Effect of Topically – Applied Carvedilol on Intraocular Pressure in Normal and Ocular Hypertensive Rabbits

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الخلاصة:
نبذة: في داء الزرقاء تقدم اعتلال العصب البصري تدريجيا دون أن يلاحظه المريض و الكشف المبكر والعلاج له أهمية قصوى في إيقافه أو السيطرة على تقدم الضرر.
هدف الدراسة: لاستكشاف آثار الكارفيديلول الموضعية على ضغط العين الداخلي (IOP)في كل من العيون ذات ضغط العين سوي وارتفاع ضغط العين في الأرانب.
المواد وطرق العمل :مجموعة 54 من ذكور الأرانب اعدت لهذه الدراسة. وقد تم استحداث ارتفاع ضغط العين عن طريق الحقن من هيدروكسي بروبيل ميثيل سيللوز في الحجرة الأمامية للعين اليمنى للأرانب. وقد تم تصميم هذه الدراسة لتقييم التأثير العلاجي الممكن (الجزء الأول) وكلا من الوقائية وكذلك التأثير علاجي (الجزء الثاني) من الدراسه، تم تقسيم الأرانب الى مجموعة الماء المقطر و مجموعات التيمولول (0.25٪ و 0.5٪) والكارفيديلول(0.25%و 0.5٪). وتم اعطاء كل من قطرات العين (بما في ذلك الماء المقطر) في العيون اليمنى 3 مرات / يوم لمدة 4 أيام وقائيا و لمدة 10 أيام علاجيا. وقد تم فحص الأرانب لقياس ضغط العين الداخلي، قطر البؤبؤ، منعكس الضوء، منعكس القرنية، واحمرار الملتحمة قبل تقطير الادوية وطوال فترة التجربة.
النتائج: كان التاثيرعلى ضغط العين من قبل قطرات العين الكارفيديلول (0.25 ٪) و (0.5٪) أكثر كفاءة من الماء المقطر .(P <0.01) وعلاوة على ذلك، تاثير قطرة العين الكارفيديلول(0.25٪) مماثل لقطرة العين التيمولول) 0.25٪ (P> 0.05) (في انخفاض ضغط العين طوال فترة التجربة.
قطرات الكارفيديلول تحتاج 4 أيام للتقطير في العيون ذات الضغط الطبيعي لتحقيق فرق معنوي عالي في التأثير الخافض للضغط (P <0.01) في حين أن هناك حاجة إلى 10 ايام للقيام بذلك في العيون ذات الضغط المرتفع ؛ التأثير الخافض لضغط العين كان أكثر كفاءة من الماء المقطر، ولكن أقل كفاءة من قطرة العين التيمولول.
في كلا جزئي هذه الدراسة والمتعلقة بكل من قطر البؤبؤ،المنعكس الضوئي،المنعكس القرني واحمرار ملحمة العين لم يلاحظ أي تأثير جانبي هام لقطرات العين الكارفيديلول(0.25 (٪ أو(0.5٪.(P> 0.05))
الاستنتاجات: قطرة العين الكارفيديلول (3مرات / يوم) كان له تأثير وقائي واضح ومفيد في ضغط العين السوي ،وكان آمن ممكن تحمله وايضا له تأثير معالج وخافض لضغط العين الداخلي مقابل هيدروكسي بروبيل –مثيل السيليلوز المسبب ارتفاع ضغط العين في الأرانب
الكلمات المفتاحية:الكارفيديلول ، ضغط العين الداخلي ، داء الزرقاء

Abstract:
Background: In glaucoma, as optic neuropathy gradually proceeds unnoticed by the patient, early detection and treatment is of paramount importance in arresting or controlling the progress of damage.
Aim of the study: To explore effects of topical carvedilol on intraocular pressure (IOP) in each of normotensive and ocular hypertensive eyes of rabbits, with assessment of drug safety.
Material and methods: A group of 54 males rabbits were included in this study. Induction of ocular hypertension was achieved by injection of hydroxy propyl methylcellulose in the anterior chamber of rabbits right eye. The present study was designed to evaluate the possible beneficial therapeutic effect (part I) and both prophylactic as well as therapeutic effect (part II). The included rabbits were divided into distilled water group, timolol (0.25% and 0.5%) groups, and carvedilol (0.25% and 0.5%). Each of drug eye drops (including distilled water) were instilled into right eyes 3 times/day for 4 days prophylactically and for 10 days therapeutically. The

rabbits had been examined for the IOP, pupil diameter, light reflex, corneal reflex, and conjunctival redness prior to instillation of drugs and along the trial period.
Results: Ocular hypotensive effects of carvedilol (0.25%) and (0.5%) eye drops were more efficient than that of distilled water (P<0.01). Furthermore, carvedilol (0.25%) eye drop simulated timolol (0.25%) eye drop (P>0.05) in its ocular hypotensive effect along the trial period .
Carvedilol eye drop required 4 days of instillation into normotensive eyes to achieve highly significant (P<0.01) ocular hypotensive effect whereas 10 days were needed to do so in ocular hypertensive eyes; such ocular hypotensive effect was more efficient than that of distilled water, but less efficient than that of timolol eye drop.
In both parts of the present study and regarding each of mean pupil diameter, light reflex, corneal reflex and conjunctival redness, carvedilol (0.25% or 0.5%) eye drops had no significant adverse effect (P > 0.05).
Conclusions: Carvedilol eye drops instilled 3 times / day had an obvious prophylactic role in normotensive and a beneficial, safe, and tolerable therapeutic ocular hypotensive effects on hydroxy propyl methyl cellulose - induced ocular hypertension in rabbits .
Keywords: Carvedilol, Intraocular Pressure, Glaucoma

Introduction

Primary glaucoma marked by an increase of intraocular pressure (IOP) which are of two main types: primary open angle glaucoma and primary angle closure glaucoma; when optic nerve damage has occurred despite a normal IOP, this is called normal tension glaucoma. Secondary glaucoma refers to any case in which another disease causes or contributes to increase eye pressure, resulting in optic nerve damage and vision loss [1].

In glaucoma, as damage gradually proceeds unnoticed by the patient, early detection and treatment is of paramount importance in arresting or controlling the progress of damage. In recent years, progress in the diagnosis and treatment of glaucoma has been remarkable, with numerous new diagnostic and therapeutic aids being introduced in the clinical setting, and the diagnosis and treatment of the disease has become multi-faceted [2].

Medical treatment of glaucoma includes topical β adrenergic antagonists(timolol, levobunolol, carteolol, metipranolol, and betaxolol) [3], carbonic anhydrase inhibitors (acetazolamide and methazolamide) [3], topical sympathomimetics (dipivefrin, apraclonidine, and brimonidine) [4], topical cholinergic agonists (pilocarpine, carbachol and ecothiophate iodide) [5], topical prostaglandin analogs (latanoprost, travoprost, bimatoprost and unprostone) [5], topical carbonic anhydrase inhibitors (dorzolamide and brinzolamid)[3], and osmotic agents (mannitol and glycerin) [6].

Carvedilol is a highly lipid soluble nonselective β-adrenergic and α_1-adrenergic antagonist that has no intrinsic sympathomimetic effect. It inhibits central sympathetic outflow and decreases presynaptic receptor neurotransmitter release. It produces negative inotropic and chronotropic effects and thus decreases myocardial oxygen consumption [7,8,9] .Carvedilol is used in treatment of hypertension, ischemic heart diseases, arrhythmias and congestive heart failure [5,6].

Materials and Methods

Materials:

The used materials in the present study are listed below with their sources accordingly:

Materials	Source
Benzalkonium chloride	SDI (supplier)
Carvedilol (Coreg) tablets (25 mg)	Emessa Labs /Homs – Syria.
Di –sodium hydrogen phosphate (Na2HPo4)	Fluka – Garantie–Switzerland
Distillator	Gesellschaft fur Labortech, Nikm. b.h. and Co., type 2016- Germany
Ethanol (70%)	Almashat-Baghdad
Hdroxypropyl methyl cellulose ophthalmic solution (2%) (United states pharmacopoeia)	Focus vision care
Ketamine hydrochloride(50 mg/ml)	HOLDEN MEDICALLelystad the Netherlands
Lidocaine hydrochloride (2%) Solution	Avenzor –Syria
pH. Meter	Friederg/Hessen-Germany
Phosphoric acid	Emscope,Laboratory Ltd .
Pupil gauge	Al-Zahrawi Private Hospital
Sartorius balance	Werke–GMBH, type 2842- Germany
Sodium chloride	Riedel – De Haen Ag seelze – Hannover
Sodium di hydrogen phosphate (NaH2Po4)	Riedel – De Haen Ag seelze – Hannover
Sodium hydroxide	Emscope,Laboratory Ltd .
Schiotz tonometer	Eichtabelle – Germany
Timolol	Pharmacia Co.- France

Animal and Housing:

A group of 54 adult male of New Zealand rabbits (Oryctologus cuniculus), aged near one year with body weight ranged 1.5-2 kg were included in the study. They were kept in Animal House at the Medical College –Al Nahrain University. Animals were kept on fresh trefoil diet, water ad libitum, suitable temperature and normal light.

Induction of ocular hypertension in rabbits

Rabbits had been injected with hydroxpropyl methylcellulose (0.4 ml) of (2% w/v) after proper anesthetization by intramuscular administration of 1ml (50mg/ml) ketamine hydrochloride. The injection of hydroxpropyl methylcellulose is done by use (27 G *1/2) needle which introduced into anterior chamber and inject 0.4 ml of (2% w/v) hydroxypropyl methyl cellulose to right eye and the left as control , the injection was under sterile condition, and the animals kept in normal light room and suitable temperature and monitored. After 48 hours the IOP increased to (20.1- 23.8 mmHg) and this elevating could persist for 10 days, after that, the IOP start to decrease gradually. The type of induced glaucoma is acute angle closure glaucoma [10, 11, 12].

Preparation of carvedilol (0.25%, 0.5 %) eye drops [13]:

Carvedilol	0.25g, 0.5 g
Benzalkonium chloride	(1%) (w / v) 1 ml
Sodium chloride	0.44 g
Ethanol	(70%) 1 ml
Phosphate buffer	to 100 ml

Treatment groups:

In the present study, the drugs were administered only to the right eyes of the rabbits whereas the left eyes were administered distilled water.

Part I: (To evaluate the possible therapeutic hypotensive effects**):**

In the 1st part of this study, the drugs (including distilled water) were administered topically 3 times/day to the right eyes of rabbits only after the ocular hypertension was definitely established, whereas the left eyes received only distilled water.

This part of study was furthermore divided into:

A- Tested agents at (0.25%) concentrations (6 rabbits for each group):

1- Distilled water (Negative control) group (i.e., Distilled water was administered to both eyes of rabbits).
2- Timolol (0.25%) (Positive control) group.
3- Carvedilol (0.25%) (Tested drug) group.

B- Tested agents at (0.5%) concentrations (6 rabbits for each group):

1- Distilled water (Negative control) group (i.e., Distilled water was administered to both eyes of rabbits).
2- Timolol (0.5%) (Positive control) group.

3- Carvedilol (0.5%) (Tested drug) group.

Part II: (To evaluate the possible prophylactic and therapeutic hypotensive effects):

In the 2nd part of this study, the drugs (including the distilled water) were administered topically 3 times/day for 4 days prior to induction of ocular hypertension (i.e. prophylactic use) and then continued thereafter for further 10 days after the disease being induced (i.e. therapeutic use).

The groups of this part (6 rabbits / group) were as follows:

1- Distilled water (Negative control) group (i.e., Distilled water was administered to both eyes of rabbits)
2- Timolol (0.5%) (Positive control) group.
3- Carvedilol (0.5%) (Tested drug) group.

Tested Parameters:

The animals had been examined for the IOP, pupil diameter, light reflex, corneal reflex, and conjunctival redness[14] prior to instillation of drugs and then daily after drugs instillation along the trial period.

Statistical methods:

In this study, 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 the 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) [15,16] .

The differences were accepted as significant if the calculated value for (t) was equal or greater than its tabulated value at (0.05) level of (P) (i.e. $0.01 < P \leq 0.05$) and highly significant if ($P \leq 0.01$). *Chi*-square (X^2) test was used whenever it was applicable (i.e. for independent qualitative data).

The differences were accepted as significant if ($0.01 < P \leq 0.05$) and highly significant if ($P \leq 0.01$) [15, 16].

Results:

PartI:

A) Carvedilol (0.25%) group:

- Post induction of ocular hypertension the IOP of right eyes was (21.9±0.002 mmHg). Treatment with carvedilol (0.25%) eye drop (3 times/day) caused a highly significant decrease in mean IOP from (19.31 ±0.53 mm Hg) to reach (16.36±0.75 mmHg) within 5 days ($P<0.01$); such decline continued like so for further 5 days of treatment to reach (12.36±0.23 mmHg) by the end of trial period (Figure 1).

- Along the trial period, the ocular hypotensive effect of carvedilol (0.25%) eye drop was more efficient than that of distilled water ($P<0.01$) and even simulated that of timolol (0.25%) eye drop ($P>0.05$) (Table 1).

- Regarding each of mean pupil diameter, light reflex, corneal reflex and conjunctival redness, carvedilol (0.25%) eye drops had no significant effect ($P > 0.05$) on them at any time during the trial period.

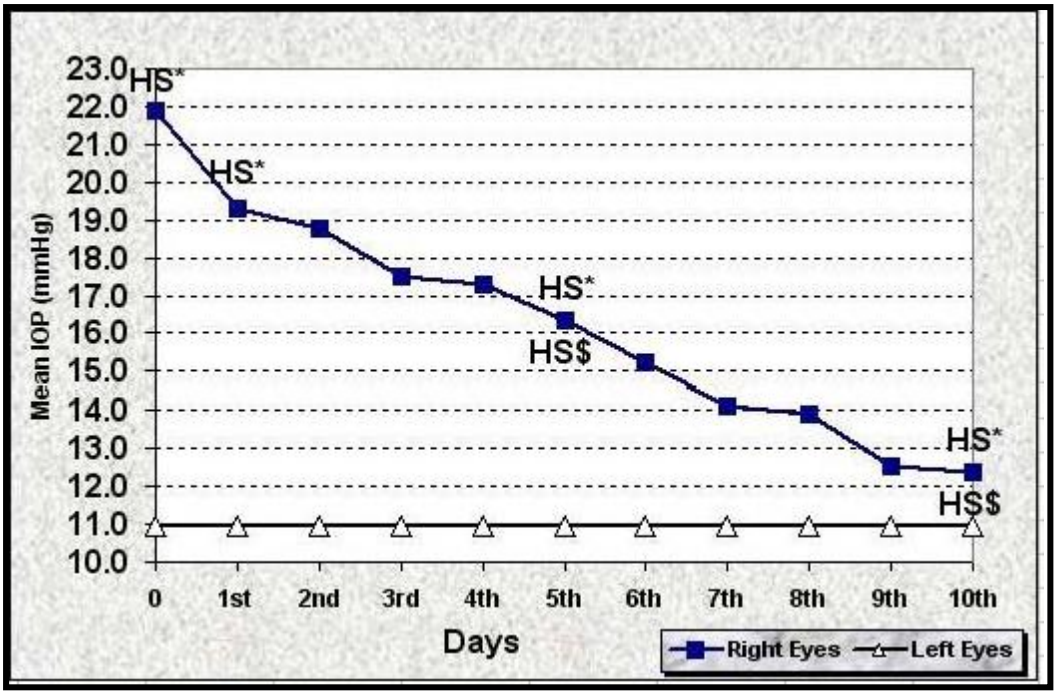

Figure (1): Effect of carvedilol (0.25%) eye drops instilled 3 times daily on mean IOP values of rabbit's right eyes (n = 6) with induced ocular hypertension.

HS = Highly significant difference (P ≤ 0.01).

*** =Compared to corresponding mean IOP values at left eyes.**

$ = Compared to corresponding mean IOP values of right eyes at first day post induction of ocular hypertension.

Table (1): Significance of differences between carvedilol (0.25%) and each of timolol (0.25%) and distilled water groups regarding the response of IOP of right eyes of rabbits.

Group	Pre induction	Post induction of ocular hypertension			
		Pretreatment	Post treatment (Day)		
			1st	5th	10th
Distilled Water	NS	NS	HS(C)	HS(C)	HS(C)
Timolol	NS	NS	NS	NS	NS

NS = No significant difference (P>0.05),
HS = Highly significant difference (P ≤ 0.01),
(T) = The lowest value of mean IOP belongs to timolol group.
***B)* Carvedilol (0.5%) group**:

- Post induction of ocular hypertension, the mean IOP of right eyes was (21.9± 0.002mmHg). Treatment with carvedilol (0.5%) eye drop (3 times/day) highly significant decreased the IOP from (18.78 ± 0.47 mmHg) to reach (14.15± 0.57 mmHg) within 5 days (P<0.01); such decline continued like so for further 5 days of treatment to reach (11.08± 0.18 mmHg), by the end of trial period (Figure 2).

- During trial period, carvedilol (0.5%) eye drop was more efficient than distilled water in its ocular hypotensive effect along the trial period (P<0.01) but less than that of timolol (0.5%) eye drop by the end of the trial period (P<0.01) (Table 2).

- Compared to distilled water group, carvedilol (0.5%) eye drop had no significant effect (P > 0.05) on each of pupil diameter, light reflex, corneal reflex and conjunctival redness at any time during the trial period.

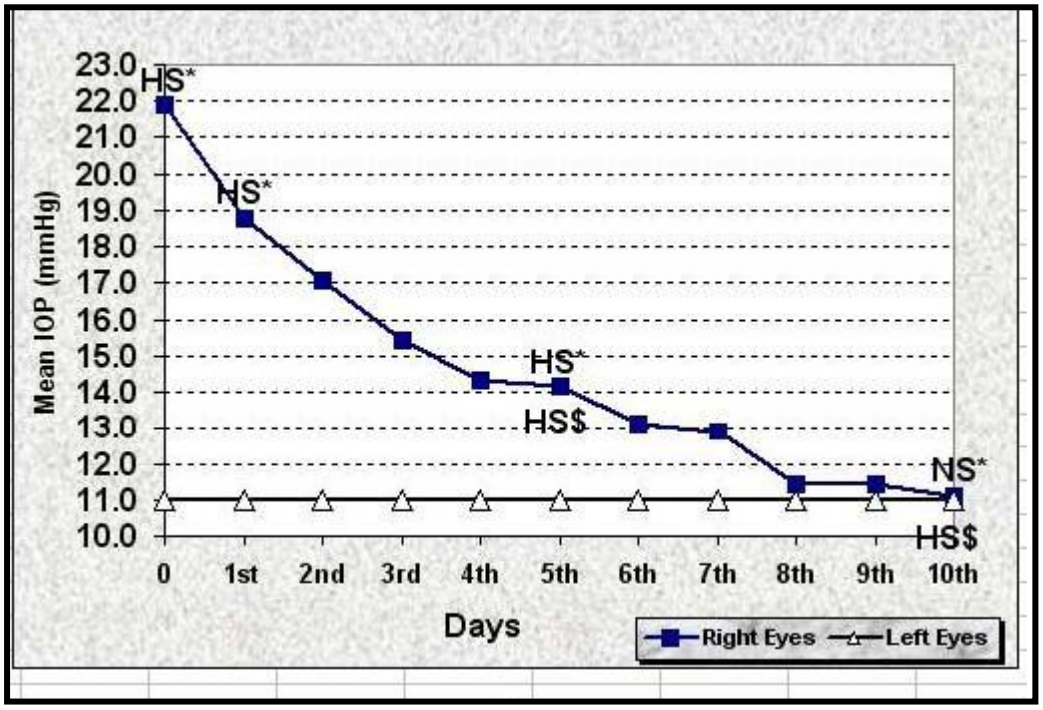

Figure (2): Effect of carvedilol (0.5%) eye drops instilled 3 times daily on mean IOP values of rabbit's right eyes (n = 6) with induced ocular hypertension.

NS = No significant difference (P>0.05),

HS = Highly significant difference (P ≤ 0.01).

*** =Compared to corresponding mean IOP values at left eyes.**

$ = Compared to corresponding mean IOP values of right eyes at first day post induction of ocular hypertension.

Table (2): Significance of differences between carvedilol (0.5%) and each of distilled water and timolol (0.5%) groups regarding the response of IOP of right eyes of rabbits.

Group	Pre induction	Post induction of ocular hypertension			
		Pretreatment	Post treatment (Day)		
			1st	5th	10th
Distilled Water	NS	NS	HS(C)	HS(C)	HS(C)
Timolol	NS	NS	S(T)	NS	HS(T)

NS = No significant difference (P>0.05),
S = Significant difference (0.01 < P ≤ 0.05)
HS = Highly significant difference (P ≤ 0.01),
(C) = The lowest value of mean IOP belongs to carvedilol group,
(T) = The lowest value of mean IOP belongs to timolol group.

Part II:

- The mean IOP value of the 6 included rabbits' right eyes prior the instillation of carvedilol (0.5%) eye drop (3 times/day) (0 time) was (12.55±0.24 mmHg). After 4 days of instillation of carvedilol eye drop, the mean IOP value decreased to reach (9.55±0.53 mmHg); such reduction was found to be highly significant (P<0.01) (Figure 3).

- After ocular hypertension being induced, carvedilol (0.5%) eye drop (3 times/day) for 5 days could insignificantly decreased IOP of right eyes from (11.63±0.36 mmHg) to reach (10.9± 0.36 mmHg) (P > 0.05) , whereas continuation such treatment for further 5 days could highly significant decrease the IOP to be (9.37±0.37mmHg) (P<0.01) (Figure 3).

- Along the trial period in the present study, carvedilol (0.5%) eye drop was found to be more efficient in its ocular hypotensive effect than distilled water, but less than timolol (0.5%) eye drop (Table 3).

- Compared to distilled water group, carvedilol (0.5%) eye drop had no significant effect (P > 0.05) on each of pupil diameter, light reflex, corneal reflex and conjunctival redness at any time during the trial period.

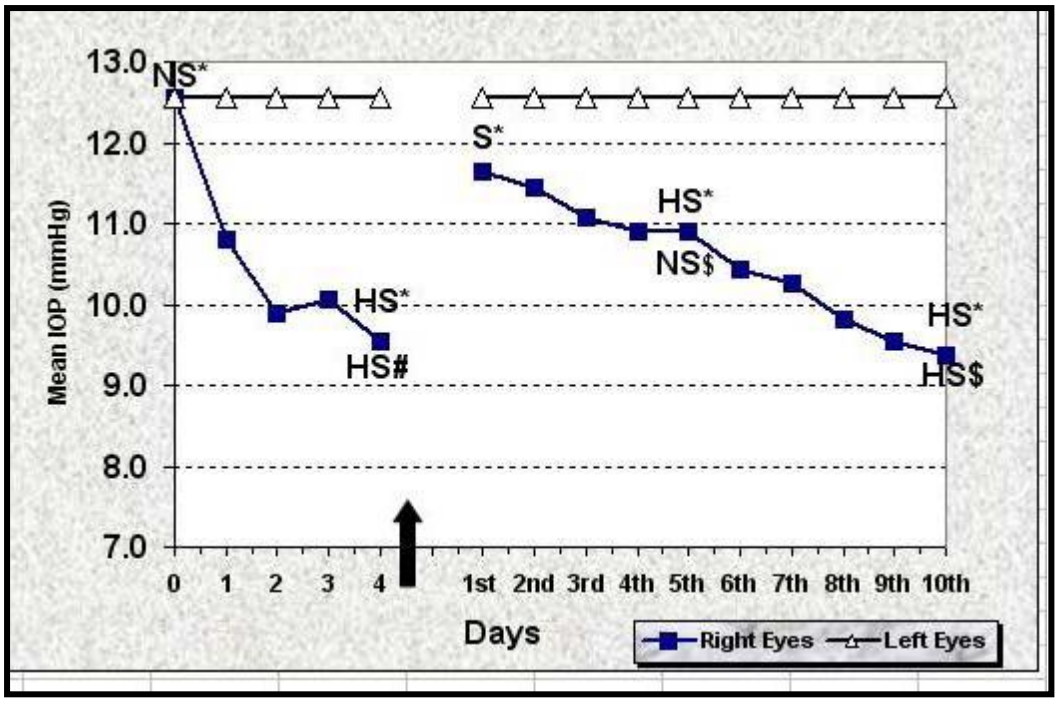

Figure (3): Effect of carvedilol (0.5%) eye drops instilled 3 times daily on mean IOP values of rabbit's right eyes (n = 6) both prior and after induction of ocular hypertension.

NS = No significant difference (P>0.05),
S = Significant difference (0.01 <P≤ 0.05),
HS = Highly significant difference (P ≤ 0.01).
⬆=Time of induction of ocular hypertension.
*** =Compared to corresponding mean IOP values at left eyes.**
 # = Compared to corresponding mean IOP values of right eyes at 0 time.
$ = Compared to corresponding mean IOP values of right eyes at first day post induction of ocular hypertension.

Table (3): Significance of differences between carvedilol (0.5%) and each of timolol (0.5%) and distilled water groups regarding the response of mean IOP of right eyes of rabbits.

	Pre induction (Day)		Post induction of ocular hypertension (Day)		
Group	**0**	**4th**	**1st**	**5th**	**10th**
Distilled water	HS(DW)	S(C)	HS (C)	HS (C)	HS (C)
Timolol	HS (T)	HS (T)	HS (T)	HS (T)	HS (T)

0 = Baseline (Pre-treatment),
S = significant difference (0.01 < P ≤ 0.05),
HS = Highly significant difference (P ≤ 0.01),
(DW) = The lowest value of mean IOP belongs to distilled water group
(C) = The lowest value of mean IOP belongs to carvedilol group,
(T) = The lowest value of mean IOP belongs to timolol group.

Discussion:

Results of the 1st part of this study, had documented the beneficial therapeutic role of carvedilol eye drops at its two tested doses (0.5% and 0.25%) when instilled 3 times/ day for 10 days since each of these concentrations could highly significant ($P < 0.01$) reduced the mean IOP along the trial period in a pattern that was more efficient ($P < 0.01$) than that of distilled water; Such results appeared to be in accordance to what was documented by Hurvitz *et al.,* (2001) [17] who reported that carvedilol had a very potent ocular hypotensive effect. Besides, the persent study determined the equivalent ocular hypotensive effect of carvedilol (0.25%) and timolol (0.25%) eye drops ($P>0.05$); a result that probably agreed with those of Kevin and Waschke, (2002)[18].

On the other hand, results of the 2nd part of the present study pointed out the obvious beneficial prophylactic as well as therapeutic ocular hypotensive effect of carvedilol (0.5%) eye drops instilled 3 times daily for 10 days since it could achieve a highly significant ($P < 0.01$) reduction in IOP after 4 days in normotensive eyes and after administration of hydroxyl propyl methylcellulose (the ocular hypertension inducing agent), it was effective in prevention of IOP from raising to its expected value (i.e. 21.3± 0.37 mmHg in distilled water group). Moreover, further 10 days of therapy with carvedilol (0.5%) eye drops (3 times/day) could also achieve highly significant effect ($P < 0.01$). Nevertheless, it was not better than that of timolol (0.5%) eye drops; this agreed with what was reported by Donoghue, (2003)[19] who proved that carvedilol was to be slightly less effective than timolol.

In agreement to what was found by Robert *et al.,* (2001) [20], the present study and along the trial period, there was no effect of carvedilol on mean IOP in the contra lateral eye after its topical administration in both ocular normotensive and hypertensive rabbits; this probably indicated that topically applied carvedilol exerted its ocular effect locally and not systemically. However**,** the results of present study conflicted with those of Osborne *et al.,* (2005) [21] who found the IOP reduction in untreated eye after unilateral topical application of carvedilol in rabbits. Sharif *et al.,* (2001) [22] noted a contralateral effect of topically applied carvedilol in ocular hypertensive patients.

Carvedilol blocks β adrenergic receptors and thus decreases IOP by reducing aqueous humor production; a mechanism that resembles that of timolol (Kevin and Waschke, 2002[18] ; Lacy *et al.,* 2004[23]). Furthermore, carvedilol has an antioxidant activity (Hoffman, 2004) [8] and probably acts by attenuation of nitrous oxide (NO) which plays a role in production of aqueous humor (Kramer and Weglicki, 1999[24]; Yoshioka et *al.,* 2000[25]).

In the present study, when being compared to results of distilled water, carvedilol eye drops seemed to be quite tolerable since there was no significant difference ($p>0.05$) between their effects on each of mean pupil diameter, light reflex, corneal reflex and conjunctival redness.

Conclusions:

1. Carvedilol (0.5%) eye drops instilled 3 times / day had an obvious prophylactic role in prevention of the hydroxyl propyl methyl cellulose from inducing the ocular hypertension in rabbits.
2. Each of carvedilol (0.25%) or (0.5%) eye drops instilled 3 times / day had a beneficial, safe, and tolerable ocular hypotensive effects on hydroxy propyl methyl cellulose - induced ocular hypertension in rabbits.

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