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The intraocular pressure (I.O.P) lowering effects of investigational anti-glaucoma drugs often need comparison with existing drugs in rabbits

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Abstract
Glaucoma is defined as heterogeneous group of disorders which manifests as chronic progressive optic neuropathy, characterized by specific morphological changes to the optic nerve head & retinal nerve fiber layer, with resultant loss of retinal ganglion cells, which results in loss of visual fields. Glaucoma is the leading cause of irreversible blindness worldwide & is second only to cataract as the most common cause of blindness over all. This study was designed to establish baseline information of the intraocular pressure lowering effect of three currently using anti - glaucoma drugs, which belongs to three different groups, so that they acts as a benchmark for the efficacy evaluation of the future anti - glaucoma drugs. The experiment was carried out in Glaucoma induced rabbits **(**Water -loaded Rabbit Model), drugs were administered in the form of eyedrops, there is decrease in I.O.P, 3.2 mmHg, 4.6 mmHg & 6.6 mmHg with timolol (0.05%), brimonidine (0.2%), & latanoprost (0.005%) respectively, showed significant (p<0.0001) decrease in the intraocular pressure. Latanoprost showed more efficacy than brimonidine and timolol in rabbits. Brimonidine has more effective than that of Timolol and less effective as compared to latanoprost.

Keywords: Glaucoma, timolol, brimonidine, latanoprost

1. Introduction
Glaucoma is defined as heterogeneous group of disorders which manifests as chronic progressive optic neuropathy, characterized by specific morphological changes to the optic nerve head & retinal nerve fiber layer, with resultant loss of retinal ganglion cells, which results in loss of visual fields [1].
The most common risk factor for Glaucoma is raise in intraocular pressure (I.O.P) due to increase in the formation of the aqueous humor, & difficulty in it's exit, or a raised pressure in the episcleral veins [2].
Normal range of I.O.P is 12.0 to 21.0 mmHg [3].

1.1 Significance & Epidemiology [4, 5].
Glaucoma is the leading cause of irreversible blindness worldwide & is second only to cataract as the most common cause of blindness over all.
Glaucoma afflicts more than 67 million people worldwide, of whom about 10% (or) 6.6 million are estimated to be blind. In United States Glaucoma affects more than 2.2 million persons & this number is projected to increase to 3.4 million persons by 2020. In India 1.2 lakh blind patients add every year due to this menace.
Although Glaucoma more commonly affects older adults, it occurs in all segments of society with significant health & economic consequences.
Glaucoma is also the second most common reason for ambulatory visits to ophthalmologists in United States by Medicare beneficiaries.
Glaucoma is diagnosed by identifying those patients who have optic nerve damage (referred to as "cupping") associated with either visual field loss or an abnormally high I.O.P.

1.2 Management [6]
The management of Glaucoma demonstrates very well the relationship between preventive medicine based in the community & treatment which is based in a hospital (or) clinic. Both are essential to prevent blindness from Glaucoma.

The treatment options for Glaucoma include
1. Medications
2. Laser therapy
3. Incisional Surgery

Currently available treatments, initiated in a stepwise process, focus on intraocular pressure reduction, and initially include topical drug therapy (single then multiple drug combinations), followed by laser therapy then surgical treatment.
Currently, there are five major classes of drugs used for the treatment of glaucoma:
1. Cholinergics
2. Adrenoceptor (beta) antagonists
3. Adrenoceptor (alpha) agonists
4. Carbonic anhydrase inhibitors
5. Prostaglandin analogues

Laser techniques for the reduction of I.O.P. include
1. Argon laser trabeculoplasty
2. Selective laser trabeculoplasty

Surgical options for glaucoma
1. Trabeculectomy
2. Glaucoma drainage tube implantation
3. Ciliary body cyclodestruction

While each of these types of procedures is effective at lowering I.O.P, therapy usually begins with medications. Medications lower I.O.P either by reducing the production or by increasing the rate of outflow of aqueous humour within the eye.
Treatment of glaucoma with the new ocular hypotensive agents, either in monotherapy or combination therapy, may provide lower I.O.P, and delay or postpone the need for surgery. Limitations to existing topical I.O.P, reducing medications include continued disease progression in glaucoma patients with normal I.O.P, treatment failure, and low rates of compliance and persistence.

1.3 Aims and Objectives
The intraocular pressure (I.O.P) lowering effects of investigational anti - glaucoma drugs often need comparison with existing drugs.
This study was designed to establish baseline information of the intraocular pressure lowering effect of three currently using anti - glaucoma drugs, which belongs to three different groups, so that they acts as a benchmark for the efficacy evaluation of the future anti - glaucoma drugs.
This study evaluates the comparison of efficacy of the intraocular pressure lowering effect of single drop instillation of Timolol (0.5%), Brimonidine (0.2%), and latanoprost (0.005%), in glaucoma induced rabbits by water loading method. The intraocular pressure is measured by Schiotz tonometer.

2. Materials and methods
The present study was conducted after obtaining approval by the ethics committee of the Narayana Medical College & General Hospital, Nellore.
The aim was to study the "Effects of Timolol (0.5%), Brimonidine (0.2%), and Latanoprost (0.005%) in Glaucoma Induced Rabbits".

2.1 Drugs used in the study
- Timolol (0.5%)
- Brimonidine (0.2%)
- Latanoprost (0.005%)
- Xylocaine (2%)

2.2 Experimental design
The experiment was carried out in Glaucoma induced rabbits

2.3 Experimental design in Rabbits
- Study was carried out on the healthy Rabbits (*Oryctolagus Cuniculus)*
- Sex: Of either sex
- Body weight : 1-2 Kgs each
- Total number of animals used : 21 (twenty one)
- Source of animals: Animal House, Narayana Medical College, Nellore.
- Housing: Rabbits are traditionally kept outdoors in hutches. Hutches are invariably made of wood, although metal and plastic hutches are available and laboratory rabbits are kept in these.
- Diet: The best diet for a rabbit is grass and good quality grass hay (eg. Timothy) The composition of grass is approximately 20-25% crude fiber, 15% crude protein and 2-3% fat, and with a small amount of a good quality high fiber (18 to 24%) commercial diet with protein levels around 15%).

Before starting the experiment, the animals were allowed to acclimatize to the laboratory environment for one week and they were provided with standard.
Diet and water in sufficient quantity, as per the recommendation of the CPCSEA (Committee for the purpose of control and supervision of experiments on Animals, 2003)
For the experiment, the animals were weighed, and randomly divided in to three (3) groups; each group consists of seven (7) rabbits

2.4 Grouping and Treatment Schedule
Group -I administered with 100 ml/kg of distilled water + Timolol (0.5%)
Group -II administered with 100 ml/kg of distilled water + Brimonidine (0.2%)
Group - III administered with 100 ml/kg of distilled water + Latanoprost (0.005%)

2.5 Glaucoma inducing method in rabbits
2.5.1 Water -loaded Rabbit Model of Glaucoma [7]
This acute model of glaucoma is widely used and established by Sugiyama *et al*. Changes in I.O.P are temporary. This model minimizes the mechanical trauma that can alter the blood aqueous barrier and it is easy to perform, it will not cause any harm to the animals.

2.5.2 Procedure
Albino rabbits of 1. 5-2 kg body weight of either sex are required for this model. They are kept fasting overnight and on the day of experiment, the baseline I.O.P was measured under corneal anesthesia by instilling 2 drops of xylocaine (2%) three times at two minutes interval. Tap water 100 ml/kg is administrated orally through an intragastric infant feeding tube within 30 sec. The I.O.P was measured at baseline, after the water loading till the I.O.P reaches the baseline values.

2.5.3 Drug administration

For evaluating the antiglaucoma activity of an agents like Timolol (0.5%), Brimonidine (0.2%), Latanoprost (0.005%), were administered in the form of eyedrops to one of the eyes of the water-loaded animal, while the vehicle is instilled in the contralateral eye (control). The difference in I.O.P was observed in the two eyes, and the intraocular measurements were made by Schiotz tonometer just prior to water loading and after the water load, for three (3) days.

2.5.4 Technical Procedure [8]

- Check tonometer before using
- Lift upper eyelid with index finger to expose sclera, and instill one drop of topical ophthalmic anesthetic at 12 o'clock position on globe.
- Wait for 30 to 60 seconds
- Tilt the rabbit snout upward toward ceiling, and retract lower eyelid with finger of one hand.
- With other hand grasp tonometer with thumb and index finger while resting other fingers on rabbit's skull just above upper eyelid.
- Place footplate of tonometer gently on central part of cornea such that tonometer is perpendicular to the cornea and to the floor.
- Note scale reading and remove tonometer.
- If scale reading is 0 to 2, add weight and take another reading.
- Repeat procedure two more times.
- Record scale reading and tonometer weight. Convert scale reading to mm Hg of intraocular pressure (I.O.P) by using table calibrated for canine eye.
- Clean tonometer thoroughly after use, disassembles, and enclose in container.
- Prevent rabbit from rubbing it's eye.

3. Results

Table 1: Treatment of glaucoma with Timolol in glaucoma induced rabbits by water loading method

S.NO	Basal I.O.P (mmHg)		I.O.P. After Water Loading		Intra ocular Pressure (mmHg) After drug administration					
					DAY-l		DAY-2		DAY-3	
	RT	LT	RT	LT	RT	LT	RT	LT	RT	LT
1	12.2	14.6	31.6	30.4	30.4	30.4	29.4	30.4	28	30.4
2	11.2	10.2	24.4	22.4	23.8	22.4	23.1	22.4	21.9	22.4
3	13.4	12.2	28	29	27.2	29	25.1	29	25.1	29
4	18.9	17.3	38.8	37.2	37.8	37.2	35.8	37.2	34.4	37.2
5	15.9	17.3	31.6	33	30.4	33	29	33	28	33
6	11.2	13.1	27.2	28	26.6	28	26.6	28	24.4	28
7	13.4	12.6	30.4	29.4	29.4	29.4	29	29.4	27.2	29.4

Table 2: Decreased I.O.P. readings with Timolol

Intra ocular Pressure (mmHg) After drug administration						
	DAY-l		DAY-2		DAY-3	
S.NO	RT	LT	RT	LT	RT	LT
1	1.20	0.00	2.20	0.00	3.60	0.00
2	0.60	0.00	1.30	0.00	2.50	0.00
3	0.80	0.00	2.90	0.00	2.90	0.00
4	1.00	0.00	3.00	0.00	4.40	0.00
5	1.20	0.00	2.60	0.00	3.60	0.00
6	0.60	0.00	0.60	0.00	2.80	0.00
7	1.00	0.00	1.40	0.00	3.20	0.00

Table 3: Decreased percentage of I.O.P. with Timolol

Intra ocular Pressure (mmHg) After drug administration					
DAY-1		DAY-2		DAY-3	
RT	LT	RT	LT	RT	LT
4	0	7	0	13	0
3	0	6	0	11	0
3	0	12	0	12	0
3	0	8	0	13	0
4	0	9	0	13	0
2	0	2	0	11	0
3	0	5	0	12	0
3	0	7	0	12	0
3		7		12	

Table 4: Treatment of glaucoma with Brimonidine in glaucoma induced rabbits by water loading method

S.NO	Basal I.O.P (mmHg)		I.O.P. After Water Loading		Intra ocular Pressure (mmHg) After drug administration					
					DAY-l		DAY-2		DAY-3	
	RT	LT	RT	LT	RT	LT	RT	LT	RT	LT
1	10.2	13.1	24.4	25.1	23.8	25.1	23.1	25.1	20.6	25.1
2	11.2	13.4	27.2	28	26.6	28	25.8	28	23.1	28
3	17.3	15.9	37.8	38.8	37.2	38.8	35.8	38.8	31.8	38.8
4	18.5	17	38.8	35.8	37.2	35.8	35.8	35.8	33	35.8
5	14.3	15.1	30.4	31.6	29.4	31.6	28	31.6	25.1	31.6
6	13.8	11.5	28	30.4	27.2	30.4	26.6	30.4	23.8	30.4
7	10.9	12.2	22.4	24.4	21.9	24.4	21.3	24.4	19.6	24.4

Table 5: Decreased I.O.P. readings with Brimonidine

Intra ocular Pressure (mmHg) After drug administration						
	DAY-l		DAY-2		DAY-3	
S.NO	RT	LT	RT	LT	RT	LT
1	0.60	0.00	1.30	0.00	3.80	0.00
2	0.60	0.00	1.40	0.00	4.10	0.00
3	0.60	0.00	2.00	0.00	6.00	0.00
4	1.60	0.00	3.00	0.00	5.80	0.00
5	1.00	0.00	2.40	0.00	5.30	0.00
6	0.80	0.00	1.40	0.00	4.20	0.00
7	0.50	0.00	1.10	0.00	2.80	0.00

Table 6: Decreased percentage of I.O.P. with Brimonidine

Intra ocular Pressure (mmHg) After drug administration					
DAY-1		DAY-2		DAY-3	
RT	LT	RT	LT	RT	LT
3	0	6	0	18	0
2	0	5	0	18	0
2	0	6	0	19	0
4	0	8	0	18	0
3	0	9	0	21	0
3	0	5	0	18	0
2	0	5	0	14	0
3	0	6	0	18	0
3		6		18	

Table 7: Treatment of glaucoma with Latanoprost in glaucoma induced rabbits by water loading method

S.NO	Basal I.O.P (mmHg)		I.O.P. After Water Loading		Intra ocular Pressure (mmHg) After drug administration					
					DAY-l		DAY-2		DAY-3	
	RT	LT	RT	LT	RT	LT	RT	LT	RT	LT
1	17.3	15.9	37.8	35.8	37.2	35.8	35.8	35.8	29.4	35.8
2	14.6	12.2	30.4	31.6	29	31.6	27.2	31.6	23.8	31.6
3	11.2	13.4	27.2	28	25.8	28	24.4	28	21.3	28
4	17	15.6	34.5	33.6	33	33.6	29.4	33.6	27.2	33.6
5	14.3	12	29.4	28	29	28	27.2	28	23.1	28
6	13.8	15.9	28	29.4	27.2	29.4	25.1	29.4	21.9	29.4
7	11.5	14.6	27.2	28	25.8	28	24.4	28	21.3	28

Table 8: Decreased I.O.P. readings with Latanoprost

Intra ocular Pressure (mmHg) After drug administration						
	DAY-l		DAY-2		DAY-3	
S.NO	RT	LT	RT	LT	RT	LT
1	0.60	0.00	2.00	0.00	8.40	0.00
2	1.40	0.00	3.20	0.00	6.60	0.00
3	1.40	0.00	2.80	0.00	5.90	0.00
4	1.50	0.00	5.10	0.00	7.30	0.00
5	0.40	0.00	2.20	0.00	6.30	0.00
6	0.80	0.00	2.90	0.00	6.10	0.00
7	1.40	0.00	2.80	0.00	5.90	0.00

Table 9: Decreased percentage of I.O.P. with Latanoprost

Intra ocular Pressure (mmHg) After drug administration					
DAY-1		DAY-2		DAY-3	
RT	LT	RT	LT	RT	LT
2	0	6	0	29	0
5	0	12	0	28	0
5	0	11	0	28	0
5	0	17	0	27	0
1	0	8	0	27	0
3	0	12	0	28	0
5	0	11	0	28	0
4	0	12	0	28	0
4		12		28	

Table 10: Treatment of glaucoma in glaucoma induced rabbits (right eye) with Timolol

		Mean	N	Std. Deviation	Std. Error Mean	
Pair 1	tb_rt_eye	30.2857	7	4.57071	1.72757	t=13.608
	ta_rt_eye	27.0000	7	3.94081	1.48949	p<0.0001

tb_rt_eye - before administration of Timolol in right eye, ta_rt_eye - after administration of Timolol in right eye

Table 11: Treatment of glaucoma in glaucoma induced rabbits (right eye) with Brimonidine

		Mean	N	Std. Deviation	Std. Error Mean	
Pair 1	bb_rt_eye	29.8571	7	6.31476	2.38675	t=10.362
	ba_rt_eye	25.2857	7	5.21614	1.97151	p<0.0001

bb_rt_eye - before administration of Brimonidine in right eye, ba_rt_eye - after administration of Brimonidine in right eye

Table 12: Treatment of glaucoma in glaucoma induced rabbits (right eye) with Latanoprost

		Mean	N	Std. Deviation	Std. Error Mean	
Pair 1	lb_rt_eye	30.6429	7	4.04963	1.53062	t=19.182
	la_rt_eye	24.0000	7	3.14219	1.18763	p<0.0001

lb_rt_eye - before administration of Latanoprost in right eye, la_rt_eye - after administration of Latanoprost in right eye

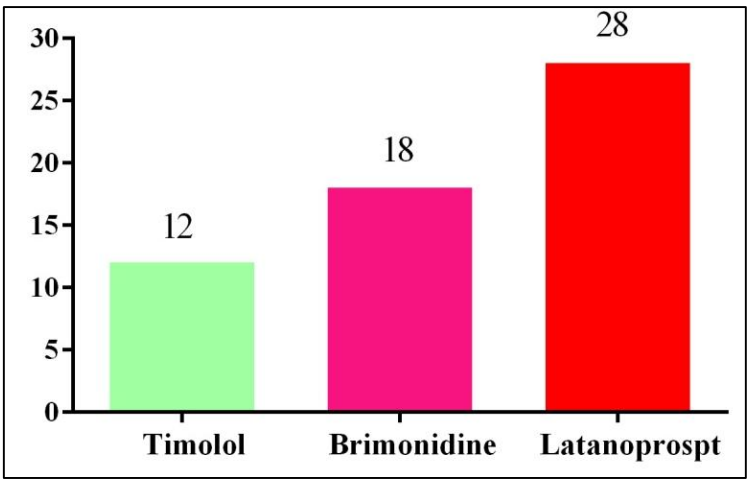

Fig 1: Bar diagrammatic representation of decreased percentage of I.O.P. with timolol, Brimonidine, Latanoprost in glaucoma induced rabbits.

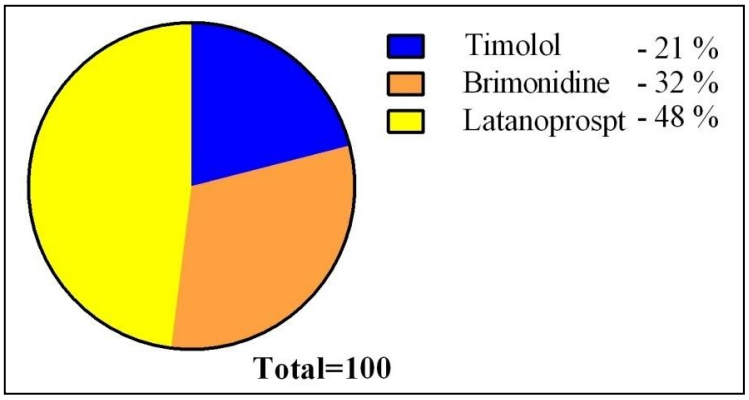

Fig 2: Pie diagrammatic representation of decreased percentage of I.O.P. with Timolol, Brimonidine, Latanoprost in glaucoma induced rabbits.

4. Discussion

The purpose of the current study was to evaluate the effects of Timolol (0.5%), Brimonidine (0.2%), Latanoprost (0.005%), In Glaucoma induced rabbits.

Glaucoma is a progressive neuropathy & clinical success in glaucoma management might ideally be defined as lack of progression of the neuropathy. However, because the disease progress slowly and measures of neuropathy and visual field loss is somewhat variable, glaucoma progression cannot be measured in a short term trail. Therefore I.O.P. reduction is used as an alternative measure of clinical success.

The present study was conducted in the glaucoma induced rabbits were divided in to three groups, each group consists of 7 rabbits. Effect of intraocular pressure was studied by using Timolol in group I, Brimonidine in group II, and Latanoprost in group III. Timolol & Brimonidine was prescribed topically twice daily as one drop in right eye & left eye maintained as a control in all rabbits. While Latanoprost was prescribed topically once daily as one drop in right eye & left eye maintained as a control in all rabbits.

In the present study results, tables - 1 & 2, gives the information about decreased I.O.P. readings with timolol in glaucoma induced rabbits. Table - 3, shows the decreased percentage of I.O.P with timolol in glaucoma induced rabbits. Tables - 4 & 5, gives the information about decreased I.O.P. readings with brimonidine in glaucoma induced rabbits. Table

- 6, shows the decreased percentage of I.O.P with brimonidine in glaucoma induced rabbits and tables - 7 & 8, gives the information about decreased I.O.P. readings with latanoprost in glaucoma induced rabbits. Table - 9, shows the decreased percentage of I.O.P with latanoprost in glaucoma induced rabbits.

Table - 10, gives the information about mean reduction in I.O.P. with timolol in rabbit's right eye & it decreases the I.O.P. by 3.2 mmHg which is highly significant.

Table - 11, gives the information about the mean reduction in I.O.P. with brimonidine in rabbit's right eye & it decreases the I.O.P. by 4.6 mmHg which is highly significant.

Table - 12, gives the information about the mean reduction in I.O.P. with latanoprost in rabbit's right eye & it decreases the 1.0.P. by 6.6 mmHg which is highly significant.

According to tables 10, 11 & 12, there is decrease in I.O.P.s, 3.2 mmHg, 4.6 mmHg & 6.6 mmHg with timolol (0.05%), brimonidine (0.2%), & latanoprost (0.005%) respectively. Such changes in I.O.P. reduction with Timolol (0.5%), Brimonidine (0.2%), & Latanoprost (0.005%) in rabbits is reported by (Gupta. S.K. *et al*, 2007) 50, & (J. Burke, 2007) 51, and accordingly there is significant decrease in the I.O.P.

Hence, by the above findings Latanoprost was found to be more effective than Brimonidine & Timolol. However Brimonidine was more effective than Timolol.

5. Conclusion

The effects of anti-glaucoma drugs which belong to different groups of classification were studied in "Effects of Timolol (0.5%), Brimonidine (0.2%), and Latanoprost (0.005%), in Glaucoma induced rabbits".

Treatment of Timolol (0.5%), Brimonidine (0.2%) & Latanoprost (0.005%) study in glaucoma induced rabbits showed significant decrease in the intraocular pressure.

From the present study, it can be seen that Latanoprost showed more efficacy than brimonidine and timolol in rabbits. Brimonidine has more effective than that of Timolol and less effective as compared to latanoprost.

Hence Latanoprost is more effective than Brimonidine and timolol, and brimonidine is more effective than Timolol.

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