



Immunopharmacology and inflammation

Impact of fexofenadine, osthole and histamine on peripheral blood mononuclear cell proliferation and cytokine secretion



Natalia Karolina Kordulewska, Elżbieta Kostyra*, Michał Matysiewicz, Anna Cieślińska, Beata Jarmołowska

Department of Biochemistry, Faculty of Biology & Biotechnology, University of Warmia and Mazury, ul. Oczapowskiego 1A, 10-719 Olsztyn, Poland

ARTICLE INFO

Article history:

Received 18 March 2015

Received in revised form

27 May 2015

Accepted 29 May 2015

Available online 3 June 2015

Key words:

Allergic disease

Antihistamine drugs

Cultures *in vitro*

Peripheral blood mononuclear cell (PBMC)

Proliferation

Cytokines secretion

ABSTRACT

This paper compares results of peripheral blood mononuclear cell (PBMC) incubation with fexofenadine (FXF) and osthole. FXF is a third-generation antihistamine drug and osthole is assumed a natural antihistamine alternative. To our best knowledge, this is the first comparative study on FXF, osthole and histamine cytokine secretion and cytotoxicity in PBMC *in vitro* cultures using cell proliferation ELISA BrdU. The cultures were treated 12, 42, 48 and 72 h with FXF and osthole at 150, 300 and 450 ng/ml concentrations and histamine at 50, 100 and 200 ng/ml. Our study results confirm that FXF, osthole and histamine exert no cytotoxic effect on PBMCs and that IL-6, IL-10 and TNF- α cytokine secretion following osthole cell stimulation was similar to that by FXF stimulation. This confirms our hypothesis that osthole is a natural histamine antagonist, and can therefore be beneficially applied in antihistamine treatment.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Allergy is one of the most common chronic diseases in Europe, and it is rapidly approaching epidemic proportions. Allergic diseases are particularly common in children, adolescents and young adults, causing a serious problem for health care systems in developing and developed countries, including Poland. Current predictions estimate that the number of allergy afflicted in 2020 will equal the healthy population. It is therefore paramount to find new pharmaceuticals which successfully combat allergic disease (Samel-Kowalik et al., 2009; Żukiewicz-Sobczak et al., 2012). Mast cells and basophils are very important in allergic reactions. Their degradation releases histamine – the important mediator of allergic and inflammatory responses – and also several active substances including Th2-type cytokines. Histamine changes vascular permeability, drives inflammatory cell infiltration, and modulates mediator production by these cells (Guzik et al., 2003; Venable and Thurmond, 2006). Histamine also modulates immune responses differently depending on the type of receptor on which it acts, and it plays a major modulatory role in physiology and in pathological conditions through its H₁, H₂, H₃, H₄ G-protein-

coupled receptors (Breunig et al., 2007; O'Mahony et al., 2011; Dy and Schneider, 2001; Gschwandtner et al., 2012). In addition, histamine's H₂R receptor stimulation suppresses many immune responses including cytokine production (Żak-Nejmark et al., 2002; Jutel et al., 2009).

Fexofenadine (FXF) is the active metabolite of terfenadine with selective peripheral H₁ receptor antagonist activity (Kasurka et al., 2011; Simpson and Jarvis, 2000; Woosley et al., 1993). It inhibits antigen-induced bronchospasm in sensitized guinea pigs and also histamine release from peritoneal mast cells in rats. FXF is a third-generation antihistamine, non-sedating, rapid and very long acting used in symptoms associated with allergic diseases such as chronic idiopathic urticaria, pruritus, allergic rhinitis, asthma and dermatitis (Aksakal et al., 2010; Kasurka et al., 2011). It is not metabolized by the liver and has no effect on cytochrome P450 (Howarth et al., 1999). In addition, human clinical and animal studies have shown that FXF is free of cardiotoxicity and nervous system effects, such as those witnessed with other non-sedating antihistamines (Hey et al., 1966; Pratt et al., 1999; Rampe et al., 1993).

Our search for anti-allergic agents from natural sources revealed promising properties in the *Cnidium monnieri* dried fruit. This is used in traditional Chinese medicine for its major active component – osthole – which has an isopentenoxycoumarin structure (Gao et al., 2014). Pharmacological studies demonstrate its wide bioactivity as an antibacterial, anticonvulsant, anti-osteoporotic, anti-carcinogenic, anti-diabetic and anti-allergic agent

* Corresponding author.

E-mail addresses: natalia.smulska@uwm.edu.pl (N. Karolina Kordulewska), elzbieta.kostyra@uwm.edu.pl (E. Kostyra), michal.matysiewicz@uwm.edu.pl (M. Matysiewicz), anna.cieslinska@uwm.edu.pl (A. Cieślińska), bj58@wp.pl (B. Jarmołowska).

(Gao et al., 2014; Lv et al., 2012; Matsuda et al., 2002; Nam et al., 2014; Shi et al., 2013; Zimecki et al., 2009). We hypothesize osthole has potential in allergy treatment; in this is first known the investigation of osthole cytotoxicity and cytokine secretion in *in vitro* cultured human peripheral blood mononuclear cells (PBMC). Here, we evaluate the precise mechanisms and effects of osthole treatment on inflammatory processes and allergic reactions. This research examines fexofenadine, osthole and histamine cytotoxicity on cultured human peripheral blood mononuclear cell proliferation and cytokine secretion.

2. Material and methods

2.1. Study participants

Peripheral blood from ten healthy 25–30 year old non-smoking male volunteers was examined. Selected participants had no allergic signs or symptoms and had not taken antihistamines in the preceding five months. The study was conducted with local Ethic Committee approval and all volunteers gave written informed consent.

2.2. Chemicals

Fexofenadine (FXF; PubChem CID: 63002), osthole (PubChem CID: 10228) and histamine (PubChem CID: 774) were obtained from Sigma-Aldrich. FXF was dissolved in 8% dimethylsulfoxide (DMSO, Sigma) to final concentration under 0.1%; thus not affecting cell viability. Osthole was dissolved in 96% ethyl alcohol (AbChem) and histamine in RPMI-1640 (Sigma-Aldrich). All solutions were sterilized through a 0.22 µg/ml filter and stored at 4 °C as stock solutions for later dilution.

2.3. PBMC isolation

Participant peripheral blood samples were collected between 9.00 a.m. and 10.00 a.m. on the first study day and concentrated in grouping tubes with K3EDTA (BD Vacutainer). PBMC isolation was initiated after tube transport to the laboratory, where monocytes, lymphocytes and dendritic cells were isolated by Ficoll density gradient centrifugation (Histopaque, 1077, Sigma). The PBMC layer was double-washed with RPMI-1640 (Sigma-Aldrich), counted, and diluted with RPMI-1640 supplemented with 1% heat inactivated human AB serum, 1% gentamycin and 0.25% phytohemagglutinin-L (PHA-L, Roche Diagnostics). The isolated cells were seeded on 96-well plates at 0.25×10^5 , and active reagents were added to each well after 24 h. The effect of 150, 300 and 450 ng/ml fexofenadine and osthole and 50, 100 and 150 ng/ml histamine on PBMC proliferation was determined. Peak FXF serum concentrations were compared with the 100% registered for unstimulated control PBMC in pure culture.

2.4. BrdU PBMC proliferation assessment

Proliferated PBMC were prepared as in the manufacturer's instructions (Roche Diagnostic, Cell Proliferation ELISA BrdU), and absorbance was measured at 492 nm λ after 12, 24, 48 and 72 h incubation with each substrate solution.

2.5. Cytokine determination

Cells were seeded at 2×10^6 cells/ml with RPMI-1640 and supplemented with 1% heat-inactivated human AB serum, 1% gentamycin and 0.25% PHA. Pure medium formed the control for each substance concentration added after 24 h incubation. Cells

were harvested after a further three days and the supernatant was collected and stored at –80 °C. Commercial ELISA kits determined interleukin IL-6, IL-10 and tumor necrosis factor (TNF- α) output, via quantitative sandwich immunoassay. Triplicate samples were run and the results were equalized by comparison with standard curves expressed in pg/ml.

2.6. Statistical analysis

Statistical analysis comprised one-way ANOVA comparisons of control and test culture proliferation. Experimental values were expressed as mean $\pm$ standard error at $P < 0.001$ significance level. GraphPad Prism 6 for Windows software was utilized (GraphPad Software, Inc., USA).

3. Results

3.1. BrdU test results

Bromodeoxyuridine (BrdU) determines cell proliferative changes over time by measuring cell DNA incorporation.

The concentrations of 150, 300 and 450 ng/ml FXF and osthole and 50, 100 and 200 ng/ml histamine were evaluated to determine PBMC toxicity. These concentrations were chosen because this is the FXF human serum level following its administration; and similar osthole concentrations were required for comparison.

The BrdU FXF test effects on PBMC are presented in Fig. 1A. Dose-dependent increases were observed in 24 h incubations, with the largest recorded for 300 ng/ml administration. No statistically significant decreases were detected when compared with controls, and cell incubation in osthole initiated no statistically significant BrdU incorporation in cellular DNA (Fig. 1B). While this was also true for all histamine administrations (Fig. 1C), PBMC proliferation in 50 ng/ml histamine only registered the same level as the control after 48 h incubation. The absence of statistically significant decrease in BrdU tests for all substances and concentrations confirmed the expected lack of PBMC cytotoxicity.

3.2. Fexofenadine, osthole and histamine effect on PBMC cytokine secretion

The influence of the above active substances on cytokine secretion was assessed: cytokines comprised IL-6 and IL-10 interleukins and TNF- α Tumor Necrosis Factor, and cytokine levels secreted by untreated PBMC in pure culture medium formed the controls.

3.2.1. Single substance application

3.2.1.1. *IL-6 secretion.* (a) PBMC incubation with 150 ng/ml FXF had the greatest IL-6 increase (Fig. 2A1 and C1), (b) histamine incubation significantly increased IL-6 at each of its 50, 100 and 200 ng/ml concentrations at $P < 0.001$ (Fig. 2 C1) and (c) no osthole concentration made any difference to control PBMC IL-6 secretion (Fig. 2A1 and B1).

3.2.1.2. *IL-10 secretion.* All substance concentrations initiated a statistically significant $P < 0.01$ increase in PBMC IL-10 secretion compared to the control effect (Fig. 2A2–C2).

3.2.1.3. *TNF- α secretion.* Incubation with all examined substances caused inhibition of PBMC TNF- α secretion compared to pure medium controls (Fig. 3A–C). (a) There was no change in TNF- α secretion in PBMC incubated in different fexofenadine concentrations. (b) 150 and 300 ng/ml osthole incubation exhibited statistical differences to controls (Fig. 3B); with 450 ng/ml having

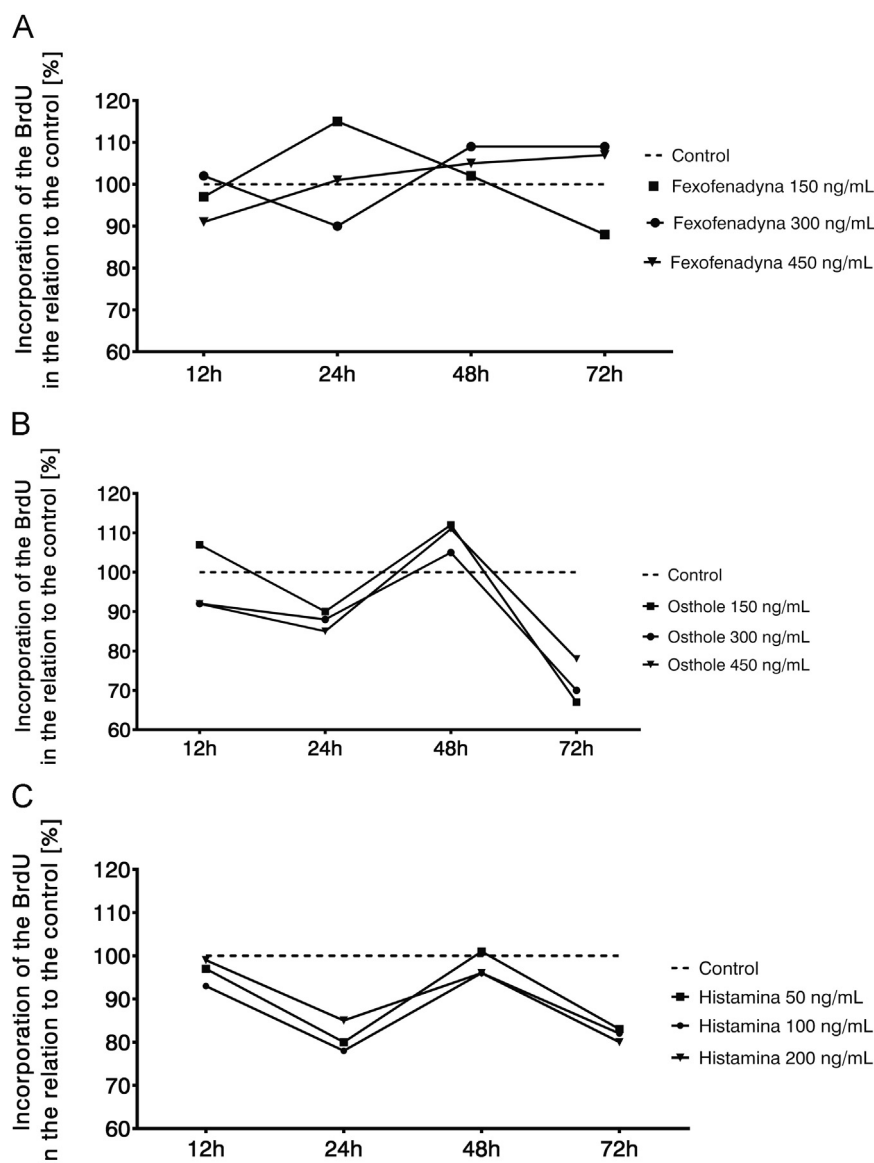


Fig. 1. Fexofenadine (A), osthole (B) and histamine (C) effect on proliferation of human peripheral blood mononuclear cells (PBMC) measured by BrdU 12, 24, 48 and 72 h after incubation. The level of PBMC proliferation in pure culture medium forms the control (100%); with BrdU incorporation expressed as a percentage of the control.

statistically significant decrease compared to the control (Fig. 3B). (c) No statistically significant differences between 50, 100 and 200 ng/ml histamine incubation and controls were noted (Fig. 3C).

3.2.2. Combined substance application

All combined substance effects are compared to control conditions in cells cultured in pure medium.

Combined 50 ng/ml histamine and 150 ng/ml fexofenadine effected the greatest PBMC IL-6 secretion increase (Fig. 4A1–C1). Combined 50 ng/ml histamine and 150 ng/ml osthole affected IL-6 secretion (Fig. 4A1). Combined 100 ng/ml histamine and 300 ng/ml osthole/fexofenadine and also 200 ng/ml histamine and 450 ng/ml osthole/fexofenadine statistically significantly increased IL-6 secretion at $P < 0.01$. All combinations increased IL-10 secretion (Fig. 4A2–C2), with no statistically significant differences noted between substance concentrations (Fig. 4B1, C1).

Combined 50 ng/ml histamine and 150 ng/ml osthole/fexofenadine, 100 ng/ml histamine and 300 ng/ml osthole/fexofenadine and 200 ng/ml histamine and 450 ng/ml osthole/fexofenadine all significantly increased IL-10 secretion at $P < 0.05$ (Fig. 4A2–C2). Combined 50 ng/ml histamine and 150 ng/ml

osthole/fexofenadine was the most effective in changing PBMC IL-6 and IL-10 secretion (Fig. 4A1, A2). All combined concentrations of osthole, fexofenadine and histamine inhibited PBMC TNF- α secretion compared to control cells cultured in pure medium (Fig. 5A–C).

Finally, all our results of osthole action convince us that osthole has great potential in the treatment of allergic diseases.

4. Discussion

The lack of literature information on FXF, osthole and histamine cytotoxicity and cytokine secretion in human peripheral blood mononuclear cells (PBMC) prompted our *in vitro* studies. While osthole and histamine PBMC cytotoxicity and cytokine secretion have not previously been studied, Kasurka et al. (2011) assert that FXF cytotoxic effect on PBMC remains unsubstantiated. In addition, sister chromatid exchange and micronucleus assays by Kasurka et al. (2011) revealed that FXF was not genotoxic at 50, 100 and 150 μ g/ml concentrations. While these authors' mitotic index and nuclear division index results showed dose-dependent decrease

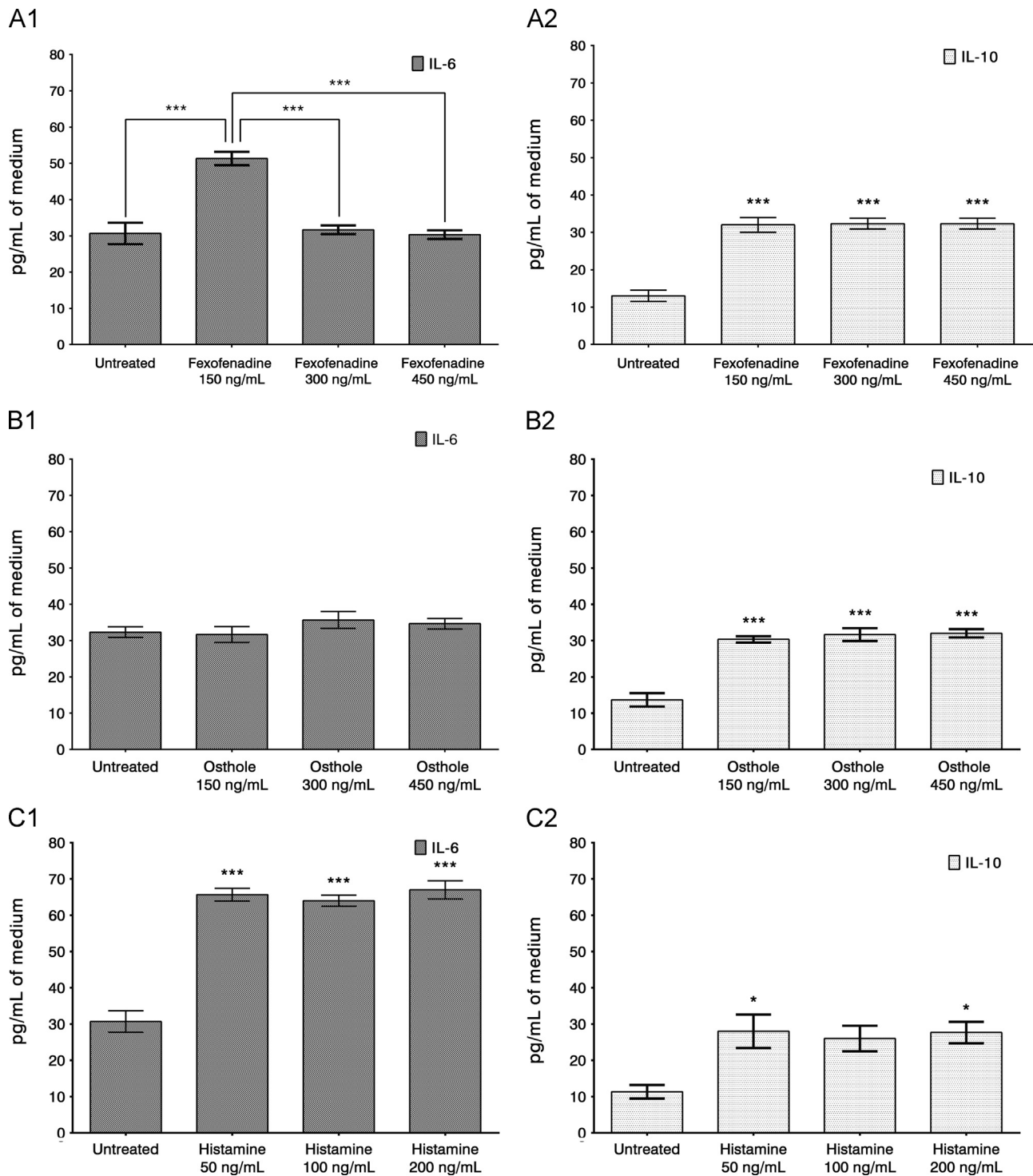


Fig. 2. The influence of test substances on PBMC IL-6 and IL-10 interleukin secretion; fexofenadine on IL-6 (A1) and IL-10 (A2); osthole on IL-6 (B1) and IL-10 (B2) and histamine on IL-6 (C1) and IL-10 (C2). Here, PBMC IL-6 and IL-10 secretion in pure medium forms the control, and test substance secretions are expressed as pg/ml $\pm$ S.E.M. Different letters indicate statistically significant differences.

and significant difference for at least one concentration, results from our PBMC incubation with FFX revealed no statistically significant cytotoxicity. These differing results may be due to dissimilar research methods, or to the different FFX concentrations used. Here, we used 150, 300 and 450 ng/ml concentrations, and Kasuraka 50, 100 and 150 ng/ml. In addition to single substance incubation, we also used various FFX, osthole and histamine combinations to determine 3-day PBMC IL-6, IL-10 and TNF- α concentrations.

Literature asserts that IL-6 and TNF- α are typical multifunctional cytokines involved in regulation of the immune response, hematopoiesis and inflammation. While IL-6 was originally identified as a B-cell differentiation factor, its major functions include (1) antibody induction and (2) Th2 effector cell increase following IL-6 secretion by innate immunity cells which play a further role in adaptive immune response and (3) inhibition of peripheral tolerance. This latter is effected by dendritic cell release of IL-6 which then inhibits the suppressive function of CD4+

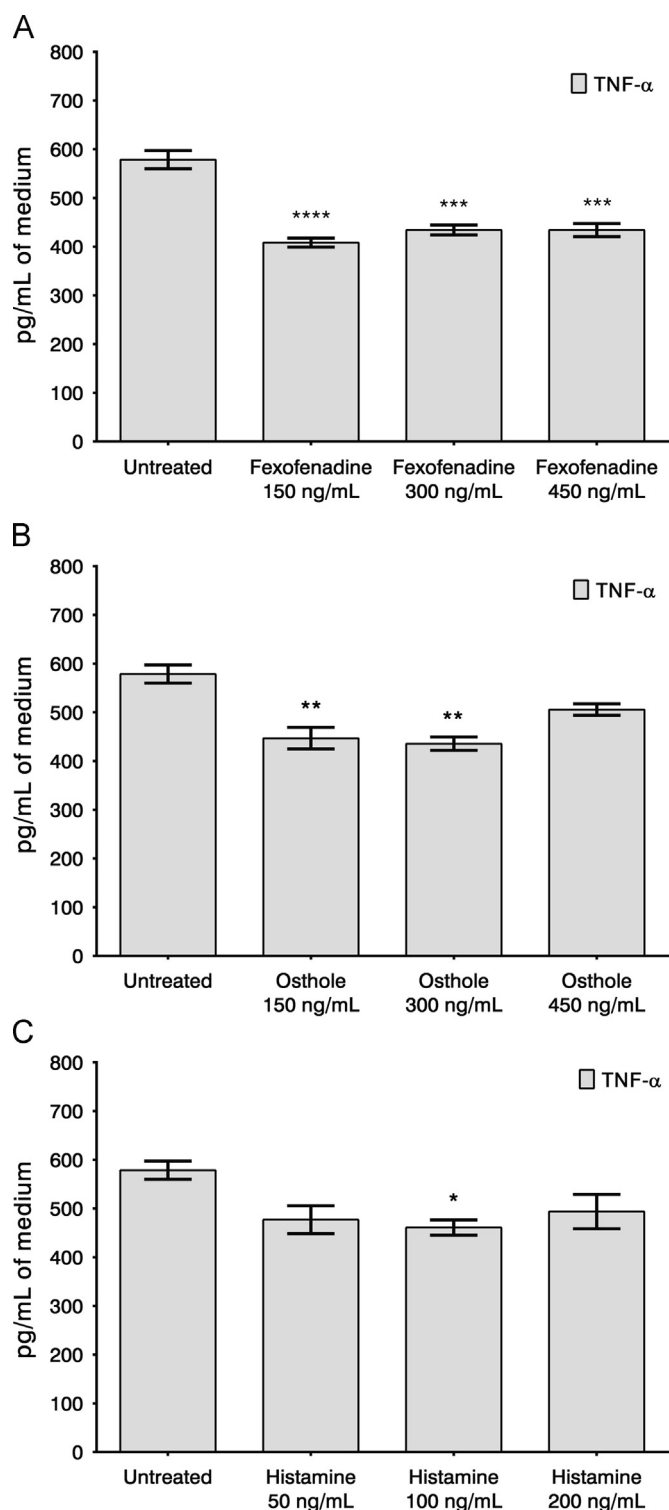


Fig. 3. Fexofenadine (A), osthole (B) and histamine (C) effect on PBMC Tumor Necrosis Factor (TNF- α) secretion. TNF- α secretion in pure medium forms the control, and test substance secretions are expressed as pg/ml $\pm$ S.E.M. Different letters indicate statistically significant differences.

CD25+ T-regulatory cells (Doganci et al., 2005).

Interleukin IL-10, meanwhile, is defined as an anti-inflammatory cytokine which suppresses secretion of pro-inflammatory cytokines. Is produced by different immune cell type including macrophages, neutrophils, B cells, mast cells, dendritic cells (DC) and several T cell subsets. When these latter are unchecked, they induce allergenic airway inflammation plus non-specific airway

responses. Additionally, IL-10 has been shown to be a feedback regulator of Th1 and Th2 (Dibra and Li, 2013). IL-10 is a downstream cytokine of IL27p28 (IL30) pathway, and the mechanism of action showed that IL27p28 is an upstream pathway to activate IL10 expression (Dibra and Li, 2013; Mitra et al., 2014).

Our major objective was to determine if osthole exhibits similar effects to FXF in blocking histamine action. While 150, 300, 450 ng/ml FXF decreased IL-6 concentration and TNF- α expression compared to controls, the anti-inflammatory IL-10 concentrations significantly increased. In contrasting placebo trials of FXF and nasal allergen challenge, FXF significantly inhibited only IL-6 and TNF- α levels. (Ciprandi et al., 2004). While there are no previous reports comparing FXF and osthole interaction with histamine, our osthole results approximate those of FXF; thus clearly indicating that osthole is a histamine antagonist.

Neveu et al. (2010) investigated IL-6's possible concurrence with other pro-inflammatory cytokine interactions in allergic symptoms and lung function. These authors measured cytokine levels in induced sputum and lung function in 16 healthy control and 18 mild-to-moderate allergic subjects. Their results indicated that levels of pro-inflammatory cytokines such as TNF- α and IL-1 β did not differ in asthmatic and control groups (Neveu et al., 2010). In our study, we clearly demonstrated that TNF- α concentration was significantly lower in the combined FXF, osthole and histamine preparations than in single substance incubation. This situation held for IL-6 secretion, where its concentration was significantly statistically higher in FXF, osthole and histamine combination than in the control group, but significantly lower compared to the healthy group. Neveu et al. (2010) reported similar results, where their IL-6 levels were specifically elevated in asthmatic subjects compared to healthy controls at $P < 0.01$. Hierarchical regression analysis in that study cohort indicated the relationship between asthma and lung function could be mediated by IL-6. Further, Lippert et al. (1995) investigated the influence of decarboethoxy-loratadine (DEL), the active metabolite in the loratadine histamine1 (H_1) blocking agent. There, ELISA determined the same mechanism employed by FXF in IL-6 release from the human HMC-1 mast cell line and the KU812 basophilic cell line. When 10^{-5} – 10^{-14} DEL concentrations were pre-incubated with the cells, DEL dose-dependently suppressed IL-6 release by up to 40%. Despite stimulating different cells, FXF and DEL interact similarly, so our results are comparable.

Herbs provide a wide variety of phytochemicals with diverse biological functions. Here, coumarins play significant roles in plant biochemistry and physiology; as antioxidants, enzyme inhibitors and precursors of toxic substances (Kostova, 2005). While it is well known that the coumarin, osthole, possesses anti-proliferative, anticonvulsant and anti-allergic properties and inhibits platelet aggregation, our study is the first to elucidate its effect in PBMC cultures. Our BrdU incorporation in cellular DNA highlighted that osthole not only engendered no cytotoxicity but it also intensified PBMC proliferation after 48 h. Additional studies have reported that osthol inhibited cancer cell viability and proliferation (Chou et al., 2007; Xu et al., 2011) and BrdU assay confirmed similar inhibited viability in RK33 and TE671 cancer lines. Our results indicate that osthole and its synthetic derivatives should prove beneficial in the treatment of allergic diseases. It is a favorable therapeutic option because it has positive direct effect on healthy cells, plus indirect benefits and no toxicity. Further research is now warranted on osthole and its synthetic derivatives' anti-allergic properties.

Ziemecki et al. (2009) tested osthole oxidation and ostholic acid effects on lipopolysaccharide-induced IL-6 and TNF- α production in human PBMC cultures. There, osthole oxidation and ostholic acid strongly inhibited these cells' TNF- α production at 10 μ g/ml concentration; but IL-6 production remained unaltered.

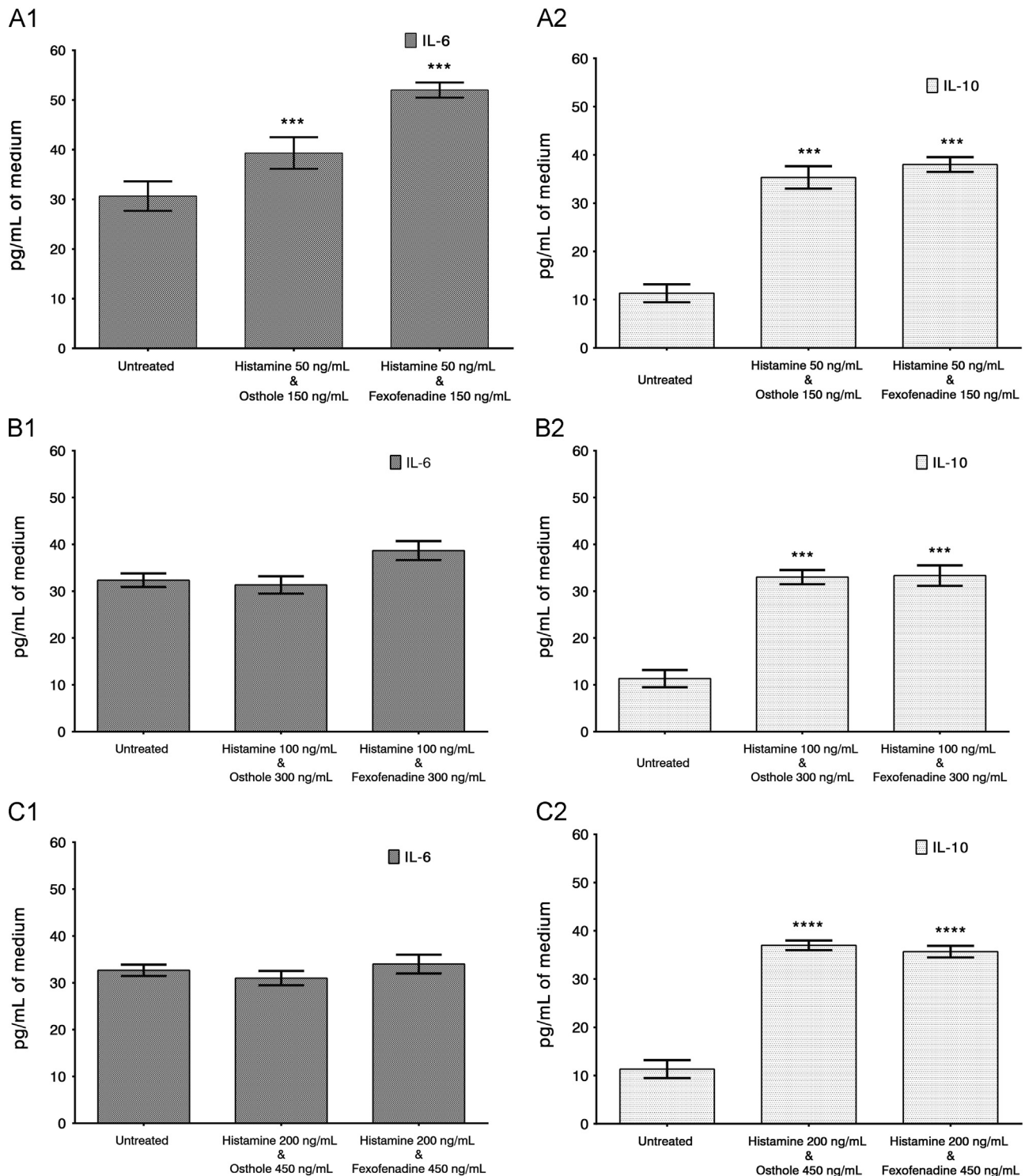


Fig. 4. Combined different fexofenadine, osthole and histamine concentration effects on PBMC IL-6 and IL-10 secretion; (1) IL-6 (A1) and IL-10 (A2) after 50 ng/ml histamine and 150 ng/ml osthole/fexofenadine. (2) IL-6 (B1) and IL-10 (B2) after 100 ng/ml histamine and 300 ng/ml osthole/fexofenadine and (3) IL-6 (C1) and IL-10 (C2) after 200 ng/ml histamine and 450 ng/ml osthole/fexofenadine. Here, PBMC IL-6 and IL-10 secretion in pure medium forms the control, and test substance secretions are expressed as pg/ml $\pm$ S.E.M. Different letters indicate statistically significant differences.

Herein, osthole's anti-inflammatory activity was determined in this first reported ELISA measurement of PBMC TNF- α , IL-6 and IL-10 cytokine levels. Histamine is an important chemical mediator in allergic inflammation, triggering asthmatic vasodilation and edema. These histamine-driven asthma symptoms and subsequent antihistamine asthma intervention substantiate histamine's inflammatory effects (Bachert, 1998; Naclerio, 1999; White, 1999;

Gschwandtner et al., 2012). While our study indicated no increased PBMC proliferation with histamine incubation, there was statistically significant increased PBMC IL-6 production; thus confirming Igaz et al.'s (2001) report that PBMC IL-6 production was influenced by histamine presence. Similar results were published by Merétey et al. (1991), who suggested that histamine contributes to the progression of allergic-inflammatory responses

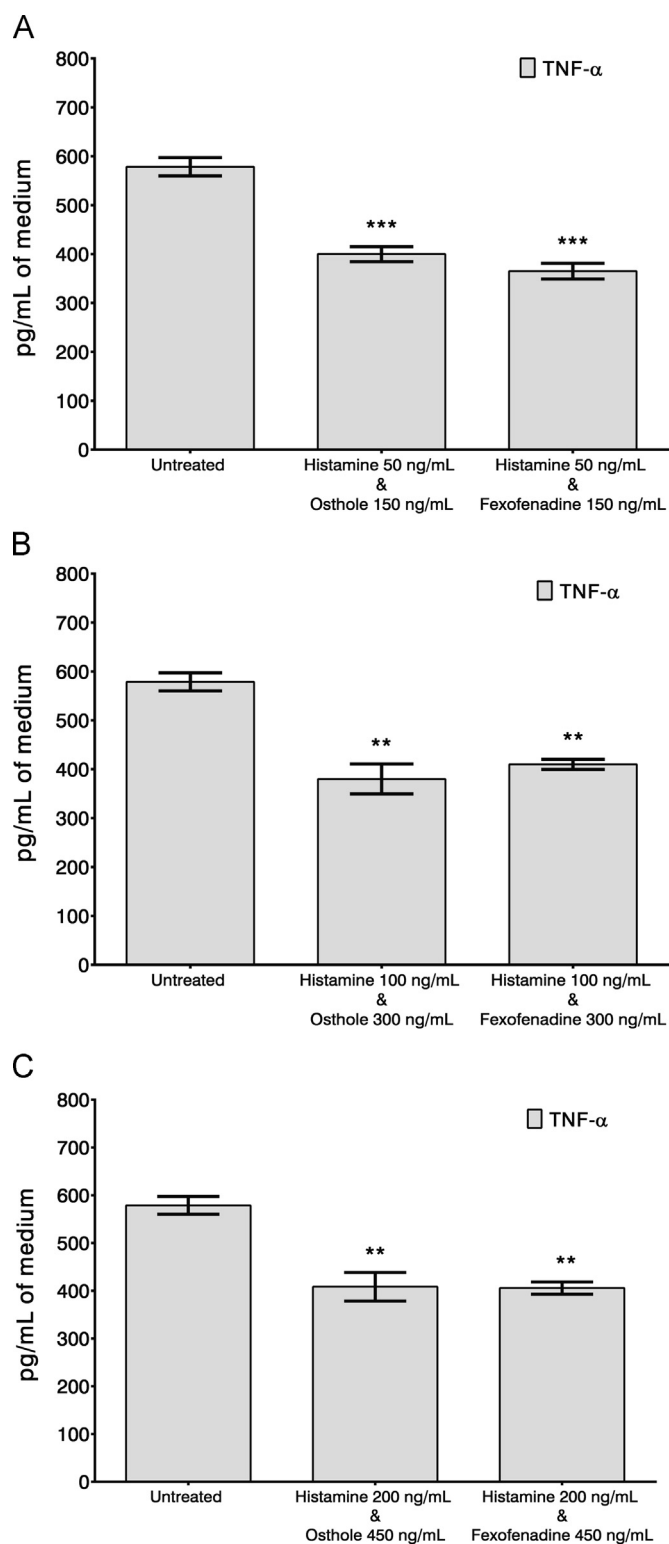


Fig. 5. Combined different fexofenadine, osthole and histamine concentration effects on PBMC TNF- α secretion. (A) 50 ng/ml histamine and 150 ng/ml osthole/fexofenadine, (B) 100 ng/ml histamine and 300 ng/ml osthole/fexofenadine and (C) 300 ng/ml histamine and 450 ng/ml osthole/fexofenadine. Here, PBMC TNF- α secretion in pure medium forms the control, and test substance secretions are expressed as pg/ml $\pm$ S.E.M. Different letters indicate statistically significant differences.

by enhanced secretion of pro-inflammatory cytokines such as IL-6. We also noted that PBMC IL-10 production stimulated histamine increase compared to the controls. This was confirmed by Elenkov

et al. (1998), where increasing histamine concentration increased dose-dependent IL-10 production by 186% at 10^{-7} M and 521% at 10^{-6} M (Elenkov et al., 1998). Further research by Sirois et al. (2015) reported alveolar macrophage treatment with histamine significantly increased IL-10 release, with concurrent significant TNF- α release inhibition when stimulated by lipopolysaccharide (LPS). While our PBMC results did not substantiate this report, augmented IL-10 release in our work was accompanied by inhibited TNF- α release. Additional histamine effects have been highlighted in its triggering of endothelial cell IL-6 and INF- γ production, its suppression of PBMC IL-12, and its induction of IL-6 and IL-10 in macrophages and IL-1 β , IL-6 and IL-8 in dendritic cells (Gelfand et al., 2002).

5. Conclusion

Our discussion compared effects of FFX, osthole and histamine administration to peripheral blood mononuclear cells with allied research of these active substances' effect on various cell types. Our study results confirm that FFX, osthole and histamine exert no cytotoxic effects on PBMC. We observed that osthole PBMC stimulation of cytokine IL-6, IL-10 and TNF- α secretion was as efficacious as that of fexofenadine; thus confirming our hypothesis that osthole is a natural histamine antagonist, and that it can therefore be beneficially utilized in antihistamine treatment of conditions such as allergies. Osthole is a favorable therapeutic option because it lacks toxicity and it bestows both direct and indirect positive effects on healthy cells.

Conflict of interest

The authors declare they have no competing interests.

References

- Aksakal, E., Ulus, T., Tas, H., Ocak, T., Sevimli, S., 2010. Prolonged QT interval after fexofenadine overdose in the presence of hypokalemia and hypocalcaemia. *Hong Kong J. Energy Med.* 17, 75–78.
- Bachert, C., 1998. Histamine: a major role in allergy? *Clin. Exp. Allergy* 28, 15–19.
- Breunig, E., Michel, K., Zeller, F., Seidl, S., Weyhern, C.W., Schemann, M., 2007. Histamine excites neurones in the human submucous plexus through activation of H₁, H₂, H₃ and H₄ receptors. *J. Physiol.* 583, 731–742.
- Chou, S.Y., Hsu, C.S., Wang, K.T., Wang, M.C., Wang, C.C., 2007. Antitumor effects of osthole from *Cnidium monnieri*: an in vitro and in vivo study. *Phytother. Res.* 22, 226–230.
- Ciprandi, G., Cirillo, I., Vizzaccaro, A., 2004. Mizolastine and fexofenadine modulate cytokine pattern after nasal allergen challenge. *Eur. Ann. Allergy Clin. Immunol.* 36, 146–150.
- Dibra, D., Li, S., 2013. The cell-to-cell coordination between activated T cells and CpG-stimulated macrophages synergistically induce elevated levels of IL-10 via NF- κ B1, STAT3, and CD40/CD154. *Cell Commun. Signal.* 11, 95.
- Doganci, A., Sauer, K., Karwot, R., Finotto, S., 2005. Pathological role of IL-6 in the experimental allergic bronchial asthma in mice. *Clin. Rev. Allergy Immunol.* 28, 257–270.
- Dy, M., Schneider, E., 2001. Histamine–cytokine connection in immunity and hematopoiesis. *Cytokine Growth Factor Rev.* 15, 393–410.
- Elenkov, I.J., Webster, E., Papanicolaou, D.A., Fleisher, T.A., Chrousos, G.P., Wilder, R. L., 1998. Histamine potently suppresses human IL-12 and stimulates IL-10 production via H₂ receptors. *J. Immunol.* 161, 2586–2593.
- Gao, Z., Wen, Q., Xia, Y., Yang, J., Gao, P., Zhang, N., 2014. Osthole augments therapeutic efficiency of neural stem cells-based therapy in experimental autoimmune encephalomyelitis. *J. Pharmacol. Sci.* 124, 54–65.
- Gelfand, E.W., Cui, Z.H., Takeda, K., Kanehiro, A., Joetham, A., 2002. Fexofenadine modulates T-cell function, preventing allergen-induced airway inflammation and hyperresponsiveness. *J. Allergy Clin. Immunol.* 110, 85–95.
- Gschwandtner, M., Bunk, H., Köther, B., Thurmond, R.L., Kietzmann, M., Werfel, T., Wolfgang Bäumer, W., Gutzmer, R., 2012. Histamine down-regulates IL-27 production in antigen-presenting cells. *J. Leukoc. Biol.* 92, 21–29.
- Guzik, T.J., Korb, R., Adamek-Guzik, T., 2003. Nitric oxide and superoxide in inflammation and immune regulation. *J. Physiol. Pharmacol.* 54, 469–487.
- Hey, J.A., Del Prado, M., Sherwood, J., Kreutner, W., Egan, R.W., 1966. Comparative

- analysis of the cardiotoxicity proclivities of second generation antihistamines in an experimental model predictive of adverse clinical ECG effects. *Arznei-mittelforschung* 46, 153–158.
- Howarth, P.H., Stern, M.A., Roi, L., Reynolds, R., Bousquet, J., 1999. Double-blind, placebo-controlled study comparing the efficacy and safety of fexofenadine hydrochloride (120 and 180 mg once daily) and cetirizine in seasonal allergic rhinitis. *J. Allergy Clin. Immunol.* 104, 927–933.
- Igaz, P., Novák, I., Lázaár, E., Horváth, B., Héninger, E., Falus, A., 2001. Bidirectional communication between histamine and cytokines. *Inflamm. Res.* 50, 123–128.
- Jutel, M., Akdis, M., Akdis, C.A., 2009. Histamine, histamine receptors and their role in immune pathology. *Clin. Exp. Allergy* 39, 1786–1800.
- Kasurka, C.B., Sekeroğlu, Z.A., Sekeroğlu, V., 2011. Evaluation of the genotoxicity and cytotoxicity of fexofenadine in cultured human peripheral blood lymphocytes. *Toxicol. In Vitro* 25, 1480–1484.
- Kostova, I., 2005. Synthetic and natural coumarins as cytotoxic agents. *Curr. Med. Chem. Anticancer Agents* 5, 29–46.
- Lippert, U., Krüger-Krasagakes, S., Möller, A., Kiessling, U., Czarnetzki, B.M., 1995. Pharmacological modulation of IL-6 and IL-8 secretion by the H₁-antagonist decarboethoxy-loratadine and dexamethasone by human mast and basophilic cell lines. *Exp. Dermatol.* 4, 272–276.
- Lv, X., Xin, X.L., Deng, S., Zhang, B.J., Hou, J., Ma, X.C., Wang, C.H., Wang, Z.B., Kuang, H.X., 2012. Biotransformation of osthole by *Mucor spinosus*. *Proc. Biochem.* 47, 2542–2546.
- Matsuda, H., Ido, Y., Hirata, A., Ino, Y., Naruto, S., Amamiya, T., Kubo, M., 2002. Antipruritic effect of *Cnidii monnieri* Fructus (fruits of *Cnidium monnieri* CUS-SON). *Biol. Pharm. Bull.* 25, 260–263.
- Merétey, K., Falus, A., Taga, T., Kishimoto, T., 1991. Histamine influences the expression of the interleukin-6 receptor on human lymphoid, monocytoid and hepatoma cell lines. *Agents Actions* 33, 189–191.
- Mitra, A., Satelli, A., Yan, J., Xueqing, X., Gagea, M., Hunter, C.A., Mishra, L., Li, S., 2014. IL-30 (IL27p28) attenuates liver fibrosis through inducing NKG2b–rae1 interaction between NKT and activated hepatic stellate cells in mice. *Hepatology* 60, 2027–2039.
- Naclerio, R., 1999. Clinical manifestations of the release of histamine and other inflammatory mediators. *J. Allergy Clin. Immunol.* 103, 382–385.
- Nam, H.H., Jun, D.W., Jeon, H.J., Lee, J.S., Saeed, W.K., Kim, E.K., 2014. Osthole attenuates hepatic steatosis via decreased triglyceride synthesis not by insulin resistance. *World J. Gastroenterol.* 20, 11753–11761.
- Neveu, W.A., Allard, J.L., Raymond, D.M., Bourassa, L.M., Burns, S.M., Bunn, J.Y., 2010. Elevation of IL-6 in the allergic asthmatic airway is independent of inflammation but associates with loss of central airway function. *Respir. Res.* 11, 28.
- O'Mahony, L., Akdis, M., Akdis, C.A., 2011. Regulation of the immune response and inflammation by histamine and histamine receptors. *J. Allergy Clin. Immunol.* 128, 1153–1162.
- Pratt, C., Brown, A.M., Rampe, D., Mason, J., Russell, T., Reynolds, R., Ahlbrandt, R., 1999. Cardiovascular safety of fexofenadine HCl. *Clin. Exp. Allergy* 29, 212–216.
- Rampe, D., Wible, B., Brown, A.M., Dage, R.C., 1993. Effects of terfenadine and its metabolites on a delayed rectifier K⁺ channel cloned from human heart. *Mol. Pharmacol.* 44, 1240–1245.
- Samel-Kowalik, P., Lipiec, A., Tomaszewska, A., Raciborski, F., Walkiewicz, A., Lu-sawa, A., Borowicz, J., Gutowska-Slesik, J., Samoliński, B., 2009. Występowanie alergii I astmy w Polsce–badanie ECAP, 3, pp. 32–34.
- Shi, J., Fu, Q., Chen, W., Yang, H.P., Liu, J., Wang, X.M., He, X., 2013. Comparative study of pharmacokinetics and tissue distribution of osthole in rats after oral administration of pure osthole and *Libanotis buchtormensis* supercritical extract. *J. Ethnopharmacol.* 145, 25–31.
- Simpson, K., Jarvis, B., 2000. Fexofenadine. *Drugs* 59, 301–321.
- Sirois, J., Ménard, G., Moses, A.S., Bissonnette, E.Y., 2015. Importance of histamine in the cytokine network in the lung through H₂ and H₃ receptors: stimulation of IL-10 production. *J. Immunol.* 164, 2964–2970.
- Venable, J.D., Thurmond, R.L., 2006. Development and chemistry of histamine H₄ receptor ligands as potential modulators in inflammatory and allergic responses. *Antiinflamm. Antiallergy Agents Med. Chem.* 5, 307–322.
- White, M., 1999. Mediators of inflammation and the inflammatory process. *J. Allergy Clin. Immunol.* 103, 378–381.
- Woosley, R.L., Chen, Y., Freiman, J.P., Gillis, R.A., 1993. Mechanism of the cardiotoxic actions of terfenadine. *J. Am. Med. Assoc.* 269, 1532–1536.
- Xu, X., Zhang, Y., Qu, D., Jiang, T., Li, S., 2011. Osthole induces G2/M arrest and apoptosis in lung cancer A549 cells by modulating PI3K/Akt pathway. *J. Exp. Clin. Cancer Res.* 30, 33.
- Żak-Nejmark, T., Kraus-Filarska, M., Małolepszy, J., Nowak, I.A., Dobosz, T., Jonkisz, A., Nadobna, G., 2002. Ekspresja mRNA receptorów histaminowych typu 1 i 2 w limfocytach krwi obwodowej osób zdrowych i z alergią atopową. *Alergia Asthma Immunol.* 8, 106–109.
- Zimecki, M., Artym, J., Cisowski, W., Mazol, I., Włodarczyk, M., Gleńsk, M., 2009. Immunomodulatory and anti-inflammatory activity of selected osthole derivatives. *Z. Naturforsch. C* 64, 361–368.
- Żukiewicz-Sobczak, W., Krasowska, E., Zwoliński, J., Sobczak, P., Chmielewska-Badora, W., Wróblewska, P., Piątek, J., Wojtyła, A., 2012. Allergic disease – current state of knowledge. *Postępy Dermatol. Allergol.* 6, 451–455.