European Review for Medical and Pharmacological Sciences 2013; 17: 1117-1122

Treatment of glaucomatous patients by means of food supplement to reduce the ocular discomfort: a double blind randomized trial

M. NEBBIOSO[1], D. RUSCIANO[2], B. PUCCI[3], A.M. ZICARI[4], R. GRENGA[1], N. PESCOCOLIDO[5]

[1]Department of Sense Organs, Sapienza University of Rome, Rome, Italy
[2]Sooft SpA, Rome, Italy
[3]Department of Pathology, IRCCS S. Raffaele Pisana, Rome, Italy
[4]Department of Pediatrics, Sapienza University of Rome, Rome, Italy
[5]Department of Cardiovascular, Respiratory, Nephrologic, Anesthetic, and Geriatric Sciences, Sapienza University of Rome, Rome, Italy

Abstract. – BACKGROUND AND AIM: Chronic use of multi-dose eye drops containing preservatives, such as it may happen in patients affected by primary open angle glaucoma, often results in a damage of the ocular surface due to the inherent toxicity of preservatives, that with time may lead to a lacrimal dysfunction syndrome and eye dryness.

PATIENTS AND METHODS: This double blind, randomized, pilot study was conducted on 38 glaucomatous patients suffering from dry eye induced by long-term use of eye drops preserved with BAK.

RESULTS: Treatment of these patients with a food supplement containing an association of forskolin, rutin and vitamins B1 and B2 for 30 days increased significantly their OPI values and improved the symptoms of dry eye with respect to a placebo-treated control group.

CONCLUSIONS: The association of forskolin, rutin and vitamins B1 and B2 appears to be protective for the ocular surface, contributing to restore a normal equilibrium of the tear film in those subjects in which toxic agents such as BAK had determined alterations of its homeostasis.

Key Words:
Benzalkonium chloride, Dry eye, Forskolin, Glaucoma, Ocular performance index, Rutin.

Abbreviations

BAK = benzalkonium chloride
OPI = ocular performance index
TrkB = tropomyosin-related kinase B
POAG = primary open angle glaucoma
EDTRS = Early Treatment of Diabetic Retinopathy Study
OSDI = ocular surface disease index
FBUT = break up time with fluorescein

Introduction

The ocular surface system is composed by corneal epithelium, conjunctiva, primary and accessory lacrimal glands, nasolacrimal duct, meibomian glands and their apical and basal matrix. All ocular surface system components are functionally and physically integrated by the nervous, endocrine and immune systems. The tear film plays an important role in maintaining ocular surface integrity. It prevents corneal epithelium dehydration, provides a uniform refractive surface to the cornea, protects the ocular surface from infections and supplies oxygen to the tissue.

The lacrimal dysfunction syndrome has been defined by the DEWS (Dry Eye Workshop, 2007) as a multifactorial disease of tears and ocular surface that results in symptoms of discomfort, visual disturbance and tear film instability, with potential damage to the ocular surface, accompanied by increased osmolarity of the tear film and inflammation of the ocular surface[1-4].

Lacrimal hyper-osmolarity (> 316 mOsm/L) may damage the epithelium by activating a cascade of inflammatory events on the ocular surface and by promoting the release of inflammatory mediators in the lacrimal secretion. These events may trigger apoptosis of epithelial and goblet cells resulting in alteration of mucin production, further increase of tear film osmolarity, and a lacrimal dysfunction syndrome[5].

Tear film instability can be induced by pathologies such as xerophthalmia, ocular allergy, prolonged use of topic preservatives and contact lenses. The epithelial damage induced by eye dryness may harm the corneal nervous terminals, which in

Corresponding Author: Marcella Nebbioso, MD; e-mail: marcella.nebbioso@uniroma1.it

turn may cause ocular discomfort, an increase in blinking frequency and an increase of reflex lacrimal secretion[6]. The decrease of natural mucins produced by goblet cells on the ocular surface increases the friction resistance between eyelid and eyeball, thus concurring to worsen the symptoms.

Glaucoma patients represent a subgroup of patients suffering from ocular discomfort. In these patients, symptoms are caused by the presence of preservatives, such as BAK, in antihypertensive eye drops used for long term therapy. BAK reduces the stability of the tear film by acting as a detergent on the lipid layer, by reducing the number of mucin secreting goblet cells, thus altering mucin presence and distribution over the ocular surface epithelium[7,8]. The consequences of BAK detergent effects on the tear film are increased tear evaporation and eye dryness. An existing lacrimal dysfunction tends to worsen in the presence of BAK[8]. The irritating effects of BAK depend on dosage and times of administration, on lacrimal secretion levels and on the severity of ocular surface disease. Such irritating effect reaches a critical threshold when several different eye drops are used at the same time as it often happens in glaucoma patients that make a chronic use of these drugs[9,10]. Moreover, in antiglaucoma treatments based on preservative-containing eye drops, patients local intolerance symptoms are from 2 to 3 times more frequent than in preservative-free therapies[10].

The current therapy for treating lacrimal dysfunction syndrome is based on lifestyle modification, a regular eyelid hygiene, and the use of artificial tears as lubricants and oral food supplements containing omega-3 fatty acids and phytoestrogens[7,11-13]. Although the most recent artificial tears formulations are similar to human tears in electrolytes composition, they do not contain most of the proteins that are normally present in natural tears and that are essential for eye protection[12,13]. The stimulation of lacrimal secretion is considered a further therapeutic strategy to decrease tear film osmolarity and alleviate the discomfort caused by eye dryness[11].

The treatment goals for lacrimal dysfunction syndrome are to improve ocular comfort, patient quality of life, and to reestablish the normal homeostatic balance of ocular surface and tear film.

Forskolin is a natural product obtained from the roots of the plant *Plectranthus barbatus*, commonly known as *Coleus forskohlii*. Forskolin is a receptor-independent activator of the enzyme adenylate cyclase (AC) that is responsible for cAMP synthesis, so that its concentration is increased in forskolin-stimulated cells. Such cAMP increase in eye tissues is known to induce conjunctiva accessory lacrimal gland secretion in rats[14]. Moreover, cAMP may also activate transcriptional factors transport to the nucleus, stimulating gene expression[14,15]. In fact, forskolin may stimulate neurotrophin (BDNF) production by astrocytes[15] and endothelial cells[16], and the expression of TrkB (BDNF receptor) by retinal ganglion cells[17].

For this reason, and because of its ability to contribute to IOP normalization[16,17], forskolin, in association with rutin and vitamins B1 and B2, is given as a food supplement to POAG patients. Rutin is a plant flavonoid with haemorheological properties[18], that may improve capillary circulation and tissue metabolism, including tissues of the eye and the lacrimal gland system[19,20].

During our daily clinical practice we noticed that our POAG patients that were under pharmacological treatment with preserved antiglaucoma eye drops, and were also taking the food supplement with forskolin and rutin, were less prone to develop symptoms related to lacrimal dysfunction. Therefore, considering that there are no clinical data in the literature on the effects of forskolin and rutin on lacrimal secretion, we set out to perform a randomized double blind pilot study with the aim to evaluate forskolin/rutin effects on patients affected by lacrimal dysfunction syndrome associated to the long-term use of anti-glaucoma eye drops containing BAK as preservative

Patients and Methods

The design of this study is randomized, double blind. It has been carried out in the Department of Ophthalmology, Policlinico Umberto I, I Faculty of Medicine and Dentistry, Sapienza University of Rome. The study was conducted in full accordance with the Declaration of Helsinki. All subjects were informed and consented to the use of their data in this study.

Patients

Thirty-eight adult patients (average age was 60 ±12 years) affected by primary open-angle glaucoma (POAG) were included[21], who suffered from lacrimal dysfunction syndrome associated with chronic use (> 6 months) of benzalkonium chloride preserved eye drops.

Patients were excluded if they had: presence of systemic (i.e. collagenopathies, cardiovascular diseases, diabetes mellitus, bronchopneu-

mopathies) or ocular (rosacea, Sjogren syndrome, chalazion, trachoma, viral or bacterial keratitis) diseases, which interfere with the ocular surface integrity; presence of an absolute central perimetric defect; filtration surgery or other ocular surgeries in the previous six months.

Enrolled patients were randomly divided in two groups, using the algorithm on the web site randomization.com. Group 1 patients were given an oral placebo supplement, containing only the excipients of the real food supplement that was given to patients of group 2.

Methods

In order to establish the severity level of ocular discomfort, patients were asked to fill up an o*cular surface disease index* (OSDI) validated questionnaire: all patients answered all the 12 questions, and were assigned an OSDI index from 0 to 100. Moreover, patients performed a series of tests in the following order: OPI, FBUT and Schirmer test 1. The subjects were then randomly divided in 2 groups: group 1 (placebo treatment) and group 2 (forskolin/rutin treatment). Tests were repeated after 30 days of treatment. When the FBUT is shorter than the interblinking interval (IBI) (OPI < 1), the eyes are exposed to focal ocular surface damage risk.

OPI is a test that quantifies the risk of ocular surface damage, and is calculated by dividing the FBUT by the IBI. IBI is the number of blinkings per minute while the patient reads an EDTRS table. This test allows to estimate the amount of time during which the ocular surface is exposed to atmospheric agents. FBUT evaluates tear film instability. After a complete blinking, as soon as the superior eyelid has completed its upward excursion, the tear film becomes thinner and finally breaks up. This breaking of the tear film generates dry spots randomly distributed. This phenomenon can be quantified as "tear film break-up time" with fluorescein (FBUT). The breaking time is measured after the instillation of fluorescein as the interval that goes from the last blinking and the appearance of the first dry spot, and measures tear film stability. A yellow barrier filter (Kodak Wratten 12) is used to enhance the visibility of fluorescent tear film breaking. FBUT cut-off to diagnose the lacrimal dysfunction syndrome is < 10 seconds. Recently, cut-off values among ≤ 5 and < 10 seconds have been adopted if small quantities of fluorescein are instillated (5 μl at 2%).

The Schirmer test 1 (without anesthesia) measures the lacrimal efflux stimulated as reflex by the insertion of an absorbent paper strip in the conjunctival sac. The strip is inserted at the medial third and lateral third of the lower lid. The patient is asked to keep the eyes shut and after 5 minutes the result can be read. The measure of the lacrimal secretion comes from the length of the portion of the wet strip. Normal values are higher than 15 mm/5', values lower or equal to 5 mm/5' indicate a clear lacrimal hypo-secretion.

The forskolin/rutin association was administrated to group 2 patients as oral supplement (2 capsules for day) for 30 days. The oral supplement contained an extract of Coleus Forskolii (150 mg titrated at 10% in forskolin: 15 mg of forskolin), rutin (200 mg), vitamin B1 (0.7 mg), vitamin B2 (0.8 mg) (Kronek®, SOOFT Italia, Montegiorgio, Italy). A placebo containing only the excipients of Kronek® was given to group 1 patients.

OSDI, FBUT, OPI and Schirmer test 1 results were calculated as average values at time 0 and after 30 days in groups 1 and 2.

Statistical Analysis

Sample size was defined based on an expectation of 10% improvement in the treated group, with 10% confidence intervals, a power of 80 and an alpha of 5. Under these conditions at least 24 patients divided in two groups should be evaluated. We enrolled 38 patients, to be sure that at the end of the observation period at least 24 would complete the study. In fact, all 38 could be evaluated throughout the whole study.

The Student's *t* test was used to compare the distribution of values between the two groups.

The analysis of variance (ANOVA) was used to compare values obtained at different times within the same group. Results were considered significantly different when $p < 0.05$.

Results

Figure 1 graphically shows the evolution of the four endpoints considered in this study. In panel A data registered at the beginning of the study are depicted, indicating that the two groups had similar enrolment values. Panel B shows the changing of the same four parameters after 30 days of either placebo or forskolin/rutin treatment. It is evident that while data did not change in the placebo control group, there was a significant improvement in the group receiving the real food supplement treatment.

Tables I and II report the actual values of the different tests respectively performed on control (placebo: group 1) and treated (group 2:

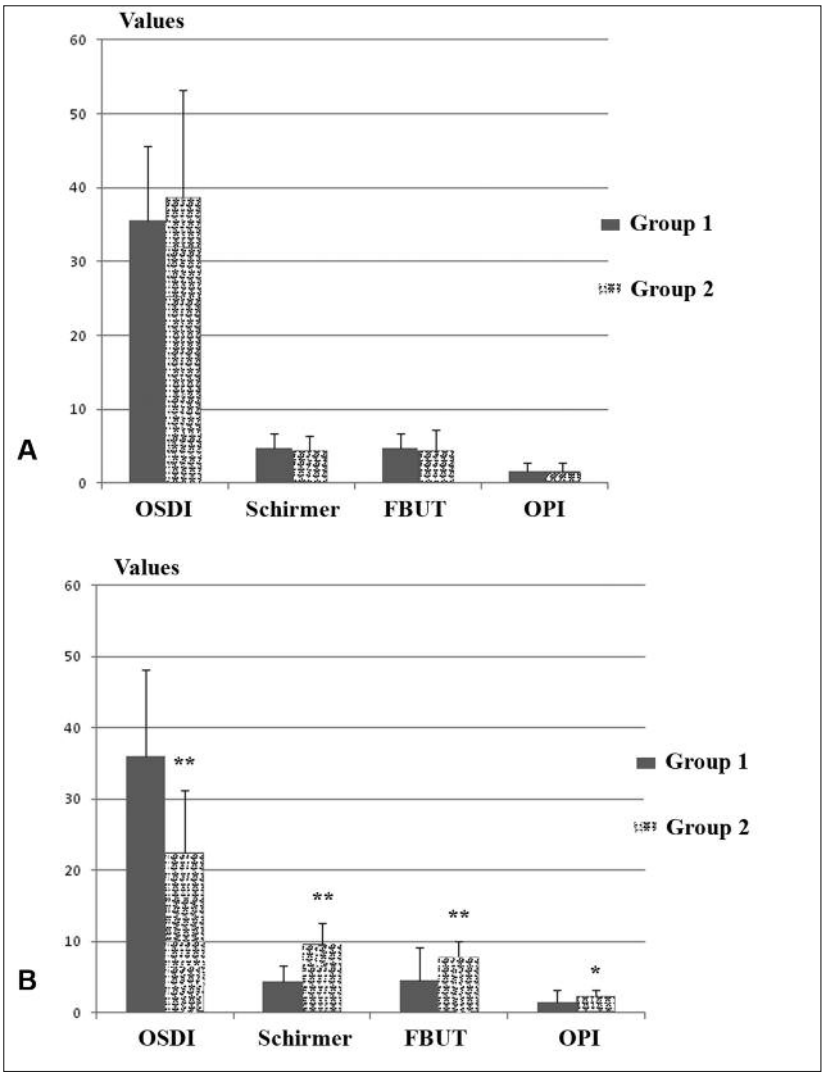

Figure 1. ***A,*** Average enrolment values ± standard deviation (SD) of patients in Group 1 (placebo) and Group 2 (forskolin/rutin). ***B,*** Average values ± standard deviation (SD) of patients in Group 1 (placebo) and group 2 (forskolin/rutin) after 30 days of treatment. Values: OSDI = ocular surface disease index questionnaire (score); Schirmer's test (mm/5 min); FBUT = break up time with fluorescein (seconds); OPI = ocular performance index (FBUT/IBI ratio). IBI: inter-blinking interval. *$p < 0.05$; **$p < 0.01$ by Student's *t* test.

forskolin/rutin) patients. All patients at enrolment had moderate to severe ocular surface discomfort: OSDI index ranged between 18 and 65 for patients in the control group, and between 22 and 65 for patients in the treatment group. Schirmer's test values ranged between 1 and 9 and 1 and 8 mm respectively for patients in the control and treatment groups. FBUT analysis showed values in the range 2-9 seconds in the placebo group and 1-10 seconds in the treatment group. Finally, the OPI score varied from 0.3 to 4.4 in the control group and from 0 to 4.4 in the treatment group. The two groups were statistically equivalent for each considered parameter.

Table I shows that after 30 days of placebo treatment no statistically significant variations in any of the measured parameters were evident: any measured change was well below 10% of the average value.

Table II reports the values of the same parameters at enrolment and after 30 days of treatment with the food supplement containing forskolin/rutin: in this case all parameters were significantly changed after treatment. We observed a 42% decrease of the OSDI score (from 38.7 to 22.5); a more than a doubling of the Schirmer's test values (from 4.4 to 9.7 mm); a 75% increase of the FBUT (from 4.5 to 7.9 seconds), and a 43% increase of the OPI score (from 1.6 to 2.3). FBUT and OPI increments indicate a greater stability of the tear film. This, in turn, decreases the risk of corneal damage, and improves patient's comfort as indicated by the decreasing OSDI score. The increase of Schirmer's values indicate a more efficient tear secretion, which should help in decreasing tears' osmolarity.

Discussion

Table I. Group 1 (placebo-treated patients) average values ± standard deviation (SD) evaluated for the indicated parameters at enrolment (T0) and after 1 month (T1) of treatment.

	Tests	T0	T1
OSDI score	Average ± SD	35.6 ± 10.1	36 ± 12.1
	Range	18-65	15-65
Schirmer's test mm/5 min	Average ± SD	4.7 ± 2.1	4.5 ± 2.2
	Range	1-9	1-10
FBUT sec.	Average ± SD	4.7 ± 2	4.6 ± 2.2
	Range	2-9	1-9
OPI	Average ± SD	1.7 ± 1.1	1.6 ± 1.1
Ratio	range	0.3-4.4	0.3-4

OSDI: ocular surface disease index questionnaire; FBUT: break up time with fluorescein; OPI: ocular performance index.

Results of this pilot study clearly indicate that treatment of POAG patients with a food supplement containing forskolin and rutin is able to blunt their ocular discomfort and dryness due to the chronic use of preserved eye drops.

Looking at the range of values before and after real treatment (Table II), it is evident that there was a generalized improvement of patients, with the lower values of the range always improving after treatment. For the OSDI score and the Schirmer's test also the upper values of the range resulted improved after treatment, whereas they remained stable for FBUT and OPI, suggesting that there was a higher improvement in the quantity (Schirmer's) than the quality (FBUT) of tears. This is coherent with the supposed action mechanism of forskolin, which – further to AC activation and stimulation of cAMP synthesis – is known to improve epithelial secretion. In fact, three separate cellular pathways are known to stimulate lacrimal gland fluid secretion, while only one inhibits its production[6,14,22,23]. In the first pathway, cAMP-dependent agonists activate the lacrimal gland by binding to receptors in the basolateral membrane of acinar cells[14]. The interaction ligand-receptor on acinar cells activates a G protein that increases the adenyl cyclase activity and consequently the intracellular cAMP synthesis from ATP. cAMP activates protein kinase A by phosphorylation, inducing protein secretion and ion channel activation[14,17,22]. In the second pathway, Ca^{2+}/diacylglycerol-dependent agonists (cholinergic agonists) stimulate acinar cells by interacting with muscarinic M3 receptors on the basolateral membrane of acinar cells. Finally, the third transduction pathway is activated by diacylglycerol (DAG)-dependent agonists (α1-adrenergic agonists) that activate acinar cells by interacting with α1-adrenergic G receptors[15].

Therefore, it is likely that forskolin improved quality and stability of the tear film through the activation of AC, which in turn increases synthesis and concentration of intracellular cAMP. This cyclic AMP may on one hand activate ion channels that increase electrolyte and water secretion from lacrimal glands, and on the other hand activate transcription factors that result in

Table II. Group 2 (forskolin/rutin-treated patients) average values ± standard deviation (SD) evaluated for the indicated parameters at enrolment (T0) and after 1 month (T1) of treatment.

	Tests	T0	T1
OSDI score	Average ± SD	38.7 ± 14.5	22.5 ± 8.7**
	Range	22-65	7-48
Schirmer's test mm/5 min	Average ± SD	4.4 ± 2	9.7 ± 2.9**
	Range	1-8	4-16
FBUT sec.	Average ± SD	4.5 ± 2.7	7.9 ± 2.1**
	Range	1-10	4-10
OPI ratio	Average ± SD	1.6 ± 1.2	2.3 ± 0.9*
	Range	0-4.4	0.9-4.4

OSDI: ocular surface disease index questionnaire; FBUT: break up time with fluorescein; OPI: ocular performance index. *$p < 0.05$ **$p < 0.01$ by ANOVA.

an enhanced protein synthesis and exocytosis into tear fluids.

Conclusions

This pilot study indicated that a forskolin/rutin oral supplement improved the stability and the quality of the tear film and induced an increase of lacrimal secretion when given for 30 days to POAG patients suffering from iatrogenic lacrimal dysfunction syndrome. This improvement of the iatrogenic ocular surface disease may also contribute to increase patients compliance during their pharmacologic treatment, beside giving a contribution to disease management thanks to the hypotonising and neuroprotective effects of forskolin.

Acknowledgements

The authors: M. N., B. P., A. M. Z., R. G., and N. P., have no proprietary interest in any materials or methods described within this article. D. R. is a full time employe of Sooft Italia S.p.A., the producer of the food supplement used in this investigation.

References

1) No authors listed. The definition and classification of dry eye disease: report of the definition and classification subcommittee of the international dry eye workshop. Ocul Surf 2007; 5: 75-92.
2) Goto E, Yagi Y, MatsumotoY, Tsubota K. Impaired functional visual acuity of dry eye patients. Am J Ophthalmol 2002; 133: 181-186.
3) Begley CG, Chalmers RL, Abetz L, Venkataraman K, Mertzanis P, Caffery BA, Snyder C, Edrington T, Nelson D, Simpson T. The relationship between habitual patient-reported symptoms and clinical signs among patients with dry eye of varying severity. Invest Ophthalmol Vis Sci 2003; 44: 4753-4761.
4) Tomlinson A, Khanal S, Ramaesh K, Diaper C, McFadyen A. Tear film osmolarity: determination of a referent for dry eye diagnosis. Invest Ophthalmol Vis Sci 2006; 47: 4309-4315.
5) Stevenson W, Chauhan SK, Dana R. Dry eye disease: an immune-mediated ocular surface disorder. Arch Ophthalmol 2012; 130: 90-100.
6) Nikkinen A, Lethosalo JI, Uusital H. The lacrimal glands of the rat and guinea pig are innervated by nerve fibers containing immunoreactivities for substance P and vasoactive intestinal peptide. Histochemistry 1984; 81: 23-27.
7) Nebbioso M, Evangelista M, Librando A, Di Blasio D, Pescosolido N. Fixed topical combinations in glaucomatous patients and ocular discomfort. Expert Opin Pharmacother 2012; 13: 1829-1835.
8) Baudouin C. The vicious circle in dry eye syndrome: a mechanistic approach. J Fr Ophtalmol 2007; 30: 239-246.
9) Baudouin C, Pisella PJ, Fillacier K, Goldschild M, Becquet F, De Saint Jean M, Béchetoille A. Ocular surface inflammatory changes induced by topical antiglaucoma drugs: human and animal studies. Ophthalmology 1999; 106: 556-563.
10) Pisella PJ, Pouliquen P, Baudouin C. Prevalence of ocular symptoms and signs with preserved and preservative free glaucoma medication. Br J Ophthalmol 2002; 86: 418-423.
11) Nebbioso M, Librando A, Plateroti AM, Pescosolido N. Iatrogenic dry eye disease: an eledoisin/carnitine and osmolyte drops study. Biomed Pharmacother 2013. In press.
12) Gilbard JP. Human tear film electrolyte concentrations in health and dry eye disease. Int Ophthalmol Clin 1994; 34: 27-36.
13) Gilbard JP, Rossi SR, Heyda KG. Ophthalmic solutions, the ocular surface, and a unique therapeutic artificial tear formulation. Am J Ophthalmol 1989; 107: 348-355.
14) Jahn R, Padel U, Porsch PH, Soling HD. Adrenocorticotropic hormone and alfa-melanocyte-stimulating hormone induce secretion and protein phosphorylation in the rat lacrimal gland activation of a cAMP-dependent pathway. Eur J Biochem 1982; 126: 623.
15) Juric DM, Loncar D, Carman-Krzan M. Noradrenergic stimulation of BDNF synthesis in astrocytes: mediation via alpha1-and beta1/beta2-adrenergic receptors. Neurochem Int 2008; 52: 297-306.
16) Nakahashi T, Fujimura H, Altar CA, Li J, Kambayashi J, Tandon NN, Sun B. Vascular endothelial cells synthesize and secrete brain-derived neurotrophic factor. FEBS Lett 2000: 24; 470: 113-117.
17) Meyer-Franke A, Wilkinson GA, Kruttgen A, Hu M, Munro E, Hanson MG Jr, Reichardt LF, Barres BA. Depolarization and cAMP elevation rapidly recruit TrkB to the plasma membrane of CNS neurons. Neuron 1998; 21: 681-693.
18) Vetrugno M, Uva MG, Russo V, Iester M, Ciancaglini M, Brusini P, Centofanti M, Rossetti LM. Oral administration of forskolin and rutin contributes to intraocular pressure control in primary open angle glaucoma patients under maximum tolerated medical therapy. J Ocul Pharmacol Ther 2012; 28: 536-541.
19) Nebbioso M, Belcaro G, Librando A, Rusciano D, Steigerwalt RD Jr, Pescosolido N. Forskolin and rutin prevent intraocular pressure spikes after Nd:YAG laser iridotomy. Panminerva Med 2012; 54(1 Suppl 4): 77-82.
20) Park YH, Chiou GC. Structure-activity relationship (SAR) between some natural flavonoids and ocular blood flow in the rabbit. J Ocul Pharmacol Ther 2004; 20: 35-42.
21) Nebbioso M, Gregorio FD, Prencipe L, Pecorella I. Psychophysical and electrophysiological testing in ocular hypertension. Optom Vis Sci 2011; 88: E928-939.
22) Dartt DA. Signal trasduction and control of lacrimal gland protein secretion: a review. Curr Eye Res 8: 619-636.