Research Article

Effect of Bosentan on the Intraocular Pressure of Normal and Ocular-induced Hypertensive Rabbits

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Received: 18-09-2017; **Revised:** 22-10-2017; **Accepted:** 05-11-2017.

ABSTRACT

Glaucoma is a multifactorial disease characterized by progressive losing of retinal ganglionic neurons that is if not treated can progress to blindness. Controlling of intraocular pressure is an essential approved medical therapy to decrease glaucoma progression. The aim of this study was to investigate the effect of topically applied (bosentan 0.05% eye drop) on the intraocular pressure (IOP) in normal and ocular-induced hypertensive of adult male rabbits. Also, to explore the possible side effects of the tested agent on eyes after instillation. For this purpose, 48 adult male rabbits were randomly divided in to 6 groups, each with 8 rabbits. For ocular-normotensive, three groups were involved where the right eyes, for seven consecutive days, received inactive ingredients, latanoprost 0.005% as positive control and bosentan 0.05% eye drops respectively. The other three groups experimentally had an induced-elevated IOP. Induction was done by injection 0.4 ml of 2% hydroxypropyl methylcellulose (HPMC) in the anterior chamber of right eyes. Distilled water, latanoprost 0.005% and bosentan 0.05% eye drops were applied respectively to the right eyes for seven days. The left eyes of all groups received distilled water. IOP was measured for both eyes with the aid of schiötz tonometer. Conjunctival redness Pupil diameter, light pupillary reflex, and corneal sensation reflex parameters were also studied. It was found that for the ocular-normotensive groups, the agents were not significantly changed the IOP nor the other studied parameters. In the induced-ocular hypertensive groups, both latanoprost, and bosentan were significantly reduced the induced elevated IOP from the second day of application till the end of experiment. Respectively, these reductions were 18.1 %, 9.95 %, on day 2 and 32.9 %, 19.32 % on day 7. Conjunctival redness was presented with both Latanoprost (12.5%) and bosentan (7%). The other parameters were not significantly changed. It was concluded that local application of bosentan 0.05% eye drop was effective in lowering IOP of HPMC induced-ocular hypertension in rabbits.

Keywords: Bosentan, Intraocular pressure, Glaucoma, Latanoprost.

INTRODUCTION

Glaucoma is a diseases characterized by progressive degeneration of retinal ganglionic cells (RGCs) that lead to cupping of optic disc and loss of peripheral vision. If it not diagnosed and treated, it can progress to blindness. Primary open angle glaucoma is the most common form of glaucoma, which is associated with a relative obstruction to aqueous humor outflow through the trabecular meshwork[1]. According to The World Health Organization, glaucoma accounted for 2 percent of visual impairment and 8 percent of global blindness in 2010 [2]. Elevation of intraocular pressure (IOP), associated with approximately two third of primary open glaucoma cases [3], is an approved modifiable risk factor of glaucoma. Increase of IOP results from disruption of equilibrium of aqueous humor production from the cilliary body, its drainage, or both. Aqueous humor returns to the venous system by two pathways. Conventional or canalicular pathway is the major one where aqueous humor passes through the trabecular meshwork, across the inner wall of Schlemm's canal. The minor one is the uveoscleral pathway where the fluid leaves through the cilliary muscle [4]. Several pharmacological groups are approved to control IOP by reduction of aqueous humor production from the ciliary body and/or increasing the outflow of the fluid through the uveoscleral or the conventional pathway. These groups are prostaglandin analogs, β-adrenoreceptor blockers, carbonic anhydrase inhibitors, adrenergic agonists and cholinergic agonists. Prostaglandin analogs and B-blockers are the current chronic first line therapy of glaucoma. The first group reduces IOP by increasing the aqueous humor outflow mainly through the uveoscleral pathway. While the second group reduces the IOP by decreasing the aqueous humor formation[5]. So entering of new drugs that reduce IOP by increasing trabecular outflow facility and can be used chronically will provide a high benefit to glaucomatous patients as the combination of these proposed drugs with the other approved anti-glaucomatous drugs that affecting aqueous humor formation or uveoscleral outflow could provide a new options of strong IOP reduction since that different mechanisms of action will be involved.

Endothelins are, 21-amino-acid peptides, produced by various types of cells throughout the body [6]. The effect of ET-1 is mediated by two G-protein-coupled receptors, endothelin receptor A (ET_A receptor) and endothelin receptor B (ET_B receptor). The signal transduction mechanism triggered by binding of ET-1 to its vascular receptors (ET_A) includes stimulation of phospholipase C,

formation of inositol triphosphate, and release of calcium from the endoplasmic reticulum, which results in vasoconstriction. Conversely, stimulation of prostaglandin I2 (PGI2) and nitric oxide (NO) synthesis (ET_B mediated) results in decreased intracellular calcium concentration and vasodilation[7]. Bosentan is an endothelin A (ET_A) and endothelin B (ET_B) receptors blocker that has been approved for treatment of arterial pulmonary hypertension[8]. A cumulative data indicates the importance role of endothelin receptor blockers in treatment of glaucoma by reduction of IOP, enhancing of ocular blood flow, and preventing or delaying RGCs loss. The first two effects are probably mediated through antagonism of ET_A receptor while the last one is probably associated with ET_B inhibition[9-10]. Using these data, bosentan will be a good candidate to be studied in animal models of glaucoma.

The aims of this study was to investigate the effect of topically applied dull endothelin receptor blocker (bosentan 0.05%) on the intraocular pressure in ocular-normotensive and induced-ocular hypertensive of adult male rabbits, and to explore the possible side effects of the tested agent on eyes after instillation.

MATERIALS AND METHODS

Materials

The tested agent, bosentan powder was provided by Cayman Chemical Company, USA. Supporting materials used in the formulation of bosentan ophthalmic solution: NaH_2PO_4 (Riedel – De Haen Ag seelze –Hannover, Germany), Na_2HPO_4 (Fluka–Garantie, Switzerland), Benzalkonium chloride solution 50% (Himedia, India), and Ethanol (Merck, Germany). Hydroxypropyl methylcellulose ophthalmic solution (2%) used for the induction was from Appasamy ocular devices, India. Drugs of anesthesia were Ketamine hydrochloride 50 mg/ml vial (Fresenius kabi, South Africa) and Diazepam ampule 10mg/2ml (Roche, Madrid). Eye drops used in this study were Latanoprost 0.005% (Pfizer, Belgium), Chloramphenicol 0.3% (Amman pharmaceutical industeries Co., Jordan) and Proparacaine HCl 0.5% (Alcon, Belgium).

Animals

Forty-eight adult male rabbits (Oryctologus cuniculus) with body weight of (1.5-2 kg) were involved in the study. The experiments were approved by Animal Ethical Committee, College of Medicine/ Al-Nahrain University, Baghdad, Iraq. They were determined to be normal on ophthalmic and general examinations. Animals had free access to fresh diet and water. They were kept under standard laboratory conditions and a light cycle of 12 hours light and 12 hours dark.

Preparation of bosentan 0.05% eye drop

The ophthalmic solution bosentan 0.05% was prepared by dissolving 5 mg of bosentan powder in 7 ml of pH 7.4 phosphate buffer solution (a buffer prepared from NaH_2PO_4 and Na_2HPO_4) and mixed well, then benzalkonium chloride solution (to concentration of 0.01% of the final volume) and ethanol (to concentration of 1% of the final volume) were added while mixing. Final volume was completed to 10 ml with phosphate buffer solution to get 0.05% (w/v) bosentan solution. Preparation done in aseptic condition and the final solution was filled in a sterile container [11].

Parameters

The intraocular pressure was measured for both eyes with the aid of schiötz tonometer (Riester, Germany) according to the instructions of manufacturer. The other examined parameters were pupil diameter, light pupillary reflex, corneal sensation reflex, and conjunctival redness. These parameters were recorded before starting the experiment and then daily before and after drug application.

Experimental animal model

The induction procedure performed under sterile condition. Anesthetization of the animals done by ketamine (50 mg/kg im) plus diazepam (2 mg/kg im)[12]. The right eye was prepared by instillation of 4% povidine iodine (AREEJ, UAE) and then washed with distilled water. Proparacaine 0.5% eye drops were instilled and then (27G X 13 mm) needle on microsyringe was inserted into the anterior chamber of the right eye at 2 o´clock on the limbus. Aqueous humor was withdrawn then without moving the needle, an equal volume of 2% (w/v) hydroxypropyl methylcellulose (HPMC) was injected into the anterior chamber with anther microsyringe. The needle was then removed without significant loss of aqueous humor. HPMC has viscoelastic properties, it is not toxic or inflammatory, and it acts by decreasing the outflow facility [13]. In this study, the injected volume of HPMC was 0.4ml. Antibiotic prophylaxis with chloramphenicol eye drop was administered 2 times daily one day before the induction and on the day of induction and ongoing later.

Study design in normotensive rabbits

In this part three groups were involved, each group consists of eight rabbits (24 rabbits). All animals had been examined for the studied parameters on the day before application of tested agent. On the seven next days, the experiment had been done by instillation of the tested agents into the right eye and distilled water instilled into the left eye twice daily at 10:00 a.m. and at 10:00 p.m. except for latanoprost, which was administered once daily. The tested parameters were recorded 30 minutes before instillation and 30, 60 minutes after instillation. The parameters were also measured in the left eyes to detect contralateral effects of the tested agent.

The studied groups were negative control group (right eye received inactive ingredients while left eye received distilled water, 8 rabbits), Latanoprost 0.005% (Positive

control group, 8 rabbits), Bosentan 0.05% group, 8 rabbits.

Study design in induced ocular hypertensive rabbits

This part also included three groups, each group of eight rabbits (24 rabbits). All animals had been examined for the tested parameters one day before induction. Forty-eight hours after induction, IOP was measured again to ensure that ocular hypertension was definitely established. Then treatment began by instillation of 1 to 2 drops of the tested agents into the right eye and distilled water instilled in the left eye twice daily at 10:00 a.m. and at 10:00 p.m. for seven days except for latanoprost, which was administered once daily. The tested parameters were recorded 30 minutes before instillation and 30, 60 minutes after instillation. The parameters were also measured in the left eyes to detect contralateral effects of the tested agent.

The three studied groups were distilled water (negative control group, 8 rabbits), Latanoprost 0.005% (Positive control group, 8 rabbits), Bosentan 0.05% group, 8 rabbits.

Statistical methods

Statistics had been done using SPSS program (version 19). The obtained quantitative data were presented as (mean ± S.E.M.) (Standard error of mean).

Student paired t-test was used for assessing the effectiveness of employed therapy for the right eyes of rabbits in a given group. While student (unpaired) t-test for independent data was used to test the significance of difference between the results of right and left eyes of rabbits in a given group or between the results of the right eyes of rabbits of any two groups.

The differences were accepted as significant if the probability was equal or below 0.05 and highly significant if the probability was equal or below than 0.01 [14].

RESULTS

Distilled water group

For the ocular-normotensive group, the IOP means prior the instillation of inactive ingredients to the right eyes and distilled water (D.W) to the left eyes were (20.6 ± 0.28 mmHg) and (20.5 ± 0.48 mmHg) respectively. The response of the IOP means on the following days of instillation were not significantly changed (P> 0.05), in compare with the day before instillation for the right and left eyes. Besides that, there was no significant difference between the IOP means of the right and left eyes on the individual days (P> 0.05). The other parameters (pupil diameter, light reflex, corneal reflex, and conjunctival redness) were not significantly changed under the effect of inactive ingredients and D.W. (P> 0.05).

For the ocular-hypertensive induced groups. Application of D.W to the right eyes was not significantly changed the moderately elevated IOP on the course of experiment (P> 0.01) (figure 1). Instillation of D.W was not significantly changed the pupil diameter of the right and left eyes on the course of experiment (P> 0.05). Nevertheless, the induction technique significantly changed (reduced) the pupil diameter where it was (8.5 ± 0.18 mm) before the induction and became (7.1 ± 0.35 mm) after the induction (P ≤ 0.05). The other parameters were not significantly changed under the effect of D.W. (P> 0.05).

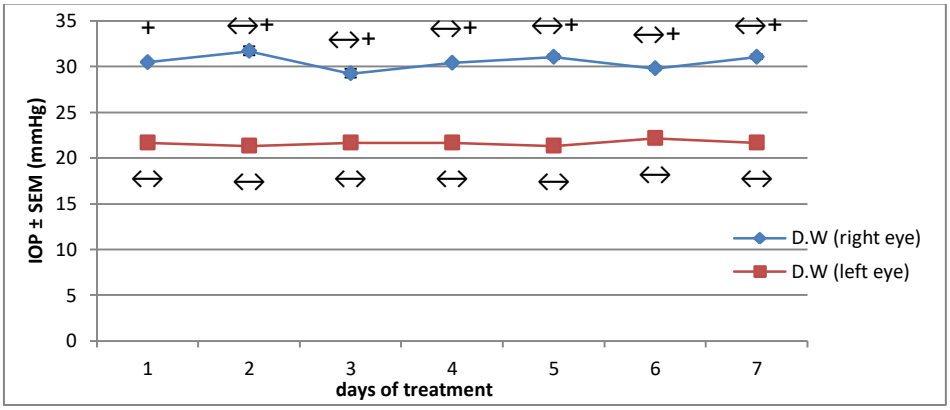

Figure 1: Effect of distilled water on IOP mean values of rabbit's right and left eyes in the ocular hypertensive eyes group. ↔ No significance difference compared to the value (21.7 ± 0.15 mmHg) before starting of treatment for the left eye, and day 1, in case of the right eye (P>0.01). + significant differences between the IOP means of the right and left eyes (P ≤ 0.01).

Latanoprost (0.005%) group

In case of the ocular-normotensive group, the IOP means before application of latanoprost 0.005% to the right eyes and D.W to the left eyes were (19.8 ± 0.52 mmHg) and (19.2 ± .85 mmHg) respectively. Those IOP means were not significantly changed on the following days of instillation (P> 0.05) compared to the day before application to the right and left eyes. Furthermore, there was no significant difference between the IOP means of the right and left eyes on the individual days (P> 0.05). The other parameters were not significantly changed under the effect of latanoprost and D.W. applied to the right and left eyes respectively (P> 0.05).

Regarding the ocular-hypertensive induced group. Application of latanoprost 0.005% to the right eyes was significantly changed (reduced) the moderately elevated IOP on the course of experiment (P ≤ 0.01) (figure 2). Instillation of latanoprost 0.005% was not significantly changed the pupil diameter of the right eyes on the course of experiment (P> 0.05). However, the induction technique significantly changed (reduced) the pupil diameter where it was (8.9 ± 0.22 mm) before the induction and became (7.8 ± 0.29 mm) after the induction (P ≤ 0.05). latanoprost caused significant conjunctival redness (P ≤ 0.01) which constituted approximately 12.5 % per day of application. The other parameters were not significantly changed under the effect of latanoprost (P> 0.05).

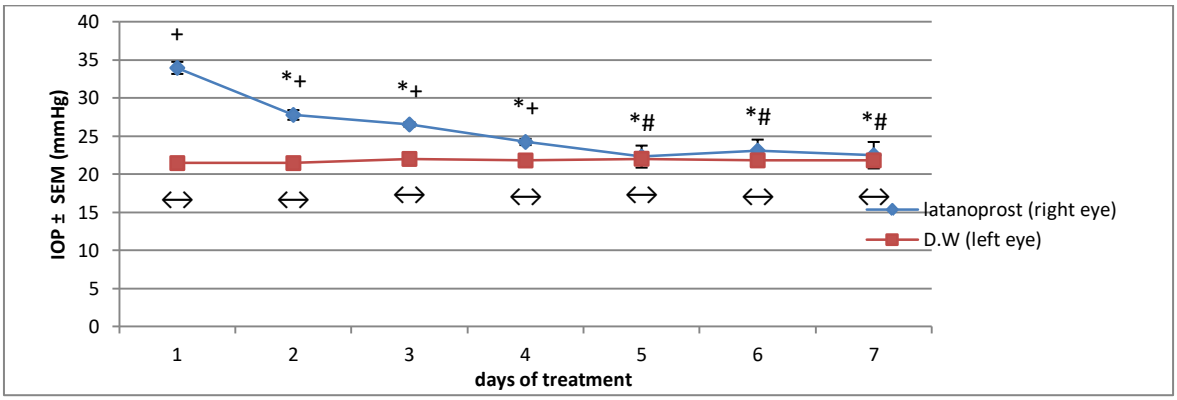

Figure 2: Effect of the latanoprost 0.005% eye drop on IOP mean values of rabbit's right eyes in induced hypertensive eyes group. ↔ No significance difference compared to the value (20.8 ± 0.33 mmHg) before starting of treatment (P>0.01) of the left eyes. + significant differences between the right and left eyes (P≤ 0.01). # No significant differences between the right and left eyes (P > 0.05). * Significantly differ (decreased) compared to day 1 (P≤ 0.01) of the right eyes.

Bosentan (0.05%) group

For the ocular-normotensive group, the IOP means before instillation bosentan 0.05% to the right eyes and D.W to the left eyes were (20.5 ± 0.54 mmHg) and (20.6 ± 0.57 mmHg) respectively. The response of the IOP means on the following days of instillation were not significantly changed (P> 0.05), in compare with the day before instillation for the right and left eyes. Besides that, there was no significant differences between the IOP means of the right and left eyes on the individual days (P> 0.05). The other parameters were not significantly changed under the effect of bosentan 0.05% and D.W. applied to the right and left eyes respectively (P> 0.05).

The results of the induced-ocular hypertensive group showed that bosentan 0.05% significantly changed (reduced) the moderately elevated IOP on the course of experiment (P ≤ 0.01) (figure 3). Instillation of bosentan 0.05% was not significantly changed the pupil diameter of the right eyes on the course of experiment (P> 0.05). However, the induction technique significantly changed (reduced) the pupil diameter where it was (8.6 ± 0.18 mm) before the induction and became (7.3 ± 0.25 mm) after the induction (P ≤ 0.05). Bosentan caused significant conjunctival redness (P ≤ 0.05) which constituted approximately 7 % per day of application. The other parameters were not significantly changed under the effect of bosentan 0.05% (P> 0.05).

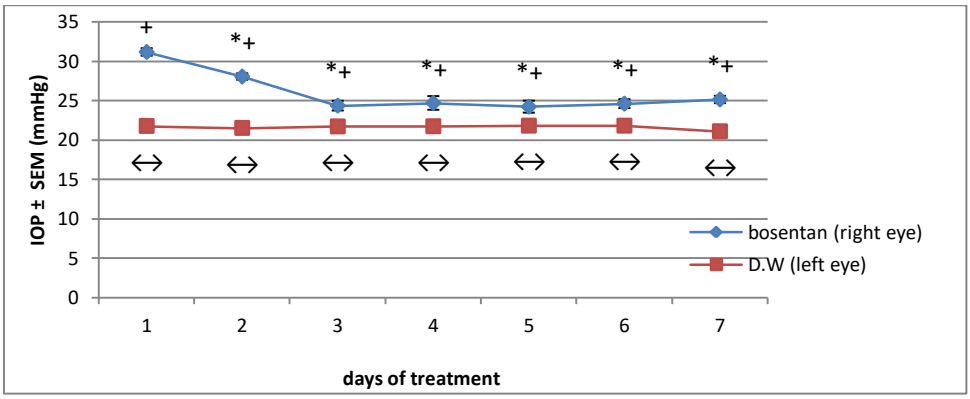

Figure 3: Effect of bosentan 0.05% eye drop on IOP mean values of rabbit's right eyes in induced hypertensive eyes group. ↔ No significance difference compared to the value (21.8 ± 0.11 mmHg) before starting of treatment (P>0.01) for the left eyes. + significant differences between the IOP means of the right and left eyes (P ≤ 0.01). * Significantly differ (decreased) compared to day 1 (P≤ 0.01) of the right eyes.

Percentages of IOP reduction by latanoprost and bosentan

The percentages of IOP reduction, compared with day 1, on the days of treatment produced by latanoprost 0.005% and bosentan 0.05% in the induced-elevated IOP right eyes groups are shown in (table 1).

Table 1: Percentages of means intraocular pressure reduction by latanoprost and bosentan in the induced-hypertensive right eye groups.

	Percentage of IOP mean reduction ± SEM (%)	
Days of treatment	**Latanoprost 0.005%**	**Bosentan 0.05%**
Day 2	18.1 ± 1.35	9.95 ± 1.43 *
Day 3	21.6 ± 1.82	21.82 ± 1.99 ↔
Day 4	28.53 ± 2.77	20.73 ± 2.82 *
Day 5	33.6 ± 4.97	22.27 ± 2.37 ↔
Day 6	31.22 ± 5.08	20.96 ± 2.18 ↔
Day 7	32.9 ± 5.83	19.32 ± 1.43 *

↔ No significant difference between percentages of IOP reduction caused by latanoprost and bosentan (P>0.05). * Significant difference between percentages of IOP reduction caused by latanoprost and bosentan. (P≤ 0.05)

The induction technique

In the present study, the injected volume of 2% HPMS was 0.4 ml. After 48 hours of the injection, there was a failure to get elevation of the IOP with a rate of 43% where the induction had been repeated on intact animals. The successful induction provided moderate elevation in the IOP (table2). This elevation was continued without significant change (P > 0.01) for the course of the experiment of the induced negative control group (figure 1). The induction technique was significantly change (reduced) (P ≤ 0.05) the pupil diameter when the results 48 hours post-induction compared to that of the pre-induction of the three studied induced groups.

Table 2: Results of the IOP of the right eyes before and 48 hours after the induction.

Groups	IOP mean ± SEM (mmHg)	
	Before induction	**48 hours after induction (day 1)**
D.W	21.6 ± 0.41	30.4 ± 0.1 ↑
Latanoprost 0.005%	21.8 ± 0.78	34 ± 0.79 ↑
Bosentan 0.05%	21.7 ± 0.12	31.2 ± 0.47 ↑

↑, significantly changed (increased) compared to that before induction (P≤0.01).

DISCUSSION

Effect of distilled water

In this study, distilled water has been used as a negative control to show the activity of the tested agents that were used for normotensive and induced-elevated hypertensive eyes groups in rabbits. The results of non-significant effect of D.W on the IOP in this study are compatible with the previous studies of distilled water effect on the IOP mean [15-16].

Effect of latanoprost 0.005% eye drop

Latanoprost had been used as positive control to study the effect of bosentan on the IOP of ocular normotensive and induced-elevated IOP in rabbits.

In the present study, there was no significant change in the IOP means (P > 0.05) occurred with application of latanoprost 0.005% drop to the normotensive eyes group compared with that of pre-application. In the previous studies of the effects on IOP of normotensive eyes rabbits, latanoprost was either caused no change in the IOP [17] or it reduced the IOP [18-19]. This difference in the observation could be related difference in the methodology as noncontact tonometer and Tonovet were used in those studies whereas schiötz tonometer was used in this study.

In the induced ocular hypertensive eyes group, latanoprost 0.005% produced significant differences (reductions) (P≤0.01) in the IOP means where these reductions constitute 18.1% of the based elevated IOP on day 2 and the reduction continuous to reach 32.9 % on day 7 (table 1). The maximum reduction was seen on day 5 where it was 33.6%. when the IOP means of the right eyes compared to that of the left, there was significant differences (P≤0.01) on the first 4 days of treatment while on the rest 3 days the IOP means of the right eyes reduced sufficiently under the effect of latanoprost to be significantly not differ from that of the left eyes (figure 2).

These results are agreed with animal and human studies, where latanoprost caused maximum reduction of 35.06 % in steroid induced model of glaucoma in rabbits [18]. In studies of human with glaucoma or ocular hypertension, IOP level reduced from 22 % to 39 % with the use of latanoprost 0.005% [20]. In this study, latanoprost had no significant effect on pupil diameter (P > 0.05). This result is similar to the effect of latanoprost on the pupil diameter in rabbits in the study of Sarchahi et al [19].

In the current study, latanoprost caused no conjunctival redness in the normotensive eyes group, but a significant redness (p≤ 0.01) had been reported in the hypertensive eyes group (12.5 %). The developed conjunctival redness could be related to localized vessel dilation associated with nitric oxide releasing under the effect of latanoprost[21].

Effects of bosentan 0.05% eye drop

In the present study, there was no significant change (P > 0.05) in the IOP means occurred with application of bosentan to the normotensive eyes group. These results are similar to that obtained with the normotensive negative and positive control groups in this study.

In the induced ocular hypertensive eyes group, bosentan 0.05% produced significant differences (reductions) (P≤0.01) in the IOP means where this reduction constitute 9.95 % of the based elevated IOP on day 2 and the reduction continuous to reach 19.32 % on day 7 (table 1). The maximum reduction was on day 5 where it was 22.27 %. When the IOP means of the right eyes compared to that of the left, there was significant differences (P≤0.01) on the days of treatment, such that the IOP means of the right eyes was not reduced sufficiently under the effect of bosentan to become significantly not differ from that of the left eyes (figure 3). The proposed mechanism of action behind IOP reduction of topical application of bosentan 0.05% drop was increased in the aqueous humor outflow through the conventional pathway by inhibition of ET_A receptors-mediated contraction of the trabecular meshwork tissues. This assumption is suggested, as ET_A receptor activation in the trabecular meshwork is associated with contraction of the meshwork that lead to increase IOP [22]. The results of this study are compatible with the effects of endothelin A receptors antagonism on the IOP where topical application of avosentan, ET_A receptor antagonist to glaucomatous monkeys eyes reduced IOP for up to 21% with the highest concentration used [23]. In human, topical application of endothelin antagonists are not studied but, a study of the effects of an oral administration bosentan on the ocular blood flow showed no effect on IOP neither in glaucoma patients nor in healthy subjects [24].

In this study, bosentan had no significant effect on pupil diameter (P > 0.05), the result similar to that of the negative and positive control groups.

In the current study, bosentan caused no conjunctival redness in the normotensive eyes group, but a significant redness (p≤ 0.05) had been reported in the hypertensive eyes group (7 %). This hyperemia could be resulted from dull inhibition of ET_A and ET_B receptors of the cojuctival blood vessels as the net result of combined ET_A and ET_B antagonism is vasodilatory in nature [25].

Related effects of latanoprost and bosentan on IOP in induced-ocular hypertensive groups

In the induced-ocular hypertensive groups, both latanoprost and bosentan reduced the IOP significantly (P≤0.01) on days of treatment (figure 2 and 3). However, the percentages of IOP reduction produced by bosentan was significantly differ (lower) than that of latanoprost on days 2, 4 and 7 but not on the rest days (table 1). Besides that, bosentan was not bringing the induced-elevated IOP of right eyes to that of the left like what had happened with latanoprost. However, both agents caused significant reduction (P≤0.01) on the second day of drug application without delay for the consequent days, besides that, both agents share the result of causing maximum IOP reduction on day 5 of the experiments. This could be explained by the work of Thieme et al. which cleared that prostaglandin agonists of FP receptor has an effect of counteracting endothelin 1-mediated contraction of an isolated bovine trabecular meshwork other than blocking of endothelin receptors and that effect is mediated through reduction of the endothelin-1 induced elevation of the intracellular calcium of cultured trabecular meshwork cells [26]. So the hypothesized sharing effect of FP prostaglandin agonists and endothelin A antagonists on the trabecular meshwork could explain the similar effect in this work regarding rapid onset of IOP reduction and the time at which maximum reduction in the IOP was seen.

CONCLUSION

It was concluded that ophthalmic solution of bosentan 0.05% and latanoprost 0.005% were significantly reduced IOP of hydroxypropyl methylcellulose induced-ocular hypertensive eyes of adult male rabbits during the course of twice daily for seven days application after the induction with latanoprost had a strong lowering effect. Conjunctival redness was recorded as a side effect with both bosentan and latanoprost, although it was less with bosentan. In addition, that neither bosentan nor latanoprost were affecting IOP of ocular-normotensive of adult male rabbits.

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Source of Support: Nil, **Conflict of Interest:** None.