Ex Vivo and In Vivo Evaluation of Topical Hesperetin Matrix Film for Back-of-the-Eye Delivery
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Purpose
The goal of the present study was to delineate the feasibility of back-of-the eye delivery of hesperetin (HT), a bioflavonoid, from a topical polymeric matrix film using ex vivo and in vivo techniques.

Methods
HT matrix film was prepared by hot melt cast method. A film of 4mm x 2mm x 0.2mm dimension was used for both the ex vivo and in vivo studies. Ex vivo studies were carried out using whole rabbit eye globes placed in 12 well Tissue Culture Plates containing phosphate buffered saline. The HT loaded films were applied at the corneal-scleral-limbus and the eyes were allowed to stand for 3h. At the end of 3h the globes were thoroughly washed and the various ocular tissues were carefully collected and analyzed for HT content. In vivo studies were carried out in anesthetized New Zealand albino rabbits following University of Mississippi Institutional Animal Care and Use Committee approved protocols. For these studies, 10% and 20% w/w HT loaded films were placed in the conjunctival sacs. At the end of the 1h/3h studies, rabbits were sacrificed with an overdose of pentobarbital given through marginal ear vein. Eye globes were excised, ocular tissues were carefully isolated and analyzed for HT content.

Results
Ocular tissue concentrations were found to be 5.8±0.1 μg/gm of aqueous humor (AH), 5.6±1.4 μg/gm of vitreous humor (VH) and 14.2±3.7 μg/gm of retina-choroid in the ex vivo studies. In vivo studies produced quantifiable amount of drug in all ocular tissues. Amount of drug in the retina-choroid was found to be 7.8±0.2 μg/gm of tissue (10% HT/1h); 27.2±3.3 μg/gm of tissue (20% HT/1h) and 3.6±0.9 μg/gm of tissue (20% HT/3h). HT levels in the VH were found to be negligible (10% HT/1h); 1.4±0.5 μg/gm of tissue (20% HT/1h) and 0.2±0.07 μg/gm of tissue (20% HT/3h).

Conclusion
The topical polymeric matrix system was able to deliver significant amounts of HT to the back-of-the eye tissues, including the vitreous humor, in vivo. The ex vivo model overestimated ocular delivery due to the absence of the conjunctival membrane and lack of the vascular and lymphatic drainage systems.