RESEARCH ARTICLE

# Comparative mydriatic study: tropicamide vs. biosimilar in normal and diabetic patients with quality control analysis

Fareena Farooqui, Sohaib Zafar Malik


## Abstract

**Objective:** To compare the effectiveness of tropicamide and its biosimilar drug in normal and diabetic patients.

**Method:** The prospective cohort study was conducted at Amanat Eye Hospital on March 5, 2023 to 5 December, 2023, and comprised individuals aged 40-50 years. Diabetic patients formed group A, while healthy controls were in group B. Tropicamide 1% was used for mydriasis in the left eye of all the subjects, and a biosimilar drug was used for mydriasis in the right eye. After administration, the size of the pupil was analysed. Data was analysed using Paired t-tests for continuous variables and McNemar's test for categorical variables.

**Results:** Of the 300 subjects (150 males, 150 females), 150(50%) were in each of the two groups. In group B, 29(19.33%) eyes were perfectly dilated in response to tropicamide compared to 23(15.43%) with biosimilar drug. In group A, the corresponding values were 53(35.33%) and 25(16.66%), respectively.

**Conclusion:** Tropicamide was found to be more effective compared to its biosimilar drug. Besides, tropicamide was more effective in healthy individuals compared to diabetic individuals.


**Key Words:** Tropicamide, Biosimilar drug, Diabetic.

(JPMA 74: 902; 2025) **DOI: https://doi.org/10.47391/JPMA.20295**

## Introduction

Mydriasis carries significant importance in the comprehensive eye examination and fundoscopy of the retina. In the United States, over 100 million pupil dilations are performed for eye examinations.[1] In addition, more than 30 million diabetic patients have annual examinations of the retina in the US for which mydriasis is very important.[2,3] The statistics of pupil dilations that are performed yearly in Pakistan are not available, but Pakistan currently ranks 4th globally in the number of adults living with diabetes, with approximately 34.5 million individuals affected in 2024. Projections indicate that by 2050, Pakistan will rise to 3rd place, with an estimated 70.2 million adults living with diabetes. Diabetes is the leading cause of diabetic retinopathies (DRs).[4] In addition, the global prevalence of glaucoma is 3.54% and is predicted to increase.[5] Furthermore, the blindness caused due to cataract is estimated to be more than 50% of all global blindness.[6]

Early diagnosis and intervention is only possible if the eye examination is performed with dilated pupils. Eye examination without pupil dilation may miss the prevailing eye disorder or disease .[7] In Pakistan, different mydriatic agents are used, such as atropine, cyclopentolate, homatropine, scopolamine and tropicamide.[8] Tropicamide is an antagonist of parasympathetic nervous system. It inhibits the parasympathetic nervous system and acts on the pupillary sphincter muscles, and results in the relaxation of these muscles. In Pakistan, the top consumers of tropicamide are two leading eye hospitals that together cater to more than 60 million patients on a monthly basis[9]. Owing to the large-scale use of tropicamide across Pakistan, various pharmaceutical companies are involved in its manufacturing.

The current study was planned to assess the effectiveness of tropicamide against its biosimilar drug in normal and diabetic patients.

## Subjects and Methods

The prospective cohort study was conducted at Amanat Eye Hospital from March 5, 2023, to December 5, 2023. After approval from the ethics review committee of Riphah International University, the sample size was calculated at a 95% confidence interval (CI), 8.83 standard deviation (SD), margin of error 1, alpha (α) score 0.05, and Z score 1.96. The value of beta (β) in the calculation was 0.2 (10). The sample size was determined using the following formula:

$n = (Z\alpha/2)^2 \times p(1-p) / d^2,$

Department of Pharmacology, Riphah International University, Islamabad, Pakistan.

**Correspondence:** Sohaib Zafar Malik. **Email:** sohaib.zafar@riphah.edu.pk

**ORCID ID:** 0000-0002-3454-4802

**Submission complete:** 06-04-2024 **First Revision received:** 07-08-2024

**Acceptance:** 05-04-2025 **Last Revision received:** 04-04-2025

where p = expected proportion, d = margin of error.[11,12]

Data was analysed using Paired t-tests for continuous variables, and McNemar's test was applied for categorical variables. P<0.05 was considered statistically significant. The sample was raised using the probability sampling technique with stratified sampling. Those included were individuals aged 40-50 years with a photopic pupil diameter ≤3.5 mm in each eye. Diabetic patients formed Group A, while healthy controls were in Group B.

Those with an allergy to tropicamide, a history of benign prostatic hyperplasia, or use of benzodiazepines, monoamine oxidase inhibitors, tricyclic antidepressants, anticonvulsants, or cholinergic drugs were excluded. Also excluded were individuals with a history of closed-angle glaucoma, anatomically narrow anterior chamber angles, ocular surgery, or laser treatment of any kind, as well as those with a history of chronic or acute uveitis. Individuals with a history of traumatic iritis or hyphaemia, traumatic mydriasis or angle recession, heterochromia, irregularly-shaped pupils secondary to ocular trauma or congenital defects, neurogenic pupil disorders, anterior chamber intraocular lens (IOL), or iris-fixated IOL were also not included. Furthermore, individuals with a history of iris surgery, iris atrophy, or iris-cornea apposition/touch, those unwilling or unable to discontinue the use of contact lenses during treatment visits, and those with current active eye disease requiring topical or systemic ophthalmic medication (except for dry eye disease managed with artificial tears) were excluded. Additionally, individuals with severe/serious ocular conditions or any other unstable medical condition that could affect the researchers' assessment were excluded, as were pregnant and lactating subjects.[13] Written informed consent was obtained from all participants. The eye drops were administered three times at five-minute intervals, with one drop in each eye.

Tropicamide 1% was used for mydriasis in the left eye of all subjects, and the biosimilar drug was used in the right eye. The standard time required to produce an effect was 15 minutes. After administration, the size of the pupil was analysed. An increase in pupil size was clearly visible to the naked eye. In addition, for confirmation, a shining light was focused on the eyes.

For quality control analysis, formal permission was obtained from Alza Pharmaceuticals, Rawalpindi, Pakistan. The control sample referred to the standard tropicamide solution provided by the manufacturer, derived from a certified reference batch, and used to benchmark the quality control analyses. This control sample served as a baseline, ensuring consistency and reliability in the analysis. Accelerated stability testing of the prepared formulation was performed for three months following relevant guidelines. The prepared formulation was kept at 40°±2°C and 75% relative humidity and analysed for changes in appearance, consistency, and potential hydrogen (pH) levels.[14] High-Performance Liquid Chromatography-Fourier Transform Infrared Spectroscopy (HPLC–FTIR) analysis was conducted to detect, identify, and determine organic impurities in commercial tropicamide, which is designed for the production of eye drops. This method is suitable for quality control testing of tropicamide during production.[15]

Data was analysed using Paired t-tests for continuous variables, and McNemar's test was applied for categorical variables. P<0.05 was considered statistically significant.

## Results

Of the 300 subjects (Mean age ± SD: X ± Y years, 150 males, 150 females), 150 (50%) were in each of the two groups. The gender distribution in Group A (diabetics) included 75 males and 75 females, while Group B (healthy) also had 75 males and 75 females. Tropicamide achieved a significantly higher proportion of perfect dilation in healthy individuals 29(19.33%) compared to the biosimilar drug 23(15.43%), p<0.05. A similar trend was observed in diabetic individuals, where tropicamide-induced dilation was 53(35.33%) compared to 25(16.66%)

**Table-1:** Comparison of tropicamide and biosimilar drug for mydriasis.

| Group | Drug | Mean Pupil Dilation (mm) ± SD | Perfect Dilatiom Proportion | (%) P-value |
|---|---|---|---|---|
| Healthy | Tropicamide | 2.5 ± 0.5 | 19.33% (29/150) | < 0.05 |
| Healthy | Biosimilar | 2.0 ± 0.4 | 15.43% (23/150) | < 0.05 |
| Diabetic | Tropicamide | 2.7 ± 0.6 | 35.33% (53/150) | < 0.01 |
| Diabetic | Biosimilar | 1.8 ± 0.5 | 16.66% (25/150) | < 0.01 |

SD: Standard deviation.

**Table-2**: Overall and group-wise data.

| Group | Drug | Mean Pupil Dilation (mm) ± SD | Proportion of Perfect Dilation (%) | P-value (Mean Difference) | P-value (Proportion Difference) |
|---|---|---|---|---|---|
| Overall | Tropicamide | 2.6 ± 0.55 | 27.33% | < 0.01 | < 0.01 |
| Overall | Biosimilar | 1.9 ± 0.45 | 16.00% | | |
| Healthy | Tropicamide | 2.5 ± 0.50 | 19.33% | < 0.05 | < 0.05 |
| Healthy | Biosimilar | 2.0 ± 0.40 | 15.43% | | |
| Diabetic | Tropicamide | 2.7 ± 0.60 | 35.33% | < 0.01 | < 0.01 |
| Diabetic | Biosimilar | 1.8 ± 0.50 | 16.66% | | |

SD: Standard deviation.

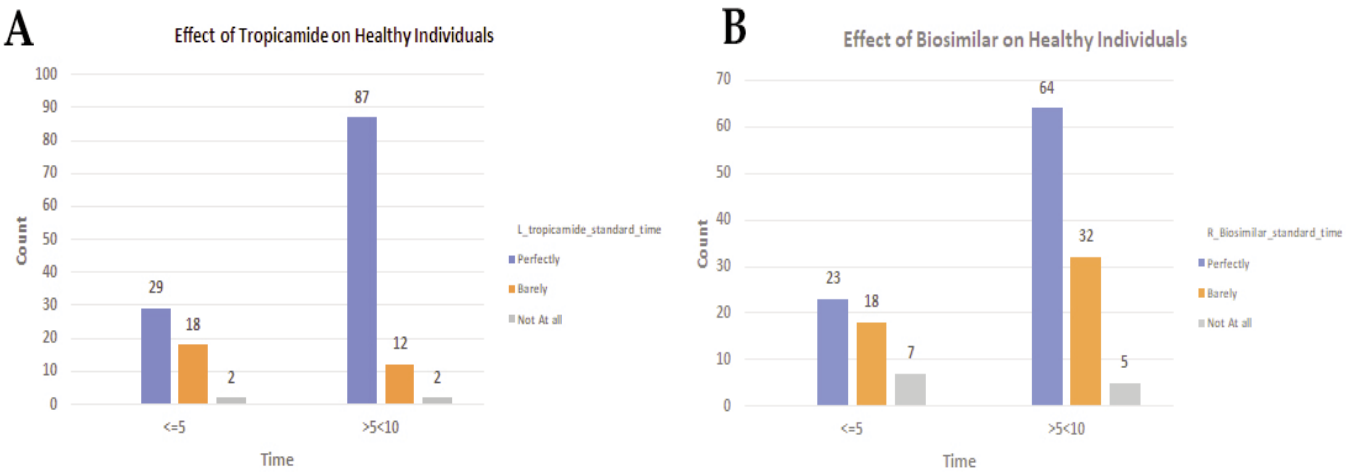


**Figure-1**: Effect of tropicamide (A) and biosimilar drug (B) in healthy individuals.

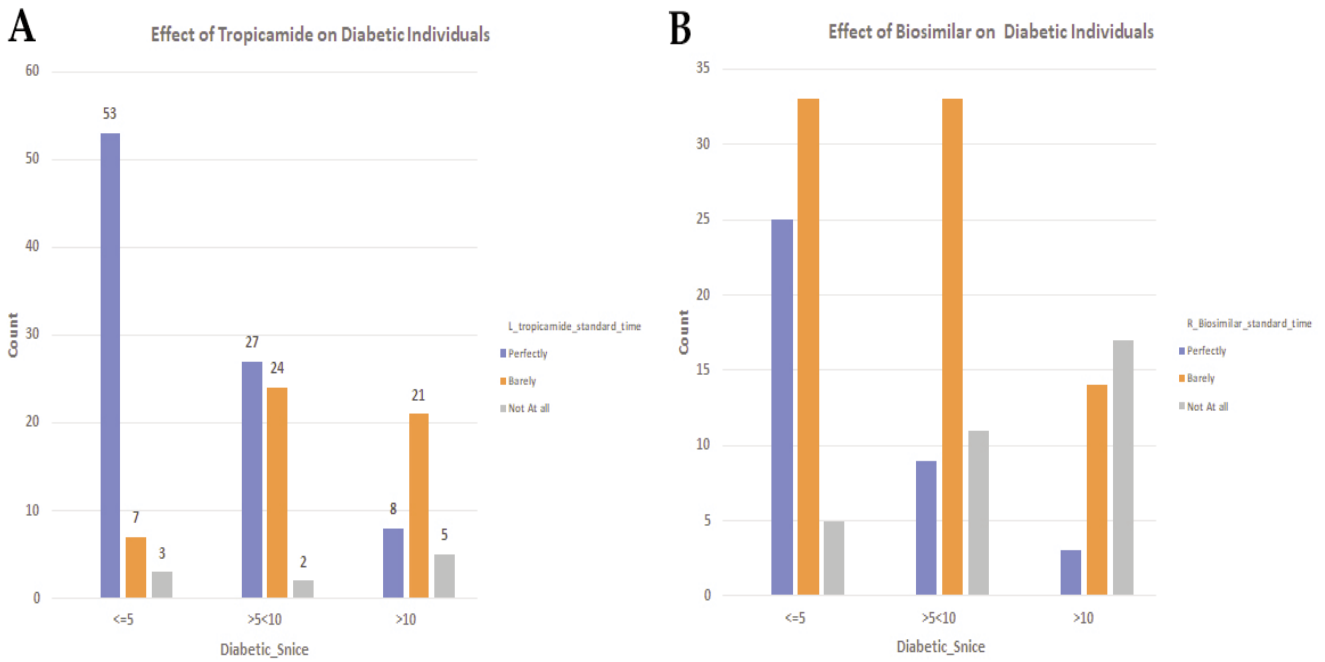


**Figure-2**: Effect of tropicamide (A) and biosimilar drug (B) on diabetic individuals

for the biosimilar drug, $p<0.01$ (Table 1). In Group A, the mean pupil dilation for tropicamide was $2.5 \pm 0.5$ mm, while for the biosimilar drug, it was $2.0 \pm 0.4$ mm. In Group B, the mean pupil dilation size for tropicamide was $2.7 \pm 0.6$ mm, compared to $1.8 \pm 0.5$ mm for the biosimilar drug. Across both groups, tropicamide demonstrated a significantly higher mean pupil dilation size compared to the biosimilar drug ($p<0.01$) (Table 2).

After ≤5 minutes of administrating tropicamide, 29 (19.33%) healthy individuals had perfectly dilated pupils, compared to 53 (35.33%) diabetic individuals. On the other hand, after ≤5 minutes of administering the biosimilar drug, 23 (15.43%) healthy individuals and 25 (16.66%) diabetic individuals had perfectly dilated pupils. Additionally, 18 (12%) healthy individuals and 7 (4.66%) diabetic individuals had barely dilated pupils after ≤5 minutes of tropicamide administration, compared to 18 (12.08%) healthy individuals and 33 (22%) diabetic individuals after ≤5 minutes of biosimilar drug administration. Among the healthy individuals, 2 (1.33%) showed no response to tropicamide within ≤5 minutes, while 3 (2%) diabetic individuals also showed no response. In the case of the biosimilar drug, 7 (4.69%) healthy individuals and 5 (3.33%) diabetic individuals

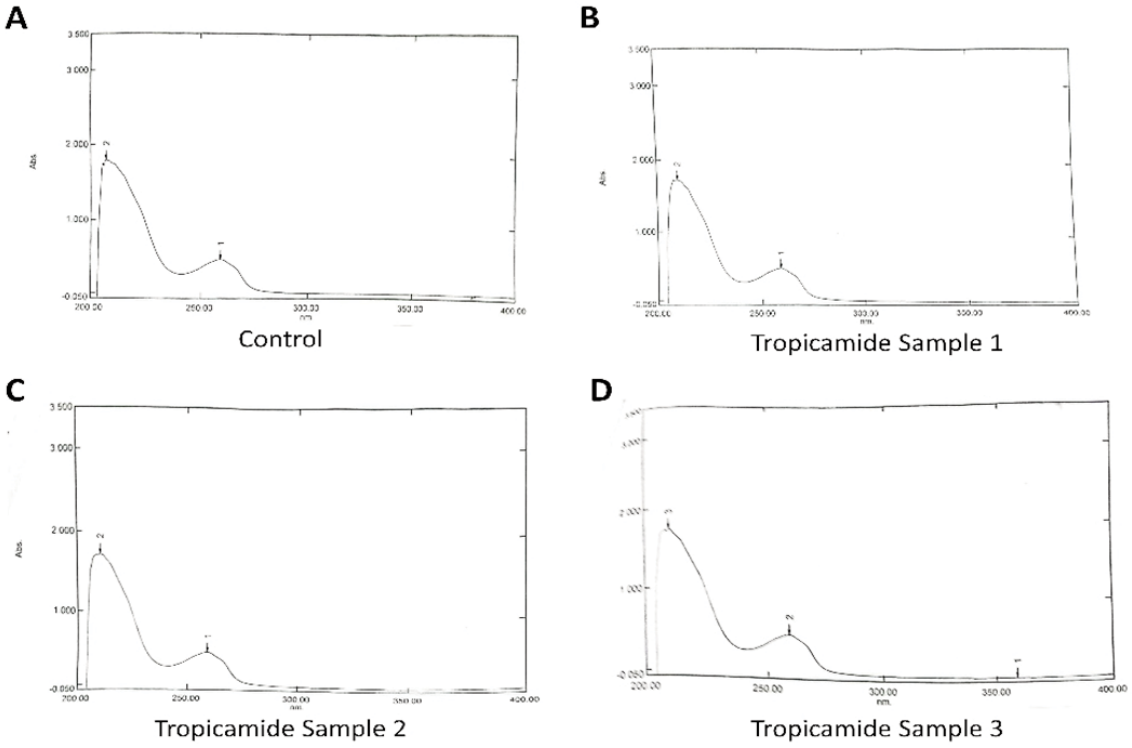


**Figure-3:** HPLC-FTIR absorption spectrum of a control sample (A) and tropicamide samples (B-D)
HPLC-FTIR: High Performance Liquid Chromatography-Fourier Transform Infrared Spectroscopy.

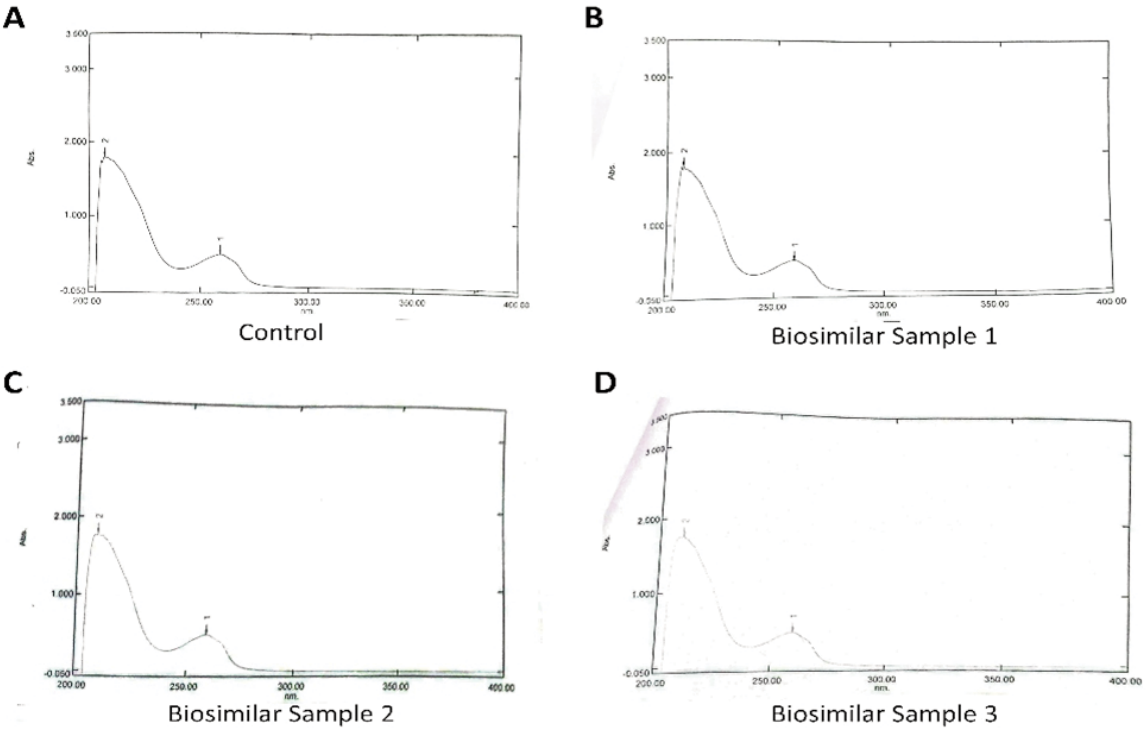


**Figure-4:** HPLC-FTIR absorption spectrum of a control sample (A) and biosimilar drug samples (B-D)
HPLC-FTIR: High Performance Liquid Chromatography-Fourier Transform Infrared Spectroscopy.

showed no response within ≤5 minutes of administration (Figures 1-2).

At 5-10 minutes post-administration, 87 (58%) healthy and 27 (18%) diabetic individuals had perfectly dilated pupils in response to tropicamide, compared to 23 (15.43%) healthy and 9 (6%) diabetic individuals with the biosimilar drug. Furthermore, 12 (8%) healthy individuals and 24 (16%) diabetic individuals had barely dilated pupils in response to tropicamide, compared to 32 (21.47%) healthy individuals and 33 (22%) diabetic individuals in response to the biosimilar drug. Two (1.33%) healthy and 2 (1.33%) diabetic individuals showed no response to tropicamide, while 5 (3.35%) healthy and 11 (7.33%) diabetic individuals showed no response to the biosimilar drug (Figures 1-2).

The effect of tropicamide and the biosimilar drug at >10 minutes of administration was only observed in diabetic patients. There were 8 (5.33%) diabetic individuals with perfectly dilated pupils at >10 minutes after tropicamide administration, compared to 3 (2%) for the biosimilar drug. In addition, 21 (14%) diabetic individuals had barely dilated pupils in response to tropicamide, compared to 14 (9.33%) for the biosimilar drug. Furthermore, 5 (3.33%) diabetic individuals did not have dilated pupils in response to tropicamide, compared to 17 (11.33%) for the biosimilar drug (Figures 1-2).

At 5-10 minutes post-administration, 87 (58%) healthy individuals had perfectly dilated pupils in the tropicamide group, compared to 64 (42.95%) in the biosimilar drug group. Among diabetic individuals, 53 (35.33%) had perfectly dilated pupils within ≤5 minutes in the tropicamide group, compared to 25 (16.66%) in the biosimilar drug group.

For quality control analysis, the pH was 4.87, 4.65, and 4.79 for the three test samples, compared to the specified range of 4.0-5.8. In addition, against the average sample volume specification of 15 mL ±3.5%, the test samples measured 15.7 mL, 16.2 mL, and 15.9 mL. HPLC-FTIR analysis found that the tropicamide assay was 102%, 102%, and 103% (Figure 3). In contrast, the assay analysis of the biosimilar drug was 100%, 102%, and 101.7%, respectively (Figure 4).

## Discussion

The current study's findings suggested that tropicamide was more effective in both healthy and diabetic individuals compared to the biosimilar drug.

Despite the high efficiency of tropicamide, it may not be as cost-effective as its biosimilar drug which can be an economical alternative for mydriasis in developing countries. The HPLC-FTIR also showed that tropicamide samples had slightly higher percentages compared to the biosimilar drug, which can be the reason behind the high efficiency of tropicamide.

Previously, studies have been conducted to determine the effect of multiple does and single dose of tropicamide on pupil dilation[16]. In addition, studies have also been conducted to determine the efficiency of single dose of tropicamide in comparison with single dose and 3 doses of tropicamide and phenylephrine.[17] However, the comparative studies of tropicamide with its' biosimilar drugs have been rarely conducted. A comparative study analysed the effect between tropicamide and phenylephrine. In agreement with the findings of the current study, it also found that tropicamide was more effective in inducing pupil dilation compared to phenylephrine.[18] Furthermore, studies have also reported a negative co-relation of diabetes with pupil dilation, which was also noted in the current study.[19, 20]

The current study has limitations. The relatively modest sample size may limit the generalisability of the findings. Moreover, variations in individual responses to mydriatic agents, influenced by factors such as age, gender and underlying health conditions, were not explored. Future research with larger and more diverse cohorts is needed to validate the current findings.

## Conclusion

Tropicamide was found to be more effective compared to its biosimilar drug. Also, tropicamide was more effective in healthy individuals compared to diabetic individuals. However, the biosimilar drug can be a good alternative of tropicamide in low-income/developing countries.

**Acknowledgements:** We are grateful to all the participants, and to the Department of Pharmacology, Riphah Institute of Pharmaceutical Sciences, Riphah International University, for providing research facilities.

**Disclaimer:** None.

**Conflict of Interest:** None.

**Source of Funding:** None.

## References

1. Karpecki PM, Foster SA, Montaquila SM, Kannarr SR, Slonim CB, Meyer AR, et al. Phentolamine eye drops reverse pharmacologically induced mydriasis in a randomized phase 2b trial. Optom Vis Sci 2021;98:234-42. doi: 10.1097/OPX.0000000000001659.
2. Robin A, Giovingo M. Screening recommendations for diabetics. Dis Mon 2021;67:101116. doi: 10.1016/j.disamonth.2020.101116.
3. Centers for Disease Control and Prevention (CDC). National Diabetes Statistics Report, 2020: Estimates of diabetes and its

burden in the United States. Atlanta, GA: U.S. Department of Health and Human Services; 2020.
4. Atlas, D . (2025). International diabetes federation. IDF Diabetes Atlas, 11th edn. Brussels, Belgium: International Diabetes Federatio. Available at https://diabetesatlas.org/resources/idf-diabetes-atlas-2025/ Cited on 15. May 2025.
5. Tham YC, Li X, Wong TY, Quigley HA, Aung T, Cheng CY. Global prevalence of glaucoma and projections of glaucoma burden through 2040: a systematic review and meta-analysis. Ophthalmology 2014;121:2081-90. doi: 10.1016/j.ophtha.2014.05.013.
6. Khairallah M, Kahloun R, Bourne R, Limburg H, Flaxman SR, Jonas JB, et al. Number of people blind or visually impaired by cataract worldwide and in world regions, 1990 to 2010. Invest Ophthalmol Vis Sci 2015;56:6762-9. doi: 10.1167/iovs.15-17201.
7. Siegel B, Thompson A, Yolton D, Reinke A, Yolton R. A comparison of diagnostic outcomes with and without pupillary dilatation. J Am Optom Assoc 1990;61:25-34.
8. Asnaashari P. Managing miotics and mydriatics. Rev Optom 2021;158:15.
9. Qureshi MB, Chaudhry I. Best Practice Integrated Approaches in Eye Care Service Delivery. In: Khanna R, Rao G, Marmamula S, eds. Innovative Approaches in the Delivery of Primary and Secondary Eye Care. Essentials in Ophthalmology. Cham, Switzerland: Springer Nature Switzerland AG; 2019. doi: 10.1007/978-3-319-98014-0_3
10. Nazish HR, Ali N, Ullah S. The possible effect of SCN1A and SCN2A genetic variants on carbamazepine response among Khyber Pakhtunkhwa epileptic patients, Pakistan. Ther Clin Risk Manag 2018;14:2305-13. doi: 10.2147/TCRM.S180827.
11. Daniel WW, Cross CL. Biostatistics: A Foundation for Analysis in the Health Sciences, 10th ed. Hoboken, NJ: John Wiley & Sons; 2018.
12. Pourhoseingholi MA, Vahedi M, Rahimzadeh M. Sample size calculation in medical studies. Gastroenterol Hepatol Bed Bench 2013;6:14-7. doi: 10.22037/ghfbb.v6i1.332.
13. Jordan JA, Oatts JT. Pharmacologic mydriasis and cycloplegia: a review of novel delivery devices. Touch Rev Ophthalmol 2023;17:89-93.
14. Mori M, Araie M, Sakurai M, Oshika T. Effects of pilocarpine and tropicamide on blood-aqueous barrier permeability in man. Invest Ophthalmol Vis Sci 1992;33:416-23.
15. Stefanowicz Z, Stefanowicz J, Mulas K. Determination of tropicamide and its major impurity in raw material by the HPLC-DAD analysis and identification of this impurity using the off-line HPLC–FT-IR coupling. J Pharm Biomed Anal 2009;49:214-20. doi: 10.1016/j.jpba.2008.10.043.
16. Siderov J, Nurse S. The mydriatic effect of multiple doses of tropicamide. Optom Vis Sci 2005;82:955-8. doi: 10.1097/01.opx.0000187847.48093.55.
17. Veranarapanit MN, Suksomboon S, Prarach KP. Comparison of single dose of 1% tropicamide, single dose of 1% tropicamide plus 10% phenylephrine and 3 doses of 1% tropicamide plus 10% phenylephrine for pupil dilatation. Rajasthan Med J 2011;22:1-4.
18. Park JH, Lee YC, Lee SY. The comparison of mydriatic effect between two drugs of different mechanism. Korean J Ophthalmol 2009;23:40-2.
19. Huber M, Smith S, Smith S. Mydriatic drugs for diabetic patients. Br J Ophthalmol 1985;69:425.
20. George A, Antony J, Thampi B. Comparison of mydriasis obtained by tropicamide and phenylephrine in type 2 diabetic and nondiabetic patients. Kerala Med J 2019;12:69-71.

**AUTHOR'S CONTRIBUTION:**

**FF & SZM:** Concept, design, data acquisition, analysis, interpretation, drafting, final approval and agreement to be accountable for all aspects of the work.