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Original Research Article

Comparative efficacy of fixed dose combination of brinzolamide 1% and timolol 0.5% without versus with benzalkonium chloride following single ocular instillation in New Zealand white rabbits

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ABSTRACT

Background: Benzalkonium chloride (BAK) is the most used preservative in topical ophthalmic formulations. BAK causes dry eye and trabecular meshwork degeneration. Hence, BAK-free formulations are desirable for chronic conditions like glaucoma. The objective of this study was to compare the efficacy of BAK-free versus BAK-containing ophthalmic suspension of fixed dose combination (FDC) of brinzolamide and timolol on their intraocular pressure (IOP) lowering effect following a single ocular instillation in New Zealand White (NZW) rabbits.
Methods: Twelve normotensive NZW rabbits (male) between 9-12 months of age (3.4-4.4 kg) received a single ocular instillation (35 µl) in left eye of either ophthalmic suspension containing FDC of brinzolamide (1% w/v)/timolol (0.5% w/v) without BAK (n=6, test) or with BAK 0.01% w/v (n=6, reference). IOP was measured before ocular instillation (baseline) and at 2, 4, 6, 8 and 24 hours after instillation using a pneumatonometer. Change in IOP from baseline were calculated and analysed using repeated measures analysis of variance (ANOVA) followed by Bonferroni post-test for pairwise comparisons.
Results: Significant IOP reduction ($p<0.05$) from baseline was seen in both test and reference groups, up to 6 hours after instillation. Maximum IOP reduction was 25.5% and 22.6% at 2 hours in the test and reference group respectively. No significant differences ($p>0.05$) were observed between the test and reference group for the change in IOP at all time points.
Conclusions: BAK-free and BAK-containing ophthalmic suspension of FDC of brinzolamide 1% and timolol 0.5% after a single ocular instillation in normotensive NZW rabbits produces similar reductions in IOP.

Keywords: Potassium sorbate, BAK, Ophthalmic suspension, IOP, Brinzolamide, Timolol

INTRODUCTION

Glaucoma is a group of optic neuropathies characterized by progressive degeneration of retinal ganglion cells, resulting in cupping of the optic disc and visual loss.[1] It is the leading cause of irreversible blindness in the world affecting more than 70 million people worldwide with about 10% being bilaterally blind.[2] On the basis of the available data in 2010, it was estimated that there were approximately 11.2 million persons aged 40 years and older with glaucoma in India.[3] The reported prevalence of glaucoma from eastern India was 2.7% in population and 3.23% in urban population.[4]

Current preferred practice pattern guidelines for primary open-angle glaucoma by the American academy of ophthalmology recommend lowering of the IOP towards a

target level of 20% to 50% from previous levels.[5] Although laser treatment and incisional surgery can be used for glaucoma treatment, reduction of IOP by use of drugs is the only proven method to treat glaucoma.[6] Four different classes of drugs are available for lowering the IOP, which include prostaglandin analogues (PGAs), beta-adrenergic blockers, carbonic anhydrase (CA) inhibitors and alpha-2 adrenergic agonists. Although miotic drugs can be used, they cause multiple side-effects and are not preferred as first-line agents.[7] Topical beta-blockers were commonly used anti-glaucomatous drugs, but their use has declined after availability of prostaglandin analogues and newer CA inhibitors.[8] Brinzolamide is a highly specific CA inhibitor which lowers IOP by reducing the rate of aqueous humour formation. Formulated as a 1% ophthalmic suspension and administered two or three times daily, brinzolamide is indicated for the topical management of primary open-angle glaucoma (POAG) and ocular hypertension (OH) as either monotherapy or adjunctive therapy with topical beta-blockers.[9] Timolol is superior to other beta blockers for glaucoma treatment.[10] Timolol causes reduction in aqueous formation through beta-receptor blockade and this action is different from the CA inhibitors. Hence, a combination of brinzolamide and timolol may have an additive effect. Timolol does not obstruct the aqueous outflow and does not affect the pupil size. The ocular hypertension treatment study and the collaborative initial glaucoma treatment study have reported that about 50-75% of patients will need combination therapy at any stage of the disease and may require two or more drugs to reach their target pressure. In such cases, a fixed combination rather than separate drugs has several potential advantages including no risk of drug washout, reduced exposures to preservatives with reduced side effects, reduced costs of treatment and ultimately better patient compliance and quality of life.[11] Hence, the FDC of brinzolamide and timolol could be potentially useful in open-angle glaucoma with no effects on the vision and minimal side effects. Prospective double-blind studies have shown benefits of ocular therapy with the fixed combination of brinzolamide-timolol in treatment of ocular hypertension.[12-14] Fixed combination of brinzolamide/timolol provides better ocular comfort than fixed combination of dorzolamide/timolol due to more physiologic pH, that may be leads to a positive patient adherence and thereby reaching the target IOP goals is more likely.[12]

Preservatives used to prevent microbial contamination in multi-dose ophthalmic medications are detergents or act by oxidative mechanisms. However, these can cause irritation and side effects in the eye.[15] BAK is the most commonly used preservative in ophthalmology and is more toxic than other or newer preservatives, such as polyquaternium-1 (Polyquad), sodium perborate, oxychloro-complex (Purite®) and SofZia.[16] The severity of BAK toxicity is dependent on the amount, concentration, and duration of topical administration. With each administration, there is disruption of the tear film lipid layer. BAK also causes cellular damage through apoptosis phenomena (free radical production) and cellular necrosis, leading to a reduction in the mucin amount which further aggravates tear film disruption.[17] Long-term treatment with BAK containing formulations for glaucoma can potentially cause severe tear film disruption and dry eye.[18] It is hypothesized that BAK helps in ocular penetration of ophthalmic drugs and that BAK-free formulations would not have as effective lowering of the IOP. However, several studies have reported effective lowering of IOP with BAK-free ophthalmic formulations.[4,19]

Ophthalmic suspensions containing FDC of brinzolamide (1%) and timolol (0.5%) are approved in India for the treatment of open-angle glaucoma or ocular hypertension. The objective of this study was to compare the efficacy of BAK-free versus BAK-containing ophthalmic suspension of FDC of brinzolamide 1% and timolol 0.5% on the IOP lowering effect following a single ocular instillation in normotensive NZW rabbits.

METHODS

This study was carried out at the department of pharmacology, Tandalja, Vadodara, Gujarat, India (Sun pharmaceutical industries Ltd.). The research protocol and study related documents were reviewed and approved by the institutional animal ethics committee (Project number IAEC#609) of Sun Pharma advanced research company Ltd., Vadodara, Gujarat, India. The study was conducted as per the CPCSEA (Committee for the purpose of control and supervision of experiments on animals) guidelines and recommendations for animal care and handling.

Twelve normotensives male NZW rabbits weighing 3.44-4.4 kg and between 9-12 months of age were selected for the study and divided into two groups of 6 each. All animals underwent veterinary health check for any abnormalities or conditions for selection of healthy animals for the study. Animals were identified by an identification number based on cage card and ear marking. One rabbit was housed per cage at ambient temperature of 18-22^{0}C with 12-hour light-dark cycle at a relative humidity of 30-70%. Animals were given a high fibre rabbit pellet feed and filtered water ad libitum.

Pre-treatment measurements of IOP were obtained for left eye of each animal at 09:00 hours and 17:00 hours for two days preceding the study day (day -2 to day -1) and at 09:00 hours on the study day (day 0) and the average IOP up to 48 hours (average of these five readings) was considered as baseline (initial) IOP.

Test group (n=6) received ocular instillation of FDC of brinzolamide 1% w/v and timolol 0.5% w/v ophthalmic suspension without BAK (Sun Pharma, India, Batch No. 26202507PF005A) using potassium sorbate 0.47% w/v as a preservative, whereas reference group (n=6) received ocular instillation of FDC of brinzolamide 1% w/v and timolol 0.5% w/v ophthalmic suspension with BAK 0.01% w/v as a preservative (Brinzotim®, Sun Pharma, India,

batch no. HKT0352A). A single 35 µl drops of either ophthalmic suspension was instilled in the left eye of the assigned animal in the test/reference group using a micropipette, at 09:00 hours on the study day (day 0) immediately after the IOP measurement. The dose was selected based on literature review of previous animal studies. Subsequent IOP measurements on the study day (day 0) were taken at 2, 4-, 6-, 8- and 24-hours post instillation.

IOP was measured using a pneumotonometer model 30 classic™ (Reichert, USA). During IOP measurements, each animal was restrained in a restrainer, without sedation. The pneumatonometer probe was placed lightly on the cornea and allowed to rest for 10-15 seconds. The probe was placed entirely on the cornea in horizontal position and five consecutive readings were recorded, each with standard deviation value <1 which was displayed on screen and an average of these 5 readings was calculated. The pneumatonometer probe filter was cleaned after each use by gently touching to cotton swab (immersed in normal saline) and just wiped with tissue paper.

The statistical analysis was carried with PRISM (GraphPad version 5.03, December 10, 2009) and $p<0.05$ was considered as statistically significant. Change in IOP was calculated with respect to baseline IOP and was analysed by using the one-way repeated measures analysis of variance (ANOVA) followed by Bonferroni post-tests (n=6) for individual measurements. IOP measurements were also analysed for differences using two-way ANOVA followed by Bonferroni post-tests (n=6) for comparison between the groups.

RESULTS

The test and reference group were similar with respect to the baseline IOP. Table 1 and Figure 1 shows the mean and standard error of mean (SEM) for the IOP at baseline and at 2, 4-, 6-, 8- and 24-hours post-instillation, in the two groups.

Single ocular instillation of either BAK-free or BAK-containing FDC of brinzolamide 1% and timolol 0.5% ophthalmic suspension showed statistically significant reduction in the IOP at 2 hours and up to 6 hours compared with the baseline IOP in normotensive NZW rabbits. However, the differences in the IOP between the test and reference group were not significant at all time points ($p>0.05$).

Table 1: Reduction in IOP in study eye.

Variables	Test (BAK-free), (n=6)				Reference (BAK-containing), (n=6)			
	IOP (mm Hg)		Change in IOP		IOP (mm Hg)		Change in IOP	
Hours	Mean	SEM	Mean change	% change	Mean	SEM	Mean change	% change
0 (baseline)	19.78	0.61	-	-	20.54	1.18	-	-
2	14.73*	0.41	-5.0	-25.5	15.90*	0.74	-4.6	-22.6
4	16.08*	0.37	-3.7	-18.7	17.73*	0.77	-2.8	-13.7
6	17.04*	0.76	-2.7	-13.8	18.75*	0.97	-1.8	-8.7
8	19.05	0.99	-0.7	-3.7	20.27	1.00	-0.3	-1.3
24	19.68	0.44	-0.1	-0.5	20.73	0.99	0.2	0.9

*P<0.001 compared to baseline (initial) IOP. (One-Way repeated measures analysis of variance). No statistically significant differences were observed between the two groups. (Two-way analysis of variance).

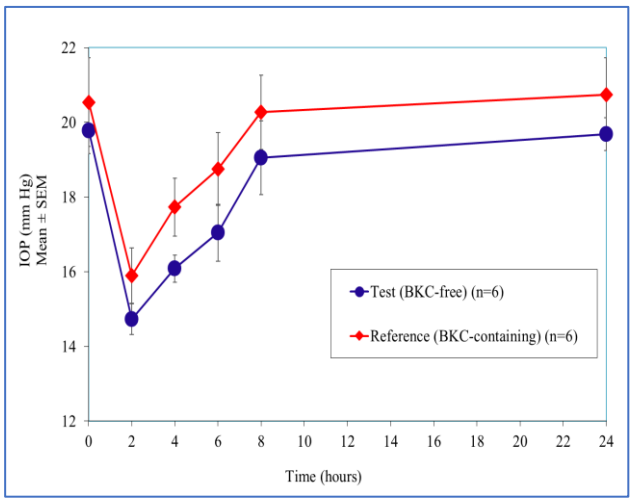

Figure 1: Mean IOP in study eye (bars indicate standard error of mean).

Table 1 also presents the mean change and percent change in IOP from baseline in the two groups at 2, 4-, 6-, 8- and 24-hours post instillation. Maximum reduction in IOP was 25.5% and 22.6% at 2 hours following single instillation in the test and reference group respectively.

DISCUSSION

The current study demonstrated comparable efficacy of BAK-free with BAK-containing ophthalmic suspension of FDC of brinzolamide 1% and timolol 0.5% on the IOP lowering effect, following a single ocular instillation in normotensive NZW rabbits. The BAK-free ophthalmic formulation containing potassium sorbate 0.47% as a preservative is approved in India for treatment of open-angle glaucoma or ocular hypertension.

Glaucoma being a chronic and progressive disease, it is critical to reduce the IOP to prevent long-term

complications including blindness. Thus, it is important to ensure good compliance to IOP reduction therapy by the patients. This is especially critical in glaucoma, since the patients may not have any symptoms due to raised IOP, which may not keep the patients motivated to continue therapy. Poor ocular tolerability due to local side effects could be one of the other important reasons of poor compliance.

BAK/BKC is used as a preservative for ophthalmic and other topical preparations. The side effects like corneal xerosis are prominent with ophthalmic drops containing BAK as a preservative.[16] BAK (0.2%) containing formulations have shown to induce severe corneal epithelial defects and 0.1% BAK once daily over 7 days have induced punctate fluorescein staining without detriment to corneal smoothness.[20] BAK disrupts the lipid layer and affects tear film stability, thus decreasing the tear break-up time (BUT).[21] BAK was found to trigger cell contraction and vacuolation, increased lactate dehydrogenase (LDH) release and elevated cell necrosis by almost four times in cultivated primary mouse corneo-limbal epithelial cells (CLEC's).[20] The toxicity of BAK has been attributed to the inhibition of human corneal epithelial cells mitochondria, and corneal cells with Leber hereditary optic neuropathy (LHON) mutations at pharmacologically relevant concentrations.[22] Use of BAK-containing eye drops are best avoided in patients with mitochondrial deficiency, including LHON patients, LHON carriers, and possibly primary open-angle glaucoma patients.[22] Also, long-term effects of BAK include a possible decrease in susceptibility of ocular bacterial flora to commonly used antibiotics and an increase in the incidence of methicillin-resistant strains.[21] Chronic exposure to BAK has been linked to an increased risk of trabeculectomy failure.[21]

There are numerous studies which report greater side effects with the use of BAK-containing eye drops compared to BAK-free eye drops for treatment of ocular conditions. In a randomized crossover study comparing topical carteolol with and without BAK, the BAK-preserved eye drops significantly decreased the tear break-up time compared to baseline values in healthy volunteers.[23] In a randomized controlled study, switching from BKC preserved latanoprost (with at least 3 months of treatment) to BKC free travoprost, significantly improved the tear film break up time.[24] In another study, the tear break-up time was significantly lower from baseline over three months of treatment with BAK preserved travoprost.[25] Also, Rossi et al reported frequent occurrences of dry eye disease in glaucoma patients on topical medications containing BAK.[26] Similarly, in another randomized clinical trial reported by Uusitalo et al a switchover from BAK-preserved latanoprost (0.02%) eye drops to a preservative-free tafluprost resulted in significant ($p<0.05$) improvement in the mean (SD) tear film break-up time from 4.5 (2.5) seconds at baseline to 7.8 (4.9) seconds at 12 weeks.[27] The Schirmer test scores improved at six weeks and the percentage of patients with abnormal Schirmer test also improved.

BAK-free ophthalmic formulations could improve tolerability and patient compliance leading to successful therapy. Potassium sorbate is one of the alternative available as a preservative for eye drops and other formulations. Sorbic acid in their salt forms like sodium sorbate and potassium sorbate are used as food preservatives in processed foods like cheese, meat, ketchup, mayonnaise, and marmalade. Potassium sorbate is a preservative with antibacterial and antifungal properties and is widely used in food and cosmetic industry.[28] Potassium sorbate 0.47% is a listed antimicrobial preservative in the US pharmacopoeia and included in the US FDA inactive ingredients database (as 0.47% w/v for ophthalmic dosage forms-emulsion, solution, drops). It is included in the Canadian list of acceptable non-medicinal ingredients and listed as generally recognized as safe (GRAS) by the US FDA.

Yanochko et al compared toxicity of BAK and other preservatives including potassium sorbate of eye drops on the monolayer and stratified air-lifted cultures of Chang conjunctival cells.[29] Eye drops containing BAK caused near complete loss of cell viability with up to 30-min exposure. Dose-response curves (DRCs) were plotted for cell-viability with use of preservatives. At 30 minutes of exposure, differences in rank order of toxicity between monolayer and stratified Chang cells were observed; monolayer: thimerosal>BAK>chlorhexidine digluconate>ethylene diaminetetraacetic acid (EDTA)> potassium sorbate; stratified: chlorhexidine digluconate >thimerosal>BAK>EDTA>potassium sorbate. However, after 24 h exposure, rank order of toxicity was same for both, monolayer and stratified: BAK>thimerosal>chlorhexidine digluconate>EDTA> potassium sorbate.

Brinzolamide is a potent inhibitor of human carbonic anhydrase II (CA-II), the predominant isoenzyme in the eye. Inhibition of CA-II in the ciliary processes of the eye decreases aqueous humour secretion, presumably by slowing the formation of bicarbonate ions with subsequent reduction in sodium and fluid transport.[30] Timolol is a non-selective beta-adrenergic receptor blocker and is generally considered to have few systemic effects when administered in the eye.[31] These two drugs reduce the IOP primarily by reducing the aqueous humour secretion, but by different mechanisms. Hence, the combined effect of these two active substances can result in additional IOP reduction compared to either of them alone. Data from clinical trials have shown that FDC of brinzolamide and timolol is as effective as combination of dorzolamide and timolol in the treatment of ocular hypertension and open-angle glaucoma, but with lesser ocular discomfort.[12-14]

There are no comparative studies of potassium sorbate containing with BAK-containing anti-glaucoma formulations. This pre-clinical study is the first study that

has compared a potassium sorbate containing (BAK-free) and a BAK-containing FDC of brinzolamide 1% and timolol 0.5% ophthalmic suspension for an IOP lowering effect.

The findings from this non-clinical study involving normotensive rabbits, and with a single instillation, cannot be extrapolated to human subjects with glaucoma. Clinical studies would be desirable for confirmation of the efficacy and ocular tolerability of BAK-free versus BAK-containing formulation in glaucoma.

CONCLUSION

FDC of brinzolamide 1% and timolol 0.5% without BAK showed comparable IOP lowering effect to the same FDC but with BAK, after a single ocular instillation in normotensive NZW rabbits. Further investigation of the ophthalmic suspension of the fixed combination of brinzolamide and timolol containing potassium sorbate as a preservative in glaucoma patients may yield favourable results.

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Ethical approval: The study was approved by the Institutional Animal Ethics Committee (IAEC) and conducted as per guidelines and recommendations of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)

REFERENCES

1. Weinreb RN, Khaw PT. Primary open-angle glaucoma. Lancet. 2004;363(9422):1711-20.
2. Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. Br J Ophthalmol. 2006;90(3):262-7.
3. George R, Ve RS, Vijaya L. Glaucoma in India: estimated burden of disease. J Glaucoma. 2010;19(6):391-7.
4. Paul C, Sengupta S, Choudhury S, Banerjee S, Sleath BL. Prevalence of glaucoma in Eastern India: The Hooghly River Glaucoma Study. Indian J Ophthalmol. 2016;64(8):578-83.
5. American Academy of Ophthalmology Preferred Practice Patterns Committee GP. Ophthalmology. Chicago, Illinois: American Academy of Ophtalmology; Preferred practice pattern: primary open-angle glaucoma. 2010.
6. Boland MV, Ervin AM, Friedman DS. Comparative effectiveness of treatments for open-angle glaucoma: a systematic review for the US Preventive Services Task Force. Ann Intern Med. 2013;158(4):271-9.
7. Faiq MA, Wollstein G, Schuman JS, Chan KC. Cholinergic nervous system and glaucoma: From basic science to clinical applications. Prog Retin Eye Res. 2019;72:100767.
8. Storgaard L, Tran TL, Freiberg JC, Hauser AS, Kolko M. Glaucoma Clinical Research: Trends in Treatment Strategies and Drug Development. Front Med (Lausanne). 2021;8:733080.
9. Cvetkovic RS, Perry CM. Brinzolamide: a review of its use in the management of primary open-angle glaucoma and ocular hypertension. Drugs Aging. 2003;20(12):919-47.
10. Singh D. Timolol for glaucoma. Indian J Ophthalmol. 1982;30(4):227-8.
11. Gopinathan U, Sharma S, Garg P, Rao GN. Review of epidemiological features, microbiological diagnosis and treatment outcome of microbial keratitis: experience of over a decade. Indian J Ophthalmol. 2009;57(4):273-9.
12. Januleviciene I. Brinzolamide 1%/timolol 0.5%: safety and efficacy of a new fixed-combination IOP-lowering product for glaucoma. Curr Med Res Opin. 2010;26(11):2575-8.
13. Sezgin Akçay Bİ, Güney E, Bozkurt KT, Unlü C, Akçali G. The safety and efficacy of brinzolamide 1%/timolol 0.5% fixed combination versus dorzolamide 2%/timolol 0.5% in patients with open-angle glaucoma or ocular hypertension. J Ocul Pharmacol Ther. 2013;29(10):882-6.
14. Manni G, Denis P, Chew P, Sharpe ED, Orengo-Nania S, Coote MA et al. The safety and efficacy of brinzolamide 1%/timolol 0.5% fixed combination versus dorzolamide 2%/timolol 0.5% in patients with open-angle glaucoma or ocular hypertension. J Glaucoma. 2009;18(4):293-300.
15. De Saint jean M, debbasch C, brignole F, Rat P, Warnet J-M, Baudouin C. Toxicity of preserved and unpreserved antiglaucoma topical drugs in an in vitro model of conjunctival cells. Am J Ophthalmol. 2000;130(2):264-5.
16. Messmer EM. Preservatives in ophthalmology. Ophthalmologe. 2012;109(11):1064-70.
17. Coroi MC, Bungau S, Tit M. Preservatives from the eye drops and the ocular surface. Rom J Ophthalmol. 2015;59(1):2-5.
18. Rosin LM, Bell NP. Preservative toxicity in glaucoma medication: clinical evaluation of benzalkonium chloride-free 0.5% timolol eye drops. Clin Ophthalmol. 2013;7:2131-5.
19. Duru Z, Ozsaygili C. Preservative-free versus preserved brimonidine %0.2 preparations in the treatment of glaucoma and ocular hypertension: short term evaluation of efficacy, safety, and potential advantages. Cutan Ocul Toxicol. 2020;39(1):21-4.
20. Zhang R, Park M, Richardson A, Tedla N, Pandzic E, de Paiva CS et al. Dose-dependent benzalkonium chloride toxicity imparts ocular surface epithelial

changes with features of dry eye disease. Ocul Surf. 2019;pii:S1542-0124(19)30285-X.
21. Rupankar Sarkar. Effects of preservatives used in ocular medications on the eye: a comparative review. Ophthalmol J. 2021;6:44-52.
22. Datta S, Baudouin C, Brignole-Baudouin F, Denoyer A, Cortopassi GA. The Eye Drop Preservative Benzalkonium Chloride Potently Induces Mitochondrial Dysfunction and Preferentially Affects LHON Mutant Cells. Invest Ophthalmol Vis Sci. 2017;58(4):2406-12.
23. Baudouin C, De Lunardo C. Short-term comparative study of topical 2% carteolol with and without benzalkonium chloride in healthy volunteers. Br J Ophthalmol. 1998;82(1):39-42.
24. Aihara M, Oshima H, Araie M. EXTraKT study group. Effects of SofZia-preserved travoprost and benzalkonium chloride-preserved latanoprost on the ocular surface-a multicentre randomized single masked study. Acta Ophthalmol. 2013;91(1):e7-14.
25. Tomić M, Kaštelan S, Soldo KM. Influence of BAK-preserved prostaglandin analog treatment on the ocular surface health in patients with newly diagnosed primary open-angle glaucoma. Biomed Res Int. 2013;2013:603782.
26. Rossi GC, Tinelli C, Pasinetti GM. Dry eye syndrome-related quality of life in glaucoma patients. Eur J Ophthalmol. 2009;19(4):572-9.
27. Uusitalo H, Chen E, Pfeiffer N. Switching from a preserved to a preservative-free prostaglandin preparation in topical glaucoma medication. Acta Ophthalmol. 2010;88(3):329-36.
28. United States Pharmacopeia (USP) and the National Formulary (NF). Available at: https://online.uspnf.com/uspnf/document/1_GUID-7DBFEDEB-80DF-44A0-BFB5-21A46A867947_8_en-US. Accessed on 28 June, 2023.
29. Gina M. Yanochko, Su Khoh-Reiter, Mark G. Evans, Bart A. Jessen. Comparison of preservative-induced toxicity on monolayer and stratified Chang conjunctival cells. Toxicol *in vitro*. 2010;24(4):1324-31.
30. DeSantis L. Preclinical overview of brinzolamide. Surv Ophthalmol. 2000;44(2):S119-29.
31. Zhuoying W, Ian D, Feng C, Lijie C, Xuecui W, Daniel RK et al. Complete atrioventricular block due to timolol eye drops: a case report and literature review. BMC Pharmacol Toxicol. 2019;20:73.

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