Clinical Nutrition Experimental 27 (2019) 21–28

Contents lists available at ScienceDirect

Clinical Nutrition Experimental

journal homepage: http://www.clinicalnutritionexperimental.com

The effects of genistein on estrogen receptor-β, IL-1β levels, and MUC5AC expression in ovariectomized rats with dry eye

Rosy Aldina [a, *], Hidayat Sujuti [a], Nur Permatasari [b], Mohammad Aris Widodo [b]

[a] *Department of Ophthalmology, Saiful Anwar General Hospital, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia*

[b] *Laboratorium of Pharmacology, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia*

ARTICLE INFO

Article history:
Received 30 June 2017
Accepted 25 December 2017
Available online 30 December 2017

Keywords:
Dry eye syndrome
Inflammation
Mucin
Estrogen
Eye drops

SUMMARY

The purpose of this study was to investigate the effects of genistein on estrogen receptor-β (ER-β) expression, IL-1β expression, and MUC5AC expression in post-menopausal dry eye syndrome model rat. A total of thirty female rats were divided into five study groups, consisting of control group (without any treatment), the dry-eye rat model group, the dry-eye rat model treated with various doses of topical genistein (50 μM, 100 μM, and 200 μM). The ER-β, IL-1β, and MUC-5AC expressions were evaluated by immunofluorescence (confocal laser scanning microscopy). The ER-β expression decreased significantly in the dry eye model group compared with the control group ($p < 0.05$), this decrease could be inhibited by all doses of genistein, but not yet achieved an expression comparable to the control group ($p < 0.05$). MUC5AC expression was significantly lower in the dry eye model compared with the control group ($p < 0.05$), this reduction could be increased by all groups treated with genistein, achieving values comparable to the control group for the groups treated with genistein of second and third doses ($p > 0.05$). The IL-1β levels in the dry eye model group were significantly higher than the control group ($p < 0.05$). This increase can be decreased by the administration of third-dose genistein. It was concluded that genistein can suppress IL-1β levels production and may trigger upregulation of ER-β and MUC5AC expression in the dry eye model due to ovariectomy.

* Corresponding author. Department of Ophthalmology, Saiful Anwar General Hospital, Faculty of Medicine, Brawijaya University, Jalan Jaksa Agung Suprapto No. 2, Klojen, Malang, East Java, 65112, Indonesia.

E-mail address: rosyaldina@yahoo.com (R. Aldina).

https://doi.org/10.1016/j.yclnex.2017.12.003

Thus, genistein may become an alternative to suppress inflammation and mucin production in dry eye syndrome.

1. Introduction

Dry eye syndrome (DES) is defined as a multifactorial disease of tears and ocular surface that may result in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface, increasing tear films osmolarity and ocular surface inflammation [1]. Until now there has been no definitive treatment for this disorder, even it is still the main cause of a patient visiting an ophthalmologist [2].

It is estimated that approximately 3.2 million women in the United States suffer from dry eye disease [3]. Hormonal status is one of the causes of dry eye disease [4], although it is still controversial. In menopausal women, there is an increased expression and secretion of proinflammatory cytokines, i.e. IL-1, IL-6, and TNF-α. Estrogen deficiency increases the cell response to some cytokines by increasing the number of cytokine receptors [5,6]. Conjunctival inflammation occurs in more than 80% of patients with dry eye syndrome. Conjunctival epithelium is a source of IL-1β involved in the pathogenesis of dry eye syndrome [7–9]. IL-1β proinflammatory cytokines increase in patients with dry eye syndrome and are fundamental in the acute phase proteins synthesis and are produced in large quantities [10]. Inflammation is the key cause and consequence of ocular surface injuries and dryness, which will trigger epithelial apoptosis as well as squamous metaplasia and apoptosis. Severe or chronic inflammation also decreases the number of goblet cells [11–14]. In dry eye syndrome there is also a change in the quantity and biochemical composition of the mucin layer, so that the average concentration of goblet cells secreting MUC5AC soluble musin decreases. The loss of goblet cells, which is a feature of dry eye syndrome, is consistent with a decrease in MUC5AC mucin gel [15,16].

Genistein is an isoflavone as an active ingredient of soybeans. The molecular weight of genistein is 270.24 g/mol [17]. Genistein has a structural similarity with 17β-estradiol and can bind to the estrogen receptor, with a higher affinity to ER-β than ER-α [18,19]. Genistein is a potentially beneficial compound for health. Its pharmacological actions include phytoestrogens, antioxidants, and can reduce symptoms in menopausal women [20]. Isoflavones are also anti-inflammatory and act as estrogens [21]. Previous studies, which have applied daidzine to dry eye disease, found that these compounds are able to protect the cornea through anti-inflammatory effects and suppression of oxidative stress [22]. Other researchers applied the genisten in suppressing IL-1β in dry-eye model rat due to castration [23]. Until now, to the best knowledge of the researcher, there have been no studies that evaluate the effects of genistein on post-menopausal dry eye syndrome. Therefore, this study aimed at investigating the effects of genistein on expression of estrogen receptor-β (ER-β), IL-1β expression, and MUC5AC expression in the dry eye syndrome model due to ovariectomy.

2. Material and methods

2.1. Animals

There were five rats groups (totally thirty rats, $n = 6$ rats each) i.e. the control group (female rats without any treatment), the dry eye model rat group, dry eye rat model group treated with the 50 μM genistein eye drops, the dry eye rat model group treated with 100 μM genistein eye drops, and the dry eye rat model group treated with 200 μM genistein eye drops. The administration of genistein was performed for a week.

2.2. Dry rat eye model due to ovariectomy

To perform ovariectomy, female strain Wistars were anesthetized using a 0.2 cc ketamine intramuscularly. The lower abdominal hair was shaved and the skin was cleaned with betadine and alcohol

70%. An 1–2 cm paralumbar lateral skin incision and a muscular incision underneath were made. Both ovaries were tied and cut, then treated with betadine. The skin and muscles were sewn apart. In the first three days after ovariectomy, the rats were intramuscularly treated with 60–80 mg/kg gentamicin daily. This procedure aims to remove prolactin secretion and cause a significant atrophy of the lacrimal gland with deficient tear production [24,25].

Schirmer test, a Ferning test, and a FBUT test were performed to determine DES model. The Schirmer test aims for assessing tear secretion, it is perform without topical anesthesia by placing a standardized folded Whatman filter paper strip over the lid margin at the junction of the medial and lateral third of the lower lid. Aqueous tear production was measured by the millimeters wetted during the test period, usually 5 min. A cutoff value of 5,5 mm was wetted in 5 min for the schirmer test diagnosed aqueous tear deficiency in 83% of DES [26].

The Ferning test was performed to evaluate the tear quality. The tear samples were collected with glass capillary and dropped on microscopy slide, and allowed to dry by evaporation at room temperature within 5–7 min, observed with light microscope type Olympus E 330-ADU12X, Japan, with 40–100$\times$ of magnification. The interpretations are (I) ferning shows uniform structures without spaces among the ferns; (II) small spaces begin to appear; (III) large spaces with poorer branches; (IV) ferning phenomenon is absent; Intermediate I/II, II/III, III/IV when the ferning morphology changed in different areas; and Abnormal value: $\geq$ type II/III [27].

Tear film stability is measured by the fluorescein tear break-up time (FBUT) Test. It is performed by placing fluorescein in the lower conjunctival sac using a fluorescein–impregnated strip wetted with non preserved saline solution. Break-up is best observed with the use of a blue exciter and yellow barrier filter. The break-up time is the time the elapses from the last blink to the first appearance of a dark spot in the fluorescein-stained film. In general, a break–up time of <10 s suggests an unstable tear film. Tear break-up time is reduced in DES [28].

2.3. Genistein preparation

Genistein 10 mg (MP Biomedicals) was dissolved in a sterile aquadest to obtain a stock solution of 1 mg/ml. The stock solution was stored at 0 °C. The stock solution would be diluted again using the sterile aquadest to the desired dose. The pipettes used in this study were 20 drops for each milliliter. Each treatment group was treated with genistein according to the prescribed doses (50 μM; 100 μM; and 200 μM) at 4 $\times$ 1 drops per day for 1 week. DES group was treated with aquadest at 4 $\times$ 1 drops per day for the week. For the control group, no drops were administered on rats eyes.

2.4. Eyeball removal

The rats were euthanized with intramuscular ketamine injection of 0.5 cc dose. Enucleation was done by cutting the extraocular muscles and connective tissues around the eyeballs, and cutting off the optic nerves. After the dienucleation, the eyeball were directly put and stored into a 10% formalin solution bottle.

2.5. Immunofluorescent staining

Paraffin blocking process was performed on conjunctival tissue. Immunofluorescence staining was made with deparaffinization where conjunctival tissues were washed with PBS for 5 min, repeated for 3 times then incubate cells with Triton-x 100 0.1%solution for 5 min. After that, the slide was washed with sterile PBS for 3 $\times$ 5 min. To maintain cell permeability, after the slide was dried and dropped with 3% hydrogen peroxide, it was incubated for 20 min. After that the slide was washed again with sterile PBS for 3 $\times$ 5 minutes. Then, antigen retrieval was performed using heat induced epitope retrieval by 95 °C heating in water bath for 20 min in buffer citrate ph 0.6. The slide was cooled down slowly. Blocking of non-specific protein was made using 5% FBS, incubation for 30 min. Afterwards, the slides were washed with sterile PBS for 3 $\times$ 5 minutes. Furthermore, specific primary antibodies were dissolved in 5% FBS at a ratio of 1: 200, then incubate for one night at 4 °C. The next day, they were washed again with sterile PBS for 3 $\times$ 5 minutes. Specific primary antibodies used were anti rat estrogen-β receptor antibody (Bioss, USA); anti rat IL-1β antibody (Bioss, USA); and anti rat MUC5AC antibody

(Bioss, USA). Afterwards, incubate them with secondary fluorescence-labeled antibodies with a ratio of 1: 200 in PBS. Secondary antibodies labeled with FITC were used for IL-1β, MUC-5AC, while TRITC (Rhodamine) labeling was for estrogen-β receptor. Incubate in the dark room for 60 min at 40C. After incubation the cells were then washed again using sterile PBS for 3 × 5 min. Visualization of proteins was examined by using the Olympus fluo FV 10-ASW confocal lasser sacnning microscopy 1.7, with a magnification of 400x in five fields of view. Units were stated in arbitrary unit.

2.6. Ethics

This research has passed the ethical approval of institutional ethics committee of Faculty of Medicine Universitas Brawijaya, Saiful Anwar Hospital, Malang, East Java, Indonesia.

2.7. Statistical analysis

Data were presented in the ±mean of standard deviation and analyzed by ANOVA test. For statistical analysis, SPSS for Windows version 17.0 statistical package were used. The p value < 0.05 was statistically significant.

3. Results

The ER-β expressions in conjuctival epithelial cells of all the study groups were shown in Fig. 1. There was a significantly decrease in estrogen receptor-β expression in the dry eye model group

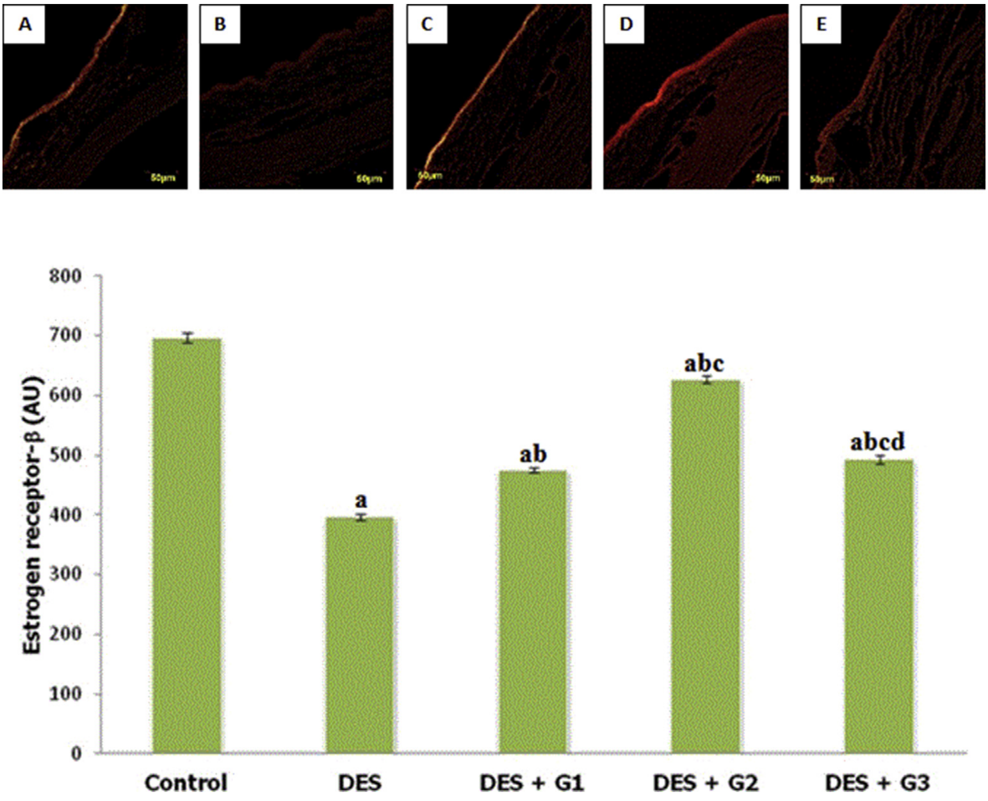


Fig. 1. Confocal micrograph of ER-β expressions in conjuctival epithelial cells, magnification x400 (A). ER-β expressions in conjuctival epithelial cells of all groups (B). Note: DES: dry eye syndrome group; DES + G1: dry eye syndrome + first dose (50 μM) of genistein group; DES + G2: dry eye syndrome + second dose (100 μM) of genistein group; DES + G3: dry eye syndrome + third dose (200 μM)) of genistein group.

compared with the control group ($p < 0.05$). Estrogen receptor-β expression increased significantly in all DES groups treated with genistein compared with the dry eye model group without the administration of genistein ($p < 0.05$), but had not achieved an expression comparable to the control group ($p < 0.05$).

MUC5AC expression in conjunctival goblet cells of various study groups was shown in Fig. 2. MUC5AC expression decreased significantly in the dry eye model compared with the control group ($p < 0.05$). MUC5AC expression increased significantly in all groups treated with genistein ($p < 0.05$), even achieving values comparable to the control group for the second and third dose genistein ($p > 0.05$) groups.

Fig. 3 presents the IL-1β expression in conjunctival epithelial cells of various research groups. The IL-1β expression in the dry eye model group was significantly higher than the control group ($p < 0.05$). The administration of three doses of genistein significantly decreased the IL-1β expression compared with the dry eye model group ($p < 0.05$), but not yet achieved the control group value ($p < 0.05$).

4. Discussion

We do not use hormonal therapy because the affinity is divided into ER-β and ER-α receptors, while the genistein affinity to the ER-β receptor [18,19]. In this study, there was a significant decrease in ER-β expression in the dry eye model group versus the control group. This indicated a downregulation of ER-β expression in the dry eye model group due to ovariectomy. This downregulation was thought to be caused by the low interaction of estrogen with ER-β due to ovariectomy, although this study did not

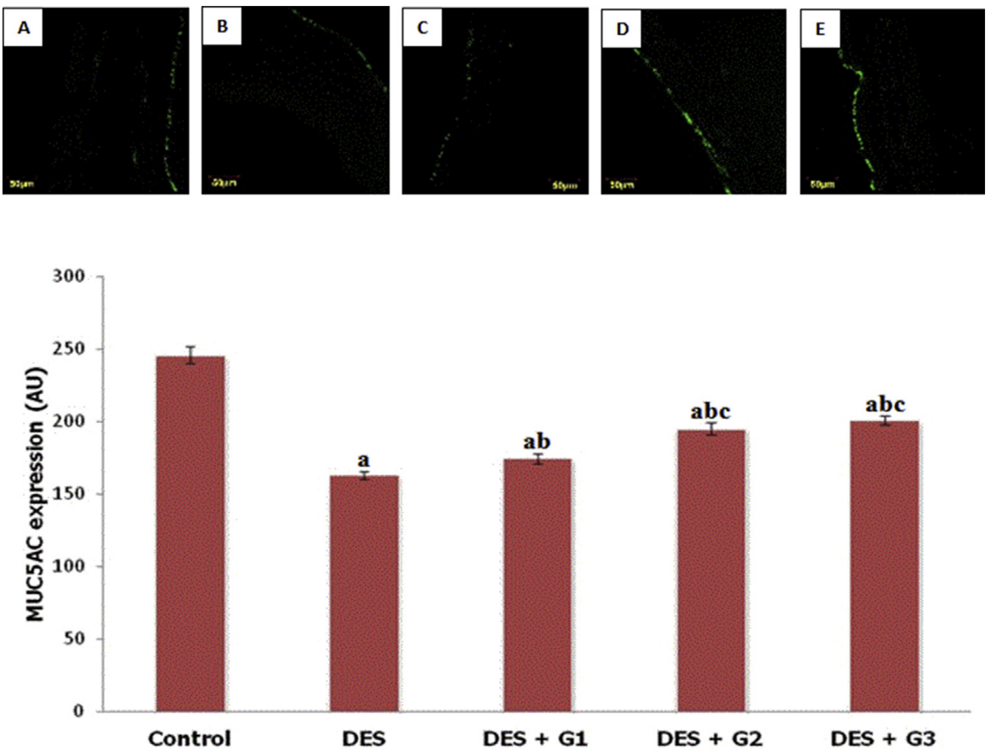
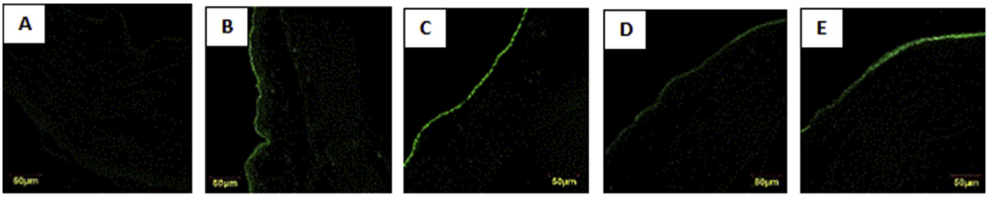


Fig. 2. Confocal micrograph of MUC5AC expression in conjunctival goblet cells, magnification x400 (A). MUC5AC expression expressions in conjuctival epithelial cells of all groups (B). Note: DES: dry eye syndrome group; DES + G1: dry eye syndrome + first dose (50 μM) of genistein group; DES + G2: dry eye syndrome + second dose (100 μM) of genistein group; DES + G3: dry eye syndrome + third dose (200 μM)) of genistein group.



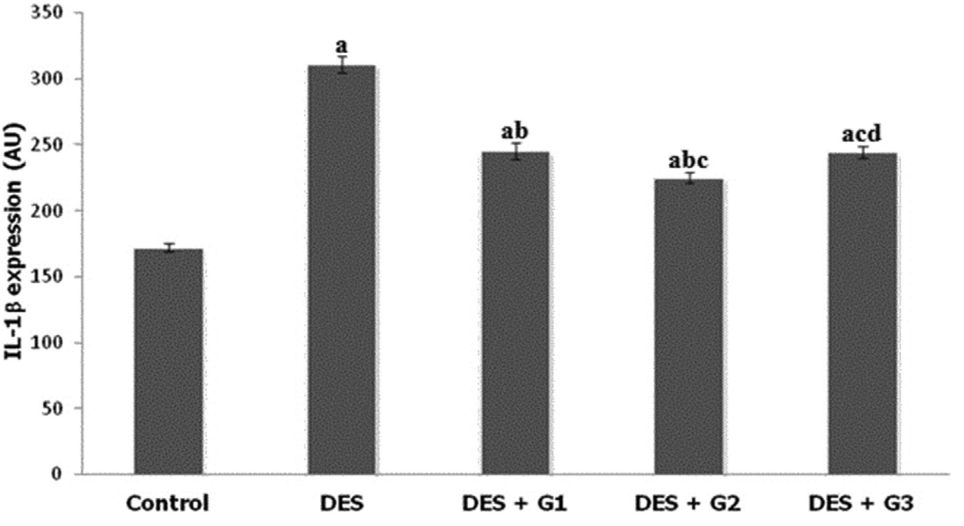


Fig. 3. Confocal micrograph of IL-1β expressions in conjuctival epithelial cells, magnification x400 (A). IL-1β expressions in conjuctival epithelial cells of all groups (B). Note: DES: dry eye syndrome group; DES + G1: dry eye syndrome + first dose (50 μM) of genistein group; DES + G2: dry eye syndrome + second dose (100 μM) of genistein group; DES + G3: dry eye syndrome + third dose (200 μM)) of genistein group.

measure estrogen levels. This study extended earlier findings that there was a decrease in downregulation of ER-β mRNA in the cornea and lacrimal gland in the dry eye model due to sex steroid deficiency [24]. The study also supported previous findings that estrogen deficiency was not the risk factor for dry eye in rats [29]. Administration of genistein in all three doses may increase ER-β expression in dry-eye model rats, although this does not achieve expression comparable to the control group. This was dependent on upregulation of ER-β due to interaction with ligand genistein. Previous studies stated that genistein may increase ER-β mRNA expression [30].

On the ocular surface, the gel-forming 5AC mucin (MUC5AC) was expressed by goblet cells to hydrate the tear layer and acted as a cleaning lubricant and molecule [31,32]. In this study, MUC5AC expression decreased significant in the dry eye model group compared with the control group. This indicated that the downregulation of MUC5AC occurred in the dry eye model group due to ovariectomy. The decreased expression of MUC5AC by goblet cells indicated the adaptive mechanism failure of goblet cells (goblet cell hyperplasia and increased MUC5AC expression). This adaptive mechanism failure will elicit symptoms of a dry eye model, which is accompanied by a decrease in goblet cells and MUC5AC [33]. The decreased estrogen or estrogen deficiency in this study is not a disadvantage given that previous findings suggested that MUC5AC in the ocular epithelia is not hormonally controlled by estrogen and is specific [34]. The three doses of genistein administered in this study were able to upregulate MUC5AC expression, even achieving values comparable to the control group for the groups treated with genistein of second and third doses. We hypothesized several mechanisms, first, through upregulation of ER-β as found in this study. The second mechanism was through the antiinflammatory properties of genistein [35].

In this study, IL-1β expression in the dry eye model group was significantly higher than in the control group. This increase was due to hyperosmolarity stress that could induce IL-1β expression [36]. Levels of IL-1β also increased in the dry eye model of the castrated male rats [23]. The administration of three doses of genistein significantly decreased the expression of IL-1β compared with the dry eye model group, but could not yet achieve the values in the control group. Previous studies had demonstrated the effect of genistein in inhibiting IL-1β mRNA [37]. This was based on the ability of genistein to inhibit Ik-kB phosphorylation and translocation of p65 NF-κβ subunit [35].

Overall, for MUC5AC expression it was found that the higher the dose of genistein the higher the effect, while this did not occur in ER-β and IL-1β. This indicates that genistein is an herb that works on critical match dose [38]. This study extends previous findings that found the effect of dose genistein response to adipocyte differentiation at doses of 20–200 μM [39].

It was concluded that genistein can suppress IL-1β production and trigger upregulation of ER-β and MUC5AC expressions in the dry eye model due to ovariectomy. Thus, genistein may be an alternative to suppress inflammation and mucin production in dry eye syndrome.

Conflict of interest

All researchers state that there is no conflict of interest in the research or publication of this article.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.yclnex.2017.12.003.

References

[1] Barabino S, Chen Y, Chauhan S, Dana R. Ocular surface immunity: homeostatic mechanisms and their disruption in dry eye disease. Prog Retin Eye Res 2012;31:271–8.
[2] Schaumberg DA, Sullivan DA, Dana MR. Epidemiology of dry eye syndrome. Adv Exp Med Biol 2002;506:989–98.
[3] Schaumberg DA, Sullivan DA, Buring JE, Dana MR. Prevalence of dry eye syndrome among US women. Am J Ophthalmol 2003;136:318–32.
[4] Barabino S, Dana MR. Dry eye syndromes. Chem Immunol Allergy 2007;92:176–84.
[5] Suzuki T, Schirra F, Richards SM, Treister NS, Lombardi MJ, Rowley P, et al. Estrogen's and progesterone's impact on gene expression in the mouse lacrimal gland. Invest Ophthalmol Vis Sci 2006;47:158–68.
[6] Pfeilschifter J, Koditz R, Pfohl M, Schatz H. Changes in proinflammatory cytokine activity after menopause. Endocr Rev 2002;23(1):90–119.
[7] Stahl JL, Cook EB, Graziano FM, Barney NP. Differential and cooperative effects of TNFα, IL-1β, and IFNγ on human conjunctival epithelial cell receptor expression and chemokine release. Invest Ophthalmol Vis Sci 2003;44:2010–5.
[8] Solomon A, Dursun D, Liu Z, Xie Y, Macri A, Pflugfelder SC. Pro- and anti-inflammatory forms of interleukin-1 in the tear fluid and conjunctiva of patients with dry-eye disease. Invest Ophthalmol Vis Sci 2001;42(10):2283–92.
[9] Sarkar FH, Li Y, Wang Z, Kong D. NF-kappaB signaling pathway and its therapeutic implications in human diseases. Int Rev Immunol 2008;27(5):293–319.
[10] Sanchez JB. Morill0-Rojas MD. Galbis-Estrada C, Pinzo-Duran MD. Determination of immune response and inflammation mediators in tears: changes in dry eye and glaucoma as compared with healthy controls. Archivos De La Sociedad Espanola De Oftamologia 2017;92(5):210–7.
[11] Ru Y, Huang Y, Liu H, Du J, Meng Z, Dou Z, et al. Alpha-melanocyte-stimulating hormone ameliorates ocular surface dysfunctions and lesions in a scopolamine-induced dry eye model via PKA-CREB and MEK-Erk pathways. Sci Rep 2015;5:18619.
[12] Pflugfelder SC, Corrales RM, de Paiva CS. T helper cytokines in dry eye disease. Exp Eye Res 2013;117:118–25.
[13] McNamara NA, Gallup M, Porco TC. Establishing PAX6 as a biomarker to detect early loss of ocular phenotype in human patients with Sjogren's syndrome. Invest Ophthalmol Vis Sci 2014;55(11):7079–84.
[14] Contreras-Ruiz L, Mir FA, Turpie B, Krauss AH, Masli S. Sjogren's syndrome associated dry eye in a mouse model is ameliorated by topical application of integrin alpha4 antagonist GW559090. Exp Eye Res 2016;143:1–8.
[15] Narayanan S, Miller WL, Prager TC, Jackson JA, Leach NE, McDermott AM, et al. The diagnosis and characteristics of moderate dry eye in non-contact lens wearers. Eye Contact Lens 2005;31(3):96–104.
[16] Dartt DA, Masli S. Conjunctival epithelial and goblet cell function in chronic inflammation and ocular allergic inflammation. Curr Opin Allergy Clin Immunol 2014;14(5):464–70.
[17] Israeli-Lev G, Pitchkhadze M, Nevo S, Fahoum L, Meyron-Holtz E, Livney YD. Harnessing proteins to control crystal size and morphology, for improved delivery performance of hydrophobic biaoctivies, using genistein as a model. Food Hydrocolloids 2017;63:97–107.

[18] Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, et al. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology 1998;139:4252–63.
[19] Morito K, Hirose T, Kinjo J, Hirakawa T, Okawa M, Nohara T, et al. Interaction of phytoestrogens with estrogen receptors alpha and beta. Biol Pharmaceut Bull 2001;24:351–6.
[20] Dixon RA, Ferreira D. Genistein. Phytochemistry 2002;60(3):205–11.
[21] Xie CI, Lin RC, Antony V, Lumeng L, Li TK, Mai K, et al. Daidzin, an antioxidant isoflavonoid, decreases blood alcohol levels and shortens sleep time induced by ethanol intoxication. Alcohol Clin Exp Res 1994;18(6):1443–7.
[22] Xiao F, Cui H, Zhong X. Beneficial effect of daidzin in dry eye rat model through the suppression of inflammation and oxidative stress in the cornea. Saudi J Biol Sci 2018;25:832–7.
[23] Yao XL, Peng QH, Peng J, Tan HY, Wu QL, Wu DL, et al. Effects of extract of Buddleja officinalis on partial inflammation of lacrimal gland in castrated rabbits with dry eye. Int J Ophthalmol 2010;3(2):114–9.
[24] Singh S, Mokhsa L, Sharma N, Titiyal JS, Biswas NR, Velpandian T. Development and evaluation of animal models for sex steroid deficient dry eye. J Pharmacol Toxicol Meth 2014;70:29–34.
[25] Andrade AS, Salomon TB, Behling CS, Mahl CD, Hackenhaar FS, Putti J, et al. Alpha-lipoic acid restores tear production in an animal model of dry eye. Exp Eye Res 2014;120:1–9.
[26] Nakamura S, Shibuya M, Nakashima H, Imagawa T, Uehara M, Tsubota K. D-b-Hydroxybutyrate protects against corneal epithelial disorders in a rat dry eye model with jogging board. Invest Ophthalmol Vis Sci 2005;27:2379–87.
[27] Masmali AM, Purslow C, Murphy PJ. The tear ferning test: a simple clinical technique to evaluate the ocular tear film. Clin Exp Ophthalmol; 97:399–406.
[28] Korb DR, Greiner JV, Herman J. Comparison of fluorescein break-up time measurement reproducibility using standard fluorescein strips versus the Dry Eye Test (DET) method. Cornea 2001;20(8):811–5.
[29] Patisaul HB, Melby M, Whitten PL, Young LJ. Genistein affects ER beta- but not ER alpha-dependent gene expression in the hypothalamus. Endocrinology 2002;143(6):2189–97.
[30] Darabad RR, Suzuki T, Richards SM, Jakobiec FA, Zakka FR, Barabino S, et al. Does estrogen deficieny cause lacrimal gland inflammation and aqueos-deficient dry eye in mice? Exp Eye Res 2003;77(1):59–68.
[31] Corrales RM, Narayanan S, Fernandez I, Mayo A, Galarreta DJ, Fuentes-Paez G, et al. Ocular mucin gene expression levels as biomarkers for the diagnosis of dry eye syndrome. Invest Ophthalmol Vis Sci 2011;52(11):8363–9.
[32] Caffery B, Heynen ML, Joyce E, Jones L, Ritter R, Senchyna M. MUC1 expression in Sjogren's syndrome, KCS, and control subjects. Mol Vis 2010;16:1720–7.
[33] Torricello AAM, Matsuda M, Novaes P, Braga ALF, Saldiva PHN, Alves MR, et al. Effects of ambient levels of traffic-derived air pollution on the ocular surface: analysis of symptoms, conjunctival goblet cell count and mucin 5AC gene expression. Environ Res 2014;131:59–63.
[34] Lange C, Fernandez J, Shim D, Spurr-Michaud S, Tisdale A, Gipson IK. Mucin gene expression is not regulated by estrogen and/or progesterone in the ocular surface epithelia of mice. Exp Eye Res 2003;77(1):59–68.
[35] Chen M, Hu DN, Pan Z, Lu CW, Xue CY, Aass I. Curcumin protects against hyperosmoticity-induced IL-1beta elevation in human corneal epithelial cell via MAPK pathways. Exp Eye Res 2010;90(3):437–43.
[36] Kim DH, Jung WS, Kim ME, Lee HW, Youn HY, Seok JK, et al. Genistein inhibits pro-inflammatory cytokines in human mast cell activation through the inhibition of the ERK pathway. Int J Mol Med 2014;34(6). 1669–1174.
[37] Ji G, Yang Q, Hao J, Guo L, Chen X, Hu J, et al. Anti-inflammatory effect of genistein on non-alcoholic steatohepatitis mice inducecd by high fat diet and its potential mechanisms. Int Immunopharm 2011;11(6):762–8.
[38] Klostranec JM, Chan WCW. Quantum dots in biological and biomedical research: recent progress and present challenges. J Adv Mater 2006;18(15):1953–64.
[39] Hwang JT, Park IJ, Shin JI, Lee YK, Lee SK, Baik HW, et al. Genistein, EGCG, and capsaicin inhibit adipocyte differentiation process via activating AMP-activated protein kinase. Biochem Biophys Res Commun 2005;338(2):694–9. 16.