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The Bioactive Effect Of Royal Jelly Against Histamine Level Induced By Lysophosphatidic Acid (Lpa)

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Abstract

Royal jelly (RJ) is a natural food that widely used to boost medical therapy for a variety of illnesses and improve physiological activity as well as enhance the body's natural immunological response. Histamine is one of the acute inflammatory and hypersensitivity reactions mediators which involved in the regulation of allergic responses and control multiple key processes in the immune response. The current study aimed to evaluate the inhibitory activity of RJ on histamine releasedfrom activated RBL-2H3 cells by lysophosphatidic acid (LPA). Royal jelly was administered to RBL-2H3 cells lines treated with LPA, and histamine expressed levels were determined using a spectrofluorometric technique. Royal jelly significantly reducedthe level of histamine produced in a time- and a dose-dependent manner ($p < 0.001$).Moreover, RJ had no significant cytotoxicity on RBL-2H3 cells.Our data suggested that RJ is an anti-allergic natural product that aids in the reduction and attenuation of allergic reactions. These effects might be connected to its ability to improve allergic symptoms by reducing histamine level.

Keywords: histamine, royal jelly,immunomodulator, lysophosphatidic acid, RBL-2H3 cells

Introduction

Apitherapy is the science of using honeybee products to preserve health and help people recover from illness. Local unfiltered honey consumed daily over a lengthy period time can maintain the patient allergy-free (Cherbuliez 2013; Sugiyama et al., 2012). Natural bee products have been more popular in both traditional and contemporary medicine, moreover, utilized to preserve human health (Pasupuleti et al., 2017).

Royal jelly (RJ) is an acidic creamy fluid secreted by honeybees thatis formed by the mandibular and hypopharyngeal glands of juvenile Apismellifera worker bees (Collazo et al., 2021; Buttstedt et al.,

2018).Royal jelly has essential active components such as RJ proteins, lipids, phenols, and flavonoids, all of which play an important part in human health maintenance. Many studies demonstrated anti-inflammatory, anti-cancer, immunological regulation activity of RJ effects (Guo et al., 2021; Sugiyama et al., 2012).Furthermore, the immune system advantages from natural products are amazing, they are engaged in the activation of antibody formation, maturation of immune cells, and stimulation of innate and adaptive immunological responses (Hu et al., 2018; Siiskonenand Harvima2019).

Mast cells play a key role in allergic responses and immunity. They are important in the development of allergy and inflammatory illnesses such as asthma, mastocytosis, inflammatory arthritis, and autoimmune diseases. Furthermore, it has been proposed that mast cells have a role in both the adaptive and innate immune responses to infections (Passante and Frankish 2009). They are either completely loaded with mediators or produce fresh mediators, which they released upon activation via a variety of processes that resulted in degranulation process(Hu et al., 2018; Siiskonen and Harvima 2019).Several factors as infections, stress, certain foods, pseudoallergens, hormones, neuropeptides, and Th2 inflammation progress are all known to activate mast cells (Church et al., 2018; Siiskonen and Harvima 2019).

Mast cells degranulatemediators that have a role in itch formation. Mast cell histamine is the most significant mediator derived from mast cell (Harvima et al., 2014).Histamine is a characteristic of allergic reactions and increases vascular permeability.Whereas released cytokines and chemokines play critical roles in starting and sustaining inflammatory responses in allergic reactions (Moiseeva and Bradding 2011; Lian et al., 2015).

RBL-2H3 cells are a model for mast cell derived mediator release and it’s a helpful tool for in vitro work since they can produce a large number of monoclonal cells rapidly using basic cell culture procedures (Passante, and Frankish 2009; Lima et al., 2020; Niu et al 2021).

Lysophosphatidic acid (LPA) is a powerful, bioactive lipid molecule whose signaling is triggeredin a variety of physiological processes (Mizuno and Kihara 2020; Ray et al., 2021).Recent studies support the notion that LPA plays a key role in asthma and allergic airway inflammation. LPA stimulates numerous cell types involved in inflammation, including eosinophils, mast cells, dendritic cells, and lymphocytes according to in vitro studies (Hashimoto et al., 2005; Georas 2021).

Given RJ's apparent anti-inflammatory properties, we speculated that RJ may have a similar impact on allergic reactions. In this work, we focused on the anti-allergic activity of royal jelly on RBL-2H3 cells in vitro.

Materials and Methods

Materials

This study was an in vitro study which was conducted on cell lines. RBL-2H3 cell line was provided by VACSERA - Cell Culture Unit, Dokky, Giza, Egypt. Mono oleoylphosphatidic acid monosodium (LPA) was got from Sigma-Aldrich (CAS Number: 268550-95-4). RJ was purchased from a local market in Egypt, except for the RJ supplied by the Egyptian Royal Jelly Farm.

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

Histamine induction by LAP

TheRBL-2H3 cell line suspension was incubated for 5 min at 37 ° C, oleoylphosphatidic acid monosodium (LAP) 10 µg /ml were introduced and incubated for 30 min. The induction was terminated by immersingthe suspension in ice-cold water. Centrifugethe tubes at 1,500 g for 10 min at 4 °C.Determination ofhistamine in the supernatant spectrofluorometricallyaccording to other studies (Hook et al., 1976; Hashimoto et al., 2005), and the percentage of the total released histamine was calculated.

Determination of histamine level after RJ challenge

Cells suspensionwas incubated at 37 °C with 10 µg/ ml of LPA for 10 min, then 100 µl /ml RJ was introduced to the same sample of RBL-2H3 cells for additional incubation time (10, 20 and 30 min). Histamine levels are calculated as a percentage of the total released histamine.

Dose course of histamine induction from RBL-2H3 cells was examined after 60 min incubation at 37 °C with 10 µl /ml of LPA and serial concentration of RJ (5, 10, 25, 50, and 100 µl/ml) of cell suspensions. Histamine levels were evaluated for comparison with LPA stimulation.

Cell Viability Assay

RBL-2H3 cells are mucosal mast cells that have been extensively studied for degranulation properties in a variety of natural product and pharmaceuticals.RBL-2H3 cells were subjected to a cell viability

experiment using the MTT colorimetric technique, as previously reported (May et al., 1970; Hashimoto et al., 2005). RBL-2H3 cells were harvested and transferred into 96-well micro-plates at a concentration of 40,000 cells per welland left overnight (37°C, 5% CO2) before dosing, then different concentrations of RJ were added (5, 10, 25, 50 and 100 µl /ml) for 48 hours at 37 °C in a humidified incubator (5% CO_2, 95% air) to evaluate the cytotoxic effect of RJ. 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 microplate reader at 570 nm.

The test samples were considered cytotoxic when the optical density of the sample-treated group was less than 80% of that in the spontaneous release(Matsuda et al., 2007Musa et al., 2014; Chansuwan et al., 2019). These concentrations showed least toxicitypercentages on cells lines as cell viability were 80%. Hence, RJ concentrations in current study is safe for evaluate anti-allergic action in RBL-2H3 cell lines.

Statistical Analysis

Analysis of Variance data was performed on the (ANOVA).For data analysis, the SPSS statistics tool (Version 16.0) was employed.The mean with standard error is used to represent the results (SEM). $P<0.05$ were considered to be significant

Results and Discussion

Mast cells are playing a crucial roles in the beginning and progression of an allergic reaction. A variety of triggers can activate them,either IgE-dependent or independent causing them to produce inflammatory mediators, as well as produce histamine and other vasoactive chemicals, resulting in hives (Krystel-Whittemoreet al., 2016; Lian et al., 2015).

The interesting areas of traditional medicine today is the use of immunomodulatory substances such as royal jelly to treat a variety of illnesses (Guendouz et al., 2017; Bouamama et al., 2021; Harwood et al., 2021). Royal jelly is widely used in the nutraceutical and cosmetic industries.Water, trace minerals, proteins, lipids,carbs, vitamins, and phenols make up the majority of its composition (Collazo et al., 2021).

RBL-2H3 cells were employed in this work to explore RJ's anti-allergic efficacy in vitro. When the cell line triggered by LAP, they can produce histamine and other inflammatory cytokines during the degranulation process(Hook et al., 1976; Hashimoto et al., 2006).After administration of lysophosphatidic acid (LPA) to induce the release of histamine, the total level of histamine level was assessed with spectrofluorometric technique.

Figure1 showed histamine release from cell lines. The spontaneous histamine release from RBL-2H3 cells was 5.45–12.32%. Whereas 10 μg/ml LPA led to increased histamine productionto the maximum level reached to 27.12 at 30 min. In contrast, RJ-treated cells significantly reduced the histamine release at 30 min ($p \leq 0.01$).

Moreover, as shown by RJ in vitro test challenge with different concentrations, the histamine level was decreased significantly with high RJ doses ($p< 0.01$) as shown in figure 2. Histamine level induced from control RBL-2H3 rat mast cells was 7± 0.75%. RJ reduced histamine release in a dose-dependent manner. Histamine reduced level at 5, 10, 25, 50 and 100 μg/ml were 16.12, 13.21, 10.04, 9.47 and 8.15 ± 0.75%, respectively. Significant differences were observed between RBL-2H3 rat mast cells induced by LPA- and RJ-treated at all concentrations examined. This result agree well with that found by Guendouz (Guendouz et al., 2017), who demonstrated that effective decrease of plasma histamine level in Balb/c mice administratedby mouth tube for 7 days with RJ with different doses. Several hypotheses have been presented to the underlying mechanisms of RJ's immune modulationand prevent an allergic responseactivity. Vucevic et al. (2007) proposed a specialized effect of RJ in histamine induction, and inflammation.

The interesting observation was the reduction response of histamine was correlated with a gradual incubation time and dose-dependent. As a result, our findings were consistent with prior research on the potential anti-allergic functions of royal jelly (Okamoto et al., 2003; Vucevic et al., 2007). On the other hand, other studies reported adverse events with some special cases that associated with allergic response from RJ due to cross-reactive reactions with causative allergens and they advise people with a history of allergic illnesses should avoid ingesting RJ products (Harwood et al 1996; Hata et al., 2020).

Another interesting finding is that the exposure of RBL-2H3 rat mast cells to different doses of RJ (5, 10, 25, 50 and 100 μl /mL)after 48 hours of incubation resulted in no significant cytotoxicity on cell lines.

Moreover, royal jelly can induce the best survival rate with 100 µl /mL under current test conditions (figure 3).

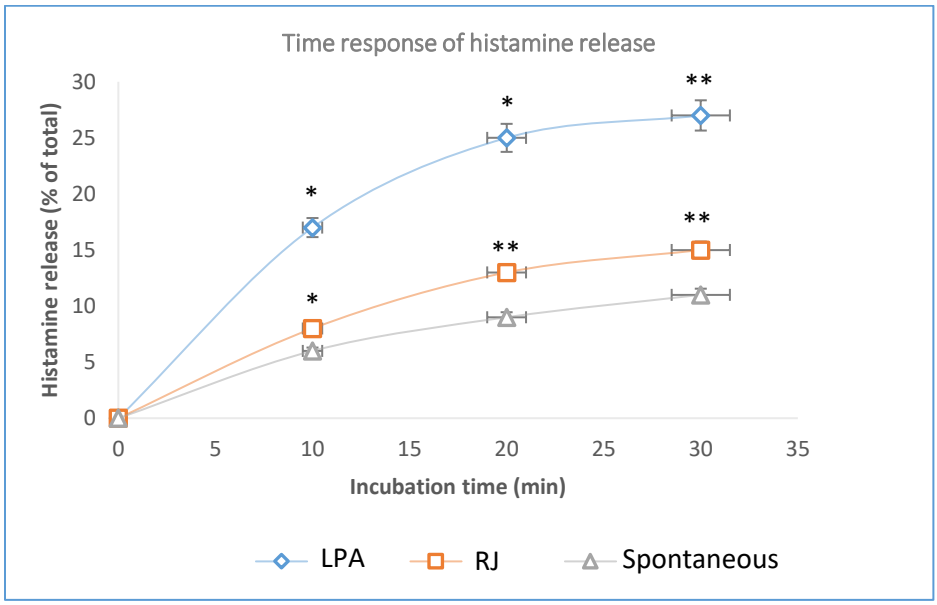

Figure 1. Time response manner of histamine induction from RBL-2H3 cells induced with 10 µg/ml LPA for 30 min. at 37 °C and inhibitory activity of RJ treatment. Histamine levels were determined using a spectrofluorometric technique.It shows statistically significant differences from the spontaneous release* $p \leq 0.05$, ** $p \leq 0.01$.

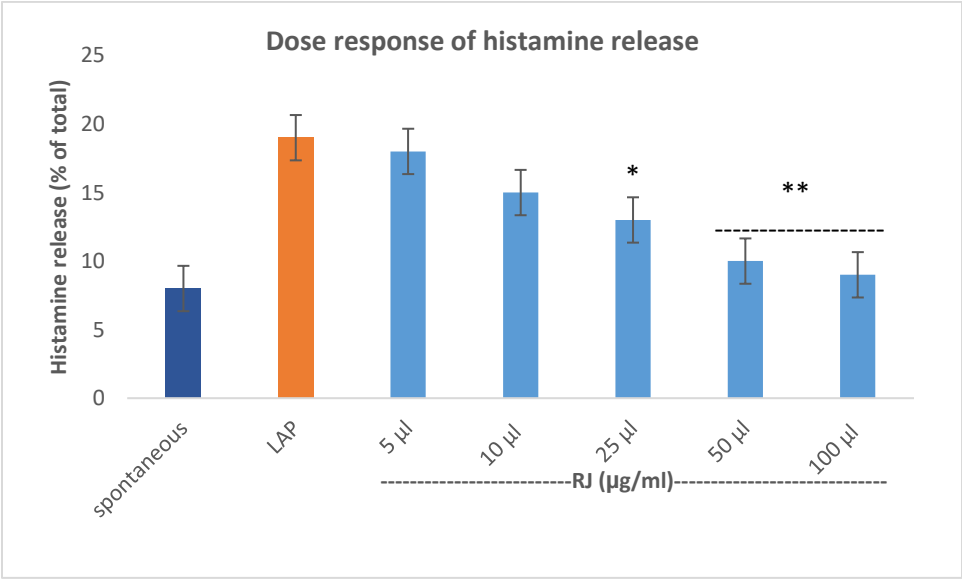

Figure 2. Histamine concentrationinduced from RBL-2H3 cells treated with 10 µg/ml LPA and serial concentration of RJ (5, 10, 25, 50 and 100 µl/ml) for 10 min. at 37 °C. RJ at dose 25µl/ml shows statistically significant differences at *p< 0.05 and greater doses (50 µl/ml and 100µl/ml) shows statistically significant differences from the spontaneous release**p< 0.01.

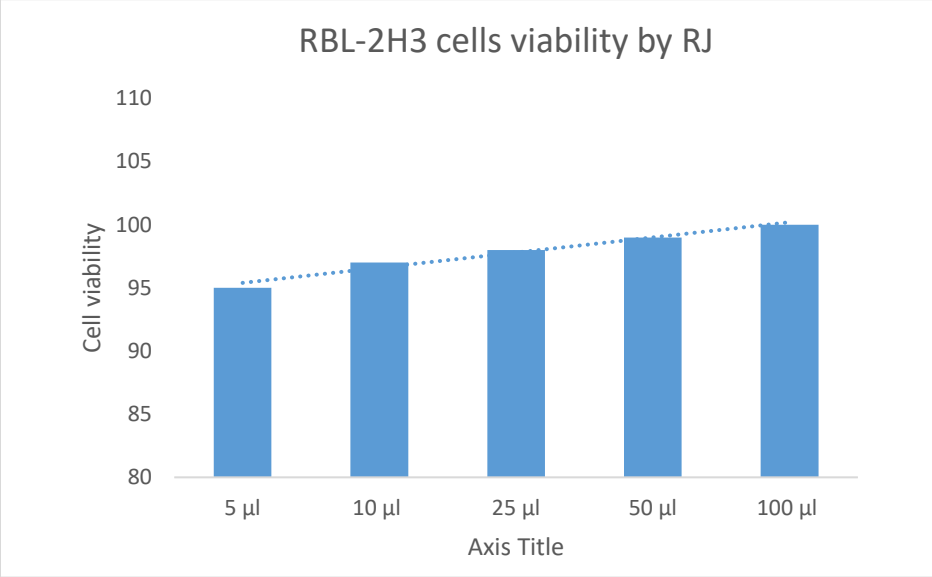

Figure 3: Effect of RJ on RBL-2H3 cells viability in terms of trypan blue staining.Linear graph showing RJ effect on cell viability in RBL-2H3 cell line.

Conclusion

In summary, we have demonstrated that RJ can reduce histamine levelsafter mast cell activation stage.According to the findings, RJ administration may have a beneficial effect on the allergy in addition to decreasing its symptoms. The mechanism of RJ attenuation to histamine level remains mostly unclear. Additional efforts to define the role of RJ interactions with histamine will potentially lead to novel clinical approaches for allergic conditions.

Conflicts of Interest

The author have no conflicts of interest to declare.

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