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EFFECT OF GINKGO BILOBA AND GREEN TEA EXTRACTS ON CORTICOSTEROID-INDUCED OCULAR HYPERTENSION IN RABBITS

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ABSTRACT

Ginkgo bilobaand green tea have attracted interest due to their neuroprotective, potent antioxidant, and blood flow regulatory activities. The current study aimed to assess the potential effects of standardized extracts of ginkgo biloba (EGb 761) and green tea on ocular hypertension induced in rabbits by a single subconjunctival administration of betamethasone (3.5 mg). The latter produced a sustainable increase in intraocular pressure (IOP), measured by tonometry, starting from day 7 post administration. Animals were randomly allocated into 10 groups in which group I served as normal. After ocular hypertension has been established namely, day 7, rabbits of group II were left untreated Groups III & IV were topically treated with timolol (0.5 %) &EGb 761 (0.05 %), respectively. Groups V & VI received EGb 761 (20 mg/kg; p.o) & green tea (300 mg/kg; p.o), respectively. Groups VII & VIII received combination of either EGb 761 (20 mg/kg; p.o) or green tea (300 mg/kg; p.o) plus timolol (0.5 %; topically), respectively. All the mentioned treatments were given once daily for 7 days. Two other groups IX & X were also included where EGb 761 (20 mg/kg; p.o) & green tea (300 mg/kg; p.o) were prophylactically given at the same day of betamethasone injection and continued for 2 weeks. IOP was measured and recorded daily. Levels of whole blood reduced glutathione (GSH), plasma malondialdehyde (MDA), and aqueous humor total antioxidant capacity (TAC) were estimated. Corneal histopathological changes were also examined. Levels of IOP and MDA were increased by betamethasone while those of TAC and GSH were decreased, as compared to normal group. Topical timolol and EGb 761 showed similar suppression of the elevated IOP as compared to betamethasone group. Oral EGb 761 and green tea improved the measured biochemical parameters as compared to betamethasone and timolol groups. The treatments improved also the histopathological features of corneal tissue. It

merits mention that groups that received combined treatments of either extracts with timolol showed the best results. Prophylactic EGb 761 and green tea prevented the elevation of IOP and improved TAC, MDA, GSH levels as compared to betamethasone group. In conclusion, EGb 761 and green tea might represent a potential avenue for the management of ocular hypertension and adjunctive supplement might be advocated to halt the progress of the disease.

KEYWORDS :*Ginkgo biloba, green tea, betamethasone, timolol, ocular hypertension, antioxidant.*

INTRODUCTION :

Glaucoma is considered the second leading cause of blindness after cataract (Quigley, 1996) and it is called the “silent blinder” (Coleman, 1999). The understanding of the pathophysiology of the optic neuropathy accompanying primary and secondary glaucomas, is so limited, that therapy in all cases is restricted to lower intraocular pressure (IOP) below a level that is likely to produce further damage to the nerve and thus preserve visual function. The treatment regimen that achieves this goal, with the lowest risk; fewest adverse effects; and least disruption of the patient's quality of life, taking into account the cost implications of treatment, should be the one employed (Noecker, 2006). Recently herbal medicines attracted interest as inexpensive and safe novel tools aiming at retarding the progression of glaucoma and blocking the deleterious mechanisms that lead to apoptosis and thus have a potential effect in management of glaucoma but this is somehow still controversial.Oxidative damage produced by reactive oxygen species is among the causative factors in IOP-induced neuronal damage in glaucoma. Oxidative stress and its consequences have been linked to the chronic activation of an inflammatory response (Chung et al., 2006). Kolko (2013) provided direct evidence associating glaucoma with low-grade inflammation.Fortunately, new methods of neuro-protection are being put into practice to target oxidative stress. The inhibition of oxidative damage by anti-oxidant treatments indicates that these agents may provide preventive or therapeutic intervention for glaucoma (Huynh et al., 2013).

Ginkgo biloba(GB) is the world’s oldest living tree species. The standardized extract of ginkgo biloba (EGb 761) contains 24% ginkgo flavone glycosides (flavonoids), 6% terpene lactones (ginkgolides and bilobalide), approximately 7% proanthocyanidines, and other uncharacterized compounds (De Feudis, 1991). Some studies have reviewed GB extract’s numerous mechanisms that may be beneficial in treating ophthalmic disorders, including its therapeutic value in ocular blood flow, anti-oxidant activity, anti-apoptotic, neuro-protective activity, platelet activating factor inhibitory activity and nitric oxide (NO) inhibition in certain forms of glaucoma (Ritch, 2000; Thiagarajan et al., 2002) particularly the non-pressure dependent type (Mozaffarieh and Flammer, 2007).

Tea is the most consumed beverage in the world. Green tea belongs to the Theacease family. The five major catechins in green tea are (-)-epigallocatechin-3-gallate (EGCG), (-)-epigallocatechin, (-)-epicatechin-3-gallate, (-)-epicatechin and (+)-catechin (Yang et al., 2000). Other Major chemical components of green tea are theanine, caffeine, and vitamin C (Gotoet al., 1996). In vivo and in vitro recent studies have shown that green tea has beneficial ocular effects and that its consumption can protect the eye against oxidative stress (Chu et al., 2010). This may be attributed to the above mentioned free radical scavenging, anti-oxidant, gene modulating activities, and vasodilator effect (Lorenz et al., 2004) together with its ability to cross the blood-brain barrier (Mandel et al., 2006).

The present study was initiated to unravel the efficacy of EGb 761 and green tea extracts, as potential anti-oxidants and neuro-protectors, on the development of ocular hypertension induced by a single subconjunctival injection of betamethasone in rabbits. Measurement of IOP was carried out.

Levels of whole blood reduced glutathione (GSH), plasma malondialdehyde (MDA), and aqueous humor total antioxidant capacity (TAC) were estimated. Corneal histopathological changes were also examined.

MATERIALS AND METHODS

ANIMALS:

EightyNew Zealand albino rabbits of either sex weighing 1.5-2.0 kg were randomly allocated into 10 experimental groups, 8 rabbits each. Animals were housed individually and kept in the animal house of October University for Modern Sciences and Arts under standard laboratory conditions with controlled temperature (about 25 ± 0.5?C), humidity (55 ± 1%), and in 12 h light-dark cycles. The study was carried out according to the guidelines of the Association for Research in Vision and Ophthalmology with approval of the ethical committees of Faculty of Pharmacy, October University for Modern Sciences and Arts and also Cairo University. Animals were allowed free access to food (standard diet pellets) and water.

DRUGS:

Drugs used in the current study were purchased from commercial sources as follows:

1.Betamethasone dipropionate/sodium phosphate salts (Diprofos depot® ampoules; 7 mg/ml) from Medical Union Pharmaceuticals, Egypt.

2.Standardized extract of ginkgo biloba (EGb 761) (TRIUM® eye drops; 0.05%) from SOOFT Italia ophthalmic pharmaceutical Co., Italy.

3.Standardized extract of green tea (green tea) (Multi-Treat® tablets; 300 mg) from Arab Co. for Pharmaceuticals and Medicinal Plants, Egypt.

4.Timolol maleate (Timolol® eye drops; 0.5%), benoxinate hydrochloride (Benox® eye drops; 0.4%), and EGb 761 (Tebonina Forte® tablets; 40 mg) from Egyptian International Pharmaceutical Industries Co., Egypt.

CHEMICALS AND DIAGNOSTIC KITS:

Malondialdehyde (MDA), reduced glutathione (GSH), and total anti-oxidant capacity (TAC) reagent kits were purchased from Biodiagnostic, Egypt. All other used chemicals were of the highest purity grade commercially available.

EXPERIMENTAL DESIGN:

I.Negative control (normal): received only sterile saline eye drops (0.9%).

II.Ocular hypertensive (Untreated): received a single subconjunctival dose of betamethasone (0.5 ml, 7 mg/ml) then left untreated.

III.Local timolol-treated ocular hypertensive: received timolol (1 drop, 0.5%).

IV.Local EGb 761-treated ocular hypertensive: received EGb 761 (1 drop, 0.05%).

V.Oral EGb 761-treated ocular hypertensive: received EGb 761 (20 mg/kg; p.o)

VI.Oral Green tea-treated ocular hypertensive: received green tea (300 mg/kg; p.o)

VII.Combined EGb 761 and timolol-treated ocular hypertensive: received a combination of EGb 761 (20 mg/kg; p.o) plus topical timolol (1 drop, 0.5 %).

VIII.Combined green tea and timolol-treated ocular hypertensive: received a combination of green tea (300 mg/kg; p.o) plus topical timolol (1 drop, 0.5%).

IX.Oral EGb 761-treated (prophylactic): received EGb 761 (20 mg/kg; p.o).

X.Oral green tea-treated (prophylactic): received green tea (300 mg/kg; p.o).
Ocular hypertension was induced in groups II to X by injecting subconjunctival betamethasone (3.5mg) on day 1. After ocular hypertension has been established namely, day 7,all thementioned treatments were given to the animals once daily for 7 days expect forgroups IX and X which were orally given the selected extracts on the same day of betamethasone injection and continued for 2 weeks.

For oral administration of either extracts, namely, EGb 761 or green tea, tablets were crushed by pestle and mortar, suspended in distilled water and the concentrations were adjusted so that each 1 kg body weight of animal received 1 ml via a stomach tube. IOP was measured in all rabbits just before betamethasone injectionand continued daily for 14 days thereafter(Gharaeiet al., 2008).

METHODS:

I.Clinical evaluation: All the eyes of the rabbits of the various experimental groups were examined before the start of the study and observed after 7 and 14 days. The corneal tissue was examined to exclude any corneal irritation and inflammation which may predispose to corneal ulceration and opacity.
II.Measurement of IOP: For the whole period of the experiment, IOP was measured on daily basis in all rabbits using RiesterSchiötz C indentation tonometer.Before each measurement, it was cleaned with ethanol and checked on a hard convex surface, supplied with the tonometer. The pointer was deflected to the zero position on the calibrated scale which means that the tonometer reading is accurate.The tonometer was placed vertically on the anaesthetized cornea (1 drop of benoxinate eye drops; 0.4%) of the laterally positioned animal, carefully the eye lids held apart with one hand, being carefully not to press on the eye ball to avoid any error in the measurement of the IOP. Three readings were taken using two weights of the plunger (5.5 and 10 g) where the second measuring was confirmatory to the first. The readings were converted to mmHg and the average of the three readings was then calculated.
III.Biochemical Determination: At the end of the experimentation period, specifically on the 14th day, blood and aqueous humor fluid samples were withdrawn for the estimation of the selected biochemical parameters viz., MDA, GSH, and TAC according to the methods of Ohkawaet al. (1979), Beutleret al. (1963), and Koracevicet al. (2001), respectively.Aliquots of blood were withdrawn from the jugular vein of the rabbits via heparinized tubes. One blood aliquot was collected to estimate the GSH content using EDTA as an anti-coagulant and the assay was done immediately on the whole blood. Plasma was prepared from the second blood aliquot using citrate as an anti-coagulant by centrifugation. Separated plasma was used to determine the MDA content.
IV.Aqueous humor fluid sampling: Aqueous humor fluid was collected for the determination of TAC (Geromettaet al., 2010). Briefly, one drop of benoxinate eye drops (0.4%) was applied to the ocular surface before the needle was inserted through the cornea. Approximately 100 µl of aqueous humor fluid was drawn from the anterior chamber of the eye with a 29-gauge needle that was inserted through the cornea near the corneoscleral junction and perpendicular to the visual axis. Withdrawn fluids were promptly transferred to eppendorf tubes and immediately assayed.
V.Histopathological examination of the corneal tissue: Animals were sacrificed on the 15th day and eyes were immediately enucleated for corneal histopathological examination. Eyes were injected with formalin to preserve the tissues, kept in 10% formalin solution for 10 days, and then examined microscopically.
VI.Statistical analysis: All values are presented as means ± S.E. Mean data from each study group were compared by one way analysis of variance (ANOVA) followed by Tukey-Kramer method as multiple comparison post ANOVA test. Statistical analysis for IOP was performed using repeated measure

ANOVA. P values <0.05 were considered significant. Statistical analysis and charts were carried out using computerized SPSS program software (Statistical Package for the Social Sciences, version 16) and STATA (version 11).

RESULTS

I.Clinical evaluation:For all the experimental groups, there were no external signs as redness of conjunctiva, tearing, or inflammation. There were also no blinking and no change in the size of the pupil. Corneal ulceration was excluded since the epithelium was found intact in all groups and there were no loss of stromal connective tissue.

II.Measurement of IOP: Tonometric measurement of IOP of the normal animals was consistent throughout the whole study and was found to be in the normal range. Induction of ocular hypertension in group II produced significant elevation of IOPstarting from day 3, established at day 7(18.30±0.2928)and was sustained till day 14 (21.51±0.3785) post injection of betamethasone. Treatment of the glaucoma model by local timolol, local and oral EGb 761, oral green tea, combination of EGb 761 or green tea with timolol (groups III, IV, V, VII, VII and VIII respectively), showed IOP values that were significantly lower than those of untreated model starting from day 8 post injection and continued till day 14. All the given treatments showed ocular hypotensive activities. As expected, the combined treatment of ocular hypertensive rabbits with local timolol and oral EGb 761 or green tea showed better outcomes than each separately. Prophylactic administration of oral EGb 761 and green tea prevented betamethasone-induced elevation in IOP starting from day 3 and 4 post injection, respectively(Table 1 and Figs. 1-4).

Table 1: Effect of local application of timolol and EGb 761, oral administration of EGb 761 and green tea, given as curative either alone or combined with local timolol, and as prophylactic treatment, on IOP in experimentally-induced ocular hypertension on days 1, 7, 8 and 14.

Groups/Days	Day 1	Day 7	Day 8	Day 14
Normal	13.10±0.3761	13.13±0.2033	13.25±0.1592	13.06±0.4297
Ocular hypertensive (Untreated)	13.11±0.8193	18.30±0.2928@	18.30±0.2928@	21.51±0.3785@
Ocular hypertensive + timolol	11.90±0.5085	19.03±0.4451@	15.86±0.8490	11.08±0.1250@#
Ocular hypertensive + local EGb 761	12.58±0.5483	19.14±0.4880@	12.96±0.4613#	10.45±0.1637@#
Ocular hypertensive + oral EGb 761	14.00±0.2268	20.41±0.4955@	16.25±0.2291@#	11.20±0.2673@#
Ocular hypertensive + timolol + oral EGb 761	11.96±0.5891	19.75±0.3213@	11.35±0.3698@#$	09.80±0.1512@#$
Ocular hypertensive + oral green tea	11.58±0.2631	18.14±0.5161@	12.95±0.3157#	10.48±0.2999@#
Ocular hypertensive + timolol + oral green tea	11.58±0.2266	19.14±0.4880@	17.35±0.4013@	10.70±0.1890@#
Ocular hypertensive + prophylactic oral EGb 761	12.98±0.3712	13.55±0.2719#	13.23±0.2711#	12.43±0.1250@#
Ocular hypertensive + prophylactic oral green tea	11.88±0.4017	13.40±0.3207#	12.10±0.2449@#	10.18±0.3150@#

-Values are presented as means ± S.E. (n=8 animals).
-@Significant difference from normal group at P<0.05.
-#Significant difference from ocular hypertensive group at P<0.05.
-$Significant difference from timolol-treated group at P<0.05.

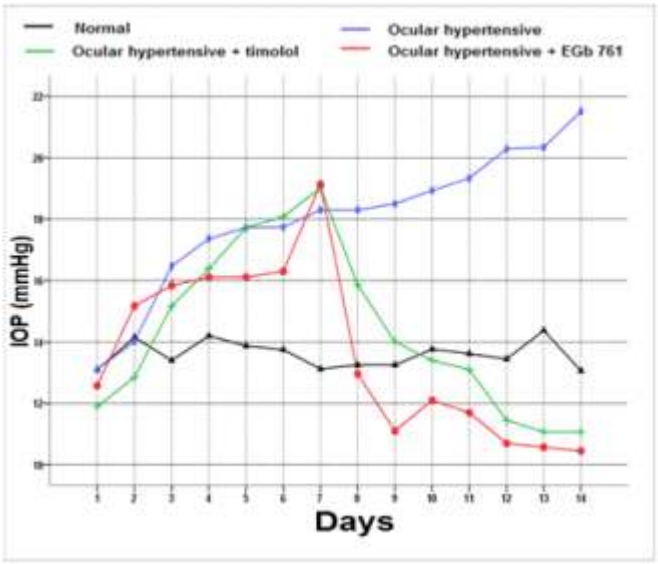

Fig. 1: Effect of local application of timolol and EGb 761 on daily measured IOP in experimentally-induced ocular hypertension.

- Ocular hypertension was induced in rabbits by a single subconjunctival injection of betamethasone (0.5 ml, 7 mg/ml) on day 1.
- Timolol (1 drop, 0.5%) and EGb 761 (1 drop, 0.05%) were instilled once daily starting from day 7 and continued for 7 consecutive days.
- Values are presented as means (n=8 animals). For simplicity, S.E.s have been omitted.

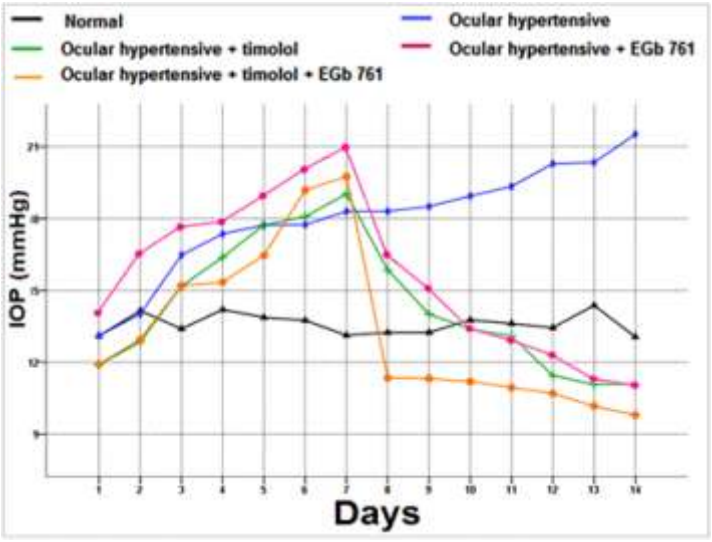

Fig. 2: Effect of oral administration of EGb 761, given either alone or combined with local timolol, on daily measured IOP in experimentally-induced ocular hypertension.

- Ocular hypertension was induced in rabbits by a single subconjunctival injection of betamethasone (0.5 ml, 7 mg/ml) on day 1.
- Egb 761 (20 mg/kg) and timolol (1 drop, 0.5%) were administered once daily starting from day 7 and

continued for 7 consecutive days.
- Values are presented as means (n=8 animals). For simplicity, S.E.s have been omitted.

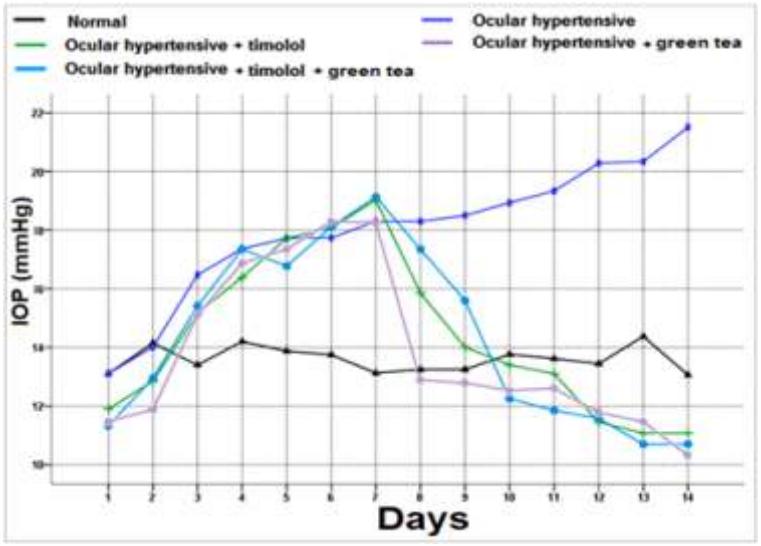

Fig. 3: Effect of oral administration of green tea, given either alone or combined with local timolol, on daily measured IOP in experimentally-induced ocular hypertension

- Ocular hypertension was induced in rabbits by a single subconjunctival injection of betamethasone (0.5 ml, 7 mg/ml) on day 1.
- Green tea (300 mg/kg) and timolol (1 drop, 0.5%) were administered once daily starting from day 7 and continued for 7 consecutive days.
- Values are presented as means (n=8 animals). For simplicity, S.E.s have been omitted.

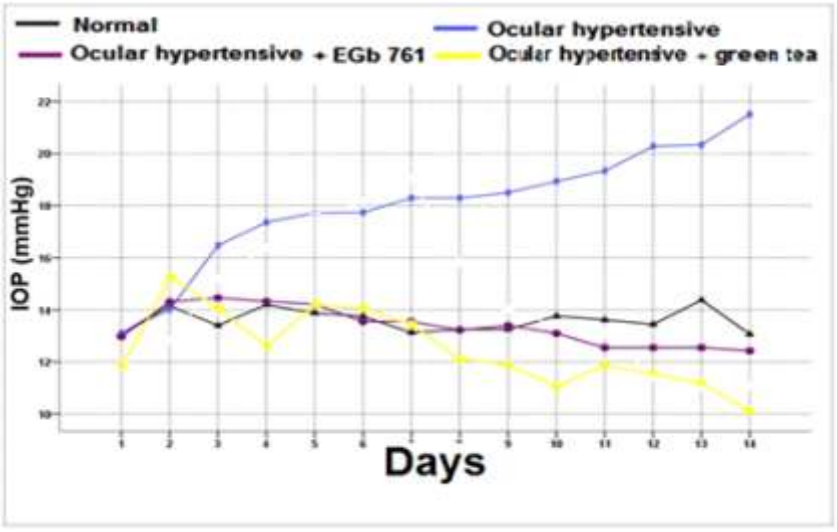

Fig. 4: Effect of oral administration of EGb 761 and green tea, given prophylactically, on daily measured IOP in experimentally-induced ocular hypertension.

- Ocular hypertension was induced in rabbits by a single subconjunctival injection of betamethasone (0.5 ml, 7 mg/ml) on day 1.

- EGb 761 (20 mg/kg) and green tea (300 mg/kg) were administered once daily on the same day of betamethasone injection and continued on daily basis for 14 consecutive days.
- Values are presented as means (n=8 animals). For simplicity, S.E.s have been omitted.

III. Biochemical determination:

The control group I showed normal levels of plasma MDA (03.515 ± 0.928), blood GSH (45.985 ± 2.302) and aqueous humor TAC (1.209 ± 0.186). In group II, induction of ocular hypertension exhibited significant elevation of MDA content and reduction in GSH level as well as TAC. Timolol treatment (Group III) failed to cause any improvement in the betamethasone-induced alterations in the levels of the measured oxidative stress biomarkers. All the treatment groups (groups IV, V, VII, VII, VIII, IX and X), caused noticeable improvement in the GSH level and TAC. Regarding MDA level, only the treatment groups (V, VII, VII, VIII, IX and X) succeeded to produce a significant improvement (Table 2 and Figs. 5-7).

Table 2: Effect of local application of timolol and EGb 761, oral administration of EGb 761 and green tea, given as curative either alone or combined with local timolol, and as prophylactic treatment, on plasma MDA and whole blood GSH contents as well as aqueous humor TAC in betamethasone-induced ocular hypertension in rabbits at day 14.

Groups	MDA (nmol/ml)	GSH (mg/dl)	TAC (mmol/l)
Normal	03.515 ± 0.928	45.985 ± 2.302	1.209 ± 0.186
Ocular hypertensive (Untreated)	10.217 ± 0.856 @	25.196 ± 3.864 @	0.530 ± 0.062 @
Ocular hypertensive + timolol	09.941 ± 1.132 @	28.647 ± 1.162 @	0.595 ± 0.040 @
Ocular hypertensive + local EGb 761	08.790 ± 2.282 @	31.860 ± 1.636 @#	0.854 ± 0.149 @#
Ocular hypertensive + oral EGb 761	03.339 ± 0.381 #	34.780 ± 2.383 @#	0.822 ± 0.060 @#
Ocular hypertensive + timolol + oral EGb 761	03.311 ± 0.437 #	49.008 ± 3.511 #	1.223 ± 0.195 #
Ocular hypertensive + oral green tea	03.560 ± 0.695 #	36.279 ± 1.036 @#	0.686 ± 0.061 @#
Ocular hypertensive + timolol + oral green tea	03.537 ± 0.552 #	45.884 ± 1.711 #	1.113 ± 0.133 #
Ocular hypertensive + prophylactic oral EGb 761	03.259 ± 0.277 #	37.782 ± 1.996 @#	1.104 ± 0.133 #
Ocular hypertensive + prophylactic green tea	03.558 ± 0.168 #	35.413 ± 0.696 @#	0.812 0.067 @#

- Values are presented as means ± S.E. (n=8 animals).
- @Significant difference from normal group at P<0.05.
- #Significant difference from ocular hypertensive group at P<0.05.

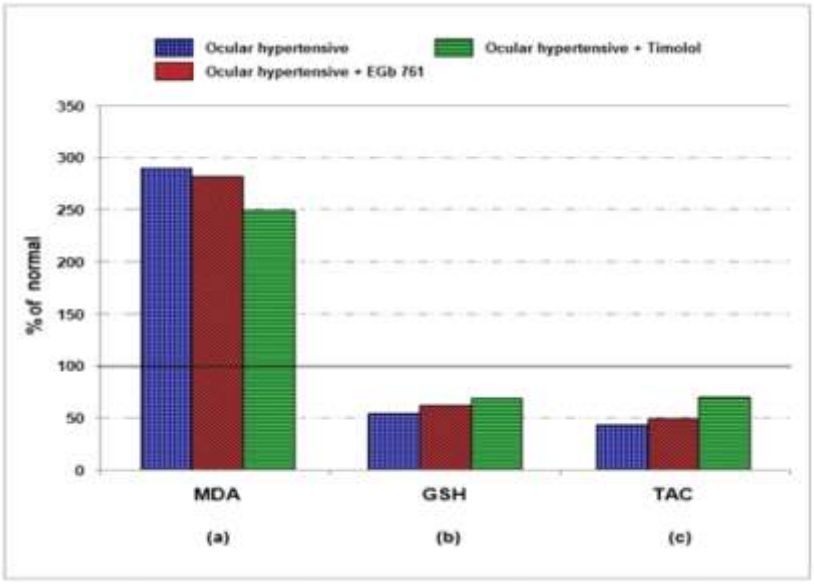

Fig. 5: Effect of local application of timolol and EGb 761 on plasma MDA (a) and whole blood GSH (b) contents as well as aqueous humor TAC (c) in experimentally-induced ocular hypertension.

-Ocular hypertension was induced in rabbits by a single subconjunctival injection of betamethasone (0.5 ml, 7 mg/ml) on day 1.
-Timolol (1 drop, 0.5%) and EGb 761 (1 drop, 0.05%) were instilled once daily starting from day 7 and continued for 7 consecutive days.
-Samples were collected on day 14.

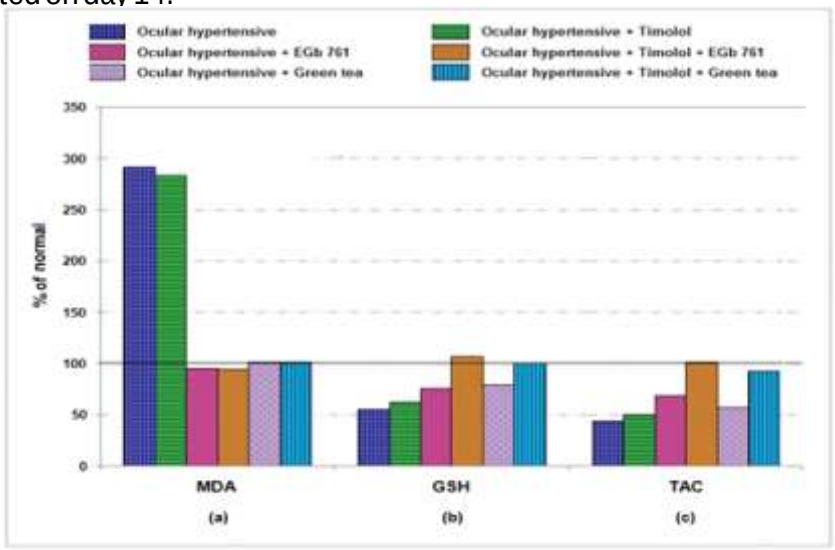

Fig. 6: Effect of oral administration of EGb 761 and green tea, given either alone or combined with local timolol, on plasma MDA (a) and whole blood GSH (b) contents as well as aqueous humor TAC (c) in experimentally-induced ocular hypertension.

- Ocular hypertension was induced in rabbits by a single subconjunctival injection of betamethasone

(0.5 ml, 7 mg/ml) on day 1.
- EGb 761 (20 mg/kg), green tea (300 mg/kg), and timolol (1 drop, 0.5%) were administered once daily starting from day 7 and continued for 7 consecutive days.
- Samples were collected on day 14.

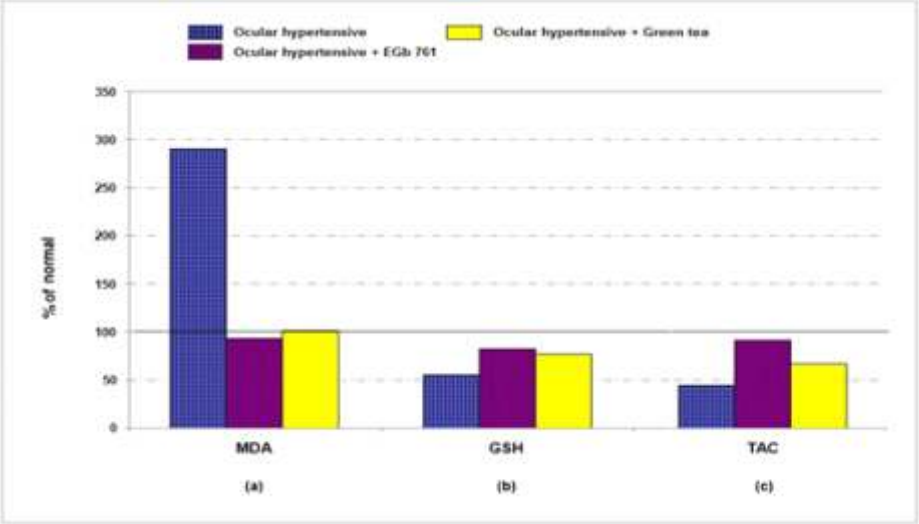

Fig. 7: Effect of oral administration of EGb 761 and green tea, given prophylactically, on plasma MDA (a) and whole blood GSH (b) contents as well as aqueous humor TAC (c) in experimentally-induced ocular hypertension.

-Ocular hypertension was induced in rabbits by a single subconjunctival injection of betamethasone (0.5 ml, 7 mg/ml) on day 1.
-EGb 761 (20 mg/kg) and green tea (300 mg/kg) were administered once daily on the same day of betamethasone injection and continued on daily basis for 14 consecutive days.
-Samples were collected on day 14.

IV. Histopathological examination of the corneal tissue

The ocular hypertension model (group II) were encountered with marked pathological changes in corneal stroma appeared in the form of severe oedema and disruption of the collagen bundles (Figure 8-b). The histopathological picture of different treated groups ranged widely. Only 1 group showed severe histopathological changes as treatment with oral green tea alone (group VI) failed to cause any improvement in the histopathological features of the corneal stroma as compared to the ocular hypertensive group (Fig. 8-g). While other groups in which treatment with timolol (group III) and oral EGb 761 (group V) causedmoderate histopathological changes (Figs. 8-c and e respectively). Treatment with local EGb 761 (group IV) and prophylactic administration of either oral EGb 761 or green tea (groups IX and X, respectively) resulted in mild stromal oedema as compared to ocular hypertensive group (Figures 8-d, i and j respectively). The corneal stroma of combined EGb 761 or green tea with timolol (groups VII and VIII, respectively) was observed to be comparable to that of the normal group (Figures 8-f and h respectively).

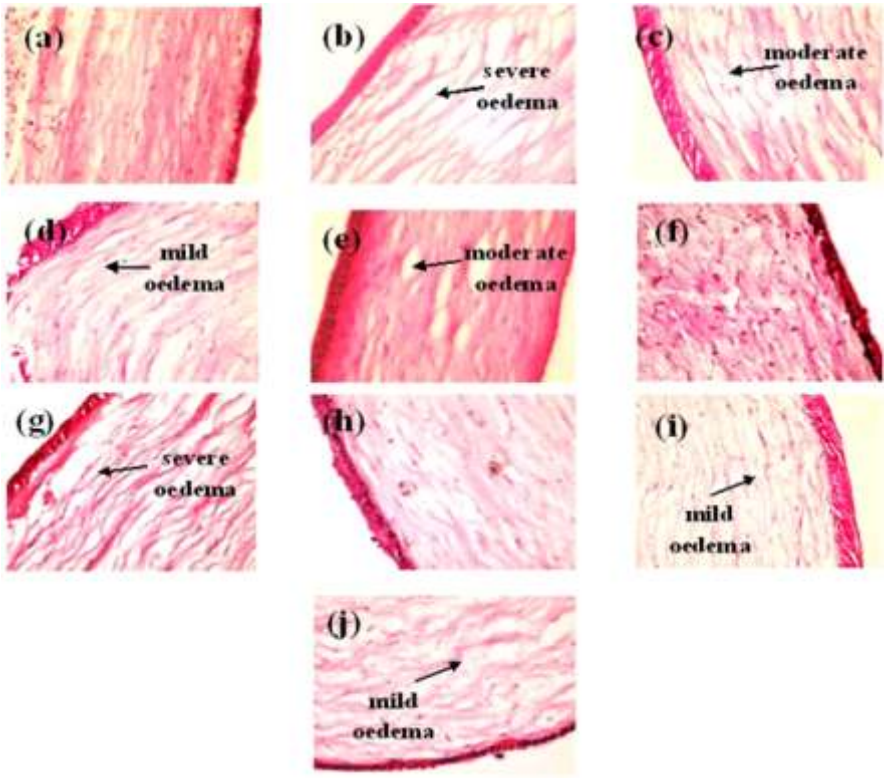

Fig. 8: Light micrograph of stained corneal sections (H&E X500) prepared from eyes of normal (a), betamethasone-induced ocular hypertensive (b), local timolol-treated ocular hypertensive (c), local EGb 761-treated ocular hypertensive (d), oral EGb 761-treated (e), oral EGb 761 combined with local timolol (f), oral green tea (g), oral green tea combined with local timolol (h),as well as rabbits prophylactically treated with oral EGb 761 (i) and oral green tea (j), given concurrently with betamethasone.

- Ocular hypertension was induced in rabbits by a single subconjunctival injection of betamethasone (0.5 ml, 7 mg/ml) on day 1.
- Timolol (1 drop, 0.5%),EGb 761 (1 drop, 0.05%), EGb 761 (20 mg/kg; p.o) and green tea (300 mg/kg; p.o) were administeredas curative treatment once daily starting from day 7 and continued for 7 consecutive days.
- EGb 761 (20 mg/kg) and green tea (300 mg/kg) were orally administered as prophylactic treatment once daily on the same day of betamethasone injection and continued on daily basis for 14 consecutive days.
- Animals were sacrificed on day 15.

DISCUSSION

Subconjunctival injections of glucocorticoids (GCs) in rabbits proved to be a viable alternative to topical application and proved a more consistent and reproducible model for the study of GC-induced ocular hypertension which may be suitable for testing short- and long-term effects of anti-glaucoma drugs. Similarly, many researchers found a more or less definite increase in IOP in experimental animals developed as a result of topical or systemic administration of GCs (Hester et al., 1987; Sawaguchiet al.,

2005; Whitlock et al., 2010). Previous clinical studies on human confirm that there is a relationship between the presence of primary open angle glaucoma and increased aqueous humor, plasma, and retinal MDA levels (Koet al., 2005; Yildirimet al., 2005) as well as decreased aqueous humor, serum, and retinal TAC (Sorkhabiet al., 2011) and plasma GSH content (Ferreira et al., 2004; Labibet al., 2010). This supports the hypothesis that betamethasone induces IOP elevation, at least in part, via an oxidative stress pathway.GC induced an increase in the expression and synthesis of extracellular matrix protein fibronectin and elastin. GCs were also found to stabilize the lysosomal membrane which causes a reduction in the degradation and depolymerization of the normally present glycosaminoglycans by hydrolases and hyaluronidase enzymes. The polymerized glycosaminoglycans may undergo hydration and produce biologic oedema and hence narrow the trabecular spaces and increase the outflow resistance (Hayasaka, 1983).Gagnon et al. (1997) found that corneal endothelial cell counts were inversely proportional to the means of IOPs in the glaucoma group. Mechanisms of cell loss may include direct damage from elevated IOP as well as alterations of endothelium in glaucoma, resulting from oxidative stress and its consequences. This is in accordance with previous speculations of stromal cell decompensation being the cause of corneal oedema. Contradictory to the current results are, however, those obtained from the study of Majzoub (1966) who investigated the influence of various GCs by instillations or subconjunctival injections on rabbit's eyes. He provided evidence that some resistance to the aqueous outflow was observed but no increase in IOP was found. He explained that the stable IOP with impairment of the outflow suggested that GC inhibited also aqueous humor production. Furthermore, other researchers experimenting on rabbits did not find any appreciable elevation in IOP under the influence of GC (Black et al., 1960). This can be attributed to the systemic administration of GC which may not cause much rise in IOP as local injection.

Timolol is the gold standard drug for glaucoma therapy, against which all new medications must be compared for their IOP-lowering ability prior to approval by the Food and Drug Administration (Marquis and Whitson, 2005). McLaughlin et al. (2001) demonstrated that in contrast to previously believed cAMP-mediated mechanism on ciliary epithelial cells, timolol acts by specifically blocking either the sodium-proton or the bicarbonate/chloride exchanger. Both of these mechanisms are considered critical in supporting the formation of aqueous humor of the eye. There were, however, few contradictory results in studies performed on albino rabbits using topical timolol (0.5%, 1% or 4.0%) where it showed no effect on IOP (Bartels et al., 1980; Boas et al., 1981; Woodward et al., 1986) but it is rather difficult to explain and reconcile with these findings. It has been documented in some early long-term studies that timolol efficacy might decrease which could be attributed to the phenomenon of "long-term drift" (Gandolfi and Vecchi, 1996). Indeed, this was in conflict with other studies which denied the development of subsensitivity to the drug (Bengtsson and Heijl, 2001).

The present study demonstrates that local EGb 761 caused suppression in the elevated IOP and increase inthe decreased GSH content and TAC in addition to improvement of the histopathological picture of the corneal tissue.However, there was no significant effect on MDA level. Oral treatment with EGb 761 to ocular hypertensive rabbits gave more or less the same pattern of response as locally administered extract. Combined treatment with EGb 761 and local timolol showed better outcomes than each separately. Prophylactic treatment with oral EGb 761 protected against the deleterious effects caused by betamethasone.

Owing to its ocular beneficial effects some authors recommended the use of EGb 761 as a supplement for ophthalmic applications particularly targeting the retina (Bartlett and Eperjesi, 2004; Gamalet al., 2011). Jiaet al. (2008) claimed that EGb 761 possesses an anti-steroid effect. In their study, they reported that EGb 761 reduced GC associated accumulation of extracellular materials within the

cribriform layers of the trabecular meshwork (TM) and achieved better TM cellularity. It has been suggested that the anti-steroid effect of EGb 761 could be mediated by downregulating GC-induced myocilin (MYOC)protein expression in TM cells.In addition to its anti-steroid effect, cholinergic and α2adrenoceptor agonistic properties might share part in the IOP-lowering effect of EGb 761. It has been reported that EGb 761 increases muscarinic cholinoceptor numbers (Taylor, 1986) and the release of acetylcholine (Kristofikovaet al., 1992) in addition to inhibiting activity of acetylcholinesterase (Das et al., 2002). It has also been proposed that EGb 761 reverses the age-related decrease in the number of a2adrenoceptors. It might thus be reasonable to assume that a adrenoceptor-induced vasoconstriction in the ciliary process could be enhanced by EGb 761 treatment leading to reduced production of aqueous humor (Huguet and Tarrade, 1992). In fact, it cannot be neglected that the IOP-lowering effect of EGb 761 can be related to its anti-oxidant activity (Szaboet al., 1997). EGb 761 was reported to readily scavenge ROS and nitrogen radicals and inhibit oxidative modifications that occur to proteins in dexamethasone-induced damage in rabbit corneal cell line (Thiagarajanet al., 2002). In close agreement with the present study, several in vitro and in vivo; studies proved the protective effect of oral administration of EGb 761 against consequences of oxidative stress damage (Seneret al., 2006; Ahmed et al,. 2009). The ginkgolides may contribute to neuro-protective properties in diseases associated with free radical generation, and the flavonoid fraction contains direct free radical scavengers(Smith et al., 1996). Bridiet al. (2001) explained that EGb 761 possesses also anti-inflammatory properties via decreasing the formation of ROS by inflammatory cells as well as scavenging NO and possibly inhibit its production (Kobuchiet al., 1997). The anti-inflammatory activity of EGb 761 should be beneficial through alleviating acute inflammatory process associated with glaucoma (Biddlestoneet al., 2007). Moreover, EGb 761 has been shown to accelerate corneal stromal wound healing in rabbits (Juarez et al., 1999) and this may in part be responsible for the improvement in the severe corneal oedema caused by betamethasone by both topical and oral EGb 761 in the present study.

The current study showed that green tea elicited significant decrease in the elevated IOP, improved the measured biochemical parameters but failed to cause any improvement in the histopathological features of the corneal stroma. The aforementioned beneficial effects were more prominent when green tea was given prophylactically as evidenced by the earlier ocular hypotensive activity and significant improvement in the histopathological picture of the cornea. Combined treatment of ocular hypertensive rabbits with green tea and local timolol gave, also, better results than each separately.

Supporting the rationale of using green tea in glaucoma, in vitro and in vivo studies in human and experimental animals documented the beneficial effects of green tea as anti-oxidant and anti-inflammatory in multiple ocular cell types (Cavetet al., 2011; Lee et al., 2011). These properties are mainly attributed to the green tea constituent, EGCG (Saffari and Sadrzadeh, 2004; Nagle et al., 2006). Chu et al. (2010) found that catechins, particularly EGCG and epigallocatechin, were selectively absorbed into the different eye components. The first documented study of how various eye tissues absorb catechins, raises the possibility that green tea may protect against glaucoma and other common eye diseases. Two possible mechanisms are postulated to explain the IOP-lowering effect of green tea. The first one is its effect on PGI2 cascade and the second one is its acetylcholinesterase inhibitory activity. It was proposed that catechins found in green tea increase PGI2 synthesis in vivo (Rhee et al., 2002) and cause its accumulation in endothelial cell culture and isolated blood vessels (Tonioloet al., 2013). The inhibition of acetylcholinesterase enzyme by green tea was previously reported in some studies (Jo et al., 2012; Okelloet al., 2012). The vasodilatory (Alvarez et al., 2006) and neuro-protective

effects of green tea contribute also to its beneficial ocular hypotensive activity (Rhone and Basu, 2008). Another proposed mechanism is that green tea reduces blood pressure (BP) before reducing IOP. Negishiet al. (2004) explained how green tea polyphenols attenuate BP through their anti-oxidant properties. Lowering of BP decreases the hydrostatic pressure for aqueous humor formation from plasma in the ciliary process capillary network. Moreover, a study conducted by Liu et al. (2007), postulated that oxidative stress is an early event in hydrostatic pressure/IOP-induced neuronal damage and hence green tea as an anti-oxidant could have a role in controlling glaucomatous damage.

Conclusively, administration of local and oral EGb 761; curative and prophylactic showed potential beneficial effects on betamethasone-induced a) rise in IOP, b) oxidative biomarkers alterations, and c) the pathological changes of the cornea. Moreover, the oral administration of green tea both curative and prophylactic treatments gave also beneficial effects on the betamethasone-induced a) elevation in IOP as well as b) oxidative biomarkers alterations c) corneal histopathological changes (prophylactic). Both extracts offer an advantage over timolol alone in treating normal tension glaucoma. In fact, among all the experimental groups used, it has been demonstrated that the combined therapy of oral EGb 761 and local timolol gave the best results. In the current study, it was also evident that prophylactic administration of both extracts gave better outcome regarding protection against the deleterious effects caused by betamethasone and the beneficial effects were more prominent. One can conclude that EGb 761 and green tea offer desirable ocular hypotensive, anti-oxidant, and cyto-protective activities that could result in better management of glaucoma and GC-induced ocular hypertension.

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