbiprofen and the arkas were formulated as gels using the polymer PF-127 30% w/w. These gel preparations were assessed for their ocular effects by determining the changes in elevated intraocular pressure elicited as a result of breakdown of blood/aqueous humour barrier (BAB). Disruption of BAB was experimentally induced by the procedure described earlier (Mengi & Deshpande 1992).

Materials and Methods

Materials

Albino rabbits of either sex, 2·5–3 kg, were housed in standard laboratory cages and allowed free access to food and water. Pluronic F-127 was a gift from BASF Corporation Chemical Division, Parsippany, NJ, USA. Cadiflur eyedrops were obtained from Cadila Chemicals Pvt. Ltd, India. Xylocaine 1% w/v was obtained from Astra-IDL Ltd. Potassium dihydrogen phosphate, sodium hydroxide and phenylmercuric acetate were of analytical grade.

Preparation of the arkas

The plant were procured from Kasara village, Maharashtra, India, in the month of September. It was authenticated at St Xavier's Blatter's Herbarium, Bombay, India. The roots and leaves were dried in air and were then individually coarsely powdered. The powders were soaked individually in water and then extracted with water in a Soxhlet extractor for 18 h. The roots and leaves extracts were then diluted. Ten millilitres of the marketed arka when dried on a water bath yielded 0·02 mg of the dry crude extract. The dilutions of the roots and leaves arka prepared were carried out so that the dry weight matched that of the marketed arka. The dilution ratio, i.e. crude drug vs water, for the roots was 1:36 and for the leaves 1:35. The preservative phenyl mercuric acetate 0·004% was added and the arkas were then autoclaved at 121°C for 30 min.

Preparation of the Pluronic F-127 (PF-127) 30% w/w gels

A weighed amount of flurbiprofen 0.03% w/w was dissolved in phosphate buffer pH 7·4. The required amount of preservative phenyl mercuric acetate 0·004% w/w was added to the drug solution as well as the arka preparations. PF-127 30% w/w was added to the above solutions and the preparations were stored at 5°C until polymer dissolution was complete (approx. 48 h). The formulations were thoroughly mixed while cold. On warming the preparations to 37°C, clear viscous gels were obtained. The pH of the gels was maintained at 7·4 ± 0·05. The gels were autoclaved at 121°C for 30 min. These gels were then evaluated for their ocular anti-inflammatory activity in rabbits.

Administration of the formulations

Drug formulations were administered using a microlitre syringe. Fifty microlitres of each of the formulations was placed in the conjunctival sac of the left eye of each animal. The contralateral right eye served as the control (positive control). Phosphate buffer pH 7·4 administered to the right eye served as the control for aqueous formulations, while non-medicated gel served as the control for the gel preparations.

Measurement of intraocular pressure

The intraocular pressure was measured using a Schiotz tonometer. Two drops of lignocaine hydrochloride (xylocaine 1% w/v) were instilled in the eye of the rabbit before intraocular pressure measurements. The intraocular recordings were performed at various time intervals beginning with 0·5 h post-administration after a single administration of each of the aqueous and gel preparations. The measurements were performed up to 5 h with the aqueous formulations and up to 28 h with the gel formulations.

In multiple dose studies the aqueous and gel formulations were administered every day for a period of 30 days. However, the aqueous preparations were administered three times a day while the gels were administered once a day. The intraocular pressure was monitored on different days post-administration beginning with day 2, followed by day 4, 5, 10, 15, 20, 25 and 30. The intraocular pressure recordings were performed every 26 h of the day post-administration of the formulations. A group consisting of a minimum of four rabbits was used to study the effect of each of the formulations included in the study.

The drug effects were expressed as mean percent reduction in intraocular pressure. The rise in intraocular pressure observed 24 h after the breakdown of BAB was considered as the baseline value. Considering this baseline value as 100%, the percent reduction in intraocular pressure was computed.

Statistical analysis

All data are presented as mean ± s.e.m. Statistical analysis was performed using one-way analysis of variance. Significance in the difference in the means was assessed by a least-significant difference procedure (LSD) with 95% confidence intervals. $P < 0.05$ was considered statistically significant.

Results

Fig. 1 reveals the comparative effect of a commercial eyedrop of flurbiprofen and arka preparations on elevated intraocular pressure. Onset of effect was seen 0·5 h post-administration with all the aqueous formulations. All the formulations revealed a peak effect by 1 h post-administra-

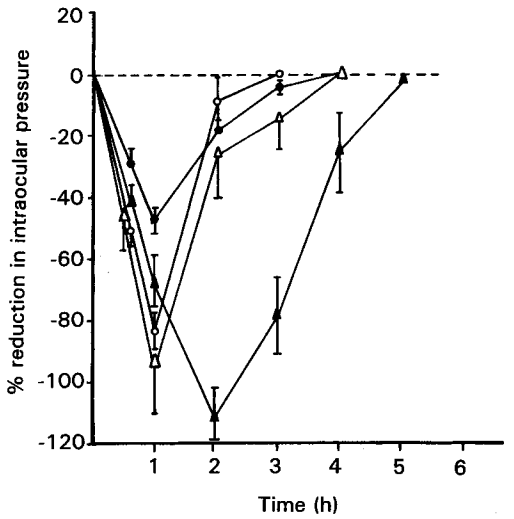

FIG. 1. Comparative effects of *Butea frondosa* arka preparations and flurbiprofen eyedrops on the rise in intraocular pressure in rabbits. ● Flurbiprofen eyedrops, ▲ leaves arka, △ root arka, ○ marketed arka, - - - baseline control. Points represent mean ± s.e.m. (n = 6–8).

tion, except *B. frondosa* leaves arka which demonstrated a peak effect by 2 h post-administration. However, by 3–5 h post-administration the recordings revealed that the intraocular pressure had returned to the original baseline value. Maximum duration of effect until 5 h was seen with *B. frondosa* leaves arka preparation. The peak effects obtained with all the aqueous formulations was subjected to statistical analysis. The results revealed statistically significant differences ($P < 0.05$) between the aqueous formulations. Maximum effect on elevated intraocular pressure was seen with *B. frondosa* leaves and root arka preparations, followed by *B. frondosa* marketed arka, followed by flurbiprofen eye-drop formulation. No change in intraocular pressure was demonstrated at any of the time points in the control eyes of rabbits.

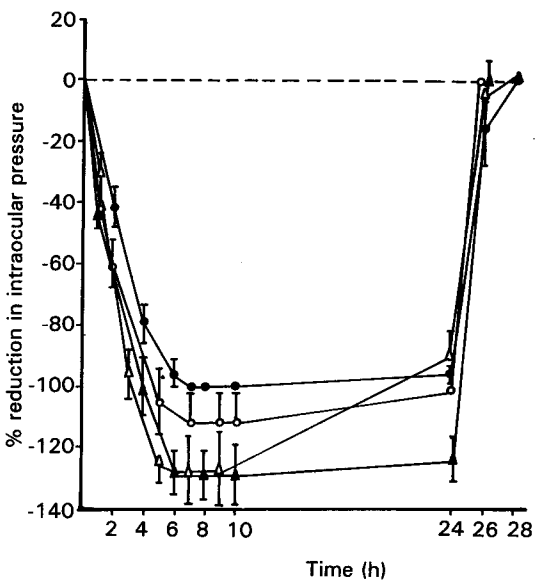

FIG. 2. Comparative effects of *Butea frondosa* arkas formulated as gels and flurbiprofen gel on the rise in intraocular pressure in rabbits. ● Flurbiprofen, ▲ leaves arka, △ root arka, ○ marketed arka, - - - baseline control. Points represent mean ± s.e.m. (n = 4–6).

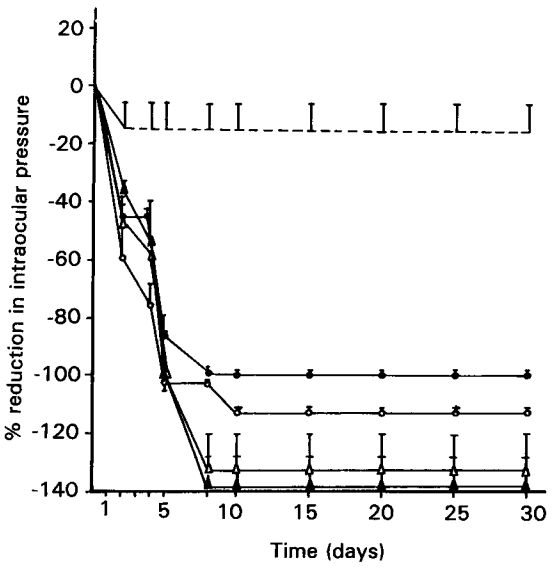

FIG. 3. Comparative effects of flurbiprofen eyedrop and *Butea frondosa* arka preparations on the elevated intraocular pressure monitored on different days. ● Flurbiprofen eyedrops, ▲ leaves arka, △ root arka, ○ marketed arka, - - - baseline control. Points represent mean ± s.e.m. (n = 4).

Fig. 2 presents the comparative effects of the gel formulations on the elevated intraocular pressure. Onset of effect with the gel preparations was seen 1 h post-administration. The maximum effect of the gel on intraocular pressure was seen 6–7 h post-administration. This effect was found to be sustained until 24 h post-administration. However, by 28 h post-administration the intraocular pressure had returned

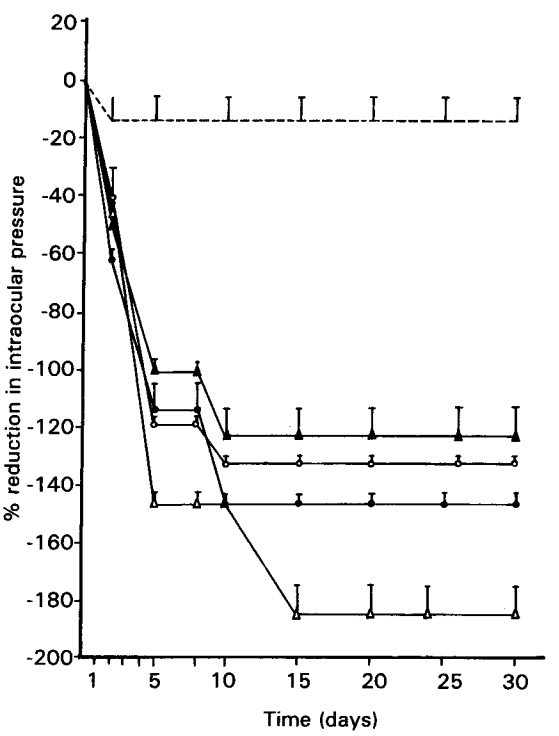

FIG. 4. Comparative effects of flurbiprofen gel and *Butea frondosa* arkas formulated as gels on the elevated intraocular pressure monitored on different days. ● Flurbiprofen, ▲ leaves arka, △ root arka, ○ marketed arka, - - - baseline control. Points represent mean ± s.e.m. (n = 4).

to the original baseline value. One-way analysis of variance performed with the data obtained at time points 4, 7 and 24 h revealed no statistically significant difference ($P > 0.05$) between flurbiprofen gel and the arkas formulated as gels. Control eyes of rabbits demonstrated no change in elevated intraocular pressure at any of time points selected for study.

Fig. 3 demonstrates the effect of aqueous preparation on elevated intraocular pressure monitored on different days post-administration. An appreciable reduction in intraocular pressure was seen on day 2 with all the aqueous formulations. A reduction of about 86–100% was seen on day 5 with these preparations. However, a maximum reduction in intraocular pressure was seen on day 8 with all the aqueous preparations except *B. frondosa* marketed arka, which revealed a maximum effect by day 10 post-administration. The reduction in intraocular pressure was maintained until day 30. The data obtained on day 2 and day 5 with the aqueous preparations, when subjected to one-way analysis of variance, revealed no statistically significant difference ($P > 0.05$) between the aqueous formulations. However, the maximum reduction in intraocular pressure achieved with these preparations on being subjected to statistical evaluation proved *B. frondosa* arka preparations to be superior to the commercial eyedrop of flurbiprofen ($P < 0.05$). However, no difference between the *B. frondosa* root arka, *B. frondosa* leaves arka and *B. frondosa* marketed arka preparation was seen.

Fig. 4 demonstrates the percent reduction in elevated intraocular pressure obtained with the gel formulation of the drug and the arkas formulated as gels on different days post-administration. The gel formulations revealed an appreciable reduction in intraocular pressure on day 2 post-administration. Significant reduction of more than 100% was seen on day 5 with all the gel formulations. Further reduction in intraocular pressure was observed either on day 10 or day 15 post-administration. Reduction in intraocular pressure was maintained until day 30. The magnitude of reduction of elevated intraocular pressure obtained with the gels when subjected to one-way analysis of variance followed by testing of significance in the difference of the means, revealed *B. frondosa* leaves arka gel superior to all the other gels in inhibiting the observed intraocular pressure. Maximum reduction in intraocular pressure was seen with the *B. frondosa* leaves arka gel formulation.

The control eyes of rabbits demonstrated a reduction of $14{\cdot}11 \pm 8{\cdot}15$ mmHg % on day 2. However, no further reduction in intraocular pressure was noted until day 30.

Discussion

Breakdown of blood/aqueous humour barrier results in mild to moderate inflammation manifested as a rise in intraocular pressure, leucocytosis and flare in the anterior chamber (Fingeret & Potter 1989). Prostaglandins have been implicated in the disruption of blood/aqueous humour barrier (Eakins 1970; Neufeld et al 1972). The anti-inflammatory activity of prostaglandin-synthesis inhibitors has been assessed by various methods. Van Haeringen et al (1982, 1983) determined the inhibitory effect of prostaglandin-synthesis inhibitors on elevated protein in the aqueous humour punctates induced by paracentesis. Podos & Becker (1976) monitored both aqueous protein and intraocular elevation produced by topical application of 5% arachidonic acid. In various models of ocular inflammation, the infiltration of leucocytes at the site of injury has been assessed (Leibowitz & Kupferman 1974). In our experiments we have monitored the elevations in intraocular pressure consequent to breakdown of BAB. The elevated intraocular pressure has been monitored at various time intervals after a single dose administration of both aqueous and gel formulations. Our findings demonstrated the reduction in elevated intraocular pressure by flurbiprofen as well as the arkas of the plant. The results suggest the presence of anti-inflammatory compounds in the arkas of the plant. However, the precise mechanism of action remains yet to be elucidated. Moreover, in the single dose study, the arka preparations proved to be more efficacious than the reference drug flurbiprofen in inhibiting the elevated intraocular pressure induced experimentally as a result of breakdown of BAB.

With regards to the gels, the intraocular pressure recordings carried out at various time intervals after a single administration demonstrated no statistically significant difference between the arkas formulated as gels and flurbiprofen gel. However, the intraocular pressure measurements performed after repeated administration up to day 30 revealed maximum reduction in intraocular pressure with *B. frondosa* leaves arka gel ($P < 0.05$). No statistically significant difference was seen between flurbiprofen gel and other arkas formulated as gels. The gels in our study were administered once a day as compared with the aqueous preparations which were administered three times a day. Reduction in frequency of administration is a definite advantage achieved with these aqueous based gels. Moreover, our studies have demonstrated a sustained action for 24 h with the gel formulations as compared with the aqueous preparations. The effect of the aqueous preparations, however, was found to be short-lasting, extending only up to 5 h. The sustained action of the gels can be attributed to the polymer PF-127. Our findings confirm those of other workers who achieved a sustained effect by the use of this polymer (Gurny 1981). The extended residence time leading to enhanced miotic effect of pilocarpine formulated as PF-127 25% w/w gel compared with an aqueous preparation was demonstrated by Miller & Donovan (1982). Prolongation of contact time with consequent reduction in nasolachrymal drainage has been suggested as the most plausible mechanism. Overall a three- to fivefold improvement in ocular bioavailability with aqueous based gels has been reported (Lee & Robinson 1986). Although we have not directly measured the aqueous humour levels, our findings corroborate the results obtained by these other workers.

Thus, in conclusion, our findings demonstrate ocular anti-inflammatory activity of the arka preparations as well as with the arkas formulated as gels. However, further studies are required to elucidate the active constituents of the arkas of the plant. Finally, the sustained action achieved with the gel formulations can be attributed to the polymer PF-127.

Acknowledgement

The authors are grateful to BASF Corporation Chemical

Division, Parsippany, NJ, USA, for providing Pluronic F-127 as a gift.

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