# Intraocular Pressure Lowering Activity of Δ⁹-Tetrahydrocannabinol in α-Chymotrypsin Induced Glaucoma Model: Dose - Effect Relationship

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## ABSTRACT

**Purpose**

To evaluate dose dependent intraocular pressure (IOP) lowering efficacy of Δ⁹-Tetrahydrocannabinol (THC) and WIN 55,212-2 (WIN-55) in α-chymotrypsin induced rabbit glaucoma model

**Methods**

Primary open-angle glaucoma was induced in New Zealand white rabbits by intravitreal injection (30G needle) of α-chymotrypsin (50 µL, 20 mg/mL). IOP was monitored daily using TonoVet® tonometer until a stable IOP value was obtained. Diurnal variation in IOP was monitored at different periods of the day (8am, 2pm and 6pm). Nanoemulsions of THC at three different doses (0.5 %w/v, 0.7 %w/v and 0.8 %w/v) and WIN-55 (0.8 %w/v) were prepared in Tocrisolve®.

Fifty microliters of each of the formulations and vehicle (control) were instilled into the test eye and IOP was measured for 2h in both normotensive and hypertensive rabbits. The change in IOP from the baseline values (%ΔIOP) was calculated. IOP lowering activity of marketed Pilocarpine HCl eye drops (2 %w/v) and Timolol maleate eye drops (0.25 %w/v) was also studied. All animal studies were conducted following IACUC approved protocols.

**Results**

Open angle glaucoma model was developed in rabbits by α-chymotrypsin and was monitored by a rise in IOP. The efficacy studies were initiated once the IOP stabilized, approximately at 30 mmHg, after about 14 days. THC exhibited IOP lowering activity in a dose dependent manner, with a peak %ΔIOP of 64% (30 min; 0.8 %w/v), which was maintained for 60 min; IOP returned to 90% of the baseline within 2 h. WIN-55 also showed a similar profile, but peak % ΔIOP was 55% at 30 min. THC did not show any effect on normotensive rabbits while WIN-55 showed peak %ΔIOP of 75% at 60 min. On the contrary, Timolol maleate showed effect in both normotensive and hypertensive rabbits. Above results suggest that the possible mechanism of IOP reduction by THC is by increased outflow of aqueous humor via the trabecular meshwork.

**Conclusion**

The rabbit α-chymotrypsin induced glaucoma model was successfully developed and employed for studying IOP lowering ability of THC and WIN-55. Additional studies to increase the duration of action and ocular retention are under investigation.

## PURPOSE

The aim of the current project is to study the dose-effect relationship of Δ⁹-Tetrahydrocannabinol (THC) on intraocular pressure (IOP) lowering activity in normotensive and α-chymotrypsin induced rabbit glaucoma model and compare it with varying doses of WIN-55,212-2(WIN-55), Timolol maleate and Pilocarpine eye drops (marketed).

## INTRODUCTION

- Glaucoma is an ocular neuropathy associated with optic nerve, characterized by progressive and irreversible loss of vision.
- Intraocular pressure (IOP) has been identified as an important risk factor in the pathogenesis of the disease. Elevated IOP leads to damage of retinal ganglion cell axons leading to progressive and irreversible vision loss.
- Cannabinoid receptors especially CB1 receptors are more prevalent in the human ciliary epithelium, corneal epithelium and endothelium, trabecular meshwork, Canal of Schlemm, ciliary muscle, ciliary blood vessels and retina.
- THC, an active ingredient of the plant *cannabis sativa*, agonist of both CB1 and CB2 receptors, could potentially be a dual acting anti-glaucoma agent: IOP lowering and neuroprotective.
- In the present study, the IOP lowering effect of THC was evaluated in normotensive (normal IOP) and α-chymotrypsin induced rabbit glaucoma model (elevated IOP).
- IOP lowering effect of THC was compared with WIN-55, Timolol maleate and Pilocarpine eye drops in normotensive and glaucoma induced rabbits.

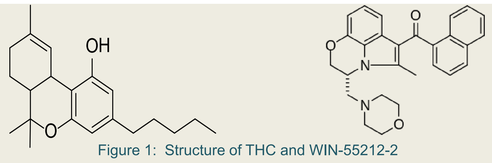


Figure 1: Structure of THC and WIN-55212-2

## METHODS

### Nano-emulsion formulations

- Nanoemulsion formulations of THC(0.4-0.8 %w/v), WIN-55(0.8 %w/v) were prepared in Tocrisolve™100 (Tocris Bioscience), by stirring and sonication.

### Glaucoma model development

All animal experiments conformed to the tenets of the Association for Research in Vision and Ophthalmology (ARVO) statement on the Use of Animals in Ophthalmic and Vision Research and followed the University of Mississippi Institutional Animal Care and Use committee approved protocols (UM IACUC Protocol No # 14-005).

- Open angle glaucoma was induced in New Zealand white rabbits by single intravitreal injection of α-chymotrypsin (50 µL, 20 mg/mL) in WFI.
- Following the intravitreal injections, animals were monitored for inflammation, ocular redness, conjunctival swelling etc.
- When the increasing IOP stabilized, studies on IOP lowering effects was initiated.
- Fifty microlitres of each of the formulations (nanoemulsions) were instilled topically into the lower *cul de sac* of the test eye and in normotensive eyes.
- IOP was measured at before instillation (baseline IOP) and every 30 min till the IOP returned to 90% of the baseline IOP, using Tonovet® tonometer (Reichert Inc.)
- The average percent change in IOP from the baseline IOP was calculated and expressed as %ΔIOP ± SEM.
- The IOP lowering effect of THC was compared with WIN-55 (nanoemulsion), Timolol maleate and Pilocarpine (marketed) eye drops.

## RESULTS

### Glaucoma model development

- Open angle rabbit glaucoma model was successfully developed and employed to study the effect of THC, WIN-55 nanoemulsions on elevated IOP and was compared against marketed Timolol maleate and Pilocarpine eye drops.
- IOP was stabilized to about 30 ± 3mm Hg in 2 weeks.

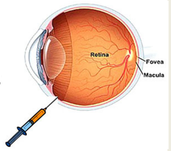


Figure 2: Structure of the eye showing path of intravitreal injections

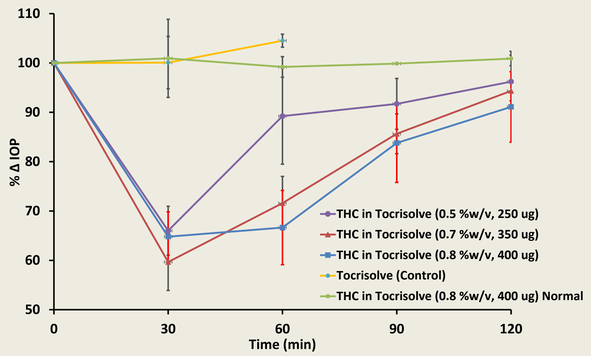


Figure 3: IOP-Time profile for THC in normotensive and α-chymotrypsin induced rabbit glaucoma model. Data represents Mean ± SEM. Numbers in brackets represent concentration and dose of THC.

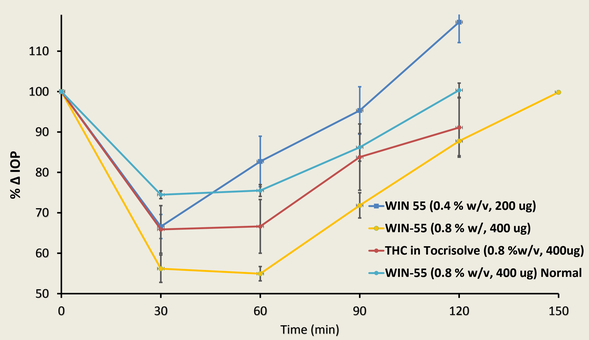


Figure 4: IOP-Time profile for WIN-55 in Tocrisolve (0.4 & 0.8 %w/v) compared with THC (0.8 %w/v) in normotensive and α-chymotrypsin induced rabbit glaucoma model. Data represents Mean ± SEM. Numbers in brackets represent concentration and dose of WIN-55 and THC.

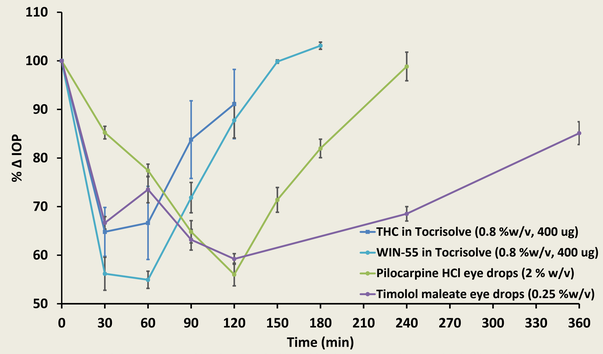


Figure 5: IOP-Time profile for THC (0.8 %w/v) and WIN-55 (0.8 %w/v) compared with Timolol maleate and Pilocarpine eye drops (marketed) in α-chymotrypsin induced rabbit glaucoma model. Data represents Mean ± SEM. Numbers in brackets represent concentration and dose of THC and WIN-55.

## DISCUSSION

- Both THC and WIN-55 showed dose dependent decrease in IOP upon topical administration in α-chymotrypsin induced glaucoma model.
- In normotensive rabbits, THC did not show any effect on IOP while WIN-55 exhibited IOP lowering effect, and WIN-55 was found to be more potent than THC.
- Timolol maleate, reduced IOP in both normotensive and glaucoma induced rabbits and is reported to decrease IOP predominantly by decreasing the production of aqueous humor.
- Pilocarpine decreased IOP in glaucoma induced rabbits, acts on trabecular meshwork by stimulating sphincter pupillae in the iris and the ciliary muscle, resulting in displacement of the scleral spur, resulting in opening of the trabecular meshwork and/or Schlemm's canal, thus increases aqueous outflow.
- Thus, the results indicate THC acts predominantly on the aqueous humor outflow facility rather on the production of aqueous humor, which is in agreement with reported literatures[4,5].

## CONCLUSIONS

- THC showed dose dependent decrease In IOP in glaucoma model, but no effect was seen in normotensive eyes.
- WIN-55 showed IOP lowering effect (dose dependent) in both normotensive and glaucoma induced eyes.
- Timolol maleate showed IOP lowering effect in normotensive and glaucoma eyes, while Pilocaprine showed effect in glaucoma eyes.
- We conclude that the IOP lowering activity of THC is by acting on outflow facility rather than aqueous humor production.

## ACKNOWLEDGEMENTS

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## REFERENCES

1. Jarvinen T, Pate DW, Laine K. Pharmacol Ther. 2002;95:203–20.
2. Green K, and M. Roth.1982. Arch Ophthalmol. 100:265-267.
3. Hingorani T, Gul. W, Elsohly. M, Repka M. A, and Majumdar S. 2012. J Pharm Sci. 101; 616-626.
4 Green K, Wynn H & Padgett D.1978. Exp Eye Res; 26(1); 65–69
5 Green K, Roth M, 1982, Arch Ophthalmol. 1982 Feb;100(2):265-7.