

# Novel Role of Topical Diltiazem in Reducing Raised Intraocular Pressure in Rabbits

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## ABSTRACT

**Objective:** The objective of the current study was to evaluate the IOP lowering of topical diltiazem which is a calcium channel blocker.

**Study Design:** Observational / descriptive study

**Place and Duration of Study:** The study was conducted at the Department of Pharmacology, Khyber medical college Peshawar, KPK-Pakistan from November 2015 to February 2016.

**Materials and Methods:** 40 healthy rabbits of a local strain weighing 1.50 to 2.00 kgs were obtained and kept at the animal house of the department of pharmacology, BMC Bannu. The study was conducted on both eyes of conscious rabbits. Three sets namely X, Y&Z were made. Topical diltiazem was injected to set X(made ocular hypertensive and glaucomatous through weekly injecting sub-conjunctival betamethasone suspension). Ocular hypertensive control set Y was also established which gets synthetic tears for a period of 28 days through the whole project. Set Z received no treatment during research and it act as normotensive control.

**Results:** Our results indicated that topical diltiazem can reduce the intraocular pressure very efficiently and quickly. Topical diltiazem yielded IOP reducing outcome in a much transitory time retro. Marvelous animal's survival was also related to it.

**Conclusion:** In future, topical diltiazem might be incorporated as a substitute anti-glaucoma drug in order to manage optical hypertensive crisis, provided its safety in human.

**Key Words:** Glaucoma, Optical Hypertension, CCB, IOP

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## INTRODUCTION

Man is borne with a nature which is non-satisfying and inquiring. That is why he is always been involved in newer researches. A worldwide research, in the same background, is ongoing to expand the management of glaucoma. To make improvement in the treatment of glaucoma and to explore the causes being involved in its onset, researchers are making extensive work on the same<sup>1</sup>. A no of drugs are made which have vasodilating and intraocular pressure lowering tendencies<sup>2</sup>. As per Glaucoma Range, medical scenario of the disease is fairly terrible and capricious.

CCB or Calcium channel blockers are assorted collection of drugs<sup>3</sup>. Keeping in view the therapeutic values of CCB's, many boulevards are still need to be discovered in order to completely understand salutary effectiveness of CCB's. In coming years, research will expectantly discover their variety in numerous therapeutic arenas additionally with ophthalmology.

CCBs are in use since 70's for their role in lowering IOP. An abundant research articles are accessible about IOP upsetting possessions of CCB's. Although more than a few contradictory information existing concerning the CCB's role on IOP<sup>4,5,6</sup> but the overall propensity is in the direction of a reduction in IOP<sup>7,8,9</sup>. Potential applications are reported about CCBs for their role in glaucoma including vasodilatation and thus refining optic nerve blood flow and neuro protection<sup>10</sup>. The technique to increase IOP through using steroids (suspension) was as described by<sup>11</sup>. The current work done is envisioned to notice the usefulness of topical diltiazem on steroid persuaded elevated intraocular pressure in rabbits. The result of the study will lead to an addition in the existing conflicting data.

## MATERIALS AND METHODS

It was an experimental study which was conceded on rabbits in two phases including phase-A& phase-B.

**Phase-A:** During this phase, ocular hypertension was created in the animals of sets X & Y excepting the standard/control set Z. The phase was continued for 3 weeks i.e. twenty one days (range 0-21).

**Phase-B:** A two days gap was given before the start of phase-B, to acquire a completely established elevated IOP (day 22 & 23).

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Through phase B, animals of set X (made ocular hypertensive during phase A), were provided a treatment with topical diltiazem ( $8.9 \times 10^{-2}$  M) solution. Set Y was in still mock tears. 4 weeks i.e. 28 days (day 24-51) were consumed during this phase. Infusion of the drugs was a single drop throughout the week.

**Animals Used:** 40 rabbits were brought for the study. The experiment was conducted on both eyes of standard and cognizant rabbits. Animals of either sex (male and female) or species (albino and colored strains) were incorporated. Their average weight was between 1500–2000 gms and their age was in between 1-2 years. Two weeks observation was done before the onset of the experiment. The animals were retained in the “Animal House, Khyber Medical University, Peshawar”. Feeding was done on fodder, wheat grains ad libitum. Fresh and nutritious water was also provided.

**Grouping of Animals:** Rabbits were organized in three sets.

**Set X:** This set contained 10 rabbits which were steroid treated and ocular hypertensive. The animals of this set were infused with topical diltiazem ( $8.9 \times 10^{-2}$  M for four weeks).

**Set Y:** 20 rabbits were included in this set. Ocular hypertension was created within this set of animals. The set worked for ocular hypertensive control. It got mock tears for a period of four weeks.

**Set Z:** 10 rabbits were retained within this set. It was aimed to serve as normal control or normotensive. No treatment was given to this set of animals.

**Chemicals:** Various chemicals were used during the study including Diltiazem HCl powder, Proparacaine HCl 0.5%, Inj. Betamethasone suspension, Fluorescein sodium 2% and artificial tears drops.

**Equipments:** Tonometer and rabbits containers were used in the study.

**Initiation of Glaucoma:** 1. Set X & Y animals were made ocular hypertensive ( $n = 30$ ). A sub conjunctival suspension of betamethasone (betamethasone sodium phosphate & betamethasone acetate 3mg/ml each)/week (0.7ml) was infused in both eyes.

2. Infusion of Betamethasone was given for three weeks.

3. Injections were given at day zero, 1, 2 & 3.

**Procedure for Injecting Beta Methasone:** Specially manufactured wooden boxes were used to keep the rabbits within them. The rabbits were infused. 5% proparacaine HCl, to persuade local anesthesia, was used. Sooner after some time, betamethasone was administered in sub conjunctival pouch of the animals. Insulin injects were incorporated to achieve the same.

**Procedure of Determining IOP:** 1. All the rabbits were tested for their IOP using tonometer for two weeks (Before the start of the study). Four readings were noted during this time. Animals showed variations more than 5mm Hg in their intraocular pressure were omitted ( $n = 5$ ) and newer animal's set was involved to swap the omitted ones.

2. To evade diurnal difference of the IOP, readings were taken at a fix time during the entire study (Ocular Pharmacology Text Book 1997).

3. Measurements of the IOP in both eyes were taken twofold in a week. Corneal epithelial damage was protected by doing this (Kanski 2004). Thursday and Monday were selected for these practices.

4. Through phase-A, 1<sup>st</sup> reading was noted prior to injecting weekly Betamethasone (Thursday) and 2<sup>nd</sup> was recorded after 3 days (Monday).

5. Base line pressure was considered after infusing 1<sup>st</sup> injection of Betamethasone. It was designated as “zero time”.

6. Before taking readings, the animals were provided with topical local anesthesia followed by fluorescein that causes stain in cornea.

7. Animals immobilization was done by placing them in specially designed wooden boxes.

8. IOP was recorded with the help of tonometer.

9. In phase-B, steroids infusion was ceased but IOP measurement was still continued. IOP was noted prior to the infusion of the drugs.

10. 2<sup>nd</sup> phase IOP readings were well-thought-out to be the initial pressure.

**Preparation of Diltiazem:** The only available form of diltiazem is the tablet form. Its ophthalmic solution is not readily available.

A strength of  $8.9 \times 10^{-2}$  M is known to possess intraocular pressure lowering effects (Juan Santafe 1997). We took the same solution and preceded our work.

**Process for Drug Therapy:** 1. Infusion of diltiazem & artificial tears was remained in progress during 2<sup>nd</sup> phase (day 24<sup>th</sup>).

2. It was completed in both eyes at a specific time.

3. Readings of the IOP were noted down prior to the infusing the drug.

## RESULTS

Readings (IOP) were taken. Similar readings of both eyes were observed. (Right eye readings mentioned only). The readings shown as \*, \*\* and NS

\* = Significant ( $P < 0.05$ ), \*\* = Highly significant ( $P < 0.05$ ), NS = Non significant ( $P > 0.05$ )

**Table No.1: Set Y and Z mean IOP during 1<sup>st</sup> and 2<sup>nd</sup> phase**

Time Interval (Weeks)	Set Y (Ocular hypertensive)	Set Z (Normotensive control)
0	19.63±0.64	20.00 ±0.31
1	22.04±0.65*	21.63± 0.60
2	22.65±0.25**	21.60±0.30
3	24.02±0.58**	22.86±0.46
4	24.64±0.24**	21.84±0.46
5	26.56±0.33**	21.80±0.56
6	26.60±0.26**	22.02±0.52
7	26.45±0.40**	22.05±0.50
8	25.45±0.30**	21.09±0.40

**Table No. 2: Sets X&Y mean IOP differences during 2<sup>nd</sup> phase**

Time Interval (Weeks)	Set X (Topical diltiazem)	Set Y (Artificial tears)
0	25.36± 0.30	25.50± 0.22
1	25.55 ±0.26**	26.54 ±0.32
2	21.29 ±0.70**	26.55± 0.25
3	21.04 ±0.80**	26.40± 0.42
4	20.52 ±0.64**	25.38 ±0.32

**Table No.3: Week wise mean IOP difference of diltiazem treated ocular hypertensive rabbits**NB: 2<sup>nd</sup> measurements of IOP has been mentioned only

	Time Interval (Weeks)	Set X (Diltiazem treated)	Mean difference
0	Starting IOP	25.40±0.32	0.91±0.22
	Week 1 / Value 2	24.40±0.26	
1	Week 1 / Value 2	25.43±0.26	3.20±0.60**
	Week 2 / Value 2	20.26±0.70	
2	Week 2 / Value 2	20.24±0.71	0.30±0.40NS
	Week 3 / Value 2	20.00±0.78	
3	Week 3 / Value 2	20.20±0.79	0.51±0.60*
	Week 4 / Value 2	19.45±0.60	

## DISCUSSION

CCB's are known to be under use for more or less than 30 years for their IOP sinking properties. Sufficient facts are accessible regarding IOP lowering potency of ccbs. Its effects are testified in man as well as in animals. Contradictory reports are there but still, there is no consensus on the same<sup>12-15</sup>.

Above all, even then, CCB's are considered to be important for the researchers due to their probable effect in glaucoma patients in lowering IOP as well as providing vasodilatation and neuro protection<sup>16-19</sup>. AGS (American Glaucoma Society) has connected the usage of iron and calcium augmentation in glaucoma victims (22<sup>nd</sup> annual meeting)<sup>20</sup>.

The aforesaid work was conceded to validate IOP dropping tendency of diltiazem topically. The results substantiate that diltiazem can lesser intraocular pressure, consequently, resulting in an ad in the current information which shows CCB's role in managing glaucoma/ocular hypertension.

Steroid headed to a fast increase in IOP of set X & Y. The improvement in IOP was established statistically momentous after 2<sup>nd</sup> dose of betamethasone showing a P value <0.05 as shown in table 1. After 4<sup>th</sup> injection, the progression became extremely significant statistically (P<0.05).

The normotensive control set Z, did not display any statistically noteworthy alteration in their IOP'S throughout the work done (P>0.05).

After interpreting the results of phase-B, it was noticed that topically smeared diltiazem abridged the IOP efficiently as shown in table 2. Set X result are highly statistically significant (P<0.00) in comparison with the control set Z (ocular hypertensive).

Looking at table 2, the alteration in IOP of set X as compared to set Y became highly significant statistically from the first 7 days of treatment (P<0.00). Topical diltiazem substantiated to be effective in sinking IOP. Topical diltiazem has the tendency to drop the IOP very energetically, predominantly between 1<sup>st</sup> and 2<sup>nd</sup> week. A constant level in IOP was observed between 3<sup>rd</sup> and 4<sup>th</sup> week. Adiredrip in the IOP noticed was 5.10±0.61 between week 0 and 2. In the last week of treatment, it showed statistically non-significance (P>0.05), when its IOP lowering effect was compared week wise (Table 3).

After steroids cessation, some natural IOP lowering effect was also seen in set Y. The IOP dip was found significant statistically (P>0.05) in comparison with the values perceived at the termination of betamethasone therapy (Week 3). After termination of steroids treatment, the IOP was checked for additional 4 weeks in both sets.

This study does not shelter mechanism of action of diltiazem & any related drug related harmfulness except steroids. In conclusion, we recommend additional laboratory and animal models studies to discover its IOP sinking action and validate any systemic or local untoward effects. Diltiazem should be tested in human volunteers and then in glaucoma/ocular hypertension patients. Topical diltiazem effect on vasodilatation and nerve protection obviously needs more high profile research work.

## CONCLUSION

It is evident that topical diltiazem is helpful to treat acute ocular hypertensive crisis because of its quick IOP dropping properties, contributing to a reduction in glaucoma related morbidity and economic costs. Dose adjustment must be mandatory on individual basis.

**Conflict of Interest:** The study has no conflict of interest to declare by any author.

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