Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.12 / Issue 69 / December / 2021 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Effect of Physostigma in Steroid Induced Glaucoma in Rabbits

T. Saravanan and Sunny Mathew*

Department of Anatomy, Vinayaka Mission's Homoeopathic Medical College and Hospital, Vinayaka Mission's Research Foundation (Deemed to be University), Salem, Tamil Nadu, India.

Received: 10 Sep 2021 Revised: 23 Sep 2021 Accepted: 21 Oct 2021

***Address for Correspondence**
Sunny Mathew
Department of Anatomy,
Vinayaka Mission's Homoeopathic Medical College and Hospital,
Vinayaka Mission's Research Foundation (Deemed to be University),
Salem, Tamil Nadu, India.
Email: drsunnymathew59@gmail.com

ABSTRACT

In Homoeopathy, Basic experimental studies in Glaucoma are not done with various potencies. Hence, this study is intended to verify the specific effect of Physostigma, 30CH, 200CH and 1M In Steroid Induced Glaucoma in Rabbits .Experimental model is proposed to achieve measurements to prove or disprove the hypothesis. To study the efficacy of physostigma30CH, 200CH and 1M in reducing glaucoma on steroid induced glaucoma models of male albino rabbits. To measure IOP after the induction of steroid which is treated on physostigma30CH, 200CH and 1M To compare and contrast the effect of different potency of physostigma.

INTRODUCTION

Glaucoma is a group of eye diseases which result in damage to the optic nerve and vision loss. Vision loss from glaucoma, once it has occurred, is permanent. About 6 to 67 million people have glaucoma globally. The World Health Organization has estimated that India has a 1% prevalence of blindness. [1] of the estimated 8.9 million blind in India, 12.8% are due to glaucoma. The problem is expected to reach alarming proportions by the turn of the century [2]. While there are excellent population-based data available from the West [3,4,5,6,7,8,9] such data from South Asia, especially India, are lacking. Risk factors for glaucoma include increased pressure in the eye, a family history of the condition, migraines, high blood pressure, and obesity. The homeopathic approach to treating eye disease is not new and there's a strong history of homeopathy in ophthalmology. The New York Ophthalmic Hospital was a homeopathic hospital in 1852 and it was under homeopathic management until 1867. In 1931 it treated over 31,000 patients. The American Homeopathic Ophthalmology and Otology Society existed from 1877, and was still in existence in 1941.Here are standard works on ophthalmology by homoeopaths. For example Homeopathic Therapeutics in Ophthalmology, published in 1916 by John L. Moffat, M.D., and Ophthalmic Diseases and Therapeutics, which was published in 1872 by A.B. Norton, M.D.

Homeopathic constitutional treatment will take good care of glaucoma cases. As glaucoma is progressive destructive disease, with homeopathic medication complaints will reduce and it will arrest the further progression of disease without any side effects. The most common type is open-angle glaucoma with less common types including closed-angle glaucoma and normal-tension glaucoma [9]. Open-angle glaucoma develops slowly over time and there is no pain.1 Side vision may begin to decrease followed by central vision resulting in blindness if not treated [9]. Closed-angle glaucoma can present gradually or suddenly.[10] the sudden presentation may involve severe eye pain, blurred vision, mid-dilated pupil, redness of the eye, and nausea. Regular eye examinations by your ophthalmologist are the best way to detect glaucoma. Your ophthalmologist will measure your eye pressure with Tonometry. Inspect the drainage angle of your eye with Gonioscopy. Evaluate your optic nerve with Ophthalmolscopy and test the visual field of each eye with Perimetry. Optic nerve evaluation and visual field testing are performed at regular intervals to monitor the effects of glaucoma. The information from these tests provides an indication of the effectiveness of the treatment being used and whether further treatments may be necessary. Eye pressure is measured in millimeters of mercury (mm Hg). Normal eye pressure ranges from 12-22 mm Hg, and eye pressure of greater than 22 mm Hg is considered higher than normal.

Suresh S, Ganesh Lakshmanan, Manonmani had done pilot study which attempts to study the direct action of homeopathic remedies on ciliary muscles of the eyes by testing the efficiency in myopic individuals. Random cases of 15 myopic individuals of different ages were selected from the primary clinic; the lowest age was 7 years, highest 35 years. An ophthalmologist tested the errors and noted them: a patient value ranging from -6 to -1 was treated with potentized physostigmavenenosum. Based on Stuart thorough protocols of potency selections were made. Repetitions of remedy based on potency were used. Parameters were verified after 24 weeks. Homeopathic physostigma showed positive changes in 11 out of 15 cases and no improvements in for 4 cases. Fair improvements were noted in 8 of 15, mild improvements were noted in 3 cases, which were free from symptoms. There was an overall improvement in 73.34% and no improvements in 26.66% of the cases. Moderate to good improvements were noted in 53.33%, mild improvement in 20% of the cases. This study clearly exhibits that potentized physostigma is most effective in treating short-sight, acting over ciliary muscles, evidence based in myopia [11].

Abed H. Pathan, Syed Ayaz Ali reported that the anti-glaucoma activity of aqueous methanolic ginger extract (*Zingiber officinale*) against carbomer induced experimental glaucoma in rabbits. Aqueous methanolic extract of *Zingiber officinale* was orally administered to carbomer induced glaucomatous rabbits. Pilocarpine 2% eye drop was used as a standard drug. Intraocular Pressure (IOP) levels were determined after oral administration of a dose of *Zingiber officinale* (200 mg/kg, p.o.) in glaucomatous rabbits. IOP were determined for four weeks after oral administration of aqueous methanolic extract of *Zingiber officinale* (200 mg/kg, p.o). An aqueous methanolic extract of *Zingiber officinale* was found to reduce intra ocular pressure in carbomer induced experimental glaucoma in rabbits. Sufficient reduction in IOP was observed from second week of administration of ginger extract. A significant decrease in IOP ($p<0.01$) was observed in animals treated with standard pilocarpine and aqueous methanolic ginger extract. The effect of extracts of *Zingiber officinale* on serum pseudocholineterase was also measured. A significant decrease in the level of pseudocholinestrase ($p<0.01$) was observed in the rabbit serum treated with aqueous methanolic extract of ginger [12].

Prabhakar Adake, H. S. Somashekar, C. G. Gokul, Abhishek Acharya, M. Naveen Kumar and R. Santosh reported that Glaucoma was induced in rabbits (N=18) by bilateral topical instillation of 1% prednisolone eye drop (10 µl) twice a day for a period of 40 days. Before the induction of glaucoma, baseline intraocular pressure (IOP) in both the eyes of all rabbits was measured under sedation (i.v midazolam) by Schiotz tonometer. At the end of 40 days induced IOP was measured for all rabbits and rabbits were divided into three groups of six rabbits in each. Right eyes of group A, B and C rabbits received 0.5% diltiazem, 0.1% verapamil, and 0.5% timolol eye drops twice daily for 12 days respectively. Whereas, left eyes of all rabbits received distilled water hence represented as control. IOP was measured in all rabbits on every 4th day till 12 days of treatment period. Intra-group comparisons of IOP changes were made by paired‘t’ test. And unpaired ‘t’ test for inter group comparisons. One way ANOVA was used for

multiple group comparisons followed by post-hoc Tukey's test for group wise comparisons. In 0.5% diltiazem treated eyes, the mean IOP significantly reduced from 22.9±1.9 mmHg (10%) on 4th day to 16.9±1.1 mmHg(S, *P*<.001) on 12th day (34%). Similarly, mean IOP in 0.1% verapamil treated eyes significantly reduced from 22.7±1.3 mmHg (7%) on 4th day to 15.5±1.4 mmHg(S, *P*<.001) on 12th day (37%). Whereas, mean IOP significantly reduced from 22.4±1.9 mmHg (14%) on 4th day to 16.4±1.4 mmHg (S, *P*=.001) on 12th day (36%) in 0.5% timolol treated eyes [13].

METHODS

Animals

Rabbits, weight 1.5 to 2.5kgs are included in this present study. The Rabbits are procured from Sri Venkateswara Enterprises Bengaluru. The Rabbits are imbared in the central Animal house of the Department of pharmacology, Vinayaka Missions College of Pharmacy, Salem. Physostigma Homoeopathic medicines are purchased from Reputed Pharmaceutical Industry which is preparing under Indian Homoeopathic pharmacopeia, 25 Rabbits are divided into 5 groups ,Each group consist of 5 Rabbits. Four Rabbits in each cage, they are randomly housing at a controlled temperature 21±3c With a 12 hours light, 12 hours dark cycle. Base line Intra Ocular Pressure (IOP) for both eyes of all Rabbits are measured before induce glaucoma, To induce glaucoma in Rabbits steroid model instilled with 10µl of I.V prednisolone eye drops twice a day for a period of 40 days.

Drugs

1% prednisolone acetate (steroid) eye drops for induce glaucoma.
Physostigma 30C, 200C, 1M for test drug for glaucoma (Group wise).
0.5% Timolol eye drops is standard drug to reduce glaucoma.(Test group of Rabbits)
4% xylocaine eye drops for anaesthetic before measuring IOP by Schiotz tono meter.
Midozolam IV for sedation of Rabbits.

Study Procedure

Before starting the research work first get clearance from Institutional Animal Ethical Committee (IAEC). Totally 25 Albino Rabbits (n=25) is using for research study. By lateral topical instillation of 1% prednisolone for induce elevation of IOP in both eyes of all Rabbits above base line level (Once a day). Induce IOP measuring at the end of 40 days by Schiotz tonometer. The cortico steroid induce glaucoma is well known in human and closely resemble the human disease in clinical feature as well as in the underline mechanism [13]. After the induction of glaucoma Rabbits were divided into 5 groups of 5 Rabbits in each potency.

Group I : Normal Group Without any medication
Group II : 2 Drops of Timolol (Standard drug) into both eyes 2 times per day in left Eye.
Group III: 2 Drops of physotigma 30CH Eye drops gives 2 times a day in left Eye.
Group IV: 2 Drops of physotigma 200CH Eye drops gives 2 times a day in left Eye.
Group V : 2 Drops of physotigma 1M Eye drops gives 2 times a day in left Eye

IOP is measure in both eyes for all Rabbits on every fourth day till end of the twelfth day of treatment period. Before measuring IOP giving sedation of all the Rabbits with intravenous (marginal ear vine) Midozolam in a dose of 0.5 – 1.0 mg/kg and cornea is anaesthetized with topical 4% xylocaine drops. For this study conversion table used to derive the IOP in millimeter of mercury (mm Hg) from the scale reading and plunger weight. To avoid Diurnal variations of IOP all Tonometeries perform at the same time of the day preferably in the morning hour (Around 9 AM).

Statistical Analysis

Collect the IOP reading are express as mean ±SD. Intra group comparison of IOP changes measuring by paired 't test and unpaired 't test for intergroup comparison. One way ANOVA is using for multiple group of comparison followed by post-hoc Tukey's test.

RESULTS & DISCUSSION

In this study there are five groups are considered with the four types of days they are as follows Group 1: Normal no any medication, Group 2: Standard drug timolol maleate Group 3: Physostigma 30ch Group 4: Physostigma 200ch Group 5: Physostigma 1m, and the days are classified as day 0, day 4, day 8 and day 12. So based on the prospective studies the samples have been collected. The number of samples collected in this study are 25 sampled observations.

Null Hypothesis H_{01}: There is no significant differences among the groups of Normal no any medication, Standard drug timolol maleate, Physostigma 30ch, Physostigma 200ch, Physostigma 1m.

Null Hypothesis H_{02}: There is no significant differences among the days of day 0, day 4, day 8 and day 12.

Alternative Hypothesis H_{11}: There is significant differences among the groups of Normal no any medication, Standard drug timolol maleate, Physostigma 30ch, Physostigma 200ch, Physostigma 1m.

Alternative Hypothesis H_{12}: There is significant differences among the days wise of drugs.

The analysis and results are tabulated and given in Table 1. It gives information regarding the descriptive statistics which gives the measures like mean, standard deviation and the number of sample observations. The table 2 gives the result of the two-way ANOVA while the study having two independent variables like group of the drugs and the different types of days of study. The mean of Groups significant value that is p-value which is lesser than the 0.05. Hence the null hypothesis of this study is rejected and it's concluded that there are significant differences among the groups of Normal no any medication, Standard drug timolol maleate, Physostigma 30ch, Physostigma 200ch, Physostigma 1m. The mean of Days significant value that is p-value which is lesser than the 0.05. Hence the null hypothesis of this study is rejected and its concluded that there are significant differences among the days of drugs.

The mean of interaction effect of Groups and Days significant value that is p-value which is less than the 0.05. Hence the null hypothesis of this study is rejected and its concluded that there are significant differences among the groups and days of drugs. There for the null hypothesis is rejected by testing the significance difference through the two-way ANOVA. So the next proceeding is to check the interaction levels through the multiple comparisons tests (Tukey test) to identify the effects of iterations in the interaction levels. The analysis and results are tabulated in Table 3. It gives the mean value 34.061 for the study with the standard error of 0.180 and the confidence interval has been built with the 95% of lower bound is 33.703 and the upper bound is 34.420.

In table 4 while considering the interactions effects with in the group while considering the Standard drug timolol maleate, Physostigma 30ch, Physostigma 200ch, Physostigma 1m having significant difference with the Normal no any medication. But there is no significant difference between Physostigma 30ch, Physostigma 200ch, Physostigma 1m having significant difference with the Normal no any medication. From the table 5 while considering the interaction effects with in the days while considering the day 0, day 4, day 8 and day 12 have the significant difference among them. Figure 1, shows the means of drugs of the Physostigma 30ch works in parallel with drugs Standard drug timolol maleate. When drugs of Physostigma 200ch and Physostigma 1m are compared with drug Standard drug timolol maleate is only a small difference.

CONCLUSION

Timolol maleate is the Standard Medicine for Glaucoma in Allopathy. In Homoeopathic System of Medicine Physostigma shows good improvement in Steroid Induced Glaucoma. In that, Physostigma 30 CH shows more improvement comparatively with other Potencies. Based on the statistical analysis Physostigma shows improvement in Glaucoma.

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Table 1. Descriptive statistics

Group	Days	Mean	Std. Deviation	N
Normal no any medication	0 Day	45.7600	1.42934	5
	4th DAY	45.7600	1.42934	5
	8th DAY	45.7600	1.42934	5
	12th DAY	45.7600	1.42934	5
	Total	45.7600	1.31165	20
Standard drug timolol maleate	0 Day	45.7600	1.42934	5
	4th DAY	34.7580	3.33284	5
	8th DAY	23.4400	1.39571	5
	12th DAY	17.9800	1.17771	5
	Total	30.4845	11.13056	20
Physostigma 30ch	0 Day	45.7600	1.42934	5
	4th DAY	36.1560	2.65643	5
	8th DAY	23.3800	1.86735	5
	12th DAY	18.1400	.96073	5
	Total	30.8590	11.22279	20
Physostigma 200ch	0 Day	45.7600	1.42934	5

	4th DAY	36.1560	2.65643	5
	8th DAY	23.3800	1.86735	5
	12th DAY	20.7000	.91924	5
	Total	31.4990	10.49242	20
Physostigma 1m	0 Day	45.7600	1.42934	5
	4th DAY	36.1560	2.65643	5
	8th DAY	23.3800	1.86735	5
	12th DAY	21.5200	.88431	5
	Total	31.7040	10.27374	20
Total	0 Day	45.7600	1.30480	25
	4th DAY	37.7972	4.74737	25
	8th DAY	27.8680	9.26147	25
	12th DAY	24.8200	10.82570	25
	Total	34.0613	11.15430	100

Table 2. Two-way Analysis of Variance from Groups and Days

Source	Sum of Squares	df	Mean Square	F	Sig.
Group	3440.601	4	860.150	264.793	.000
Days	6864.378	3	2288.126	704.389	.000
Group * Days	1752.583	12	146.049	44.960	.000
Error	259.871	80	3.248		
Total	128334.649	100			

Table 3. Estimated Marginal Means

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
34.061	.180	33.703	34.420

Table 4. Multiple Comparisons from the Groups

Group	Group	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Normal no any medication	Standard drug timolol maleate	15.2755*	.56995	.000	13.6848	16.8662
	Physostigma 30ch	14.9010*	.56995	.000	13.3103	16.4917
	Physostigma 200ch	14.2610*	.56995	.000	12.6703	15.8517
	Physostigma 1m	14.0560*	.56995	.000	12.4653	15.6467
Standard drug timolol maleate	Normal no any medication	-15.2755*	.56995	.000	-16.8662	-13.6848
	Physostigma 30ch	-.3745	.56995	.965	-1.9652	1.2162
	Physostigma 200ch	-1.0145	.56995	.392	-2.6052	.5762
	Physostigma 1m	-1.2195	.56995	.214	-2.8102	.3712
Physostigma 30ch	Normal no any medication	-14.9010*	.56995	.000	-16.4917	-13.3103
	Standard drug timolol maleate	.3745	.56995	.965	-1.2162	1.9652
	Physostigma 200ch	-.6400	.56995	.794	-2.2307	.9507
	Physostigma 1m	-.8450	.56995	.577	-2.4357	.7457
Physostigma 200ch	Normal no any medication	-14.2610*	.56995	.000	-15.8517	-12.6703
	Standard drug timolol maleate	1.0145	.56995	.392	-.5762	2.6052
	Physostigma 30ch	.6400	.56995	.794	-.9507	2.2307

	Physostigma 1m	-.2050	.56995	.996	-1.7957	1.3857
Physostigma 1m	Normal no any medication	-14.0560*	.56995	.000	-15.6467	-12.4653
	Standard drug timolol maleate	1.2195	.56995	.214	-.3712	2.8102
	Physostigma 30ch	.8450	.56995	.577	-.7457	2.4357
	Physostigma 200ch	.2050	.56995	.996	-1.3857	1.7957
**. The mean difference is significant at the .05 level.*						

Table 5. Multiple Comparisons from the Days

Days	Days	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0 DAY	4th DAY	7.9628*	.50978	.000	6.6252	9.3004
	8th DAY	17.8920*	.50978	.000	16.5544	19.2296
	12th DAY	20.9400*	.50978	.000	19.6024	22.2776
4th DAY	0 DAY	-7.9628*	.50978	.000	-9.3004	-6.6252
	8th DAY	9.9292*	.50978	.000	8.5916	11.2668
	12th DAY	12.9772*	.50978	.000	11.6396	14.3148
8th DAY	0 DAY	-17.8920*	.50978	.000	-19.2296	-16.5544
	4th DAY	-9.9292*	.50978	.000	-11.2668	-8.5916
	12th DAY	3.0480*	.50978	.000	1.7104	4.3856
12th DAY	0 DAY	-20.9400*	.50978	.000	-22.2776	-19.6024
	4th DAY	-12.9772*	.50978	.000	-14.3148	-11.6396
	8th DAY	-3.0480*	.50978	.000	-4.3856	-1.7104
**. The mean difference is significant at the .05 level.*						

Table 6. Mean Drugs of Groups and Days

Days	0 Day	4th Day	8th Day	12th Day
Normal no any medication	45.75	45.75	45.75	45.75
Standard drug timolol maleate	45.75	34.76	23.44	17.98
Physostigma 30ch	45.75	36.16	23.38	18.14
Physostigma 200ch	45.75	36.16	23.38	20.7
Physostigma 1m	45.75	36.16	23.38	21.52

Table 7. Comparison on 12th Day

Rabbit Sl. No	Group: A (Normal No Any Medication)	Group: B (Standard Drug Timolol Maleate)	Group: C (Physostigma 30 CH)	Group: D (Physostigma 200 CH)	Group: E (Physostigma 1M)
01	46.9	18.0	17.3	21.3	21.9
02	45.8	19.6	18.5	19.6	20.1
03	45.8	16.5	17.3	20.1	21.3
04	46.9	17.3	19.6	21.9	21.9
05	43.4	18.5	18.0	20.6	22.4

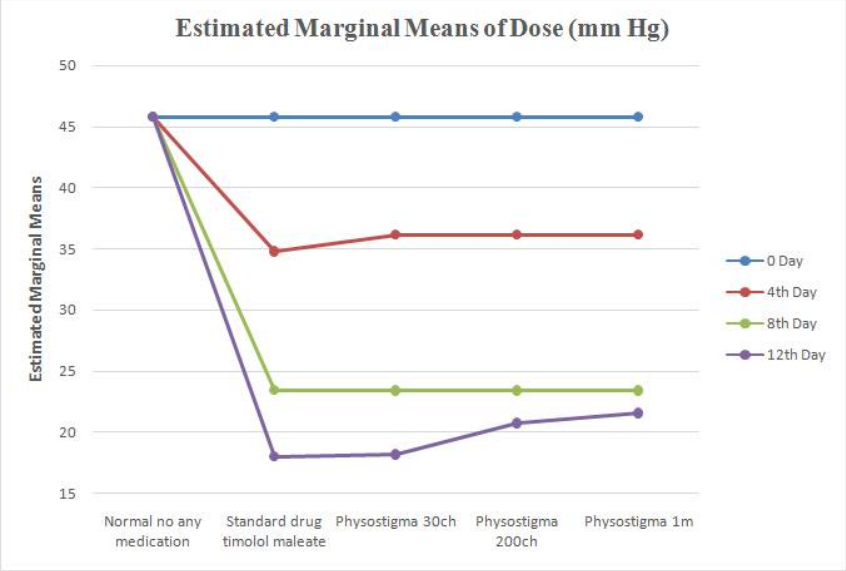

Figure 1. Estimated Marginal Means of Dose (mm Hg)