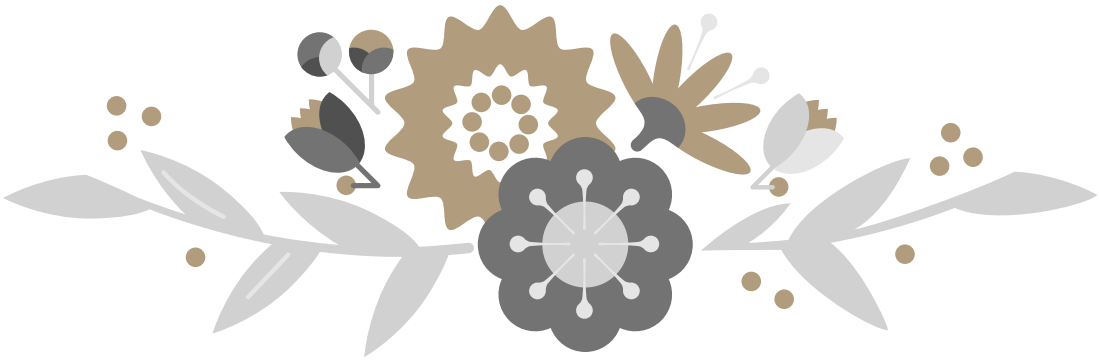

Chapter-5

CHAPTER - 5

TO DEVELOP CINNAMALDEHYDE INDUCED ALLERGIC CONTACT DERMATITIS IN C57BL/6 MICE UNDER THE UVB EXPOSURE

5.1. INTRODUCTION

Allergic contact dermatitis is a frequently occurring skin problem, occurs due to the exposure of chemicals. ACD leads to activation of T cell mediated adaptive immune responses categorized as type 4 delayed type hypersensitivity. Pathogenic symptoms of ACD occurs in two phases one is induction phase in which sensitization occurs and another is elicitation phase in which inflammation is induced upon re-exposure to the same antigen. As the fragrances applied dermally contains ring structure absorbs the sunlight containing UV radiation which may enhance its allergic contact dermatitis potential. Although the allergic contact dermatitis (ACD)–inducing potential of cinnamaldehyde (CA) as is well known, however, its photoallergic potential has remained largely unexplored in the literature. Our *in-silico, in-chemico, in-vitro* and proteomics-based study has established CA as a potent photosensitizer. So, to check phenotypic manifestations of ACD upon CA and UVB co-exposure, we have used C57BL/6J mice. We used C57BL/6J female mice shaved it and applied CA dermally and exposed to UVB irradiation for photoallergy development upto 28 days. Symptoms were noted weekly and H&E histopathology was performed to explore the photoallergic potential.

5.2. RESULTS

5.2.1 Macroscopic features of photoallergy

The clinical scoring results of dryness or scaling, lesion severity, edema and erythema in C57BL/6 female mice treated with cinnamaldehyde (CA; 0.002% and 0.02%) and DNCB (4µg/ml), under dark and UVB exposure conditions was done.

5.2.1.1 Erythema

Vehicle-treated mice exhibited no visible erythema under either dark or UVB conditions. CA 0.002% (dark) induced minimal erythema, whereas CA 0.002% + UVB produced a significant increase in erythema intensity, indicating UVB-enhanced inflammatory reactivity. CA 0.02% (dark) caused moderate erythema, while CA 0.02% + UVB resulted in severe, persistent erythema, comparable to the DNCB positive control, reflecting robust allergic inflammation.

5.2.1.2 Edema

No edema was observed in vehicle controls. CA 0.002% (dark) elicited negligible to mild edema, which was significantly exacerbated under UVB exposure. CA 0.002% + UVB CA 0.02% (dark) induced clear edema, while CA 0.02% + UVB caused marked swelling, consistent with enhanced vascular permeability and inflammatory cell influx. DNCB-treated mice showed pronounced edema, validating the sensitization model.

5.2.1.3 Dryness and scaling

Skin dryness and scaling were absent in vehicle controls. CA 0.002% (dark) produced minimal surface dryness, whereas CA 0.002% + UVB led to visible scaling, suggesting barrier disruption. CA 0.02% (dark) induced moderate dryness and flaking, which progressed to severe scaling and roughened skin texture under UVB exposure, CA 0.02% + UVB indicative of epidermal hyperproliferation and barrier impairment. DNCB caused extensive dryness and scaling, consistent with chronic dermatitis.

5.2.1.4 Lesion severity

Composite lesion severity scores (integrating erythema, edema, and scaling) remained low in vehicle controls. CA 0.002% (dark) showed mild lesion development, significantly intensified by UVB exposure. CA 0.02% (dark) resulted in moderate lesions, while CA 0.02% + UVB produced severe lesions, characterized by thickened skin, scaling, and occasional excoriation, approaching the severity observed with DNCB treatment.

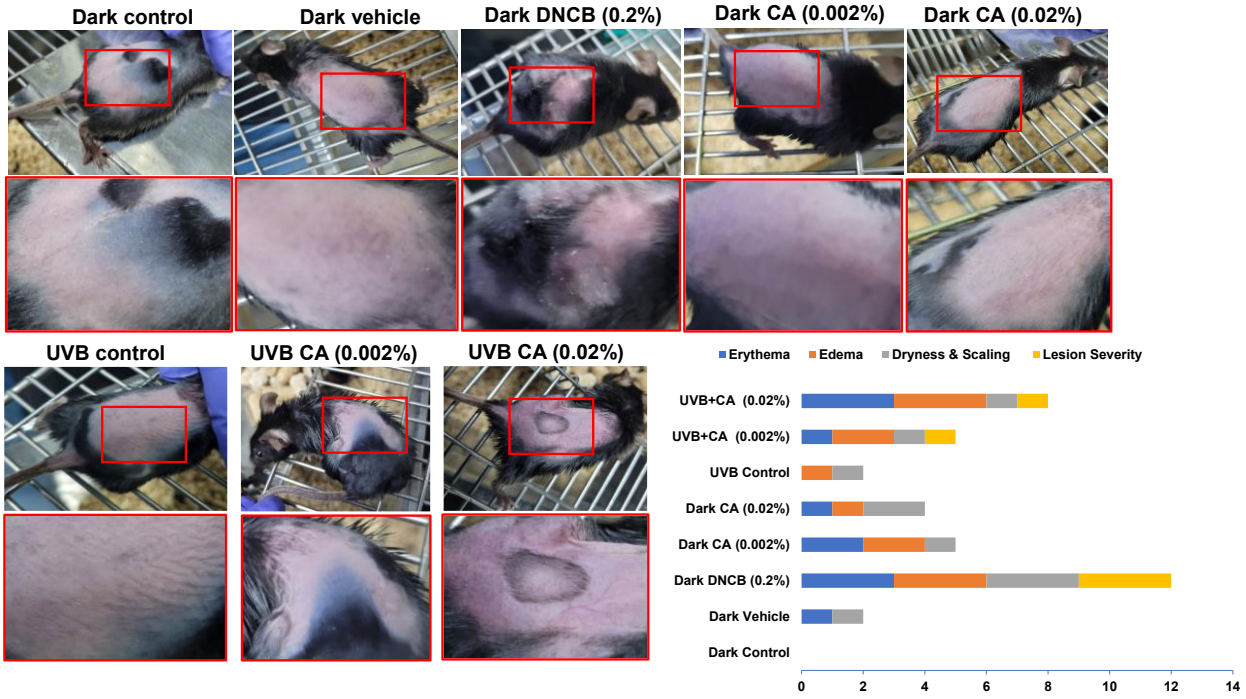

Figure 5.1. *Histopathological Evaluation of Skin, Ear Pinna, and Draining Lymph Nodes*

5.2.2 Skin histopathology (dorsal skin)

Vehicle control (acetone: olive oil, 4:1) under both dark and UVB conditions showed normal epidermal architecture, with a thin, well-organized epidermis, intact dermo-epidermal junction, and absence of inflammatory cell infiltration. CA 0.002% (Dark exposure) induced minimal epidermal thickening, with mild spongiosis and sparse perivascular inflammatory infiltrates, indicating weak irritant or sub-threshold sensitization effects.

CA 0.002% and UVB exposure resulted in marked epidermal hyperplasia, enhanced spongiosis, and moderate dermal inflammatory cell infiltration dominated by mononuclear cells. These changes indicate UVB-amplified sensitization, exceeding that observed under dark conditions. CA 0.02% (Dark exposure) produced pronounced epidermal thickening, focal parakeratosis, and moderate dermal inflammation, consistent with dose-dependent skin sensitization. CA 0.02% and UVB exposure demonstrated the most severe pathological alterations, including robust epidermal hyperplasia and acanthosis, disruption of epidermal integrity, dense dermal inflammatory infiltrates and early tissue remodeling features. These findings indicate photo-enhanced allergic skin inflammation. DNCB (positive control) elicited severe epidermal hyperplasia, spongiosis, and dense inflammatory infiltrates, validating the experimental sensitization model.

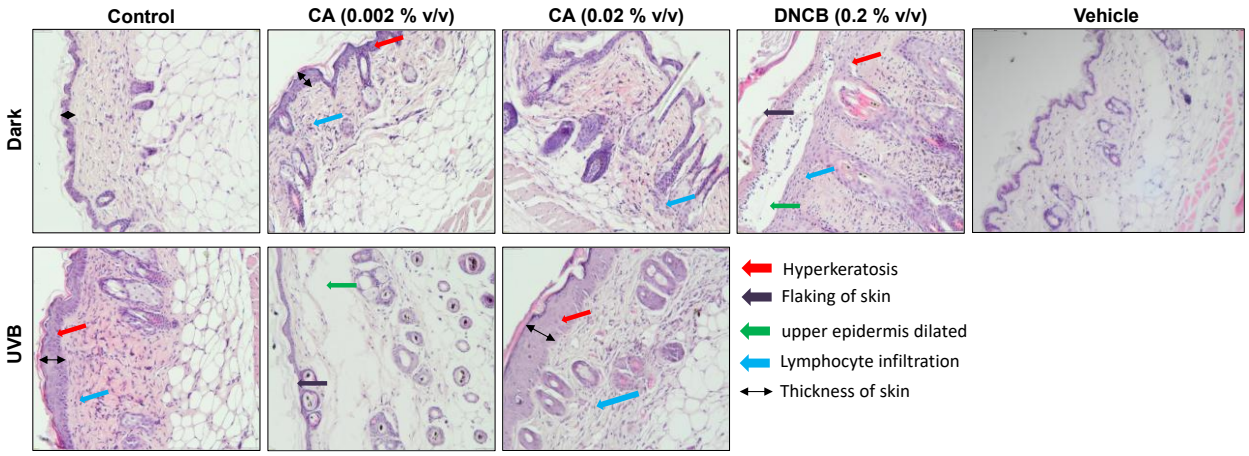

Figure 5.2. *H & E staining of in dissected skin tissues after treating with CA (0.002%, 0.02% v/v), DNCB (0.2% v/v), vehicle control (acetone: olive oil) in dark and UVB exposure.*

(5.2.3 Ear pinna histopathology

Vehicle-treated mice exhibited normal ear pinna thickness with no edema or cellular infiltration. CA 0.002% (Dark) caused negligible histological changes, whereas CA 0.002% + UVB induced mild-to-moderate edema, epidermal thickening, and inflammatory cell accumulation. CA 0.02% (Dark) showed clear dermal edema and inflammatory infiltration, while CA 0.02% and UVB resulted in Marked ear pinna thickening, Severe edema, Dense leukocyte infiltration. These changes correlate with enhanced ear swelling responses, a classical endpoint of allergic contact dermatitis. DNCB-treated ears displayed extensive edema, epidermal disruption, and massive inflammatory infiltrates, confirming robust ACD induction.

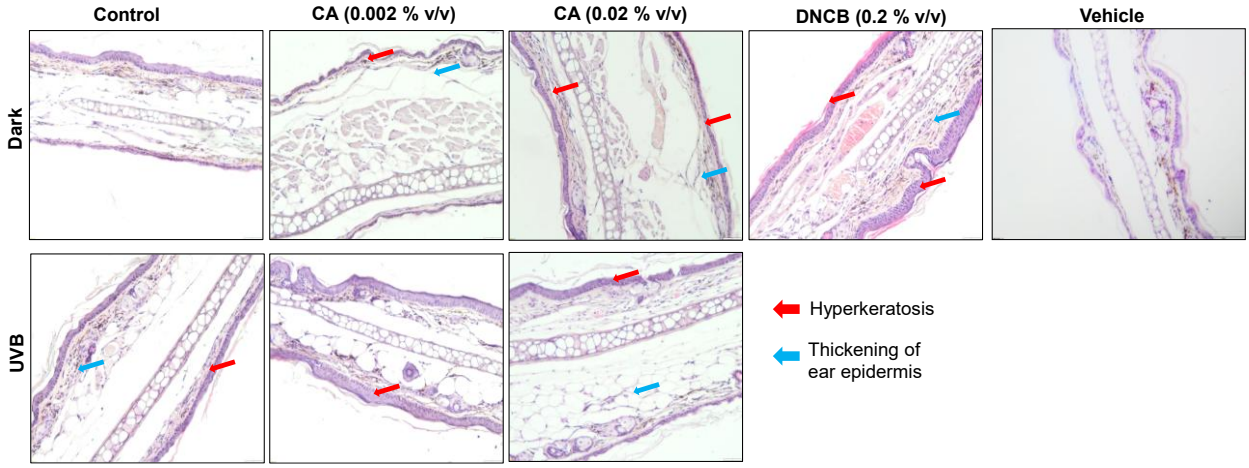

Figure 5.3. *H & E staining of in dissected ear pinna tissues after challenge with CA (0.5% v/v), DNCB (0.2% v/v), vehicle control (acetone: olive oil) in dark and UVB exposure.*

5.2.4 Draining lymph node histopathology

Lymph nodes from vehicle-treated mice maintained normal cortical and medullary architecture, with no evidence of hyperplasia. CA 0.002% (Dark) showed minimal lymphoid activation, whereas CA 0.002% and UVB demonstrated moderate cortical thickening and increased cellularity, indicating early immune activation. CA 0.02% (Dark) induced distinct lymphoid hyperplasia, while CA 0.02% and UVB exposure exhibit pale-staining areas indicating cortical expansion, disrupted paracortical cellularity, features consistent with antigen-driven T-cell activation followed by lymphocyte exhaustion. DNCB treatment resulted in pronounced lymph node enlargement and hyperplasia, serving as a strong positive control for sensitization-induced immune activation.

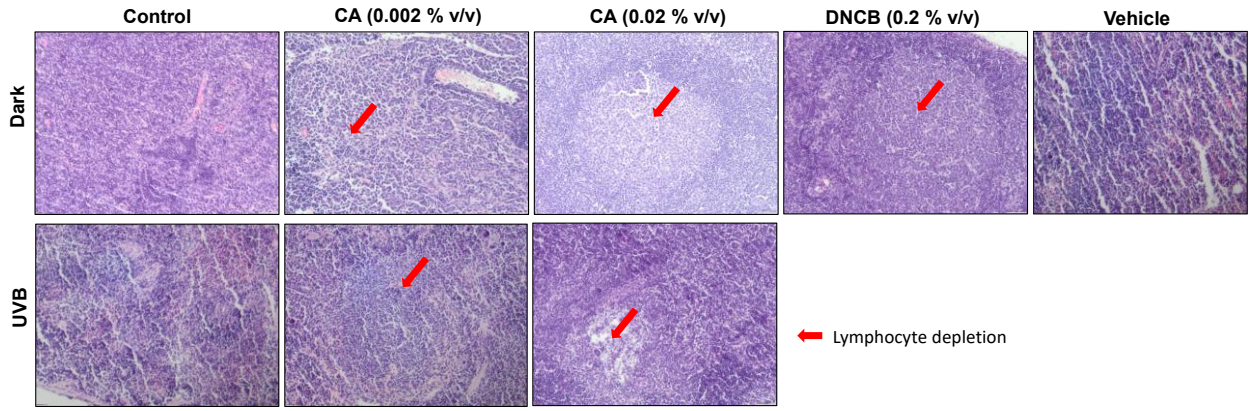

Figure 5.4. *H & E staining of lymph node tissues after treating with CA (0.002%, 0.02%), DNCB (4 µg/ml), vehicle control (acetone: olive oil) in dark and UVB exposure.*

5.3. DISCUSSION

ACD is a multicellular interacting pathogenic condition. Haptens, or low-molecular-weight (<1,000 Da) sensitizers such as fragrances applied topically to the skin, bind to proteins and form

complete antigens and these antigens are taken up by the antigen-presenting cells (Romani et al., 2010). Hapten-loaded APCs go into local lymph nodes, where they deliver the hapten to particular TCR possessing T cells which later begin to multiply and move into the skin. However, a range of cells are engaged in the elicitation phase, such as mast cells, NK cells, neutrophils, and CD8+ cytotoxic T cells. Increased ACD was observed when CD4+ T cells were depleted (Ring et al., 2009; Xu et al., 1996). This suggests that after sensitization, not only are T effector cells generated but also CD4+ T cells. T helper-1 (Th1) cells play important role in the skin sensitization or ACD involve a number of cytokines, such as IFN-g, TNF, IL-6, IL-8, IL-12, IL-18 GM-CSF, and IL-1b (Martin, 2015). Since ACD have inter-intra cellular signaling and its pathology can be easily observed in the animal model. C57BL/6 and Balb/C mice are generally used for the immunological response study. Balb/c mice show the prominent Th2 cell and IgE mediated responses. while the C57BL/6 mice have the melanin in skin and shows the Th1 cells mediated inflammatory responses which made it more relevant model for the ACD/ photoallergic studies. Classical contact allergens such as 2,4-dinitrochlorobenzene (DNCB) and dinitrofluorobenzene (DNFB) have been shown to induce ear swelling, epidermal hyperplasia, spongiosis, and dermal leukocyte infiltration when combined with UVB exposure, compared with hapten treatment alone, alongside enhanced activation and remodeling of draining lymph nodes (Toews et al., 2004; Schwarz et al., 2005). Similarly, fragrance-related photoallergens including ketoprofen, fenticlor, and musk ambrette have been reported to elicit minimal dermatitis under dark conditions but robust inflammatory responses following UV irradiation, characterized by barrier disruption, keratinocyte damage, and amplified T-cell–mediated immune responses in murine skin (Karlsson et al., 2013; Tokura et al., 1994). UVB-exposed skin has also been shown to increase hapten penetration and antigen presentation, thereby converting weak sensitizers into strong elicitors of dermatitis and producing pathology comparable to potent chemical allergens (Basketter et al., 2014). Collectively, these in-vivo studies establish that UV radiation acts as a critical co-factor in photoallergic and photo-

aggravated contact dermatitis by intensifying cutaneous inflammation and systemic immune activation. Overall, clinical scoring demonstrates that cinnamaldehyde induces dose-dependent cutaneous inflammation, and ambient UVB exposure markedly amplifies erythema, edema, barrier disruption (dryness/scaling), and lesion severity. The CA 0.02% and UVB group exhibits near–positive-control–level pathology, providing strong phenotypic evidence of photo-augmented allergic contact dermatitis. Collectively, H&E histopathological analysis reveals that Cinnamaldehyde induces dose-dependent skin sensitization, UVB exposure significantly amplifies CA-induced epidermal damage, inflammation, and lymphoid activation, CA 0.02% and UVB produces pathology comparable to classical sensitizer DNCB. These findings provide morphological evidence supporting photo-augmented allergic contact dermatitis, linking cutaneous inflammation with systemic immune activation via draining lymph nodes.