

Effect of Telmisartan on Intra-Ocular Pressure in induced Open Angle Glaucoma in Rabbits

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Abstract: *The present study was designed to evaluate the possible intraocular pressure lowering effect of corneal instillation of telmisartan in experimentally corticosteroid induced ocular hypertensive eyes of rabbits, and to explore the possible side effects of the tested drugs on eyes after instillation. Group of (40) adult male of New Zealand rabbits, were divided to 3 groups: isotonic buffer group (8 normotensive rabbits), this group was instilled with isotonic buffer solution in the right eye and DW in the left eye to show if there is any effect of the vehicle (isotonic solution) on the eye, timolol group (8 rabbits) were both eyes of this group have been induced for ocular hypertensive, the right eyes instilled with timolol (0.5%) drop twice daily which considered as a positive control group, while the left eyes instilled with DW twice daily which considered as a negative control group., telmisartan group (24 rabbits), divided in to (3) subgroups 8 rabbits in each to evaluate the effect of telmisartan (0.25%, 0.5% & 1%) drop that instilled once daily. The present study clearly demonstrated that single drop of telmisartan (0.25%) able to reduce mean IOP by (2.5%) in hypertensive rabbits eyes after one day of instillation and the peak mean IOP decline that achieved after 7 days of instillation was (6.25%). Also the present study clearly demonstrated that single drop of telmisartan (0.5%) able to reduce mean IOP by (2.8%) in hypertensive rabbits eyes after one hour of instillation and peak mean IOP decline achieved after 7 days of instillation was (11.83%). Telmisartan (1%) clearly reduced mean IOP by (12%) in hypertensive rabbits eyes after one day of instillation and peak mean IOP decline achieved after 7 days of instillation (20.33%).*

Keywords: Telmisartan, Timolol and intra-ocular pressure

1. Introduction

Glaucoma is a disease that damages eye's optic nerve. It usually happens when fluid builds up in the front part of the eye. That extra fluid increases the pressure in the eye, damaging the optic nerve¹. Glaucoma is an irreversible neurodegenerative disease of the visual system and is the second leading cause of blindness in the world, Glaucoma may be congenital (developmental) or acquired². It is a family of related diseases frequently associated with elevated IOP which exceeds 21 mmHg and may be as high as 70 or 80 mmHg during the attack³, if untreated, leading to optic nerve damage and loss of vision. Glaucoma is an extensive clinical and healthcare problem responsible for blindness, it accounts for 15% of all global blindness, the World Health Organization has estimated that globally there are 12.5 million people blind from glaucoma with the total number affected by this condition at around 66 million⁴. Currently, the only clinically proven glaucoma intervention aims to lower intraocular pressure (IOP) in afflicted individuals. However, glaucoma may continue to progress in some patients even after lowering IOP to normal levels, which indicates that other key factors may be contributing to the disease⁵. Reducing (IOP) can slow the progression of disease in patients with glaucoma and normalizing (IOP) in patients with ocular hypertension can delay or even prevent the development of open-angle glaucoma⁶. It is characterized by the ongoing deterioration of the retinal ganglion layer and worsening of visual field defects, accompanied changes in the optic nerve head. High intraocular pressure (IOP) has long been considered the most important risk factor for the onset and progression of glaucoma, and therefore pharmacological and surgical

treatments have focused on lowering the IOP. However, even with treatment to lower IOP and even in normal tension glaucoma optic nerve damage may progress⁷. Telmisartan block the AT₁ receptors by binding reversibly and selectively to the receptors, decreasing the activation of AT₁ receptors by angiotensin II. Its pharmacologic effects are similar to those of ACE inhibitors in that they produce arteriolar and venous dilation. Telmisartan is an angiotensin II receptor blocker that shows high affinity for the angiotensin II receptor type 1 (AT₁), with a binding affinity 3000 times greater for AT₁ than AT₂. In addition telmisartan acts as a selective modulator of peroxisome proliferator-activated receptor gamma (PPAR-γ), a central regulator of insulin and glucose metabolism⁸. Recently, existence of angiotensin receptors has been demonstrated in the eyes of arterial hypertensive rats⁹. Angiotensin II has been implicated in the pathogenesis of glaucoma by causing vasoconstriction and vascular remodeling¹⁰.

2. Methods

Group of (40) adult male of New Zealand rabbits weighing 1.5 to 2 kg with no signs of ocular inflammation or gross abnormality were used in this study. Isotonic buffer group (8 normotensive rabbits), this group was instilled with isotonic buffer solution in the right eye and DW in the left eye to show if there is any effect of the vehicle (isotonic solution) on the eye. Timolol group (8 rabbits) both eyes of this group have been induced for ocular hypertensive, the right eyes instilled with timolol 0.5% drop (1 drop) twice daily which considered as a positive control group, While the left eyes instilled with DW which considered as a negative control

group. Telmisartan group (24 rabbits), divided into (3) subgroups.

(8 rabbits): instilled with telmisartan 0.25% drop.

(8 rabbits): instilled with telmisartan 0.5% drop.

(8 rabbits): instilled with telmisartan 1% drop.

The right eyes of these groups were induced for ocular hypertensive and instilled with (1 drop) of telmisartan drop once daily for 7 days. These concentrations were chosen after doing a pilot study on 6 animals using different concentrations of the tested drugs and the used concentrations were chosen depend on the effect and adverse effect. Animals were housed individually in a plastic cage; all rabbits were maintained conventionally during the study with regulated air temperature (15-21 °C), an artificial light cycle (12 hours light /12 hours darkness) and good ventilation. They fed a standard rabbit diet and had free access to drinking water¹¹.

2.1 Induction of Ocular Hypertension in Rabbits

Ocular hypertension induced according to Melana¹² and co-workers who found that this model of induction is mimic human chronic open angle glaucoma. After proper anesthetization of eyes by local instillation of 2% lidocaine HCL, a subconjunctival injection (by using a micro-fine syringes, 30 gauge × 1/2 inches) of 0.7 ml of betamethasone suspension containing betamethasone sodium phosphate (3 mg/ml) and betamethasone acetate (3 mg/ml). This formulation provides a readily accessible (sodium phosphate) and a sustained release (acetate) fraction of betamethasone. The value observed at zero time (first betamethasone injection) was considered the starting pressure. The animals received weekly (for 4 weeks) subconjunctival injections of betamethasone in both eyes over a period of 21 days. The instillation of the tested drugs was started at the 24th day of corticosteroid treatment (3 days after the fourth subconjunctival injection), a time at which the betamethasone-induced ocular hypertension turned out to be stable, and was prolonged up to 25 days.

2.2 Preparation of telmisartan ophthalmic solution

Ophthalmic drops are sterile aqueous or oily solutions, suspensions, or emulsions intended for instillation into the conjunctival sac. Ophthalmic drops should be clear and practically free from particles when examined under suitable conditions of visibility. Ophthalmic solutions are isotonic, sterile, free from foreign particles, and specially prepared for instillation in the eye^{13, 14}.

2.3 IOP measurement

After local anesthetization of the cornea with 1-2 drops of 2% lidocaine HCL ophthalmic solution, the animal was held on its back and Schiotz tonometer is placed on the cornea. A control or zero time value of IOP was taken 15 minutes (min) before the administration of tested drug. One drop of freshly prepared tested drug was instilled in the middle of inferior conjunctival sac followed by lid closure. Thereafter, IOP was measured after (1 hour) of topical application. Telmisartan instilled as one drop (50 µl) for 7 days once daily and IOP measured daily at about the same time to

avoid diurnal IOP fluctuation. After each measurement the instrument was properly cleaned with diethyl ether. Right eyes of the rabbits were used for evaluation of the tested drugs. To suppress growth of bacteria and other microorganism being introduced by the device or during drug administration, ophthalmic eye drop preparation containing a suitable antibacterial agent (chloramphenicol) were instilled in the eye rabbits at the end of each experiment¹⁵.

2.3.1 Pupil diameter

The measuring of pupil diameter was done by using the pupil gauge. The obtained results represented in millimeter unit¹⁶.

2.3.2 Light reflex

The light reflex or pupillary response of both eyes was tested by swinging flashlight to detect a relative afferent pupillary defect. The obtained results would be presented as either it was intact or absent¹⁷.

2.3.3 Corneal reflex

It could be tested for both eyes by using a wisp of cotton wool it applied from the side and away of its approach. The obtained results would be presented as either it was intact or absent¹⁶.

2.3.4 Conjunctival redness

It could be detected by inspection of conjunctiva of both eyes. The obtained results would be presented as either it was present or not¹⁸.

2.3.5 Lacrimation

It could be detected by inspection of conjunctiva of both eyes. The obtained results would be presented as either it was present or not¹⁸.

2.3.6 Statistical design and analysis

The results were presented by means of means ± standard error of mean (SEM). One way analysis of variance (ANOVA) followed by Tukey test comparison *t*-test (2-tailed) was utilized to compare between groups. The differences between the means are studied as significant at the 0.05 confidence level. The concentration that decreases 50% of the IOP this value was analyzed by linear regression equation and logarithmic equation. The statistical analysis was done by using Windows SSPS 16.0 (SPSS Inc. Chicago, IL), the level of significance was set at $P < 0.05$ as significant¹⁹.

3. Results

Effect of Isotonic buffer solution and DW on normotensive eyes

Response of mean IOP

The effect of isotonic phosphate buffer (vehicle) used for preparation of ophthalmic solution of the tested drugs on mean IOP of rabbits right eyes did not reach the level of statistical significance ($P \leq 0.05$) during the time course of the experiment (7 days).

Effect of Distilled Water

Response of mean IOP

Effect of DW on mean IOP of rabbits left eyes nearly remained constant during the time course of experiment ($P = 0.949$).

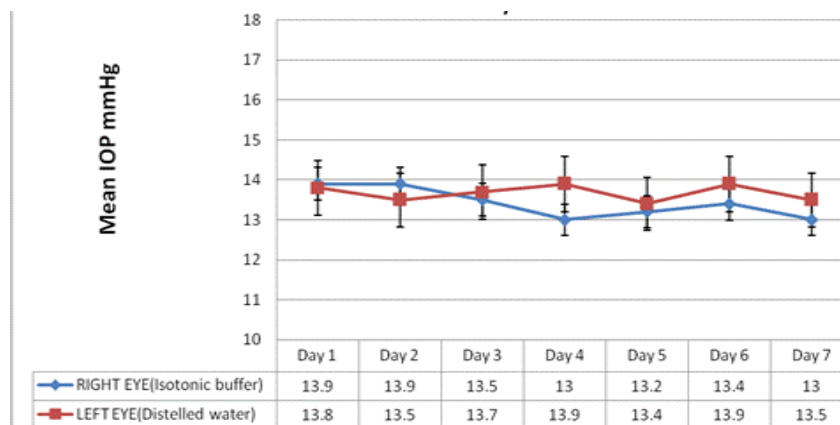


Figure 1: Effect of Isotonic Buffer & Distilled water groups regarding the response of mean IOP in ocular normotensive rabbits

Isotonic buffer solution and DW application in the present study had no effect on pupil diameter and no effect regarding other possible side effect (i.e. light reflex, corneal reflex, conjunctival redness and lacrimation).

Effect of Timolol (0.5%) Drop

Response of mean IOP

At post induction of ocular hypertension, the mean IOP was (23.9 ± 0.85 mmHg). After one hour of drug application the

mean IOP decreased by (0.9 mmHg) with no significant effect, and after four days of drug use the mean IOP reduced by (3.7 mmHg) which was highly significant, while the maximum reduction occurred after seven days of (timolol 0.5%) drop instillation (twice daily), the reduction in mean IOP was (7.1 ± 0 mmHg) which was found to be highly significant ($P=0.01$) comparing with DW.

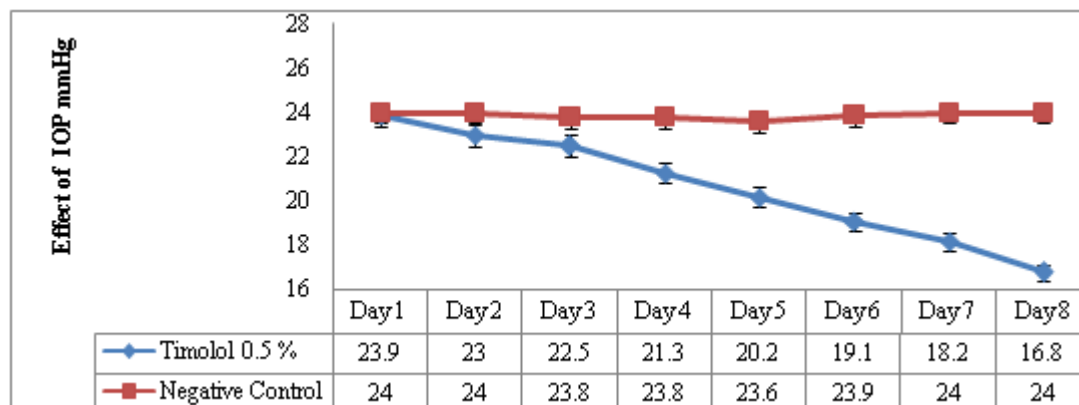


Figure 2: Effect of Timolol(0.5%) and DW on mean IOP of ocular hypertensive rabbits

Timolol drop application in the present study had no effect on pupil diameter and no effect regarding other possible side effect (i.e. light reflex, corneal reflex, conjunctival redness and lacrimation).

Effect of Telmisartan (0.25%, 0.5%, 1%) Ophthalmic drop

Response of mean IOP

The mean IOP of telmisartan (0.25%): Post induction of ocular hypertension, the mean IOP was (24 ± 0.19 mmHg).

After one hour of drug instillation the mean IOP non-significantly decreased by (0.2 mmHg), and after four days of drug used the mean IOP significantly decreased by (1.2 mmHg). The maximum reduction of mean IOP occurred after seven days of telmisartan (0.25%) drop instillation (once daily), the reduction in mean IOP was (1.5 ± 0 mmHg) which was found to be significant ($P<0.05$) comparing with DW, and there is highly significant difference when compared with timolol (0.5%) ($p=0.01$).

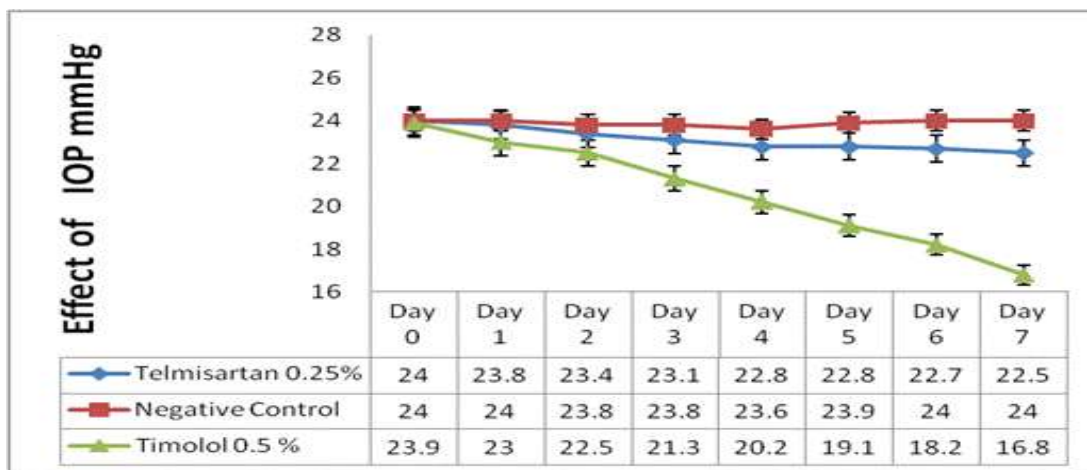


Figure 3: Effect of Telmisartan (0.25%),Timolol(0.5%)and DW on mean IOP of ocular hypertensive rabbits N=8

The mean IOP of telmisartan (0.5%): Post induction of ocular hypertension, the mean IOP was (24.5 ± 0.316 mmHg). After one hour of drug instillation the mean IOP decreased by (0.7 mmHg), after four days the mean IOP decreased by (1.7mmHg).The maximum reduction reached after seven days of telmisartan (0.5%) drop instillation (once

daily), the reduction in mean IOP was (2.3 ± 0 mmHg) which was found to be significant ($P < 0.05$) comparing with DW, and there is significant difference when compared with timolol (0.5%)($p=0.036$).

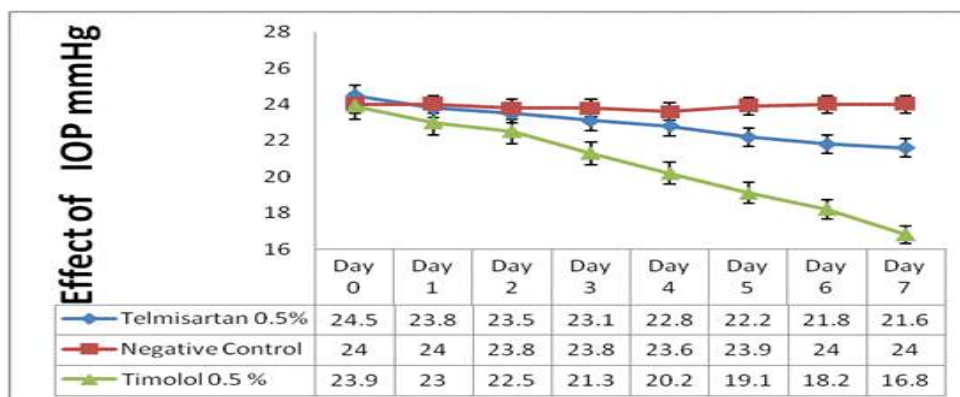


Figure 4: Effect of Telmisartan (0.5%), Timolol(0.5%)and DW on mean IOP of ocular hypertensive rabbits N=8

The mean IOP of telmisartan (1%): Post induction of ocular hypertension, the mean IOP was (24.1 ± 0.60 mmHg). After one hour of drug instillation the mean IOP decreased significantly by (1.9 mmHg), after four days of drug application the mean IOP reduced significantly by (3.9 mmHg). The maximum reduction of IOP occurred after

seven days of telmisartan (1%) drop instillation (once daily), the reduction in mean IOP was (4.9 ± 0 mmHg) which was found to be highly significant ($P = 0.008$) comparing with DW, and there is non-significant difference when compared with timolol (0.5%)($p=0.995$).

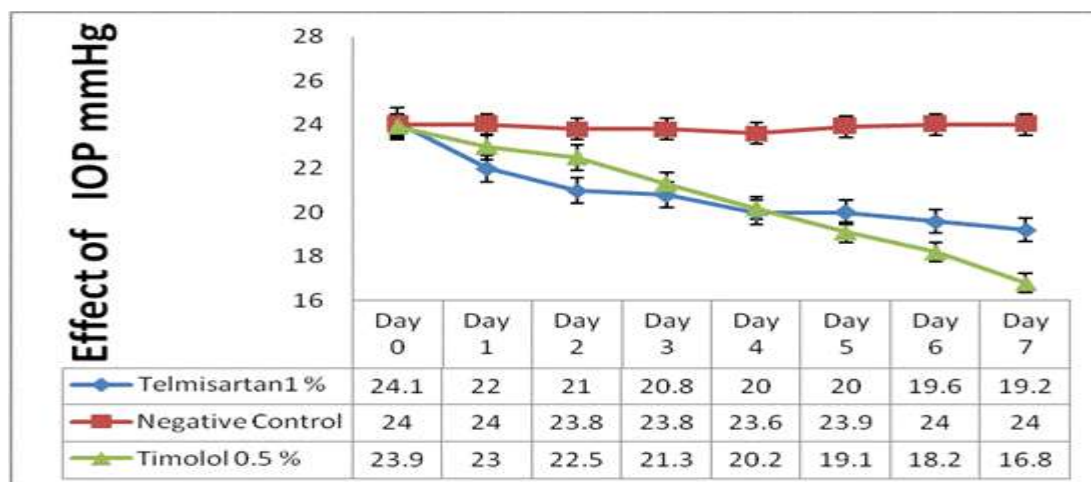


Figure 5: Effect of Telmisartan (1%),Timolol(0.5%)and DW on mean IOP of ocular hypertensive rabbits N=8.

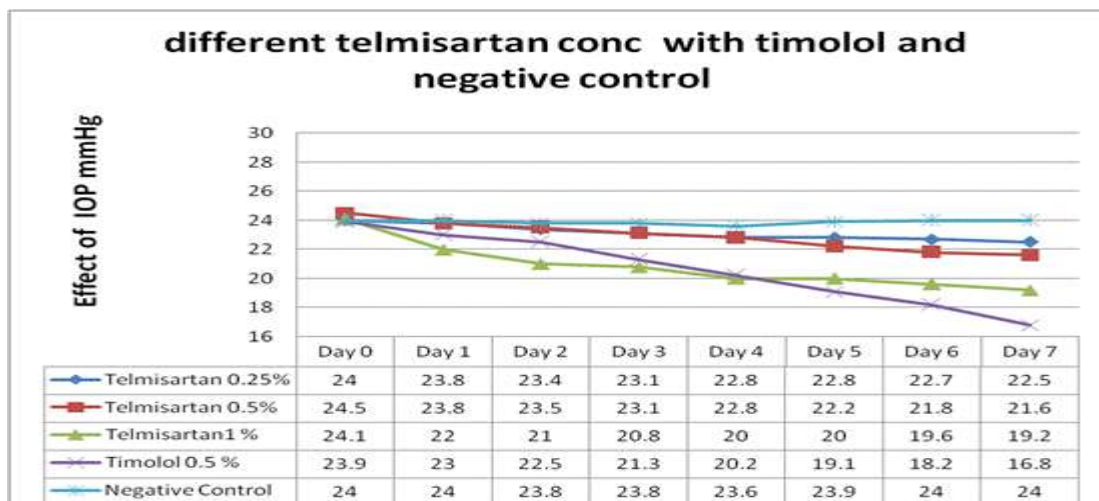


Figure 6: Comparing the effect of different concentrations of telmisartan and DW with timolol(0.5%) on ocular hypertensive rabbits n=8

4. Discussion

In the present study, inactive ingredients were used in the formulation for preparing eye drops of (telmisartan) and these inactive ingredients included benzalkonium chloride, sodium chloride, ethanol, phosphate buffer. Furthermore, these inactive ingredients could not change the mean of IOP in normotensive eyes of the rabbits after 7 days of inactive ingredients instillation, and there was no significant effect when compared mean of IOP during trial period with pre-treatment mean of IOP. Also, there was no significant effect when compared between distilled water and inactive ingredients on mean IOP of the rabbit eyes during trial period. In present study, the distilled water could not counteract the effectiveness of betamethasone as an inducing agent for ocular hypertension. Furthermore, distilled water had no effect on the tested parameters in this study thus; it could be accepted as a negative control group regards study of effect of tested drugs. This effect confirmed by²⁰. Timolol eye drop was used as positive control to test the ocular hypotensive effect of most experimented drugs and its preferred in chronic open glaucoma²¹. In present study, timolol had a noticeable ocular hypotensive effect on induced hypertensive eyes. Timolol (0.5%) had shown highly significant ($P < 0.01$) reduction in mean IOP of hypertensive eyes after one day of its instillation, furthermore, such effect showed highly significant differences ($P < 0.01$) comparing with distilled water group. The therapeutic role of timolol (i.e. after chronic induced ocular hypertension) was detected in dose (0.5%) when applied for 7 days, which is highly significantly ($P < 0.01$) reduced the mean IOP along the trial period. Therapeutically, timolol (0.5%) at 7th day post treatment of chronic induced ocular hypertension caused noticeable reduction in the mean IOP which was comparable to the normal IOP and there was no significant difference ($P > 0.05$). Timolol (0.5%) produced (29.71%) reduction in mean IOP at day 7, these results are strengthened by²² reported that timolol (0.5%) produced (20%) to (35%) reduction in mean IOP in ocular hypertensive eyes. Timolol drop application in the present study had no effect on pupil diameter and no effect regarding other possible side effect (i.e. light reflex, corneal reflex, conjunctival redness and lacrimation). The present

study clearly demonstrated that single drop of telmisartan (0.25%) able to reduce mean IOP by (2.5%) in hypertensive rabbits eyes after one day of instillation. Peak mean IOP decline achieved after 7 days of instillation (6.25%). There is an obvious hypotensive effect of telmisartan (0.25%) when compared with that of DW group. There is a comparable hypotensive when compared telmisartan(0.25%) to that of timolol group along all periods of experiment in hypertensive eyes. Also the present study clearly demonstrated that single drop of telmisartan (0.5%) able to reduce mean IOP by (2.8%) in hypertensive rabbits eyes after one hour of instillation. Peak mean IOP decline achieved after 7 days of instillation (11.83%). Telmisartan (1%) clearly reduced mean IOP by (12%) in hypertensive rabbits eyes after one day of instillation. Peak mean IOP decline achieved after 7 days of instillation (20.33%). Telmisartan drop application in the present study had no effect on pupil diameter and no effect regarding other possible side effect (i.e. light reflex, corneal reflex, conjunctival redness and lacrimation).

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