Effect of Diltiazem on Intraocular Pressure in Normal and Ocular Hypertensive Rabbits

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الخلاصة

داء الزرقاء مرض متعدد الاسباب يتضمن حدوث ضرر مرضي في العصب البصري للعين المصابة بسبب ارتفاع ضغط العين وقد يؤدي للعمى اذا لم يتم خفض ضغط العين في العين المصابة.

الهدف من هذه الدراسة بيان امكانية استخدام قطرات عينية تحتوي مادة الدلتيازم وبيان تاثيرها على مستوى ضغط العين في الارانب المصابة بارتفاع ضغط العين وايضا على الارانب الغير مصابة بهذا الداء .

الدراسة شملت اربع وخمسون ارنبا وكانت النتيجة حدوث انخفاض في ضغط العين بعد استخدام مادة الدلتيازم ثلاث مرات يوميا لمدة اربعة ايام في كلا النوعين (الارانب المصابة والغير مصابة)

Abstract

Background; Glaucoma is a multifactorial disease involving progressive optic neuropathy and altered intraocular hemodynamics; furthermore, glaucoma can cause blindness if it is left untreated.

Aim of the study: To explore effects of topical diltiazem on intraocular pressure (IOP) in each of normal and ocular hypertensive rabbits.

Materials and methods: A group of 54 males of the rabbits were included in the present study. Induction of ocular hypertension was achieved by injection of hydroxy propyl methylcellulose in the anterior chamber of rabbits right eye. In addition to distilled water (as negative control), each of timolol (as positive control) and diltiazem (the tested drug) eye drops were instilled 3 times daily to right eye prophylactically for 4 days and therapeutically

Results: Part I: Diltiazem (0.5%) caused highly significant ($P < 0.01$) reduction in intraocular pressure of right eye .

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Part II: diltiazem, at both concentrations (0.5% and 0.25%) caused highly significant reduction ($P < 0.01$) in IOP at 5th and 10th days post treatment.
Conclusions: Diltiazem exerted a detectable ocular hypotensive effect on the eye of rabbits when applied at concentration (0.5%) or (0.25%) 3 times daily.
Key words: Diltiazem, Intraocular Pressure, Glaucoma

Introduction

Glaucoma is a group of diseases of the optic nerve involving loss of retinal ganglionT cells in a characteristic pattern of optic neuropathy. Although raised IOP is a significant risk factor for developing glaucoma, there is no set threshold for IOP that causes glaucoma [1].One person may develop nerve damage at a relatively low pressure, while another person may have high eye pressure for years and yet never develop damage. Untreated glaucoma leads to permanent damage of the optic nerve and resultant visual field loss, which can progress to blindness [2].

The two main types of glaucoma are primary open angle glaucoma and primary angle closure glaucoma. These are marked by an increase of IOP. 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]

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

Diltiazem hydrochloride is a benzothiazepine calcium channel blockers (CCBs) with peripheral and coronary vasodilator effect; it lowers blood pressure and has effect on cardiac conduction [7]. Diltiazem inhibits passage of calcium through voltage gated L- (large) type calcium channel which is the dominant type in the cardiac and smooth muscles and is known to contain severeal drug receptors[8, 9].

Orally, diltiazem is used in treatment of angina pectoris due to coronary artery spasm, chronic stable angina, essential hypertension, prevention of reinfarction of non-Q-wave myocardial infarction (MI), Raynaud's syndrome

and migraine headache prophylaxis. Parenterally, diltiazem is used in aterial fibrillation or flutter and in paroxysmal supraventricular tachycardia [5, 10].

Aim of the study

This study was designed in order to: explore effect of topically applied diltiazem on mean IOP value of normal rabbits' eyes.

1- evaluate the possible beneficial effect of various doses of topically applied diltiazem on mean IOP values of experimentally induced ocular hypertension in rabbits.
2- explore the possible local adverse effects of the tested drug in an attempt to assess its safety.

Materials and Methods

Materials:

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

Materials	Source
Diltiazim hydrochloride (Cardizem) tablets (60 mg)	Emessa Labs /Homs – Syria.
Benzalkonium chloride	SDI (supplier)
Sodium chloride	Riedel – De Haen Ag seelze –Hannover
Di –sodium hydrogen phosphate (Na2HPo4)	Fluka – Garantie–Switzerland
Sodium di hydrogen phosphate (Na H2Po4)	Riedel – De Haen Ag seelze –Hannover
Hdroxypropyl methyl cellulose ophthalmic solution (2%) (United states pharmacopoeia)	Focus vision care
Phosphoric acid	Emscope,Laboratory Ltd .
Sodium hydroxide	Emscope,Laboratory Ltd .
Lidocaine hydrochloride (2%) Solution	Almashat-Baghdad.
Ketamine hydrochloride(50 mg/ml)	HOLDEN MEDICALLelystad the Netherlands
Distillator	Gesellschaft fur Labortech, Nikm. b.h. and Co. , type 2016 - Germany
pH. meter	Friederg/Hessen-Germany
Sartorius balance	Werke – GMBH, type 2842-Germany
Sehiotz tonometer	Eichtabelle – Germany
Pupil gauge	Al-Zahrawi Private Hospital

Animals 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 Collage –Al Nahrain University. Animals were kept on fresh trefoil diet, water ad libitum, suitable temperature and normal light.

Tested Parameters:

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

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 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 [11,12,13].

Preparation of diltiazem (0.25%, 0.5 %) eye drops

Diltiazim hydrochloride	0.25g, 0.5 g
Benzalkonium chloride	1% (w / v) 1 ml
Sodium chlorid	0.44 g
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

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

1- Distilled water (Negative control) group.
2- Timolol 0.5% (Positive control) group.
3- Diltiazem (0.5%) (Tested drug) group.

The drugs (including the distilled water) were administered topically 3 times/day for four 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).

Part II

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

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

1- Distilled water (Negative control) group.
2- Timolol (0.5%) (Positive control) group.
3- Timolol (0.25%) (Positive control) group.
4- Diltiazem (0.5%) (Tested drug) group.
5- Diltiazem (0.25%) (Tested drug) group.

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).

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 ($P \leq 0.01$) [14, 15].

Results

Part I

- The mean IOP value of the 6 included rabbits' right eyes prior the instillation of diltiazem (0.5%) eye drop (3 times/day) (0 time) was (11.81±0.33 mmHg). After 4 days of instillation of diltiazem eye drop, the mean IOP value decreased to reach(10.1± 0.71 mmHg); such reduction was found to be highly significant ($P<0.01$).

- After ocular hypertension being induced, diltiazem (0.5%) eye drop (3 times/day) for 5 days could insignificantly decreased IOP of right eyes from (11.20±0.72 mmHg) to reach (10.85±0.76 mmHg) ($P > 0.05$) , whereas continuation such treatment for further 5 days could significantly decrease the IOP to be (10.32±0.61 mmHg) ($0.01 < P < 0.05$) (Figure 1).

- Along the trial period in the present study, diltiazem (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 1).

- Regarding each of pupil diameter, light reflex, corneal reflex and conjunctival redness, diltiazem (0.5%) had no significant effect ($P > 0.05$) on them at any time during the trial period.

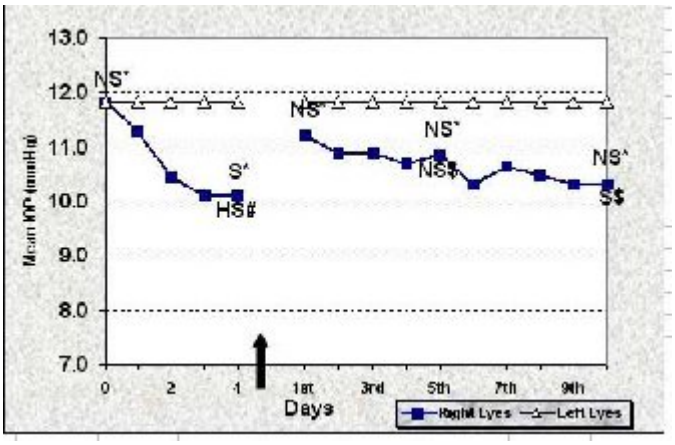

Figure (1): Effect of diltiazem (0.5%) eye drops instilled 3 times daily on mean IOP values of rabbit's right eyes (n = 6) both prior and after induction of ocular hypertension.

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 (1): Significance of differences between diltiazem (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	4	1st	5th	10th
Distilled water	NS	NS	HS (Dilt)	HS (Dilt)	HS (Dilt)
Timolol	HS (T)	S (T)	HS (T)	HS (T)	HS (T)

0 = Baseline (Pre-treatment),
NS = No significant difference (P>0.05),
S = significant difference (0.01 < P ≤ 0.05),
HS = Highly significant difference (P ≤ 0.01),
(Dilt) = The lowest value of mean IOP belongs to diltiazem group,
(T) = The lowest value of mean IOP belongs to timolol group.

PartII

A) Diltiazem (0.5%) group

- Post induction of ocular hypertension the mean IOP of right eyes was (22.21± 0.31 mmHg). Treatment with diltiazem (0.5%) eye drop (3 times/day) highly significant decreased the IOP from (19.87 ± 0.51 mmHg) to reach (16.33± 0.6 mmHg) within 5 days (P<0.01); such decline continued like so for further 5 days of treatment to reach (11.82± 0.33 mmHg) by the end of trial period (Figure 2).

- During trial period, diltiazem (0.5%) eye drop was more efficient than distilled water in its ocular hypotensive effect but less than that of timolol (0.5%) eye drop (Table 2).

- Regarding each of mean pupil diameter, light reflex, corneal reflex and conjunctival redness, diltiazem (0.5%) had no significant effect (P > 0.05) on them at any time during the trial period.

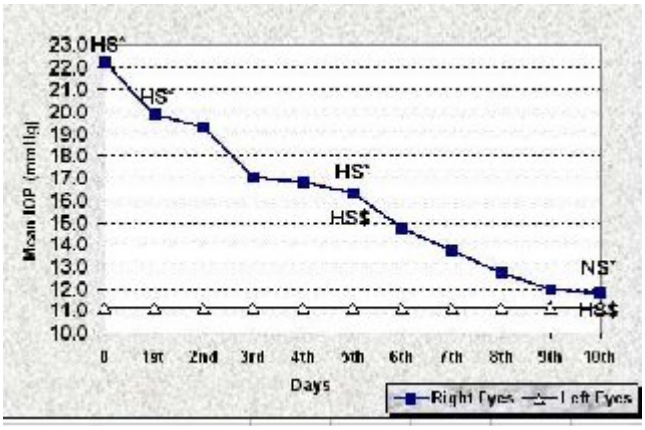

Figure (2): Effect of diltiazem (0.5%) eye drops instilled 3 times daily on mean IOP values of rabbit's right eyes (n = 6) with induced ocular hypertension.

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 diltiazem (0.5%) and each of timolol (0.5%) and distilled water groups regarding the response of IOP of right eyes of rabbits.

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

NS = No significant difference (P>0.05),
HS = Highly significant difference (P ≤ 0.01),
(Dilt) = The lowest value of mean IOP belongs to diltiazem group,
(T) = The lowest value of mean IOP belongs to timolol group.

B) Diltiazem (0.25%) group

- Post induction of ocular hypertension the IOP of right eyes was (21.9±0.0015mmHg). Treatment with diltiazem (0.25%) eye drop (3 times/day) caused a highly significant decrease in mean IOP from (19.6 ± 0.56 mm Hg) to reach (16.78±0.44 mmHg) within 5 days (P<0.01); such decline continued like so for further 5 days of treatment to reach (12.93±0.35 mmHg) by the end of trial period (Figure 3).

- The diltiazem (0.25%) eye drop was more efficient than distilled water in its ocular hypotensive effect during trial period and even it simulated that of timolol (0.25%) eye drop on 10th day post treatment (Table 3).

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

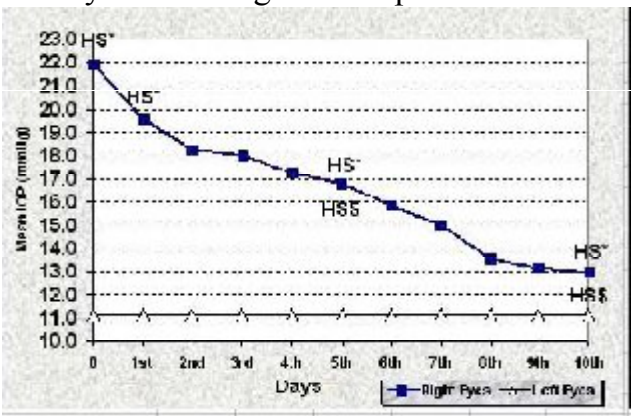

Figure (3): Effect of diltiazem (0.25%) eye drops instilled 3 times daily on mean IOP values of rabbit's right eyes (n = 6) with induced ocular hypertension.

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 (3): Significance of differences between diltiazem (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 (Dilt)	HS (Dilt)	HS (Dilt)
Timolol	NS	NS	S (T)	S (T)	NS

NS = No significant difference (P>0.05),

S = significant difference (0.01 < P ≤ 0.05),

HS = Highly significant difference (P ≤ 0.01),

(Dilt) = The lowest value of mean IOP belongs to diltiazem group,

(T) = The lowest value of mean IOP belongs to timolol group.

Discussion

Prophylactically the (0.5%) concentration of diltiazem (3 times/day) caused highly significant ($P < 0.01$) reduction in IOP after 4 days in normotensive eyes and was effective in prevention of IOP from raising to its expected value (i.e. 21.3± 0.37 mmHg in distilled water group) after administration of inducing agent (hydroxyl propyl methylcellulose); besides, it could achieve significant ($0.01 < P < 0.05$) ocular hypotensive effect but required further 10 days to do so. Diltiazem (0.5%) caused reduction in of IOP below the normal value and there was significant ($0.01 < P < 0.05$) difference on the 4th day pre induction in comparison to left eye. On 10th day caused lowering of IOP below the normal value but there was no significant ($P > 0.05$) difference in comparison to left eye. Diltiazem effect was better ($P < 0.01$) than that of distilled water group in comparison with corresponding values, but not than that of the timolol group. These results pointed out to the beneficial prophylactic and therapeutic hypotensive effect of diltiazem (0.5%) 3 times/day for 10 days. Therapeutically diltiazem used in two doses (0.5% and 0.25%) and instilled 3 times daily for 10 days after induction of disease, both concentrations caused highly significant ($P < 0.01$) reduction in of IOP, at the (0.5%) concentration diltiazem was more efficient than distilled water ($P < 0.01$) but less than timolol (0.5%) ($P < 0.01$). At the concentration (0.25%) diltiazem simulated the hypotensive effect of timolol (0.25%) on10th day, but on 1st and 5th days timolol was more effective ($0.01 < P < 0.05$).

Diltiazem (0.5%) could reduce the IOP to be normal on 10th day post induction of ocular hypertension, and there was no significant ($p > 0.05$) difference compared to left eye. At dose (0.25%), diltiazem caused reduction in the IOP of right eye along trial period but not reached to normal and there was highly significant ($P < 0.01$) difference in comparison with normal left eye on 10th day post induction of disease. There was no effect of diltiazem on the pupil diameter, and in comparison to distilled water group there was no significant difference ($p > 0.05$) regarding other possible side effect (i.e. light reflex, corneal reflex and conjunctival redness).

This refer to that diltiazem have tolerable ocular hypotensive effect in normal and hypertensive eyes, after the ocular hypertension had been induced. There was no detectable effect on IOP in untreated left eyes and there was no contralateral effect after instillation of diltiazem eye drops.

Melena *et al.*[16] described the ocular hypotensive effect of CCBs in rabbit model for glaucoma. Santafe *et al.* [17] showed that single doses of verapamil, nifedipine and diltiazem produced a dose-dependent decrease in IOP in ocular normotensive rabbits after topical application but not after

intravenous administration. Furthermore, the ocular hypotensive effect of diltiazem was remarkable due to its duration, thus permitting the administration frequency used in the work of Santafe *et* al. [17].

In humans, topical verapamil, diltiazem and nifedipine have been found to significantly lower IOP in normal and ocular hypertensive subjects. A single topical application of CCBs prompted IOP decrease in ocular hypertensive patients [18, 19]. Netland *et al.* [20] also found that verapamil and diltiazem significantly lowers the IOP in normal human volunteers.

After topical application of verapamil and diltiazem for 2 weeks, decrease in IOP has been measured in ocular hypertensive subjects [21].

Yet, in the present study the hypotensive effect of diltiazem on hydroxyl propyl methylcellulose induced ocular hypertensive rabbits could be detected in to their effects on normotnsive eyes which appeared to in agreement to results of the studies just mentioned.

On the other hand, conflict with those of Beatty ***et al.***[22], who found an increase in IOP after intravenous and topical application of verapamil, nifedipine and diltiazem in rabbits and after topical verapamil in humans. Because the doses of these drugs used by Beatty ***et al.,*** [22] were higher than those applied in most of the beforementioned studies, Abelson *et* ***al.*** [18] proposed that CCBs may have a biphasic effect on IOP, with an ocular hypotensive action at low and an ocular hypertensive action at high concentrations. However, Melena *et al.* [16] results did not support this hypothesis because a decrease in IOP was noted even at very high concentrations of diltiazem; furthermore, and in agreement to results of the present study, Melena *et al.* [16] have not found a clear bilateral effect of diltiazem when administered to only one eye. Suggesting that diltiazem and other CCBs lack a contralateral effect in betamethasone induced rise in IOP in rabbits, and this agreed with Jani *et al.* [23] who found that not clear bilateral effect of verapamil and diltiazem when administered to only one eye, suggesting that these CCBs lack a contralateral effect in these animal models for glaucoma. Appeared in contrast to those of Segarra ***et al***. [24] who found an IOP reduction, although not dose-related, in the contralateral eye after unilateral topical application of verapamil and diltiazem in albino rabbits. Abelson *et al.* [18] Mooshian ***et al.***[19] also noted a contralateral effect of topically applied verapamil , diltiazem and nifedipine in ocular hypertensive subjects, whereas Netland ***et al.***[20] reported no effect of diltiazem and nifedipine on IOP in the contralateral eye after topical administration in normal subjects.

Possible mechanism

1- CCBs cause reduction in aqueous humor production by effecting on ultra filtration of aqueous, due to CCBs cause relaxation of blood vessels in ciliary epithelium and decrease the hydrostatic pressure which is one of the factors that cause passing of fluid into ciliary process[25, 26, 27]

2- The (Gap) junctions which are possibly regulated by calcium, exist between non pigmented and pigmented ciliary epithelial cells, CCBs may interfere with these (Gap) junctions, altering cellular permeability of the ciliary epithelium and thus inhibiting normal aqueous humor formation [28].

3- The potassium channel is important in formation of aqueous humor in ciliary epithelium, and this channel depends on the calcium ion, also when topical administration of the calcium ion had been shown to increase the IOP [29]. For that the CCBs cause reduction in aqueous formation [30].

4- Elevating of intracellular calcium accelerates the activity of several adenosine triphosphate (ATP) consuming enzymes and one of these enzymes is the enzyme complex (in pigmented and nonpigmented ciliary epithelial cells) that energy depend which transport sodium and potassium ions that are important in aqueous humor formation, thus the using of CCBs play important role in impairment of these process[8, 31].

5- The trabecular meshwork cells have contractile properties which may be influenced by calcium ion influx through voltage-dependent L-type calcium channels, thus the relaxation by CCBs can increase the trabecular outflow facility[32]. The perfusion studies in dissected human eyes showed dose related increase in outflow facility after verapamil, diltiazem and nifedipine administration[33, 34]. In addition to that, the outflow of aqueous humor influenced by episcleral venous pressure may be directly affected by calcium inhibition[35].

Conclusions

1-Diltiazem, exerted a detectable ocular hypotensive effect on the normal eyes of rabbits when applied at concentrations (0.5%) or (0.25%) 3 times daily.

2-Diltiazem at concentration (0.5%) 3 times daily could prevent development of ocular hypertension after administration of the inducing agent (hydroxyl propyl methyl cellulose).

3- Diltiazem, had beneficial ocular hypotensive effect on hydroxy propyl methyl cellulose-induced ocular hypertension in rabbits when each of them given topically at concentrations (0.5%) or (0.25%) 3 times daily.

3- Compared to effect of timolol (0.5%) eye drops, diltiazem (0.5%) eye drops given 3 times daily had accepted ocular hypotensive effect.

5- In comparison with timolol (0.25%) eye drops, diltiazem (0.25%) eye drops given 3 times daily had a comparable efficient ocular hypotensive effect.
4- The tested drug in their applied doses was found to be apparently safe and tolerable along the trial period.

Recommendations

1- Detailed controlled clinical studies using diltiazem (0.25%) or (0.5%) eye drops for patients with glaucoma seemed to be recommended.
2- Results of the present study could be a guide line for selection the cardiovascular acting drugs in glaucomatous patients undergo cardiovascular problem such as hypertension, ischemic heard disease or arrhythmias.

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