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Immunohistochemical investigation of galectin-3 in the skin of mice applied with *Origanum hypericifolium* essential oil and irradiated with ultraviolet B

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Aim: To demonstrate galectin-3-immunoreactivity in the undiluted essential oil of *Origanum hypericifolium* when applied to the ultraviolet B (UVB) irradiated skin of mice.

Materials and methods: Female BALB/c mice were allocated to 4 groups, each comprising 6 mice (Group 1: control; Group 2: UVB irradiated control; Group 3: undiluted *O. hypericifolium* essential oil applied; Group 4: undiluted *O. hypericifolium* essential oil applied before UVB irradiation). One week prior to UVB irradiation, the undiluted *O. hypericifolium* essential oil was applied to the shaved dorsal skin of mice 3 times a week. Subsequently, the mice were irradiated 3 times per week with UVB (week 1: 50 mJ/cm^2, week 2: 70 mJ/cm^2, and weeks 3 and 4: 80 mJ/cm^2) for 4 weeks. At the end of this period, immunohistochemical staining for galectin-3 was performed on frozen sections of skin specimens, and then they were photographed.

Results: Numerous galectin-3–immunoreactive cells, which were considered to be immune system cells, were observed in the dermis of Group 3.

Conclusion: It is suggested that undiluted *O. hypericifolium* essential oil may cause an increase in the galectin-3–immunoreactive cells. However, there is a need to research these findings with further molecular analyses.

Key words: *Origanum hypericifolium*, skin, UVB, galectin-3

1. Introduction

Galectins, which show both intracellular and extracellular activity and thereby control the viability and death of cells (1) and interact with glycosylated proteins at the cell surface or within the extracellular matrix (2), are members of a large family of β-galactoside–binding animal lectins (3). Based on their carbohydrate-recognition domains of primary structural homology, galectins are classified into 3 groups, referred to as prototype, chimeric, and tandem-repeat galectins (4).

The only chimeric galectin described in mammals, galectin-3, is an antiapoptotic molecule (4). It is mainly found in the cytoplasm, although, depending on cell type and proliferative state, it can also be detected in the nucleus, on the cell surface, or in the extracellular region (5). This protein is involved in the cell cycle, cell growth, cell adhesion and migration, apoptosis, pre-mRNA splicing, and various immune and inflammatory responses, and also in several physiological and pathological processes, including cancer (3,6–9). It is also involved in the pathogenesis of inflammatory skin diseases through its effects on immune cell functions, and it has been suggested as a new therapeutic target for various skin diseases (10).

Exposure to ultraviolet (UV) light of the sun, particularly UVB (280–320 nm), results in the development of erythema, edema, hyperplasia, hyperpigmentation, formation of sunburn cells, photoaging, immunosuppression, inflammation, gene mutations, and skin cancer (11,12). It has been reported that UVB radiation promotes epidermal proliferation and differentiation and increases the number of mast cells and neutrophils in the skin (13,14).

Recent research has provided new data on the effects of plants on various skin diseases (15). One of the approaches adopted for the protection of human skin against the damage of UV radiation is the use of antioxidants as photoprotectors. In the past few years, natural agents of plant origin, such as phenolic acids, flavonoids, and high molecular weight polyphenols, have received great attention as protective agents (16).

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Origanum hypericifolium is an endemic plant also found in Denizli Province and its vicinity (17). The essential oil of the plant contains monoterpenes, including mainly p-cymene, carvacrol, thymol, and γ-terpinene (18,19). It is well known that monoterpenes have antifungal, antibacterial, antioxidant, anticarcinogenic, antispasmodic, hypotensive, and vasorelaxant effects (20). Nonetheless, it has been reported that 2 of the main components of *O. hypericifolium* essential oil, thymol and carvacrol, induce dose-dependent irritation (21,22).

In this study, it was aimed to demonstrate the effect of undiluted *O. hypericifolium* essential oil on galectin-3 immunoreactivity in the UVB irradiated skin of mice.

2. Materials and methods

2.1. Collection of the plants and extraction of the essential oil

The aerial parts of the *O. hypericifolium* plant were collected in August 2009 during the flowering period from Sandras Mountain, Beyağaç, Denizli, Turkey. The plants were dried in the shade at room temperature. The essential oil was extracted by hydrodistillation for 3 h using a Clevenger-type apparatus. The essential oil was dried over anhydrous sodium sulfate and stored at 4 °C.

2.2. Animals

Female BALB/c mice, 8–10 weeks old, were fed with standard food and water ad libitum and were kept on a 12 h light/12 h dark cycle. They were randomly housed into 4 groups (6 per group): Group 1: control; Group 2: UVB irradiated control; Group 3: undiluted *O. hypericifolium* essential oil applied; Group 4: undiluted *O. hypericifolium* essential oil applied before UVB irradiation. The dorsal skin of the mice was shaved under ether anesthesia 2 days before the experiment. The study was approved by the Pamukkale University Animal Ethics Committee (No: PAUHDEK2008/024).

2.3. Exposure to UVB

UVB exposure was performed as described by Kim et al. (23). Accordingly, undiluted *O. hypericifolium* essential oil was applied topically 1 week prior to UVB exposure to the shaved dorsal skin of the mice 3 times a week. During the following 4 weeks, the dorsal skin of mice was exposed to UVB 3 times a week (week 1, 50 mJ/cm^2; week 2, 70 mJ/cm^2; week 3, 80 mJ/cm^2; week 4, 80 mJ/cm^2). UVB irradiation was performed using 3 UVB lamps (T-15.M, UVItec UVIlite UV Lamps, Cambridge, UK) and the UV dose was measured with a UV meter (WLX-3W, UVItec). The mice were sacrificed under deep anesthesia 3 days after the end of UVB irradiation.

2.4. Tissue preparation and galectin-3 immunohistochemistry

For cryosectioning, dorsal skin samples taken from the mice were embedded in an optimum cutting temperature medium, snap-frozen in liquid nitrogen, and stored at –86 °C. For the immunostaining of galectin-3, biotinylated antimouse galectin-3 antibody (BAF 1197, R&D Systems, Minneapolis, MN, USA) and tissue staining kit (CTS 002, R&D Systems) procedures were performed on frozen tissue sections (6 μm). The slides were then photographed using an Olympus BX50 light microscope and a DP2-BSW microscope digital camera system.

3. Results

In all groups, galectin-3–positive reactions were observed in the keratin layer, epidermal cells, and dermal fibrillar structures. Galectin-3–immunoreactive cells, which were considered to be immune system cells, were extensively observed in the dermis of Group 3 when compared to Group 4. Additionally, numerous galectin-3–positive granules, which were considered to be mast cell secretory granules, were seen particularly in the dermis of Group 3 (Figure).

4. Discussion

Skin inflammation caused by UV light (24) is known to result in erythema (sunburn), production of inflammatory mediators, alterations in vascular responses, and an inflammatory cell infiltration (25). Reports in the literature indicate that, in the dermis of skin exposed to UVB, the number of mast cells increases (26) dose-dependently (27) and that these cells have an important role in UVB-induced inflammation (28). It is also known that monoterpenes (carvacrol, eugenol, and thymol) are skin sensitizers and allergen substances (29). It has been reported that *Thymus vulgaris* essential oil and carvacrol inhibit leukocyte migration in carrageenan-induced pleurisy. In ear edema, carvacrol reduced edema formation, exerting a topical antiinflammatory effect; however, thymol did not reduce edema formation but presented an irritative response, probably dependent on histamine and prostanoid release (30). It has been shown that galectin-3, expressed by all immune and inflammatory cell types (31), has a role in allergic inflammation (32). In view of all these data, in this study it is suggested that the galectin-3–positive cells observed in the dermis could be immune system cells. However, the higher intensity of these cells observed in Group 3 compared to Group 4 was considered to be an indicator of the effect of *O. hypericifolium* essential oil

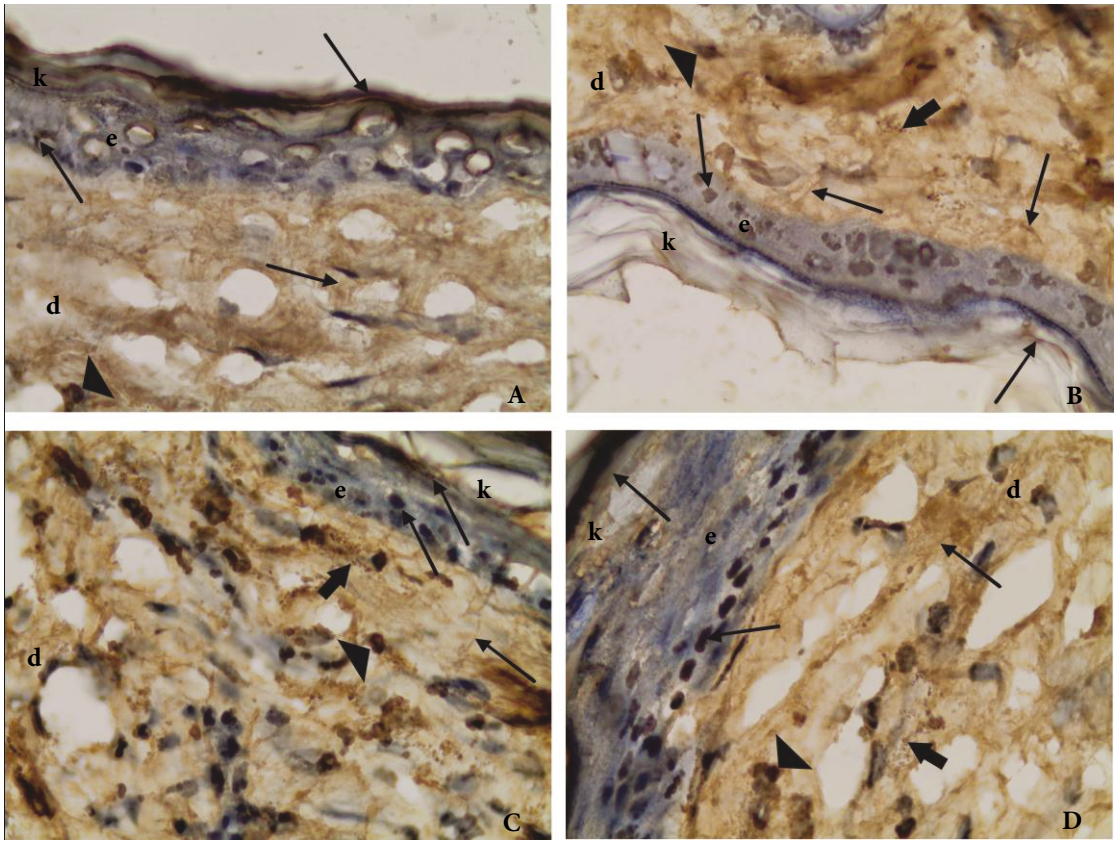

Figure. Galectin-3–positive reactions in the keratin layer (k), epidermal cells (e), and dermal fibrillar structures (d) in the groups (arrows). Galectin-3–positive cells considered to be immune system cells (arrow heads) and granules considered to be mast cell secretory granules (bold arrows) were extensively observed in the dermis of Group 3 (C). A: Group 1, B: Group 2, C: Group 3, D: Group 4; galectin-3 immunohistochemistry + hematoxylin; 1000×.

on galectin-3–immunoreactive cells. On the other hand, it is also known that exposure to UV results in immune suppression (33–35). For example, it was demonstrated that when mouse ear skin was applied with hapten prior to UVB irradiation, 10 weeks after the first application, infiltrations of $CD8^+$ T cells were observed in the region, while in the event of UVB irradiation prior to hapten application, these cells were absent, suggesting an impaired development of peripheral memory T cells (36). Similarly, it was determined that UV irradiation can directly (independently of antigen-presenting cells and suppressor T cells) inhibit T cell activation and also suppress preactivated T cells (37). Accordingly, in the group that was exposed to UVB and had the essential oil applied (Group 4), the intensity of galectin-3–positive cells in the dermis was lower than that of the group that had the essential oil alone applied (Group 3). This could be attributed to the immunosuppressive effect of UVB on these cells. Therefore, the high cell population in Group 3 may be related to the population of inflammatory cells; however, the lower cell population in Group 4 was considered to be related to the immunosuppressive effect of UVB on some of the cells of this cell population.

Furthermore, it has been reported that the localization of galectin-3 in the secretory granules of human mast cells and basophils suggests that these cells may release this lectin when activated to degranulate (38). Therefore, in the present study, the galectin-3–positive granules observed in the dermis in the groups, particularly in Group 3, were considered to be the granules of degranulated mast cells.

In conclusion, undiluted *O. hypericifolium* essential oil alone may increase galectin-3–immunoreactive cells, which were considered to be immune system cells, in the dermis. On the other hand, the observed decrease of the galectin-3–immunoreactive cells in the group that was exposed to UVB and had the undiluted *O. hypericifolium* essential oil applied may be due to the immunosuppressive effect of UVB on some of the cells of this cell population. Further molecular analyses are necessary to research and develop the data obtained from this study.

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