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Regulation of Immune and Nonimmune Mast Cell Activation by Phenols from Olive Oil

Alicia Beatriz Penissi

Abstract

The purpose of this study is to establish if hydroxytyrosol and oleuropein, the most significant phenols found in olive oil and olives, can inhibit the activation of mast cells induced by immune and nonimmune pathways. Preincubation of purified peritoneal mast cells was carried out in the presence of hydroxytyrosol or oleuropein compounds and, prior to incubation, with concanavalin A, compound 48/80, or calcium ionophore A23187. Dose-response and time-dependence were studied. Comparative studies were performed using sodium cromoglycate, a well-known mast cell stabilizer. The supernatants and pellets were analyzed for β-hexosaminidase content via colorimetric reaction after incubation. The percentage of β-hexosaminidase obtained in each tube was measured and taken as a referent mast cell activation indicator. Other cell pellet samples were studied for cell viability, by means of the trypan blue exclusion method, or analyzed with light and electron microscopy. For the first time, biochemical and morphological results have shown that hydroxytyrosol and oleuropein inhibit degranulation of mast cells triggered by both immune and nonimmune causes. These findings suggest that olive phenols, specifically hydroxytyrosol and oleuropein, may be set the bases for developing practical tools not only to prevent and treat mast cell-mediated disorders but also to improve olive oil industrialization.

Keywords: β-hexosaminidase, degranulation, hydroxytyrosol, mast cell, oleuropein, olive

1. Introduction

Mast cells are key effector cells that clearly play both physiological and pathophysiological functions in the body [1]. In vertebrates, mast cells are widely distributed in the tissues, especially near surfaces exposed to the environment, where pathogens, allergens, and other environmental agents are frequently found. Due to this distribution pattern, they are some of the first cells involved in the immune response which interact with environmental antigens and allergens, invading pathogens or environmentally derived toxins [2]. Mast cells have been involved in the pathogenesis of a number of disorders including contact dermatitis, allergic rhinitis, asthma, atopic dermatitis, bullous pemphigoid, fibrotic lung disease, cancer, multiple sclerosis, neurofibromatosis, psoriasis, scleroderma, rheumatoid arthritis,

interstitial cystitis, ulcerative colitis, peptic ulcer, and Crohn's disease [1, 3–7]. Understanding mast cells is essential for the pathophysiological bases of this type of disorders since these cells release varied inflammatory mediators prompted by both immune and nonimmune causes. Among mast cell mediators, preformed molecules can be mentioned, for example, histamine and proteases, which are accumulated in secretory granules [7–9]. The immediate response upon mast cell activation to an appropriate stimulus is called degranulation, characterized by the extrusion of cytoplasmic granule contents into the extracellular space by a process called exocytosis [10]. In this context, the exploration of interactions of mast cells with molecules capable of modulating mediator release from cell granules is a promising field for the treatment of *mast cell-mediated diseases*.

In vitro and in vivo studies have shown that several plant products with antioxidant properties inhibit mast cell activation induced by both immune and nonimmune secretagogues [11–14]. Recent scientific evidence from preclinical studies and clinical trials including humans has highlighted different nutritional interventions, such as dietary polyphenols, as promising agents able to alleviate symptoms associated with mast cell activation [15]. Dietary polyphenols are a class of bioactive compounds found in abundance in plants and fruits which have been studied thoroughly in several disease models [15]. The pulp of olives contains these compounds, which are hydrophilic, and they are also found in the oil. The class of phenols includes numerous substances, such as simple phenolic compounds like hydroxytyrosol and more complex compounds like oleuropein [16]. Hydroxytyrosol and oleuropein, the major phenols found in olives, are used in disease prevention because they have important antioxidant and anti-inflammatory properties [17, 18]. Several in vitro and in vivo studies have shown that oleuropein and its derivate hydroxytyrosol possess a wide range of biochemical and pharmacological properties. However, no studies have been published on the effects of these molecules on mast cell degranulation.

We carried out a series of experiments to determine the effects of both phenolic compounds on mast cell degranulation and thus explore the possibility that oleuropein and hydroxytyrosol might inhibit in vitro mast cell activation.

The biochemical and morphological findings of the present study showed for the first time that hydroxytyrosol and oleuropein inhibit the degranulation of mast cells induced by both immune and nonimmune pathways.

2. Material and methods

2.1 Chemicals and reagents

The compound molecules, hydroxytyrosol and oleuropein, were provided by Extrasynthèse (Lyon, France). **Figure 1** shows the polyphenol chemical structure. A solution containing 6.7 mM Na_2HPO_4, 6.7 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, 0.8 mM $CaCl_2$, 0.5 g/l albumin, and 1 g/l glucose was used for dissolving the polyphenols, which was adjusted to pH 7.2 and stored at −20°C. The same solution was used for diluting the stock solutions until the final concentration was reached. Bovine serum albumin (fraction V), concanavalin A, compound 48/80, calcium ionophore A23187, sodium cromoglycate, 4-nitrophenyl-N-acetyl-β-D-glucosaminide, toluidine blue, trypan blue, glutaraldehyde, formaldehyde, and osmium tetroxide were acquired from Sigma (St. Louis, MO, USA). Percoll was purchased from GE Healthcare (Munich, Germany). All other substances were provided by Merck (Darmstadt, Germany). The highest quality available is guaranteed in all the chemicals used for this study.
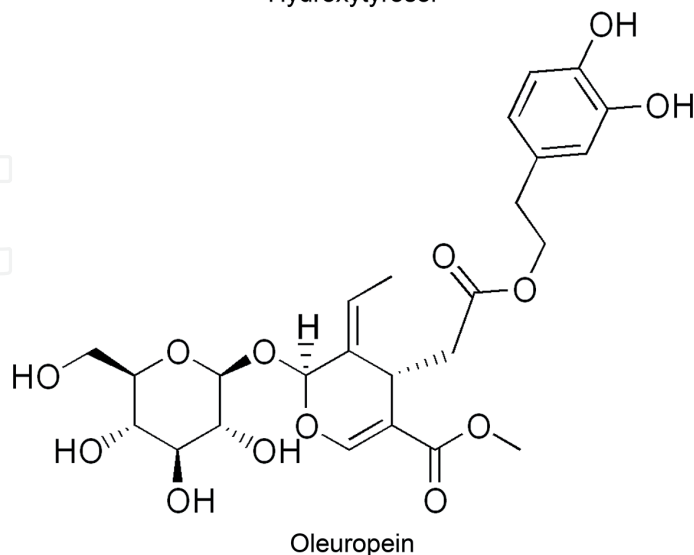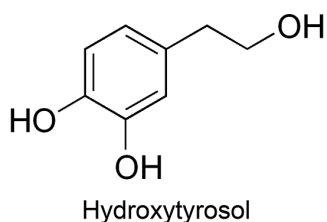
Figure 1.
Structural formulas of polyphenols used in this study.

2.2 Animals

Adult male 300–500 g Wistar rats free from infections were used for the study. They were kept under a 12-h dark/light cycle in a room set at 24–25°C with free access to laboratory food and drinking water. Animals experiments were carried out according with the standards contained in the *Guide for the Care and Use of Laboratory Animals*, published by the National Academy of Sciences, National Academies Press, Washington, DC, and accepted by the Institutional Committee for Care and Use of Laboratory Animals (CICUAL, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza, Argentina).

2.3 Mast cell isolation and purification

Isolation of mast cells was performed by means of peritoneal lavage as described before [19] with some modifications. Rats were killed by CO_2 inhalation and then injected with 20 ml of a pH 7.2 solution containing 6.7 mM Na_2HPO_4, 6.7 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, 0.8 mM $CaCl_2$, 0.5 g/l albumin, and 1 g/l glucose, into the peritoneal cavity. A gentle massage was applied on the abdomen for about 3 min. After the peritoneal cavity was opened with care, a Pasteur pipette was used for aspirating the fluid with peritoneal cells, which were then purified via centrifugation through a discontinuous gradient of Percoll per reports by MacGlashan and Guo [20]. It was easy to harvest the mast cells since they precipitated to the bottom of the tube and formed a layer, different from the other cells that formed a rather compact layer on top of the gradient and were simply removed by aspiration. Cell metachromatic staining was carried out with toluidine blue (0.1% w/v, pH 1.0), and quantification required a Neubauer hemocytometer under a Nikon microscope (magnification 200×). Mast cells were present in the crude peritoneal suspended content by 3%, and, after gradient centrifugation, their purity rose to over 95%. After washing purified mast cells, resuspension was performed in a balanced salt

solution with 6.7 mM Na_2HPO_4, 6.7 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, 0.8 mM $CaCl_2$, 0.5 g/l albumin, and 1 g/l glucose, adjusted to pH 7.2 (cell density of 1×10^6/ml) and kept at 4°C for 30 min at the most. Both the ability of mast cells to exclude trypan blue and the amount of β-hexosaminidase in the supernatant established mast cell viability. The trypan blue exclusion test determined that the mast cells were viable by over 95%. Basal β-Hexosaminidase release remained always below 4%.

2.4 General protocol

Purified peritoneal mast cells (cell density of 1×10^6/ml) were balanced at 37°C for 10 min. Preincubation of 30-μL aliquots of the balanced cells required polypropylene tubes at 37°C with hydroxytyrosol or oleuropein. Then, incubation was carried out at 37°C for 10 min using concanavalin A (final concentration was 200 μg/ml, with 50 μg/ml phosphatidylserine as a co-stimulator), compound 48/80 (concentrated at 10 g/ml), or calcium ionophore A23187 (final concentration 50 μg/ml). Positive and negative controls, that is, with and without mast cell secretagogues stimulation, respectively, were included. Studies on dose–response (hydroxytyrosol and oleuropein concentrations of 10, 50, 100, 200, and 400 M) and time-dependence (hydroxytyrosol and oleuropein preincubation for 5, 10, 20, and 45 min) were performed. Comparative studies were performed using sodium cromoglycate, a well-known mast cell stabilizer, with identical concentrations and time ranges. Each tube's final incubation volume reached 100 l. During incubations, the average total number of mast cells was 4×10^4/ml per tube. Secretion was halted by cooling the tubes in an ice-cold water bath. Cells and supernatants were separated by centrifugation (180 g, 5 min, 4°C). The supernatants helped determine the β-hexosaminidase content by colorimetric reaction, which was a parameter to measure β-hexosaminidase release. The cell pellets were lysated with 1% Triton X-100 to release the remaining β-hexosaminidase, which was quantified by colorimetric reaction and taken as a measure of the residual β-hexosaminidase. Other cell pellet samples were studied for cell viability, by means of the trypan blue exclusion method, or analyzed with light and electron microscopy. The purpose of cell viability studies was to ascertain that changes in β-hexosaminidase release were not caused by cell death. The percentage of β-hexosaminidase obtained in each tube was measured. All the tests were performed at least five times in duplicate.

2.5 β-Hexosaminidase assay

β-Hexosaminidase release, as an index of mast cell degranulation, was assayed by a colorimetric assay as before explained [21] with some changes. In brief, 50 μL of the supernatant was combined with an equal volume of 2 mM substrate solution (p-nitrophenyl-N-acetyl-β-D-glucosaminide in 0.2 M citrate, pH 4.5) and then incubated for 3 h at 37°C. The reaction was halted by adding 250 μL of stopping buffer (0.4 M glycine in Na_2CO_3/$NaHCO_3$, pH 9). Absorbance was studied with a microplate reader at 405 nm (Thermo Scientific Multiskan FC, Helsinki, Finland). Results were stated as the percentage of β-hexosaminidase activity released over the total (enzyme released plus intracellular enzyme).

2.6 Light microscopy and morphometry

Mast cells were fixed in 2% glutaraldehyde for 2 h. Then, the suspended cells were stained with toluidine blue (0.1% w/v, pH 3.0), put between slides and cover

slides, and analyzed under a Nikon Optiphot 2 microscope. Using a magnification of 400×, percentage of degranulated mast cells was quantified. Mast cell was considered active due to the presence of extruded granules near the surface of the cell in question or a toluidine blue stain in half or less of the cell.

2.7 Transmission electron microscopy

Karnovsky's fixative (2% formaldehyde, freshly prepared from paraformaldehyde, 2.5% glutaraldehyde, 0.025% $CaCl_2$, 0.1 M cacodylate buffer, pH 7.4) was used for mast cell fixation. After 1 h in the fixative at 20°C, mast cells were rinsed in 0.2 M cacodylate buffer and postfixed in 1% OsO_4 in 0.1 M cacodylate buffer for at least 2 h at room temperature and dehydrated in ethanol. Next, suspended cells were embedded in Spurr (Pelco, USA). An automatic ultramicrotome (Leica Ultracut R, Austria) was used to cut semithin transverse sections (1 μm), which were stained with filtered 1% toluidine blue. Ultrathin sections (60 nm) were cut with diamond knives, stained with uranyl acetate and lead citrate, mounted on grids (Pelco, USA), and examined in a transmission electron microscope (Zeiss EM 902, Germany).

2.8 Statistical analysis

Results obtained from biochemical and morphometric analyses are presented as ±SEM. Variance analysis was used to determine differences between groups, followed by Tukey-Kramer multiple comparisons test. $P < 0.05$ was considered statistically significant.

3. Results and discussion

Figure 2 shows the effect of varying concentrations of hydroxytyrosol and oleuropein on the mast cells β-hexosaminidase release, induced by concanavalin A, compound 48/80, or calcium ionophore A23187. β-Hexosaminidase release was significantly increased by incubating mast cells with 200 μg/ml concanavalin A or 10 μg/ml compound 48/80 or 50 μg/ml calcium ionophore A23187 solutions, as compared with the corresponding value from the basal group (basal release was always less than 4%). The concanavalin A-induced effects were inhibited by preincubation of mast cells with hydroxytyrosol (10, 50 and 100 μM) or oleuropein (100 μM). Mast cell activation induced by compound 48/80 was inhibited by hydroxytyrosol (100 μM) and oleuropein (100 μM). The calcium ionophore A23187-induced effects were inhibited by preincubation of mast cells with hydroxytyrosol (100 μM) and oleuropein (10, 50 and 100 μM). The inhibitory action of the polyphenols was not accompanied by changes in cell viability (the trypan blue exclusion test indicated a viability of greater than 80%), except for polyphenol concentrations higher than 100 μM (data not shown).

Figure 3 shows a dose-response comparative study with sodium cromoglycate, a mast cell stabilizer, which was used to evaluate the potency of hydroxytyrosol and oleuropein. The inhibitory effect of hydroxytyrosol was higher than that obtained with sodium cromoglycate at the same concentration (100 μM) when mast cells were challenged with concanavalin A. No significant differences were observed when mast cells were challenged with compound 48/80. The oleuropein inhibitory effect was higher than the one obtained with sodium cromoglycate at the same concentrations (10, 50 and 100 μM) when the calcium ionophore A23187 was used to challenge mast cells.
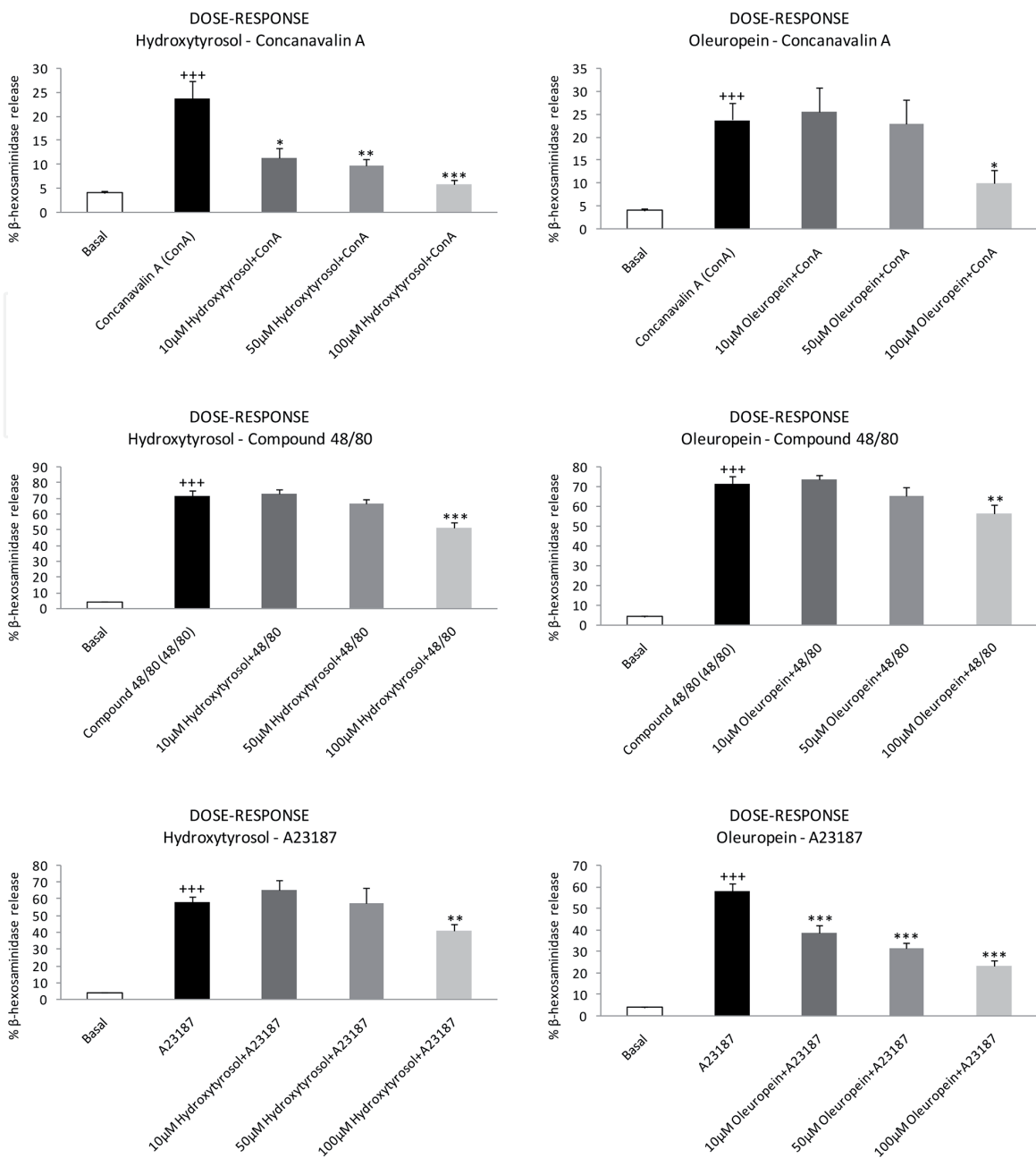
Figure 2.
Effect of varying concentrations of hydroxytyrosol and oleuropein on the concanavalin A-, compound 48/80-, and calcium ionophore A23187-induced β-hexosaminidase release from peritoneal mast cells. Mast cells were preincubated with increasing concentrations of hydroxytyrosol or oleuropein for 10 min and then stimulated with 200 μg/ml concanavalin A, 10 μg/ml compound 48/80, or 50 μg/ml calcium ionophore A23187, for 10 min at 37°C. β-Hexosaminidase release was measured by colorimetric reaction with the chromogenic substrate p-nitrophenyl-N-acetyl-β-D-glucosaminide. Results are expressed as percentage release of β-hexosaminidase. Values are presented as means ± SEM $^{+++}P < 0.001$ versus basal, $^{*}P < 0.05$ versus secretagogue, $^{**}P < 0.01$ versus secretagogue, and $^{***}P < 0.001$ versus secretagogue.

The kinetic study results connected with the effect of hydroxytyrosol and oleuropein on β-hexosaminidase release from mast cell are described in **Figure 4**.

The concanavalin A-induced effect was inhibited by hydroxytyrosol (10 min), oleuropein (10 min), and sodium cromoglycate (10 min). Mast cell activation induced by compound 48/80 was only inhibited by sodium cromoglycate (5, 10, and 20 min). The ionophore A23187-induced effect was inhibited by hydroxytyrosol (10 min), oleuropein (5, 10, and 20 min), and sodium cromoglycate (10 and 20 min). The inhibitory action of the test compounds was not accompanied by changes in cell viability (the trypan blue exclusion test indicated a viability of greater than 80%), except for incubation times higher than 20 min (data not shown).
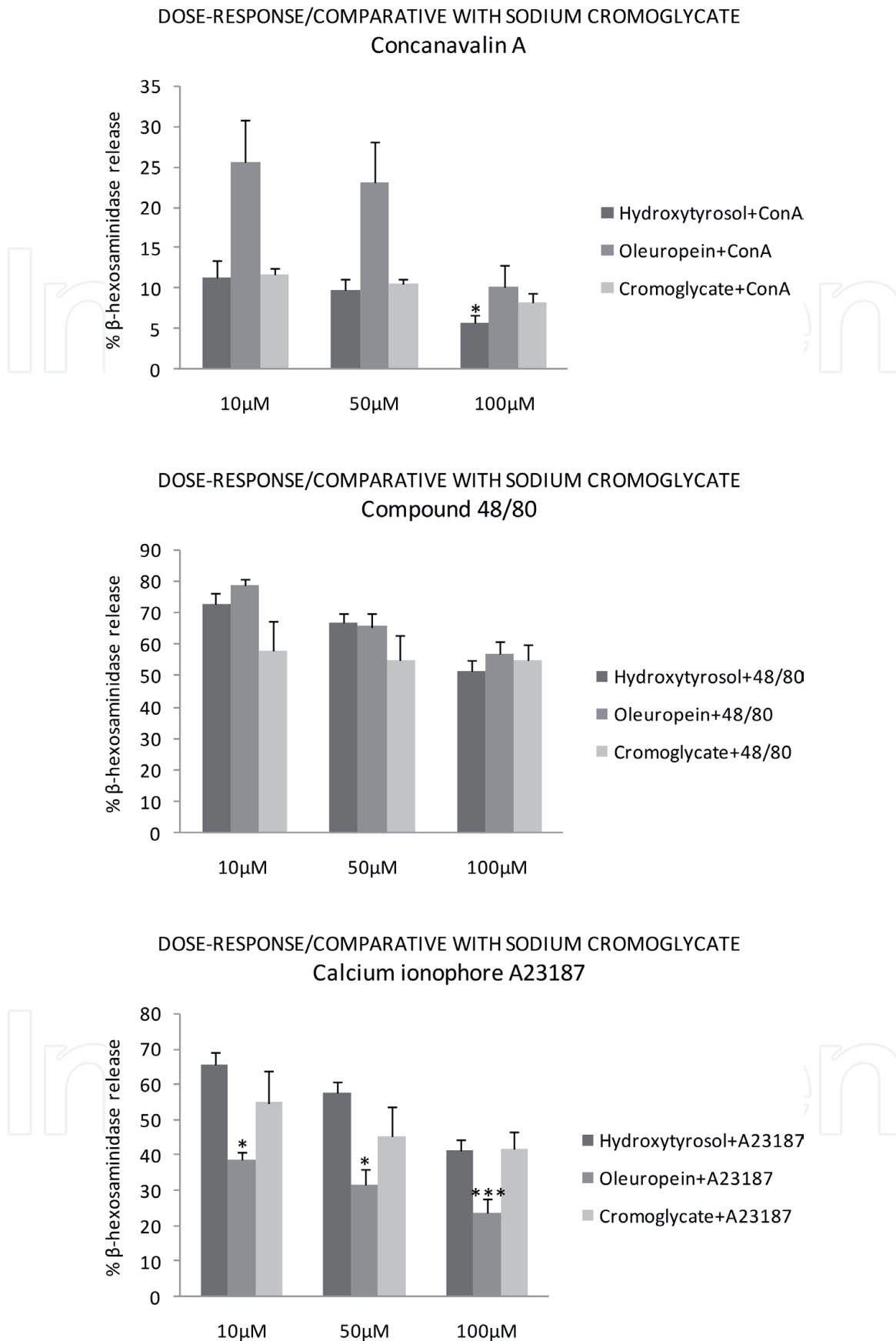
Figure 3.
Effect of hydroxytyrosol and oleuropein on β-hexosaminidase release from rat peritoneal mast cells, compared with a reference compound, the mast cell stabilizer sodium cromoglycate. Purified mast cells were preincubated with increasing concentrations of hydroxytyrosol, oleuropein, or sodium cromoglycate for 10 min and stimulated with 200 μg/ml concanavalin A, 10 μg/ml compound 48/80, or 50 μg/ml calcium ionophore A23187 for another 10 min at 37°C. β-Hexosaminidase release was measured by colorimetric reaction with the chromogenic substrate p-nitrophenyl-N-acetyl-β-D-glucosaminide. Results are expressed as percentage release of β-hexosaminidase. Values are presented as means ± SEM [*]P < 0.05 versus sodium cromoglycate and [***]P < 0.001 versus sodium cromoglycate.
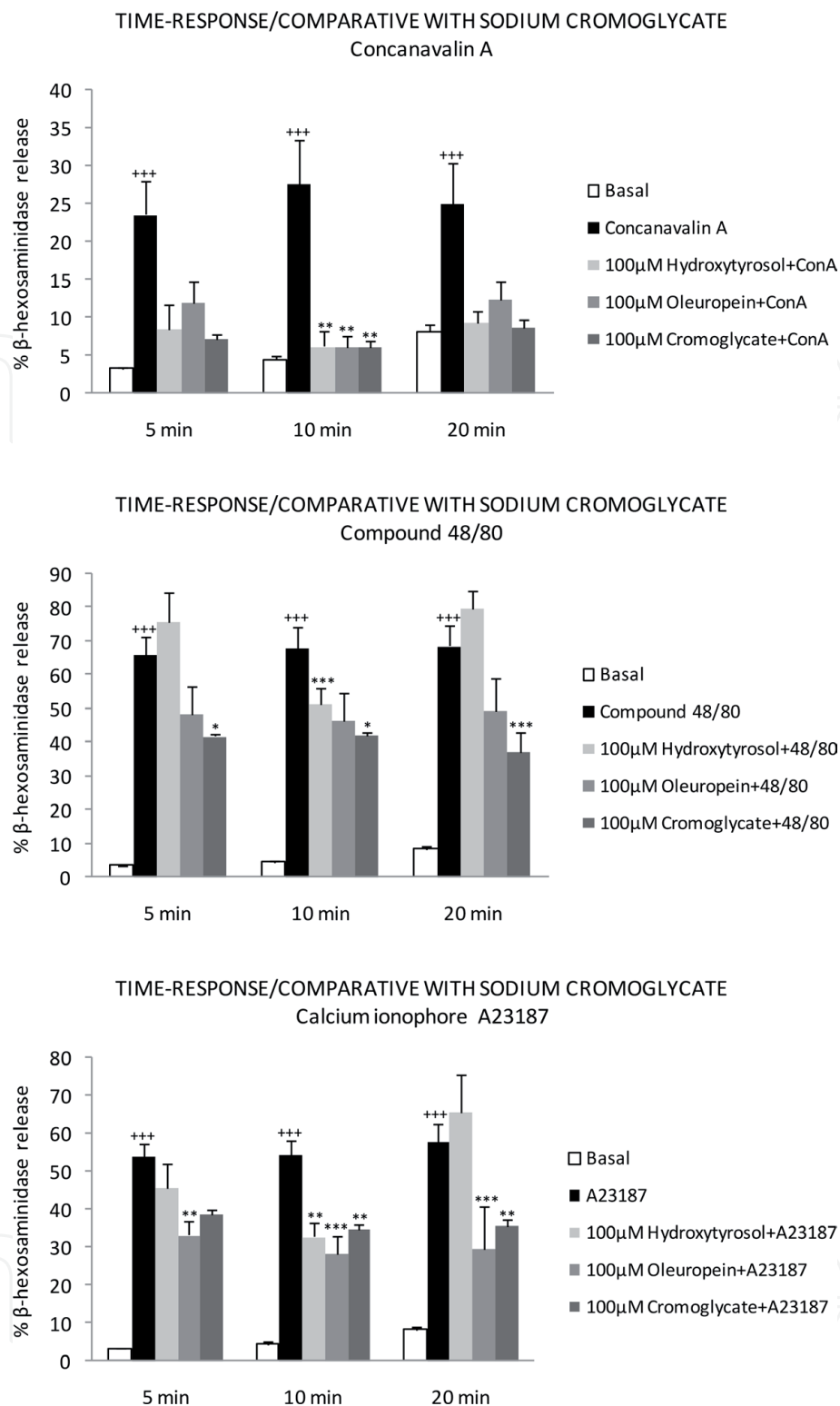
Figure 4.
Kinetic study related to the effect of hydroxytyrosol or oleuropein preincubation on mast cell β-hexosaminidase release, induced by concanavalin A, compound 48/80, or calcium ionophore A23187. Peritoneal mast cells were preincubated for increasing times with 100 μM hydroxytyrosol or oleuropein and then stimulated with 200 μg/ml concanavalin A, 10 μg/ml compound 48/80, or 50 μg/ml calcium ionophore A23187 for 10 min at 37°C. β-Hexosaminidase release was measured by colorimetric reaction with the chromogenic substrate p-nitrophenyl-N-acetyl-β-D-glucosaminide. Results are expressed as percentage release of β-hexosaminidase. Values are presented as means ± SEM [+++]P < 0.001 versus basal, [*]P < 0.05 versus secretagogue, [**]P < 0.01 versus secretagogue, and [***]P < 0.001 versus secretagogue.

As a matter of interest, the main findings of this study evidenced that hydroxytyrosol and oleuropein, at non-cytotoxic concentrations, inhibit β-hexosaminidase release from peritoneal mast cells stimulated by different triggers, acting then as mast cell stabilizers.

Mast cell activation is inhibited by several dietary polyphenols, as shown in other in vitro studies [11, 15]. A well-studied immunological effect of polyphenols such as quercetin is their inhibitory action on degranulation, particularly histamine release from mast cells [11, 15, 22]. Quercetin is able to regulate the entry of calcium into mast cells; also the role of apple and grape quercetin and polyphenols in inhibiting mast cell degranulation has been thoroughly documented using in vitro systems such as the RBL-2H3 assay [11, 15]. Epigallocatechin gallate was found to be the active ingredient in green tea extracts for protection against cutaneous inflammation. This compound may also inhibit mast cell histamine release stimulated with both a calcium ionophore and an IgE-antigen complex [23]. Kaempferol, myricetin, phloretin, and luteolin also proved to be effective inhibitors of histamine release [11, 15]. However, hydroxytyrosol and oleuropein were never studied on mast cell mediator release in response to either immune or nonimmune triggers.

In addition, we have also proven that the inhibitory effects by hydroxytyrosol were stronger than those of oleuropein or the reference compound sodium cromoglycate when mast cells were activated by concanavalin A. On the contrary, oleuropein resulted to be a more efficient inhibitor of mast cell degranulation than hydroxytyrosol or sodium cromoglycate when cells were challenged with the calcium ionophore A23187. **Table 1** presents EC_{50} values for each compound. These results strongly suggest that the inhibitory activity might be defined by the nature of the stimulus for β-hexosaminidase release and the chemical structure of both polyphenols. Each of the secretagogues takes a different mast cell activation pathway, and these pathways may be differentially sensitive to the action of hydroxytyrosol or oleuropein. Mast cells may be activated via several different mechanisms, among which is the classical pathway known as immunological or IgE-mediated mast cell activation, triggered by the cross-linking of FcεRI receptors. Mast cell activation may also be completed in an IgE-independent manner using commercially available activators, such as basic secretagogues and calcium ionophores. Concanavalin A is a glucose-/mannose-specific lectin that activates mast cells by a mechanism similar to the antigen-antibody reaction. This lectin causes FcεRI receptors to cluster on cell membranes. In turn, this triggers a series of intracellular events leading to the secretion of mast cell mediators by exocytosis. Phosphatidylserine markedly enhances the release of mediators from rat mast cells by concanavalin A [24–26]. The synthetic compound 48/80, a basic secretagogue, activates heterotrimeric G proteins by enhancing the dissociation of GDP from Gα subunits, thus accelerating the event considered as a rate-limiting step in conventional G protein activation by receptors coupled to heterotrimeric G proteins [27]. Calcium ionophore A23187, a mobile carrier of divalent cations such as Ca^{2+}, Mg^{2+}, and double H^+, may reduce the level of calcium stored in mitochondria or increase the inflow from the extracellular medium, resulting in a cytosolic calcium increase, which may induce mast cell exocytosis and preformed mediator release. Calcium release from internal stores has been shown to be related to some second messengers, including phospholipase C, phospholipase D, inositol

	Hydroxytyrosol	Oleuropein	Sodium cromoglycate
Concanavalin A	7.58 ± 4.9	67 ± 5.1	95 ± 8.9
Compound 48/80	61 ± 5.0	59 ± 5.0	97 ± 9.1
Calcium ionophore A23187	64 ± 5.0	22 ± 1.8	54 ± 5.1

Table 1.
EC_{50} (µM) ± SEM values for inhibitory activity of hydroxytyrosol, oleuropein, and sodium cromoglycate on concanavalin A-, compound 48/80-, and calcium ionophore A23187-induced mast cell activation.

1,4,5-triphosphate, and diacylglycerol. Degranulation dependent on the influx of extracellular calcium may be associated to the members of the SNARE (soluble NSF attachment protein receptor) family, such as synaptosome-associated protein of 23 kDa (SNAP-23), syntaxin, synaptotagmin, and molecules of the vesicle-associated membrane protein (VAMP) family which regulate the granule-to-granule or granule-to-plasma membrane fusion process [28]. Sodium cromoglycate is a compound commonly used in the treatment of allergic diseases. The effect of sodium cromoglycate is due to its ability to stabilize the mast cell membrane and to prevent the release of histamine and inflammatory mediators [29]. Mechanisms of the action of sodium cromoglycate include blocking of the influx of calcium into mast cells, inhibition of phosphodiesterase, and regulation of phosphorylation of mast cell proteins [30].

The findings of this study suggest that hydroxytyrosol and oleuropein act at different molecular sites. It seems that the hydroxytyrosol inhibitory mechanism may be related, at least partially, to inhibition of the cross-linking of high-affinity receptors for IgE (FcεRI) or to a probable interaction of the polyphenol with concanavalin A. Reports show that polyphenols form soluble and insoluble complexes with proteins and may cause them to become hypoallergenic by either modifying the structure of the allergenic protein or making it less bioavailable [15]. Moreover, two mechanisms are proposed for the mast cell inhibitory action of quercetin and other polyphenols: one where polyphenols impact allergen-IgE complex formation and another one where the polyphenols impact on the complex binding to their receptor (FcεRI) on mast cells [15].

Our results also suggest that oleuropein seems to block signaling pathways downstream of cytosolic calcium increase. However, further research is needed in order to explain the exact molecular mechanisms of these actions.

Despite the strong biochemical evidence, we considered a morphological evaluation necessary to reinforce the validity of our initial findings. Thus, a second set of experiments was designed to analyze the effect of the hydroxytyrosol and oleuropein on mast cell morphology by light and electron microscopy.

Peritoneal mast cells were easily identified by the presence of a cytoplasm dominated by distinctive secretory granules which stain metachromatically (**Figure 5A–J**). A representative mast cell from the basal group is shown in **Figure 5A**. Tightly packed secretory granules dominate the cytoplasm. Characteristic mast cells stimulated with concanavalin A, compound 48/80, and calcium ionophore A23187 are shown in **Figure 5B–D**, respectively. Cell surface disruption, typical of degranulating mast cells, may be observed. **Figure 5E–G** show mast cells treated with 100 μM hydroxytyrosol, for 10 min, prior to the challenge with concanavalin A, compound 48/80, and calcium ionophore A23187, respectively. **Figure 5H–J** show mast cells treated with 100 μM oleuropein, for 10 min, prior to the challenge with concanavalin A, compound 48/80, and calcium ionophore A23187, respectively. The morphology of the cells treated with the polyphenols shows a lower degree of degranulation than that of secretagogue samples.

Figure 6A–J shows mast cells observed under transmission electron microscope. **Figure 6A** shows a characteristic view of the basal mast cell population. These cells are characterized by a non-segmented, irregular nucleus with only moderate nuclear chromatin condensation, narrow surface folds, and numerous secretory granules regularly distributed throughout the cell cytoplasm. Almost all granules display either round or oval profiles and appear homogenously dense. Representative mast cells from the concanavalin A-, compound 48/80-, and calcium ionophore A23187-treated group are shown in **Figure 6B–D**, respectively.
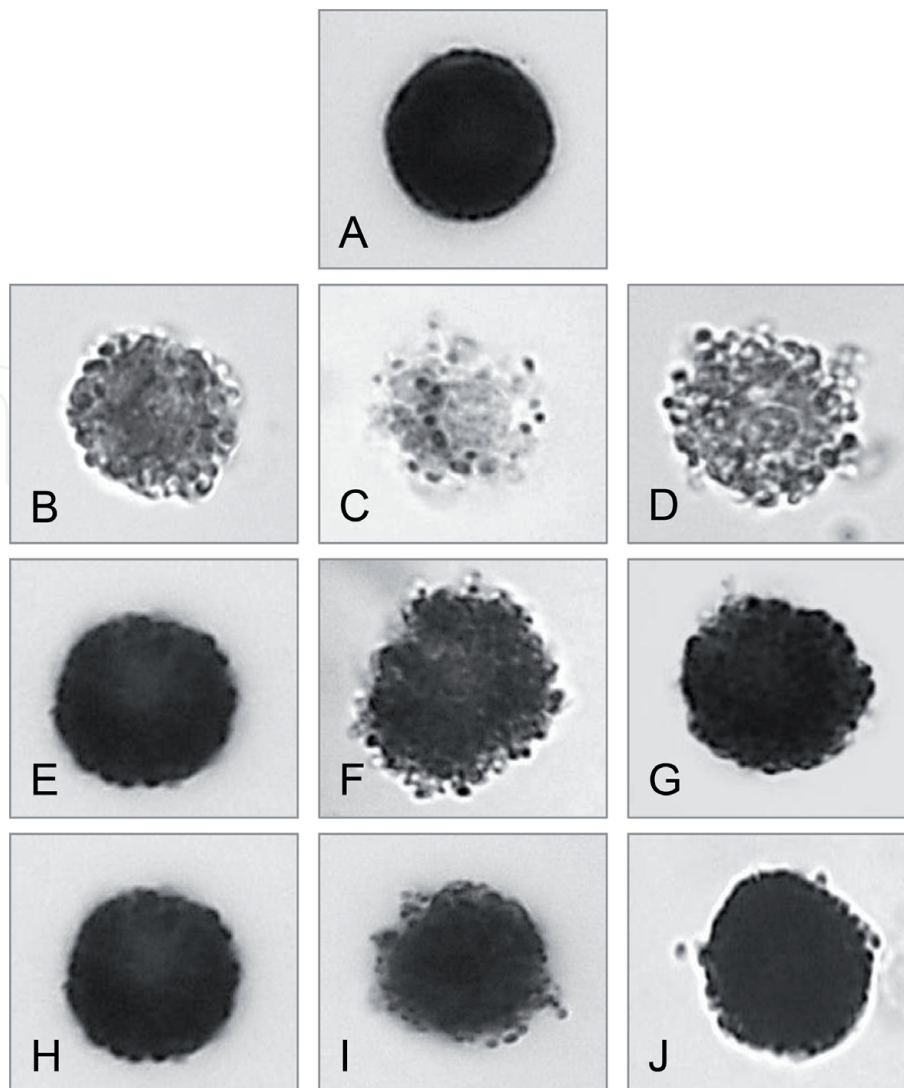
Figure 5.
Light microscopic photographs of peritoneal mast cells (toluidine blue stain). Mast cells were preincubated with 100 μM hydroxytyrosol or oleuropein for 10 min and then stimulated with 200 μg/ml concanavalin A, 10 μg/ml compound 48/80, or 50 μg/ml calcium ionophore A23187, for 10 min at 37°C. After incubations, cells were fixed and stained for light microscopy. (A) Basal. The cytoplasm is dominated by closely packed secretory granules. (B–D) Concanavalin A (ConA), compound 48/80 (48/80), and calcium ionophore A23187 (A23187), respectively. Degranulating cells may be seen. (E–G) hydroxytyrosol + ConA, hydroxytyrosol + 48/80, and hydroxytyrosol + A23187, respectively. (H–J) Oleuropein + ConA, oleuropein + 48/80, and oleuropein + A23187, respectively. The morphology of the polyphenol-treated cells shows a lower degree of degranulation than that of secretagogue samples. 600×.

These cells display obvious morphological changes and show evidence of increased granule release by exocytosis compared to basal cells. Mast cells exhibit a cytoplasm with irregular secretory granules showing various degrees of electron densities. Perigranular dilated and electron-lucent spaces surround some granules. Some of these spaces look fused, forming multiple cavities and intracytoplasmic channels. **Figure 6E–G** show mast cells treated with 100 μM hydroxytyrosol, for 10 min, prior to the challenge with concanavalin A, compound 48/80, and calcium ionophore A23187, respectively. **Figure 6H–J** show mast cells treated with 100 μM oleuropein, for 10 min, prior to the challenge with concanavalin A, compound 48/80, and calcium ionophore A23187, respectively. The morphology of the polyphenol-treated cells shows a lower degree of degranulation than that of secretagogue samples.

Our biochemical results about β-hexosaminidase release are consistent with those obtained by light and electron microscopy.
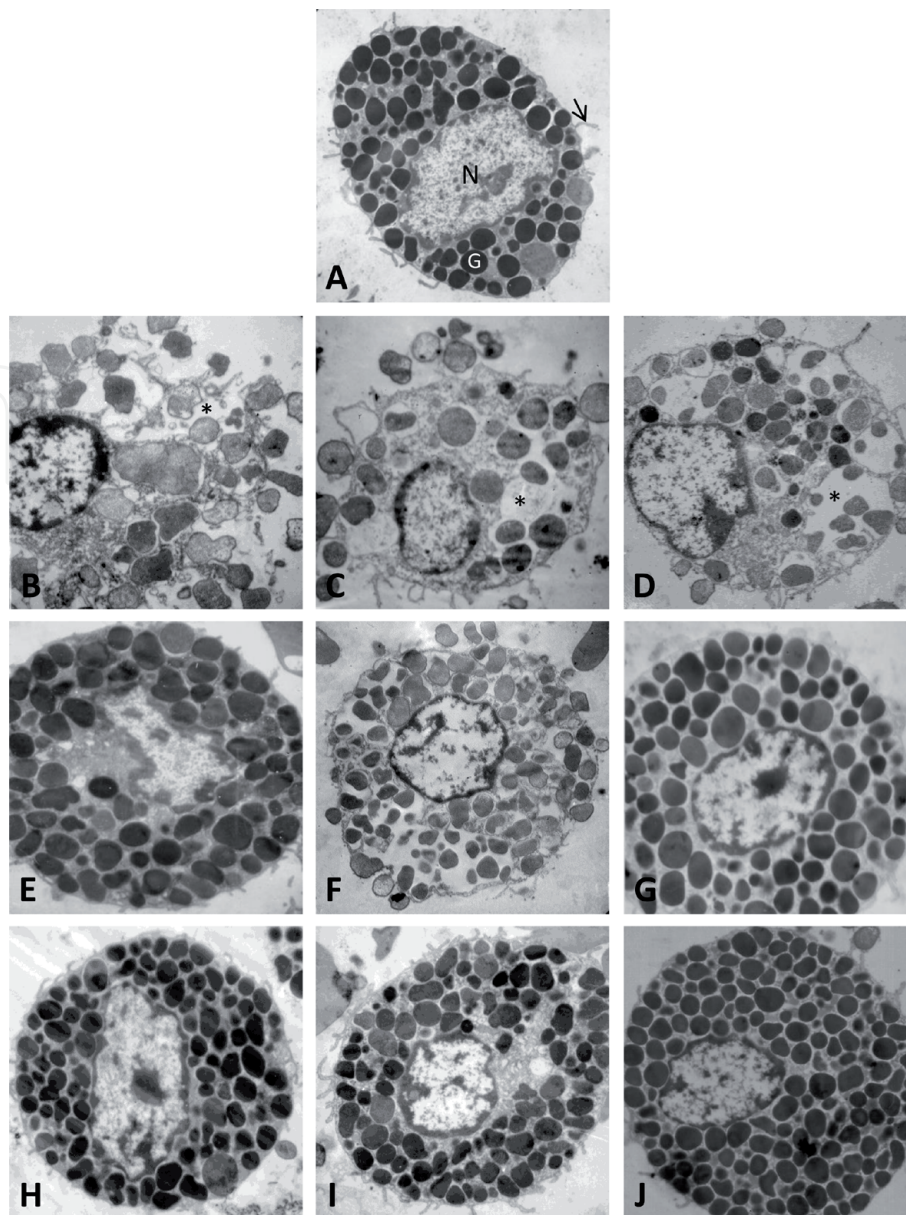
Figure 6.
Transmission electron micrographs showing peritoneal mast cells. Mast cells were preincubated with 100 μM hydroxytyrosol or oleuropein for 10 min and then stimulated with 200 μg/ml concanavalin A, 10 μg/ml compound 48/80, or 50 μg/ml calcium ionophore A23187, for 10 min at 37°C. After incubations, cells were processed for electron microscopy. (A) Basal. A non-segmented nucleus (N), narrow surface folds (red arrows), and numerous secretory granules (G) distributed throughout the cell cytoplasm are observed. (B–D) Concanavalin A (ConA), compound 48/80 (48/80), and calcium ionophore A23187 (A23187), respectively. Granules surrounded by perigranular dilated and electron-lucent spaces are evident. Some granules are seen out of mast cell cytoplasm, and others appear fused, forming cavities and intracytoplasmic channels (asterisk). (E–G) Hydroxytyrosol + ConA, hydroxytyrosol + 48/80, and hydroxytyrosol + A23187, respectively. (H–J) Oleuropein + ConA, oleuropein + 48/80, and oleuropein + A23187, respectively. Polyphenol-treated cells show minimal degranulation. 5000×.

4. Conclusions

In conclusion, our present findings reveal for the first time that hydroxytyrosol and oleuropein, the major phenolic compounds in olive, inhibit mast cell degranulation triggered by both immune and nonimmune pathways. Our discoveries also suggest that olive polyphenols, especially hydroxytyrosol and oleuropein, may provide insights to develop useful tools to prevent and treat mast cell-mediated disorders. These findings may be of interest to the immunopharmacology industry or could even lead in determining the ideal concentrations of each component in

virgin olive oils to be able to label them as healthy oils. However, further tests are needed in order to generate hypoallergenic products via polyphenol treatment.

Acknowledgements

Conflict of interest

The author declares no conflict of interest.

Abbreviations

ConA	concanavalin A
48/80	compound 48/80
A23187	calcium ionophore A23187
FcεRI	high-affinity receptor for IgE
IgE	immunoglobulin E

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