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Immunomodulatory Potential Effects Of Pomegranate On Rbl-2h3 Cells

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Abstract

Mast cells have a role in a variety of inflammatory and immunological responses and release a variety of mediators, including histamine.Pomegranate fruit is utilized in traditional medicine of several Asian cultures to cure a number of diseases.Pomegranate biological effects has been extensively studied, including in vitro, in vivo, and in clinical trials.The aim of this study was to evaluate whether pomegranate modulates histamine production using RBL-2H3 cell lines after treated by lysophosphatidic acid (LPA).Histamine levels were measured using a spectrofluorometric approach after whole squeezed pomegranate juice was administered to RBL-2H3 cell lines treated with LPA.We found a significant reduction in histamine level with pomegranate juice treatment in dose dependent manner ($p \leq 0.01$).Moreover,there was a dose-dependent slight increase in cytotoxic effects in RBL-2H3 cell lines after pomegranate treatment, but no significant decrease in cell viabilitywas found.Our finding suggests the potential role of pomegranate on the reduction of histamine level induced from mast cell, which indicate to the implication of pomegranate in allergy therapeutic and pharmacology industryfuture work.

Key words: histamine, lysophosphatidic acid, pomegranate, RBL-2H3

Introduction

Allergy is one of diseases negatively affect people life quality worldwide. Several elements implicated in developing allergic disease include environment, food. Allergy can be developed at any phase of human life especially in childhood. Moreover, allergen substances inhalationcan stimulate immune system leading toallergic reaction (Elizabeth et al., 2005). The immunoglobulin E (IgE) induced in immune system particularly to eliminate allergen from the body (Galli, 2010; Metcalfe, 2008). IgE antibody interact and relocate the allergen to mast cells (da Silva et al., 2014). Activated mast cells degranulate and release inflammatory chemical molecules causing induction of allergic reactions such as wheezing, swelling and redness (Valent, 2013). Mast cells recognized within

inflammatory process and allergic condition as tissue-fixed effector cells (Galli and Tsai, 2012; Bossi et al., 2011). Several different mast cell mediators were reported, for instant: cytokines, histamine, proteases, leukotrienes, and heparin (Valent, 2013; Chad, 2005; Kraneveld et al., 2004). These mediators expressed from granulated mast cells playing a significant role in triggering allergic rhinitis, asthma, and eczema reaction (Kawa, 2012; Méndez-Enríquez and Hallgren, 2019). Histamine is the major mediator that enhance allergic reaction and exhibiting clear symptoms. Furthermore, the secretion of IgE antibody in blood stream is a markable element to allergic reaction as well(Kawa, 2012).

Recent studies showed a considerable demand to establish an adequate active natural product that be safe and inexpensive to use as treatment for different disease aiding to improve people life. Many natural components derived from different fruits and vegetables were examined in medical research aiming to control and down regulate inflammatory progress within several patho- logical conditions (Zhaoet al., 2017; Salas-Salvado et al; 2018). Ginger, curcumin, green tea, blueberries, pomegranate, apple, olive oil and more other natural sources have been identified as an active factor that suppress the development of different illness (Wu and Xiao, 2016; Yoo et al., 2014; Yamprasertet al., 2020). Most fruits and vegetables contain powerful molecules capable to inhibit inflammatory, oxidation and infection process as well asautoimmune diseases and asthma(Wu and Xiao, 2016; Rengasamy et al., 2019; Zhang et al., 2021).

Punica granatum L, (pomegranate) trees distributed throughout the world which used in science research as antimicrobial, anticancer, and anti-inflammatory source (Wang et al., 2018;Achrafet al., 2017). Pomegranate is one of the essential sources of flavonoids, presenting a dominant natural aspect that able to control inflammation, infection, and oxidant progression. Researchers investigate the influence of each part of pomegranate on different disease. Pomegranate fruit has three parts: seeds, peel and aril which tested separately in clinical research. Organic acids (i.e. malic and ascorbic acids), lipids, isoflavones, and polyphenols were detected in seeds, while punicalagins, ellagitannins, hydrolysable tannins, minerals (i.e. magnesium, nitrogen, potassium and sodium) and flavonoids obtain within aril part (Fourati et al., 2020; Li et al., 2020; El-Hadary and Ramadan, 2019; Singh et al., 2018).In addition, several types of polyphenols identified in peel include ellagic acid, gallotannins, proanthocyanidins, ellagitannins, and anthocyanins that provide red color for pomegranate (Akhtar et al., 2015; Díaz-Mula et al., 2019; Li et al., 2009). All pomegranate parts exhibit downregulation of oxidant and inflammatory pathway using different type of cell lines and rat model as well. The existence of flavonoids and other bioactive compounds in pomegranate increase the interest of individuals to use it as fresh fruit and encourage researchers to evaluate the significant role of their

properties that may suppress the progression of different diseases (Karimi et al., 2017; Viuda-Martos et al., 2010). On the other hand, few previous research was indicated pomegranate as a causing matter to develop allergic reaction with specific health cases in some patients (Bolla et al., 2014; Petersen et al., 2011).

Importantly, Lysophosphatidic acid (LAP)exist in human bodyas a bioactive molecule located in blood and tissues of eukaryotes, involved in cell migration and proliferation (Hashimoto et al., 2006; Lundequist and Boyce, 2011). LAPnormally associate and appear with high concentration of tumor growth and inflammation place (Xiao et al., 2001; Goetzl et al., 2000). High amount of LAP was detected in ovarian cancer serum sample which consider as an early sign for this cancer type (Lygia and Amal, 2002). In addition, LAP play an essential role in another cancer mode include prostate and breast cancer (Kue and Daaka, 2000; Im et al., 2000). Previous studies illustrated the association of high level of LAP with the presence of the proinflammatory pathway, bronchoalveo- lar lavage fluid, and the increase of airway smooth muscles concentration (Tager et al., 2008; Montesi et al., 2014; Georas et al., 2007). The aim of the present investigation is to assess the role of pomegranate juice containing seeds, peel and aril on mast cell derived histamine using LAP as an activator element.

Materials and Methods

Materials

RBL-2H3 cell line was provided by VACSERA - Cell Culture Unit, Dokky, Giza, Egypt. Mono oleoyl phosphatidic acid monosodium (LPA) was obtained from Sigma-Aldrich (CAS Number: 268550-95-4). Pomegranate fruits were obtained from Egyptian market. The whole fruits (peel, seeds, and aril) squeezed to prepare natural fruits juices used in our study.

The RBL-2H3 cells was cultured in MEM supplementedpurchased from Sigma Chemical with 15% fetal calf serum, 0.434 mg/ml glutamine, and an antibiotic-antimycotic mixture containing 100 U/ml penicillin, 100 µg/ml streptomycin was added. The cells were incubated at 37°C in 5% CO2/air for culture.

Stimulation of histamine release

Thecell suspension was pre-warmed for 5 min at 37 ° C, oleoyl phosphatidic acid monosodium (LAP) 10 µg /ml were introduced and incubated for 30 min. The reaction was terminated by placement the test tube in ice-cold water. After centrifugation at 1,500 g for 10 min at 4 °C, histamine in the supernatant was determined spectrofluorometrically according to (Hashimoto et al 2005). The percentage of total released histamine calculated.

Determination of pomegranate effect on histamine level

Cell suspensions was incubated at 37 °C with 10 µg / ml of LPA for 10 min, then 150 µl /ml of pomegranate juicesintroduced to the same sample of RBL-2H3 cells for additional incubation time (15, 30 and 60 min). Histamine levels calculated as percentage of the total released histamine.Dose course of histamine release from RBL-2H3 cells treated with 10 µl /ml of LPA was analyzedusing spectrophotometer after 60 min incubation at 37 °C.Serial concentration of pomegranate juices(25, 50, 100, 150 and 200 µl/ml) were added to RBL-2H3 treated cells, and then histamine levels evaluated and compare with LPA stimulation.

Cell viability assay

A cell viability assay was performed with RBL-2H3 cells using the MTT colorimetric method according to previously described (May et al 1970). RBL-2H3 cells were harvested and transferred into 96-well micro-plates at a density of 40 000 cells per welland left to adhere overnight (37°C, 5% CO2) before dosing, then different concentrations of pomegranate juices were added (25, 50, 100, 150 and 200 µl /ml) for 48 hours at 37 °C in a humidified incubator (5% CO_2, 95% air) in order to evaluate the cytotoxic effect of pomegranate. After incubation MTT solution (10 µl, 5 mg/ml in PBS) was added to the wells and the cells were incubated for another 4 h at 37 °Cand 100 µl of DMSO was added to dissolve the formazan crystals. The optical density of the solution was measured with a micro-plate reader at 570 nm.

Statistical Analysis

Data were subjected to Analysis of Variance (ANOVA). The SPSS statistics program (Version 16.0) was used for data analysis. Results are presented as the mean with standard error (SEM). $P<0.05$ were established to significant statistical difference.

Results and Discussion

Cytotoxic assay

The impact of substance under investigation on cell function and appearance is a key aspect to build up experiment outcomes on accurate basic. The test samples considered as a cytotoxic when the optical density of the sample-treated group less than 80% of that in the spontaneous release (Musa et al 2014).Pomegranate concentrations in the current study showed minimal toxicity on RBL-2H3 cells as the percentages of cell viability were all over 80%. Consequently, pomegranate juices at those concentrations are safe for assessing anti-allergic activity in RBL-2H3 mast cells.The maximum cytotoxic impact was observed at the highest dosage (200 µg/l) utilised in current investigation. Moreover, we observed that pomegranate juice treatment resulted in a non-significant decrease in

cell viability in RBL-2H3 cells (figure 1).Pomegranate extract has been found not toxic on other cell type in previous work which support our result (Nahta, 2011).

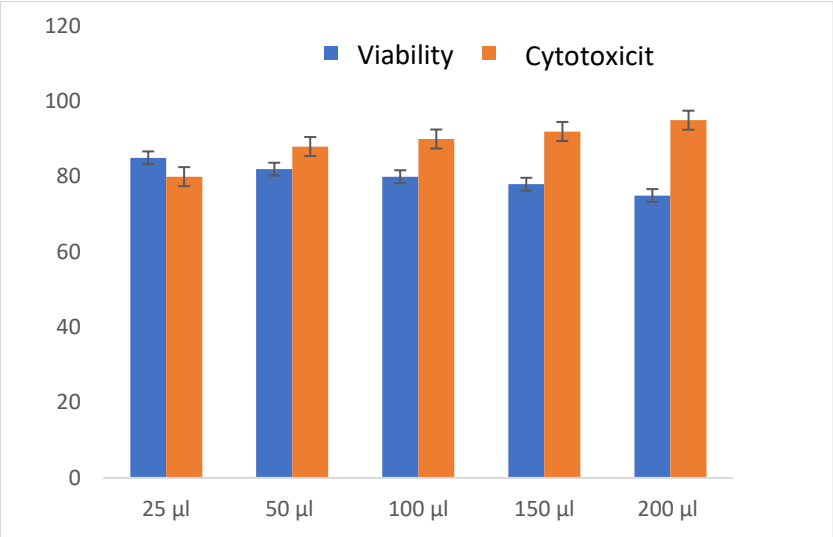

Figure 1: Effect of pomegranate juice (PJ) on RBL-2H3 cell viability and cytotoxicity.

Effect of PJ on histamine production correlated with incubation time

Mast cells contains granules of several types of mediators such as: proteases, cytokines, growth factors heparin and histamine that identified as associated molecules with different diseases (Galli and Tsai, 2012). Allergic reaction starts when allergens influence IgE in immune system that trigger histamine expression in human body (Galli and Tsai, 2012; Bot et al., 2013). IgE and LPA are both normally used in science research lab to stimulate mast cell degranulate to release different mediators in culture experiment (da Silva et al., 2014; Bot et al., 2013).

Few in vitro studies on the entire pomegranate fruit or its juice have been conducted to investigate anti-allergic activity. Our in vitro study was to evaluate pomegranate juice (PJ) effect on RBL-2H3 cell line treated with LAP. The PJ has the ability to lower histamine level, which is thought to be the primary mediator responsible for allergic reactions. Herein, the induction of spontaneous histamine from mast cells was ranged between 8.12 and 10.52 percent, whereas 10 µg/ml of LPA boosted releasedhistamine to a high level of 38.12 at 60 minutes (figure 2). Our data exhibited similar effect of LAP on mast cells as pervious reported works (Kondoa et al., 2021; da Silva et al., 2014).

On the other hand, pomegranate juice-treated cells lead to a significant decreased of released histamine at 60 minutes ($p \leq 0.05$), which indicated to considerable different with the spontaneous production (figure 2). The incubation stages of PJ with activated RBL-2H3 cells were assessed at

different time. A substantial decline of histamine amount particularly at 60 minutes was achieved indicating to a link between histamine level and PJ incubation period. Figure 2 clearly exhibit a high level of histamine suppressed with long PJ incubation period achieved. This finding may will be reported for the first time linking the effect of pomegranate juice on mast cells and histamine induction using LPA as activator element. However, many studies have been identified the effect of pomegranate fruit parts (seeds, peel, and aril) on several type of cells within oxidant and inflammatory conditions but not exactly on histamine (Wang et al., 2018; Achraf et al., 2017; Li et al., 2020; El-Hadary and Ramadan, 2019). Rasheed and his group (2009) investigated the influence of flavonoids extracted from pomegranate fruit on pro-inflammatory cytokine gene expression using human basophilic cell line KU812 (Rasheed et al., 2009). They reported the ability of pomegranate fruit extraction to inhibit inflammatory development throw basophil. Importantly, PJ and mast cell-derived histamine require more future research to clarify the relation between them.

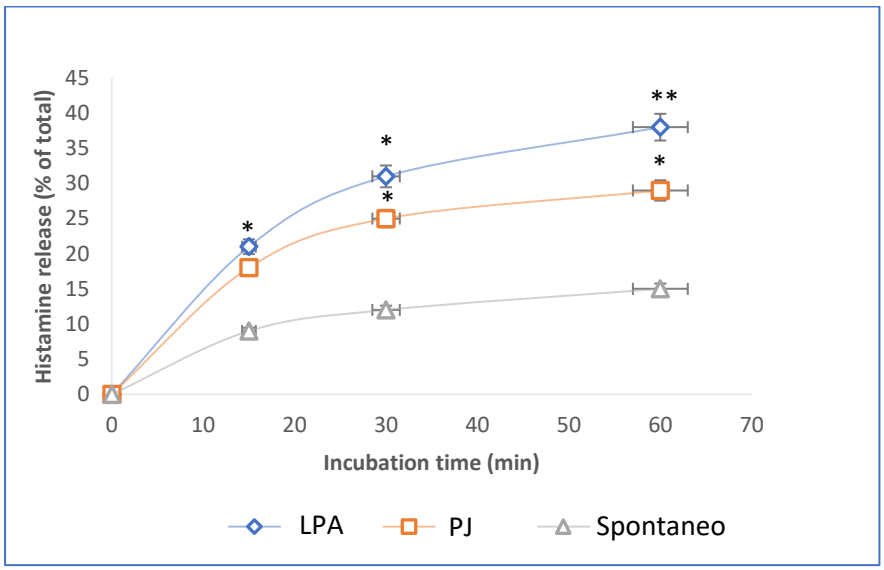

Figure 2. The effect of pomegranate juice incubation period on histaminelevel.RBL-2H3 cells induced histamine after treated with 10 µg/ml LPA for 60 min at 37 °C.The inhibition of histamine level increased significantly (* $p \leq 0.05$, ** $p \leq 0.01$) when incubation time increase as well.

Effect of PJ on histamine production correlated with dosemanner

In response to LAP stimuli, RBL-2H3 cells produced histamine which have the potential to cause allergy. PJ reduced histamine in RBL-2H3 cells treated with LAP, confirming a decrease in overall allergy activity and validating PJsuppression of mast cell activity. Five PJ concentrations were carried out ranging from 25 to 200 µl/ml (figure 3) in the present study. All PJ concentrations were incubated with activated mast cell for 10 minutes, then histamine level was evaluated (figure 3).

Histamine level declined significantly with the highest PJ concentration (200 µl/ml) ($p \leq 0.01$). Gradual suppression of histamine level depending on PJ dose concentration was detected in our study. Previous research illustrates a potential reduction in histamineexpression from human basophilic cell line KU812 after polyphenol-rich pomegranate fruit extract treatment, which reliable with our results (Rasheed et al., 2009).Our studyis novel compared with the other works; no mast cell derived histamine was investigated using PJ in previous studies. Whereas the involvement of pomegranate extractions and juice were tested with other cells and diseases (Wang et al., 2018; Achraf et al., 2017; Li et al., 2020; El-Hadary and Ramadan, 2019). Within cancer studies, pomegranate have been reported as a promising anti-cancer element in pharmacology industry (Wang et al., 2018; Achraf et al., 2017). Other study reported that the production of pro-inflammatory cytokines was considerably decreased mainly with high pomegranatepeel extraction (PPE) concentration (Mastrogiovanni et al., 2019). The consistent between their data and our finding provide strong evidence of pomegranate ability to down regulate inflammatory pathway through cytokines in cancer and histamine in allergy that improve immunomodulatory effect.

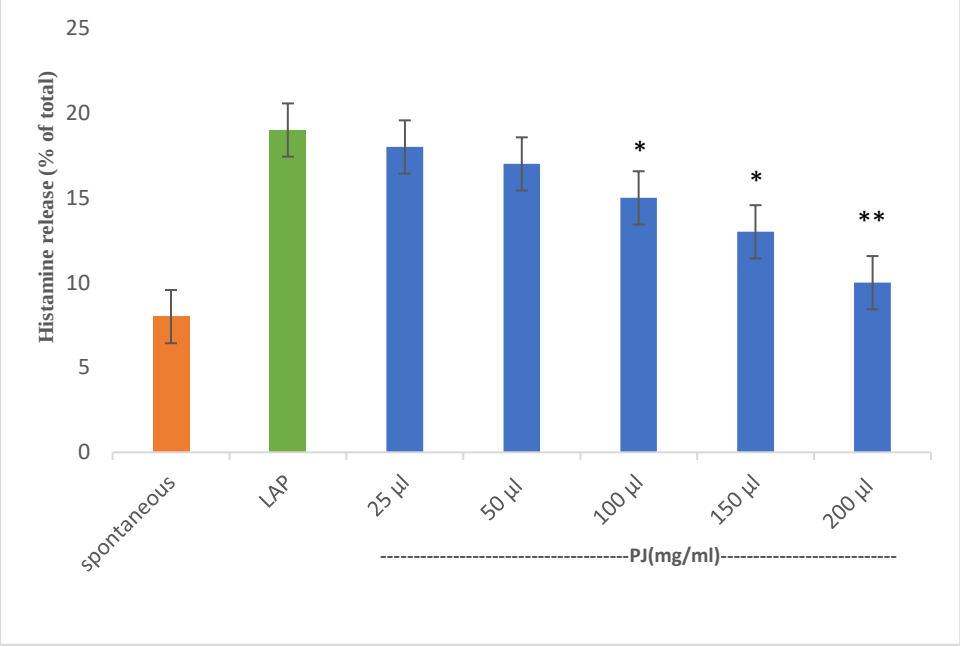

Figure 3. Influence of different pomegranate juice concentrations on histamine production.RBL-2H3 cells induced histamine after treated with 10 µg/ml LPA for 60 min at 37 °C. Then RBL-2H3 cells incubated for 10 min with several concentrations of pomegranate juice (25, 50, 100, 150 and 200 µl/ml) at 37 °C. Histamine level decreased significantly (* $p \leq 0.05$, ** $p \leq 0.01$) with high PJ concentrations

Conclusion

Punica granatum L, (pomegranate)is an excellent natural source for bioactive molecules include proanthocyanidin, vitamin C, phenolics, ellagitannins, flavonoids, and different fibers as well. Pomegranate have been reported previously as an anti-inflammatory, antioxidant, and anti-microbial agent. Current study investigates the role of pomegranate juice containing seeds, peel, and aril on allergic reaction especially histamine production level. To the best of our knowledge pomegranate have not been tested with mast cells within allergic disease. The present study demonstrates a dramatic decrease of histamine production under PJ treatment condition. In addition, the incubation period and PJ concentration showed dose and time dependent manner. Further work needs to be carried out regarding to pomegranate role with other mast cell mediators in addition to histamine as well. Our finding provides evidence of Punica granatum L ability to suppress allergic reaction that may obtain a future source for therapeuticand pharmacology industryregarding allergy.

Conflicts of Interest

The author have no conflicts of interest to declare.

References

Achraf Ammar, Mouna Turki, Omar Hammouda,Hamdi Chtourou, Khaled Trabelsi, Mohamed Bouaziz , Osama Abdelkarim, Anita Hoekelmann, Fatma Ayadi, Nizar Souissi , Stephen J. Bailey, Tarak Driss and Sourour Yaich. (2017). Effects of pomegranate juice supplementation on oxidativestress biomarkers following weightlifting exercise, Nutrients, 9, 819.

Akhtar, S., Ismail, T., Fraternale, D., & Sestili, P. (2015). Pomegranate peel and peel extracts: Chemistry and food features. Food Chemistry, 174, 417–425.

Bolla M.Zenoni S. Scheurer S. Vieths S. San Miguel Moncin M.d.M.Olivieri M.Antico A. Ferrer M. Berroa F. Enrique E.Avesani L.Marsano F. Zoccatelli G. (2014). Pomegranate (Punica granatum L.) Expresses Several nsLTP Isoforms Characterized by Different Immunoglobulin E-Binding Properties. Int Arch Allergy Immunol, 164:112-121.

Bossi F, Frossi B, Radillo O, Cugno M, Tedeschi A, Riboldi P, Asero R, Tedesco F, Pucillo C. (2011). Mast cells are critically involved in serum-mediated vascu- lar leakage in chronic urticaria beyond high- affinity IgE receptor stimulation. Allergy, 66:1538–1545.

Bot M, Saskia C. A. de Jager, Luke MacAleese, H. MaximeLagraauw,Theo J. C. van Berkel, Paul H. A. Quax, Johan Kuiper, Ron M. A. Heeren, Erik A. L. Biessen, and Ilze Bo. (2013). Lysophosphatidic acid triggers mast cell-driven atherosclerotic plaque destabilization by increasing vascular inflammation. Journal of lipid research, 54(5), 1265–1274.

Chad K Oh. (2005). Mast cell mediators in airway remodeling. Chem Immunol Allergy, 87:85e100.

da Silva, E.Z.; Jamur, M.C.; Oliver, C. (2014). Mast Cell Function: A New Vision of an Old Cell. Journal of Histochem. Cytochem, 62, 698–738

Díaz-Mula, H. M., Tom´as-Barber´a n, F. A., & García-Villalba, R. (2019). Pomegranate Fruit and Juice (cv. Mollar), Rich in ellagitannins and anthocyanins, also provide a significant content of a wide range of proanthocyanidins. Journal of Agricultural and Food Chemistry, 67(33), 9160–9167

El-Hadary AE and Ramadan MF. (2019). Phenolic profiles, antihy- perglycemic, antihyperlipidemic, and antioxidant properties of pomegranate (Punica granatum) peel extract. J Food Biochem, 43, e12803.

Elizabeth A Erwin, Kristin Wickens, Natalie J Custis, Robert Siebers, Judith Woodfolk, David Barry, Julian Crane, Thomas A E Platts-Mills. (2005). Cat and dust mite sensitivity and tolerance in relation to wheezing among children raised with high exposure to both allergens. J AlergyClinImmunol, 115(1):74-9.

Erika Méndez-Enríquez and Jenny Hallgren. (2019). Mast Cells and Their Progenitors in Allergic Asthma. Frontiers in Immunology, 10 (821)

Fourati M, Smaoui S, Hlima HB, Elhadef K, Braïek OB, Ennouri K, Mtibaa AC and Mellouli L. (2020). Bioactive compounds and pharmacological potential of pomegranate (Punica granatum) seeds - a review. Plant Foods Hum Nutr, 75: 477-486.

Galli SJ, Tsai M. (2010). Mast cells in allergy and infection: versatile effector and regulatory cells in innate and adaptive immunity. Eur J Immunol, 40:1843–1851.

Galli SJ, Tsai M. (2012). IgE and mast cells in aller- gic disease. Nat Med,18:693–704.

Georas S N, Berdyshev E, Hubbard W, Gorshkova I A, Usatyuk P V, Saatian B, Myers A C, Williams M A, Xiao H Q, Liu M, Natarajan V. (2007). Lyso- phosphatidic acid is detectable in human

bronchoalveolar lavage fluids at baseline and increased after segmental allergen challenge. Clin Exp Allergy,37(3):311–22.

Goetzl, E. J., H. Lee, H. Dolezalova, K. R. Kalli, C. A. Conover, Y. Hu, T. Azuma, T. P. Stossel, J. S. Karliner, and R. B. Jaffe. (2000). Mechanisms of lysolipid phosphate effects on cellular survival and proliferation. Ann. N.Y. Acad. Sci, 905: 177–187.

Hashimoto T, Ohata H, Honda K. (2006). Lysophosphatidic acid (LPA) induces plasma exudation and histamine release in mice via LPA receptors. J PharmacolSci, 100(1):82-7.

Hashimoto T, Ohata H, Momose K, Honda K. (2005). Lysophosphatidic acid induces histamine release from mast cells and skin fragments. Pharmacology, 75(1):13-20.

Im DS, Heise CE, Harding MA, George SR, O'Dowd BF, Theodo- rescu D, Lynch KR. (2000). Molecular cloning and characterization of a ly- sophosphatidic acid receptor, edg-7, expressed in prostate. Mol Phar- macol, 57:753–759.

Karimi M, Sadeghi R, Kokini J. (2017). Pomegranate as a promising opportunity in medicine and nanotechnology. Trends Food Sci Technol, 69:59–73.

Kawa Amin. (2012). The role of mast cells in allergic inflammation. Respiratory Medicine, 106,9e14

Kondoa M, Tezukaa T, Ogawab H, Koyamaa K,Bandoa H, Azumaa, M, Nishioka Y. Lysophosphatidic Acid Regulates the Differentiation of Th2 Cells, and Its Antagonist Suppresses Allergic Airway Inflammation. Int Arch Allergy Immunol, 182(1):1-13.

Kraneveld AD, James DE, de Vries A, Nijkamp FP. (2004). Excitatory non-adrenergic-non-cholinergic neuropeptides: key players in asthma. Eur J Pharmacol, 405:113e29.

Kue PF, Daaka Y. (2000). Essential role for G proteins in prostate cancer cell growth and signaling. J Urol, 164:2162–2167.

Li G, ChenM, ChenJ, ShangY, LianX, WangP, LeiHand Ma Q. (2020). Chemical composition analysis of pomegranate seeds based on ultra-high-performance liquid chromatography coupled with quadrupole-Orbitrap high-resolution mass spectrometry. J Pharm Biomed Anal, 187: 113357.

Li, J. K., Li, G. X., Zhao, Y. H., & Yu, C. Z. (2009). Composition of pomegranate peel polyphenols and its antioxidant activities. Scientia Agricultura Sinica, 42(11), 4035–4041.

Lundequist A and Boyce J A. (2011). LPA5 Is Abundantly Expressed by Human Mast Cells and Important for Lysophosphatidic Acid Induced MIP-1b Release. PLoS ONE, 6 (3). e18192.

Lygia T. Budnikand Amal K. Mukhopadhyay. (2002). Lysophosphatidic Acid and Its Role in Reproduction. Biology of Reproduction, 66, 859–865.

Mastrogiovanni F, Mukhopadhya A, Lacetera N, RyanM T, Romani A, Bernini R, and Sweeney T. (2019). Anti-Inflammatory Effects of Pomegranate Peel Extracts on In Vitro Human Intestinal Caco-2 Cells and Ex Vivo Porcine Colonic Tissue Explants. Nutrients,11, 548

May CD, Lyman M, Alberto R, Cheng J. (1970). Procedures for immunochemical study of histamine release from leukocytes with small volume of blood. J Allergy, 45: 12–20.

Metcalfe DD. (2008). Mast cells and mastocytosis. Blood, 112:946–956.

Montesi SB, Mathai SK, Brenner LN, Gorsh- kova IA, Berdyshev EV, Tager AM. (2014). Docosatetraenoyl LPA is elevated in exhaled breath condensate in idiopathic pulmonary fibrosis. BMC Pulm Med, 14:5.

Musa M, Nasir NF, Thirumulu KP. (2014). Evaluation of royal jelly as an alternative to fetal bovine serum in cell culture using cell proliferation assays and live cell imaging. Afr J Tradit Complement Altern Med,**11**(1):148-55.

NahtaOTa. (2011). Delphinidin Inhibits HER2 and Erk1/2 Signaling and Suppresses Growth of HER2-Overexpressing and Triple Negative Breast Cancer Cell Lines. Breast Cancer: Basic and Clinical Research,5:143–154.

Petersen A, Kleinheinz A,Jappe U. (2011). Anaphylactic reactions to pomegranate: identification and characterization of eliciting IgE-reactive components. Clinical and Translational Allergy, 1,P88.

Rasheed Z Akhtar N, Anbazhagan A N, Ramamurthy S, Shukla M and Haqqi T M. (2009). Polyphenol-rich pomegranate fruit extract (POMx) suppresses PMACI-induced expression of pro-inflammatory

cytokines by inhibiting the activation of MAP Kinases and NF-κB in human KU812 cells. Journal of Inflammation, 6:1

Rengasamy KRR, Khan H, Gowrishankar S, LagoaRJL, Mahomoodally FM, Khan Z, SuroowanS, TewariD, ZenginG, Hassan STS, Pandian SK. (2019). The role of flavonoids in autoimmune diseases: Therapeutic updates. PharmacolTher, 194:107-131.

Salas-Salvadó J1, Becerra-Tomás N1, García-Gavilán JF1, Bulló M1, Barrubés L. (2018). Mediterranean diet and cardiovascular disease prevention: What do we know? ProgCardiovasc.Dis, 61, 62–67

Singh B, Singh JP, Kaur A and Singh N. (2018). Phenolic compounds as beneficial phytochemicals in pomegranate (Punica granatum L.) peel: A review. Food Chem, 261: 75-86.

TagerMA , LaCamera P, Shea BS, Campanella GS, Selman M, Zhao Z, Polosukhin V, Wain J, Karimi-Shah BA, Kim ND, Xu Y, Chun J, Luster AD. (2008). The lyso- phosphatidic acid receptor LPA1 links pul- monary fibrosis to lung injury by mediating fibroblast recruitment and vascular leak. Nat Med, 14(1):45–54.

Valent P.(2013). Mast cell activation syndromes: definition and classification. Allergy, 68: 417–424.

Viuda-Martos M, Fernández-López J and Pérez-Álvarez JA. (2010). Pomegranate and its many functional components as related to human health: A review. Compr Rev Food Sci Food Saf, 9: 635-654.

Wang D, Özen C, Abu-Reidah IM, Chigurupati S, Patra JK, Horbanczuk JO, Jóźwik A, Tzvetkov NT, Uhrin P and Atanasov AG.(2018). Vasculoprotective Effects of Pomegranate (Punica granatum L.). Front Pharmacol, 9: 544.

Wu S, Xiao D. (2016). Effect of curcumin on nasal symptoms and airflow in patients with perennial allergic rhinitis. Ann Allergy Asthma Immunol, 117(6):697-702.e1.

Xiao Y. J B. Schwartz, M. Washington, A. Kennedy, K. Webster, J. Belinson, and Y. Xu. (2001). Electrospray ionization mass spectrometry analysis of lyso- phospholipids in human ascitic fluids: comparison of the lysophospholipid con- tents in malignant vs nonmalignant ascitic fluids. Anal Biochem, 290: 302–313.

Yamprasert R. Chanvimalueng W. Mukkasombut N. and Itharat A. (2020). Ginger extract versus Loratadine in the treatment of allergic rhinitis: a randomized controlled trial. BMC Complementary Medicine and Therapies,20**,** 119.

Yoo JM, Sok DE, Kim MR. (2014). Anti-allergic action of aged black garlic extract in RBL-2H3 cells and passive cutaneous anaphylaxis reaction in mice. J Med Food, 17(1):92-102.

Zhang Z, Shi J, Nice EC, HuangC, Shi Z. (2021). The Multifaceted Role of Flavonoids in Cancer Therapy: Leveraging Autophagy with a Double-Edged Sword. Antioxidants, 10, 1138.

Zhao CN, Meng X, Li Y, Li S, Liu Q, TangGY, Li HB. (2017). Fruits for prevention and treatment of cardiovascular diseases. Nutrients, 9, 598