Development of an irritant-induced allergy model to characterise the anti-inflammatory properties of honeybush *(C. subternata)* in skin *in vitro*

R. Makgato[1], T. Magcwebeba[1], E. Joubert[2], M. Lilly[1]

[1]Applied microbial and health biotechnology institute (AMHBI), Symphony way, Bellville, 7530, South Africa. [2]Department of Food Science, Stellenbosch University, Private Bag X1, Matieland (Stellenbosch) 7602, South Africa; Plant Bioactives Group, Post-Harvest & Agro-Processing Technologies, Agricultural Research Council (Infruitec-Nietvoorbij), Private Bag X5026, Stellenbosch 7599, South Africa

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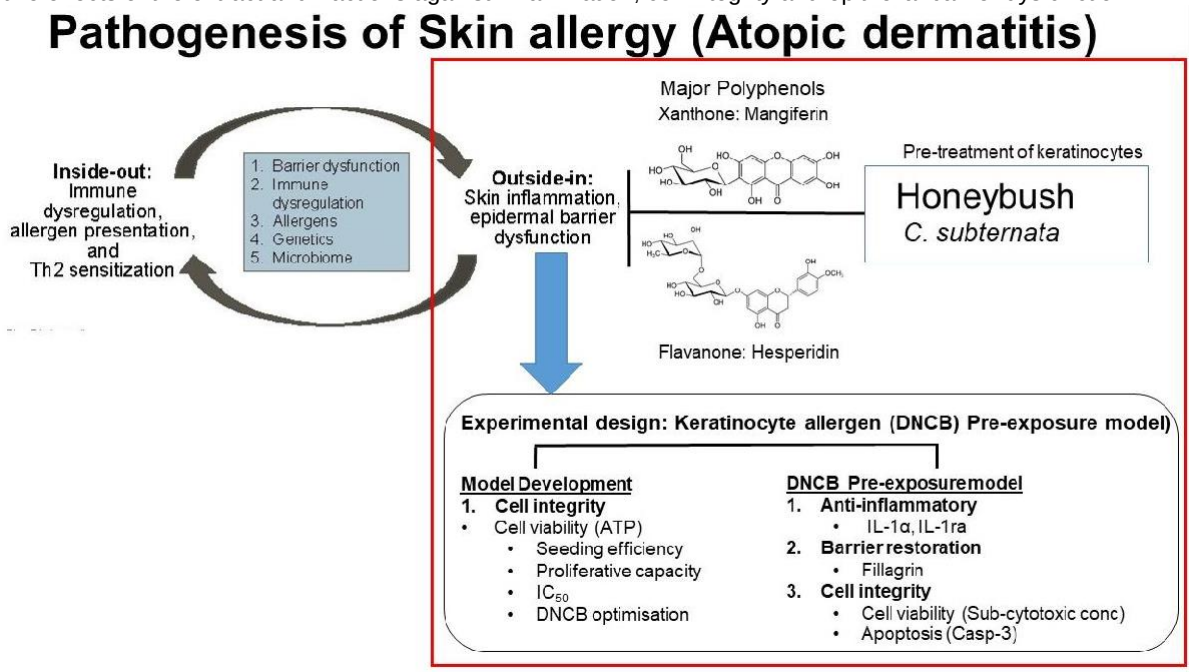
Introduction: The prevalence of allergies associated with epithelial barrier dysfunction has increased at an alarming rate (2-3 fold) with skin allergies forming part of the major atopies in children and adults (1,2). The most frequently diagnosed skin allergies include atopic dermatitis, urticaria, angioedema and contact dermatitis and they are caused by various factors ranging from irritants, allergens, pathogens to medical conditions (1-3). Current treatment options for skin allergy have limitations and adverse side effects. Consequently, natural treatments such as honeybush (*Cyclopia spp*.) have been considered as a source of natural products, specifically phenolic compounds for use as a safer alternative. The antioxidants in honeybush tea have a wide range of health benefits, including health, as well as antidiabetic and anticancer properties, however their underlying mechanisms against epithelial barrier dysfunction and inflammation stillneeds further characterisation (4-6).
Aim: Develop an irritant (2,4-Dinitrochlorobenzene, DNCB)-induced allergy model with keratinocytes (HaCaTs) to characterise the barrier restorative and anti-inflammatory properties of *C. subternata* extract/fractions.
Methodology: A pre-exposure skin cell model was optimized for seeding efficiency (6 hrs vs 24 hrs), proliferative capacity (+/- FBS), DNCB concentration (160-20µM) for cytokine production (IL-1α, IL-1ra, IL-6), cell integrity (Casp- 3, ATP) and filaggrin expression. *C. subternata* extract and fractions were screened for biological activity (IC_{50} concentration) using cell viability. Anti-inflammatory (IL-1α, IL-1ra, IL-6) and cytoprotective effect (Caspase-3) was determined by ELISA.
Results: Optimum seeding efficiency for HaCaT cells in the pre-exposure model was acquired at 24 hrs with 10% FBS. DNCB induced minimal cytotoxic effect at 20-80µM. *Cyclopia subternata* extract and fractions reduced cell viability in a dose-dependent manner with Fraction-2 and Fraction-3, enriched in xanthones and other compounds, amongst others the flavone, scolymoside, respectively, exhibiting the strongest activity with regards to cell viability. The *C. subternata* extract exhibited differential effects against IL-1ra, IL-1α, IL-6 and Casp-3.
Discussion and conclusion: In the pre-exposure irritant-induced allergy model, HaCaT cells require longer incubation periods for optimal seeding efficiency, shorter periods render cells more sensitive to toxic effects of DNCB and *C. subternata*. This may distort the protective effects of the extract and fractions against inflammation, cell integrity and epithelial barrier dysfunction.



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