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Steroid Induced Short Term Ocular Hypertensive Glaucoma Model in New Zealand White Rabbits - A Pilot Study

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Abstract: This pilot study evaluated the efficacy of three commonly prescribed topical steroid eye drops in inducing a minimally invasive, short-term ocular hypertensive (OHT) glaucoma model in New Zealand White rabbits. The agents tested included commercial betamethasone, dexamethasone and prednisolone eyedrops solutions. Their effectiveness was assessed based on the consistency of intraocular pressure (IOP) elevation and the presence of clinically significant side effects. IOP was measured daily using both Tonopen and Schiotz tonometers following topical analgesia. The progression of IOP over a three-week period was recorded and analyzed to determine the suitability of each steroid for inducing short-term OHT. Prednisolone administration resulted in marked fluctuations in body condition scores, protein loss, and body weight, although no mortality was observed. Dexamethasone induced higher IOP spikes but lacked consistency. In contrast, betamethasone produced a sustained and consistent elevation in IOP without notable adverse effects, making it the most promising candidate for short-term glaucoma modelling. This minimally invasive model provides a reliable platform for short-term glaucoma research, with strong potential for translational applications in diagnostics and therapeutic evaluation.

Keywords: *Steroid, Eyedrops, Ocular Hypertension, Glaucoma Model, Rabbits, Intraocular Pressure*.

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I. INTRODUCTION

Glaucoma is a progressive and often insidious ocular disorder characterized by the degeneration of retinal ganglion cells and optic nerve damage, which may occur with or without clinically detectable ocular hypertension. New Zealand White rabbits, genetically predisposed to glaucoma (bu/bu), have long served as valuable models in ophthalmic research and surgical interventions [20].

Numerous methods have been developed to induce glaucoma in laboratory animals, including: Steroid-induced models[1,6,17], Argon laser photocoagulation[8],Water loading techniques[3], Intracameral viscoelastic injection[7], Intravitreal hypertonic saline [11], Intracameral polystyrene microbeads [15], Subconjunctival betamethasone injection [9], Vacuum-induced deformation using suction cup oculopressor [16], Intravitreal chymotrypsin injection [12], Radiofrequency ablation of the ciliary body [5], Limbal buckling [18] and Trypsin-induced ocular hypertension [10].

While effective in establishing sustained ocular hypertension, many of these methods result in irreversible structural and functional damage to ocular tissues, compromising animal welfare and limiting opportunities for reuse and rehabilitation.

Given the widespread clinical use of topical steroid eye drops—particularly postoperatively (e.g., following cataract surgery) and as over-the-counter medications—there is a pressing need to develop a minimally invasive, short-term, and clinically translatable glaucoma model. This study aimed to evaluate the efficacy of three commonly prescribed steroid eye drops in inducing reversible ocular hypertension without

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compromising systemic health or ocular integrity. The goal was to identify a steroid capable of producing sustained intraocular pressure (IOP) elevation with minimal side effects, thereby establishing a reliable short-term glaucoma model.

Over the years, various experimental approaches have been developed to induce glaucoma in laboratory animals. These include pharmacological, mechanical, and surgical techniques, each with varying degrees of invasiveness and efficacy. While many of these methods successfully establish sustained ocular hypertension, they often result in irreversible structural damage to ocular tissues and compromise animal welfare. This limits the potential for rehabilitation and reuse of the animals, raising ethical concerns and reducing clinical translatability. The current study helped in providing first hand information as a pilot study in the clinical setting

Given the widespread clinical use of topical steroid eye drops—particularly in postoperative care and as over-the-counter medications—there is a growing need for a minimally invasive, short-term glaucoma model that mimics steroid-induced ocular hypertension. Such a model would allow for controlled induction of elevated intraocular pressure (IOP) without systemic side effects or permanent ocular damage. This study aimed to evaluate the efficacy of three commonly prescribed steroid eye drops in inducing reversible ocular hypertension in New Zealand White rabbits, with the goal of identifying a reliable and ethically sound model for short-term glaucoma research.

II. MATERIALS AND METHODS

A. Ethical Approval and Animal Welfare

This in vivo pilot study was designed to evaluate the efficacy of three commonly prescribed topical steroid eye drops in inducing ocular hypertension in adult New Zealand White rabbits. The objective was to identify a pharmacological agent capable of reliably elevating intraocular pressure (IOP), thereby establishing a minimally invasive steroid-induced glaucoma model.The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) of the College of Veterinary and Animal Sciences, Mannuthy, Thrissur (Project No. CVAS/MTY/IAEC/23/38), and the Committee for the Control and Supervision of Experiments on Animals (CCSEA), New Delhi. All procedures adhered to the guidelines outlined in the CCSEA Compendium (2018) and the Prevention of Cruelty to Animals Act (1960, amended 1998).

All rabbits were procured from a CCSEA-registered breeder and housed in the institute's registered animal facility. A two-week acclimatization period preceded the study. Each rabbit was housed individually in wire-floored cages designed to prevent injury and ensure comfort. The animals were provided with standard rabbit pellet feed, ad libitum access to hay, grass, and potable water, and UV-sterilized soft hay bedding. Enrichment was provided through UV-sterilized wooden blocks, and seasonal fruits and vegetables were included in the diet in limited quantities.

Environmental conditions were strictly controlled, with room temperatures maintained between 18–24°C, relative humidity kept below 70%, and 10–15 air changes per hour to ensure air quality. Lighting followed an automated 12-hour light/dark cycle to support natural circadian rhythms.

B. Experimental Design

Three clinically normal adult New Zealand White rabbits (two females and one male), each weighing at least 2.5 kg and aged between 6–8 months.A thorough physical examination was performed to examine and document general appearance, behaviour, body condition score and body weight prior to start of study. For physical examinations, each rabbit was placed in an individual play pen or tray and handled with minimal or no restraint. Vital parameters like core body temperature (per rectal in °C), pulse rate per minute and, respiration were documented prior start of study and as a part of weekly routine physical examination. The visible mucous membranes, skin and fur, ambulation, feeding, drinking and grooming habits, oral examination and systematic physical examination were also performed. Routine ophthalmic examination was performed on all rabbits prior to commencement of study and also at weekly intervals. All rabbits were subjected to conscious neuro-ophthalmic examination including menace response, pupillary light reflex, palpebral reflex, corneal reflex and visual placing response. Extra ocular and adnexal structures were examined using a slit lamp biomicroscope. The anterior segment and fundus were examined with direct ophthalmoscope (Heine mini-3000, Gilching, Germany). The intra ocular pressure was recorded prior to start of study and weekly for all rabbits using a Tonopen Avia Vet® (Reichert Inc., NY, USA) tonometer upon desensitisation of cornea with proparacaine eyedrops, between 9am-12pm. The IOP was also subsequently recorded with a Schiotz tonometer (Reister, Germany) to increase precision.

Each rabbit was randomly assigned to receive one of the following steroid eye drops:

- Prednisolone acetate 1% (Predfort®, Allergan, Piramal Ltd., MP, India):
  Administered as one drop daily in the left eye for 22 days. IOP was measured twice daily (9 AM and 9 PM) for 26 days using a Tonopen.
- Dexamethasone sodium phosphate 0.1% (Dexadrops®, Chethana Pharmaceuticals, Kerala, India):
  Administered as one drop twice daily (12-hour interval) in the left eye for 22 days. IOP readings were recorded twice daily and plotted for analysis.
- Betamethasone sodium phosphate 0.1% (Betnesol-N®, GlaxoSmithKline Ltd., Mumbai, India):
  Administered as one drop thrice daily in the left eye for 22 days. IOP was measured twice daily using both a Tonopen and Schiotz tonometer.

Only the left eye (OS) was treated and monitored. A consistent elevation in IOP above 23 mmHg was considered indicative of successful induction of ocular hypertension. The data collected were used to determine the most effective steroid for establishing a short-term glaucoma model.

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III. RESULTS AND DISCUSSION

This pilot study evaluated the efficacy of three topical steroid eye drops—prednisolone, dexamethasone, and betamethasone—in inducing ocular hypertension (OHT) in New Zealand White rabbits.

➢ *Prednisolone Acetate 1%*

The rabbit treated with prednisolone acetate 1% exhibited an initial IOP spike on day 4, reaching 30.4 mmHg in the left eye. However, this elevation was not sustained. The animal showed a marked decline in body condition, with scores dropping from 3 to below 2 by the third week, despite being on a standard maintenance diet. These systemic effects suggest that prednisolone may exert broader physiological impacts beyond ocular pressure modulation.

➢ *Dexamethasone Sodium Phosphate 0.1%*

The rabbit receiving dexamethasone demonstrated an IOP peak of 26 mmHg on day 7, followed by a solitary spike of 58 mmHg on day 16. Despite these elevations, the pattern was inconsistent, and no sustained hypertensive state was observed. Importantly, no signs of weight loss or behavioral changes were noted, indicating better systemic tolerance compared to prednisolone.

➢ *Betamethasone Sodium Phosphate 0.1%*

The most promising results were observed in the rabbit treated with betamethasone. This animal exhibited a consistent rise in IOP throughout the 22-day treatment period, beginning on day 9 with an IOP of 29 mmHg and peaking at 52 mmHg on day 20. No adverse changes in body condition, behavior, or vital parameters were recorded, suggesting that betamethasone effectively induced ocular hypertension without systemic compromise.

➢ *Tonometry Comparison*

Readings from both the Schiotz and Tonopen tonometers were comparable for dexamethasone and betamethasone-treated rabbits. However, the Tonopen was preferred for future studies due to its minimally invasive, transcorneal method and ease of use. The Schiotz tonometer, while congruent with the corneal surface of adult rabbits, required skillful handling, head and neck restraint, and a clear cornea for accurate readings—factors that could affect animal comfort and welfare. In contrast, the Tonopen allowed IOP estimation with minimal restraint and topical analgesia (proparacaine), making it more suitable for routine use.

➢ *Model Validation and Literature Comparison*

Betamethasone (Betnesol-N®) was identified as the most suitable agent for inducing OHT in this rabbit model. Its ability to produce sustained IOP elevation without systemic morbidity makes it ideal for focused ocular studies. The consistent rise in IOP aligns with previous findings that topical corticosteroids can induce ocular hypertension by altering aqueous humor dynamics and increasing outflow resistance. Betamethasone's potent anti-inflammatory properties and deeper tissue penetration likely contribute to its reliability.

All tonometric measurements were taken between 9 AM and 12 PM, with peak IOP values typically recorded between 9 AM and 11:30 AM. Pereira et al. [13] reported similar diurnal variations in rabbit IOP, regardless of the tonometer used. Yuschenkof et al. [19] noted that clinical glaucoma in pet rabbits typically presents with IOP above 25 mmHg. In this study, a sustained rise above 23 mmHg for more than 72 hours was considered indicative of OHT.

Bonomi et al. [2] used subconjunctival betamethasone (4 mg) to induce OHT, but results lacked consistency. Subconjunctival injections are painful and require restraint and sedation. In contrast, topical eye drops used in this study were administered with minimal or no restraint, avoiding stress and distress.

Gupta et al. [4] used topical betamethasone thrice daily for six weeks, along with weekly subconjunctival cortisone injections. Another group received systemic prednisone. The first group showed minimal IOP elevation (≤1.2 mmHg), while the second group experienced mortality and weight loss without significant IOP changes. In comparison, this study demonstrated that topical betamethasone alone induced a sustained IOP rise from 13 mmHg in week one to 52 mmHg by week three, with no mortality or decline in body condition.

➢ *Ethical and Translational Relevance*

Unlike invasive models such as intracameral microbead injection or laser ablation, which often cause irreversible damage and stress, the topical steroid model preserves ocular integrity and systemic health. This supports the ethical principles of the 3Rs—Replacement, Reduction, and Refinement—by minimizing animal suffering and enabling repeatable, non-terminal studies.

The absence of systemic side effects in the betamethasone-treated rabbit further supports its suitability. Systemic corticosteroids are known to cause weight loss, immunosuppression, and behavioral changes, which were notably absent in this model.

The variability observed with dexamethasone and prednisolone mirrors earlier reports that not all corticosteroids induce OHT consistently. This underscores the importance of selecting the appropriate agent for reliable model development.

By establishing a minimally invasive, reproducible, and clinically relevant glaucoma model, this study lays the groundwork for future research into the pathophysiology of steroid-induced glaucoma and the evaluation of therapeutic interventions..

IV. CONCLUSION

Topical steroid administration offers several advantages over invasive methods. Unlike techniques such as limbal buckling, laser ablation, or intracameral injections—which are painful and disruptive—topical eye drops are minimally invasive and better aligned with animal welfare principles. This approach supports the 3Rs of animal research[14]:

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repeatability, reduction in animal use, and refinement of induction techniques. Importantly, no irreversible ocular damage was observed in the betamethasone-treated rabbit, reinforcing the model's suitability for short-term glaucoma research. By preserving systemic and ocular integrity, this method enhances the accuracy, reproducibility, and clinical translatability of experimental outcomes. It also facilitates downstream applications such as sonographic analysis of orbital vascular supply and etiopathological investigations in glaucoma models. This pilot study successfully established a minimally invasive, steroid-induced OHT model in rabbits, paving the way for larger-scale studies and translational research in glaucoma diagnostics and therapeutics.

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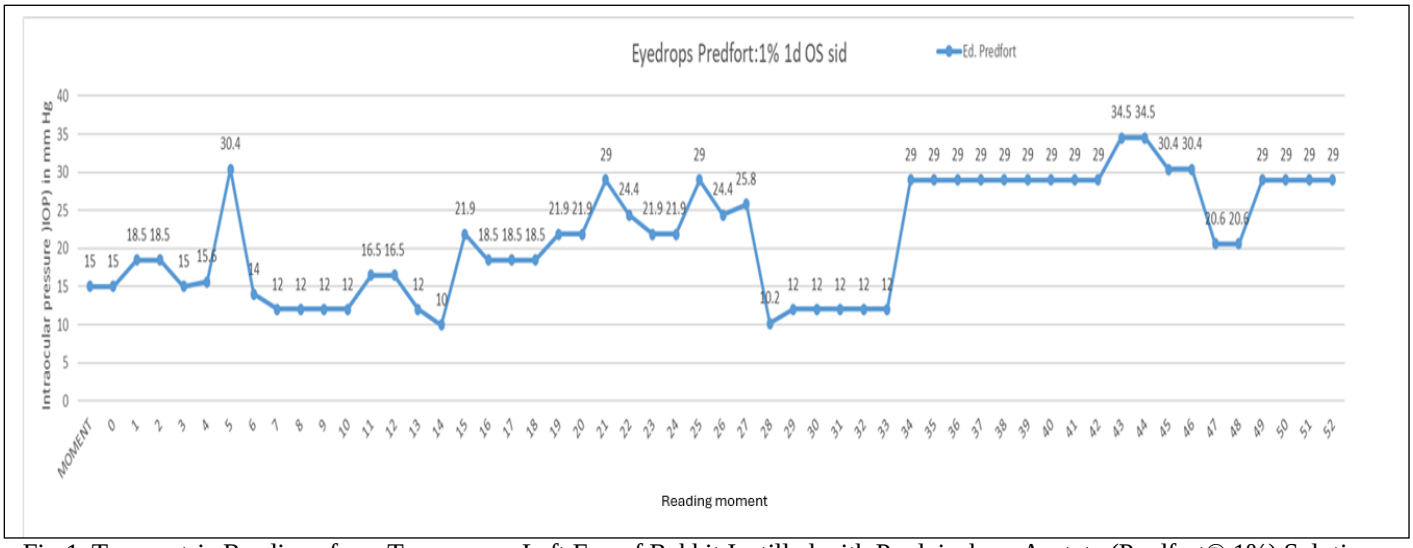
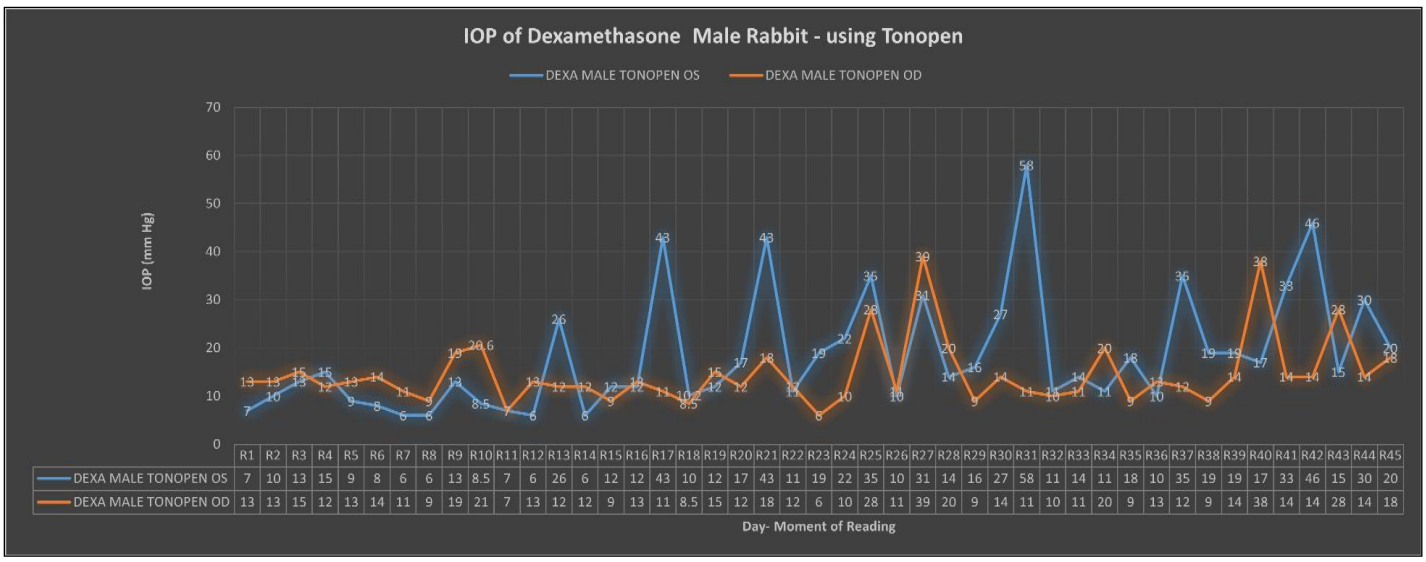
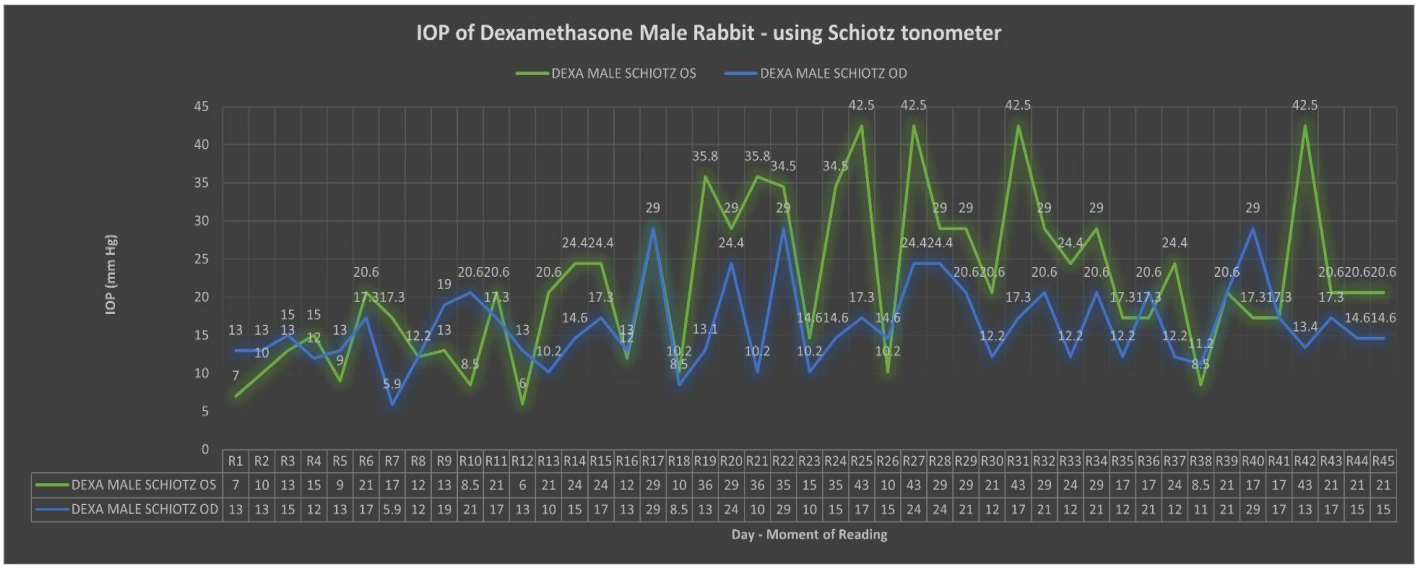


Fig 1. Tonometric Readings from Tonopen on Left Eye of Rabbit Instilled with Prednisolone Acetate (Predfort® 1%) Solution Once Daily for 22 Days and Twice a Day Readings as Moments were Documented for 26 Days



	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15	R16	R17	R18	R19	R20	R21	R22	R23	R24	R25	R26	R27	R28	R29	R30	R31	R32	R33	R34	R35	R36	R37	R38	R39	R40	R41	R42	R43	R44	R45
DEXA MALE TONOPEN OS	7	10	13	15	9	8	6	6	13	8.5	7	6	26	6	12	12	43	10	12	17	43	11	19	22	35	10	31	14	16	27	58	11	14	11	18	10	35	19	19	17	33	46	15	30	20
DEXA MALE TONOPEN OD	13	13	15	12	13	14	11	9	19	21	7	13	12	12	9	13	11	8.5	15	12	18	12	6	10	28	11	39	20	9	14	11	10	11	20	9	13	12	9	14	38	14	14	28	14	18

Fig 2. IOP Readings Using Tonopen from Both Eyes of Rabbit Instilled with Ocular Dexamethasone Eyedrops for 22 Days



	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15	R16	R17	R18	R19	R20	R21	R22	R23	R24	R25	R26	R27	R28	R29	R30	R31	R32	R33	R34	R35	R36	R37	R38	R39	R40	R41	R42	R43	R44	R45
DEXA MALE SCHIOTZ OS	7	10	13	15	9	21	17	12	13	8.5	21	6	21	24	24	12	29	10	36	29	36	35	15	35	43	10	43	29	29	21	43	29	24	29	17	17	24	8.5	21	17	17	43	21	21	21
DEXA MALE SCHIOTZ OD	13	13	15	12	13	17	5.9	12	19	21	17	13	10	15	17	13	29	8.5	13	24	10	29	10	15	17	15	24	24	21	12	17	21	12	21	12	21	12	11	21	29	17	13	17	15	15

Fig 3. IOP Readings Using Schiotz Tonometer from Both Eyes of Rabbit Instilled with Ocular Dexamethasone Eyedrops for 22 Days

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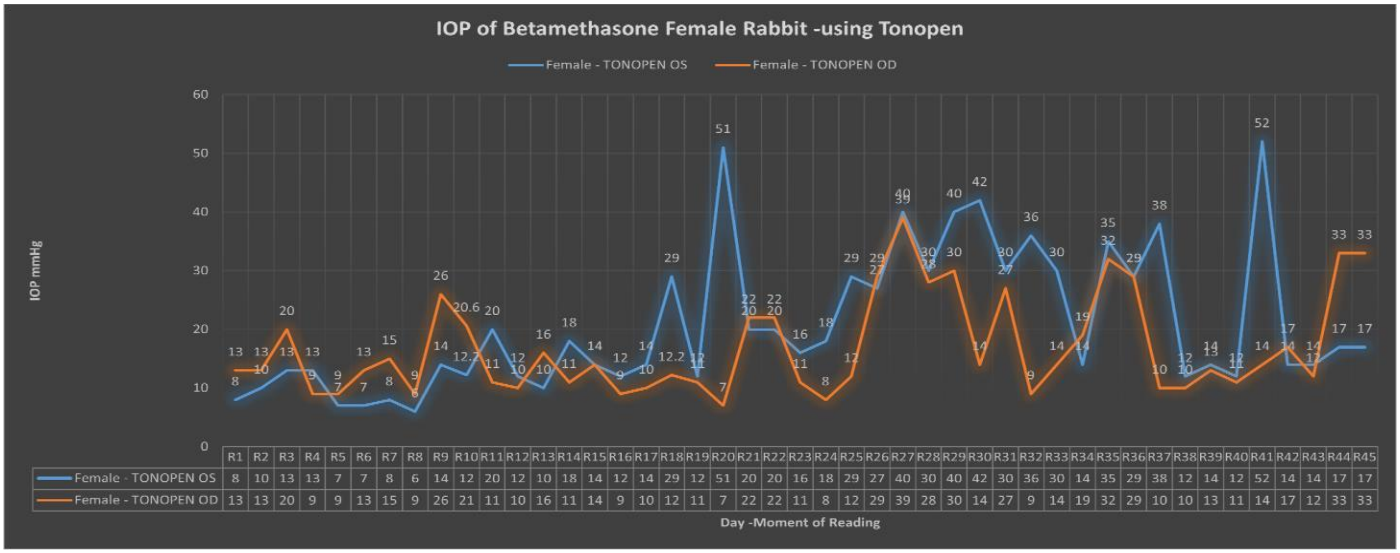
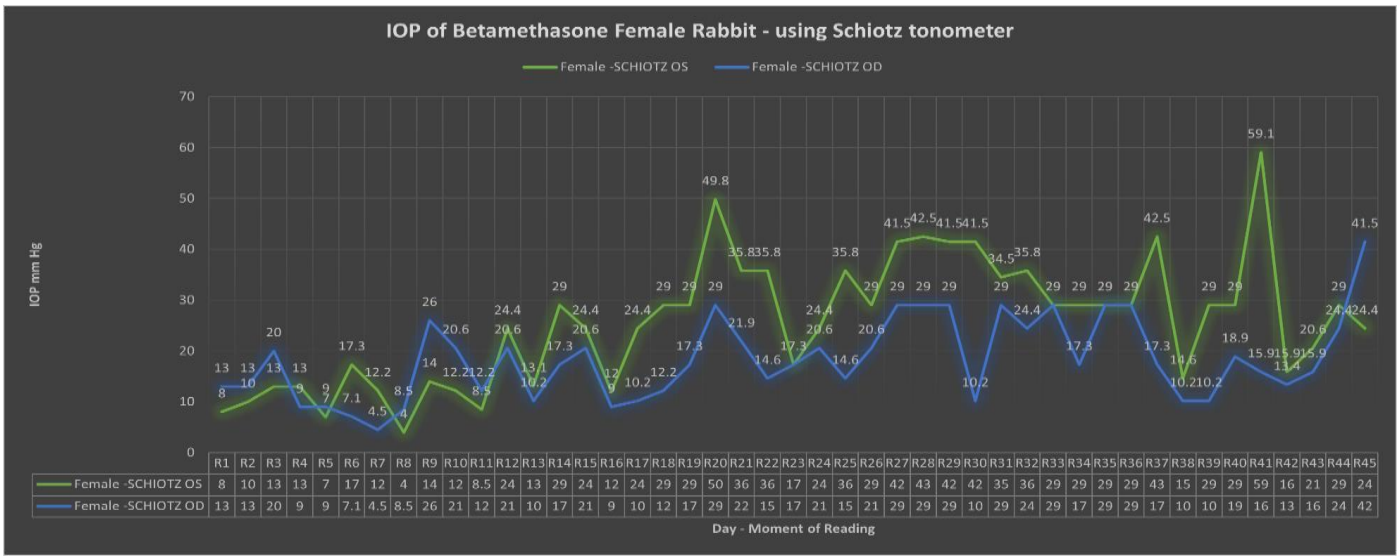


	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15	R16	R17	R18	R19	R20	R21	R22	R23	R24	R25	R26	R27	R28	R29	R30	R31	R32	R33	R34	R35	R36	R37	R38	R39	R40	R41	R42	R43	R44	R45
Female - TONOPEN OS	8	10	13	13	7	7	8	6	14	12	20	12	10	18	14	12	14	29	12	51	20	20	16	18	29	27	40	30	40	42	30	36	30	14	35	29	38	12	14	12	52	14	14	17	17
Female - TONOPEN OD	13	13	20	9	9	13	15	9	26	21	11	10	16	11	14	9	10	12	11	7	22	22	11	8	12	29	39	28	30	14	27	9	14	19	32	29	10	10	13	11	14	17	12	33	33

Fig 4. IOP Readings Using Tonopen from Both Eyes of Rabbit Instilled with Ocular Betamethasone Eyedrops for 22 Days



	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15	R16	R17	R18	R19	R20	R21	R22	R23	R24	R25	R26	R27	R28	R29	R30	R31	R32	R33	R34	R35	R36	R37	R38	R39	R40	R41	R42	R43	R44	R45
Female -SCHIOTZ OS	8	10	13	13	7	17	12	4	14	12	8.5	24	13	29	24	12	24	29	29	50	36	36	17	24	36	29	42	43	42	42	35	36	29	29	29	29	43	15	29	29	59	16	21	29	24
Female -SCHIOTZ OD	13	13	20	9	9	7.1	4.5	8.5	26	21	12	21	10	17	21	9	10	12	17	29	22	15	17	21	15	21	29	29	29	10	29	24	29	17	29	29	17	10	10	19	16	13	16	24	42

Fig 5. Fig. 27. IOP Readings Using Schiotz Tonometer from Both Eyes of Rabbit Instilled with Ocular Betamethasone Eyedrops for 22 Days