**In vitro activity of alkylamides and ethanolic extracts from Echinacea**

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**INTRODUCTION**

Ethanolic extracts of Echinacea have been shown to have immunomodulatory activity [1]. Standardisation of many Echinacea extracts to cichoric acid implies that this is responsible for therapeutic activity and not the alkylamides also present. Alkylamides have been shown to be readily bioavailable [2,3] unlike cichoric acid which has poor bioavailability in vitro [2] and is therefore unlikely to contribute to the immune activity of an oral Echinacea product.

We have investigated the activity of an ethanolic Echinacea extract as well as several components in three in vitro measures of immune function - NFkB, TNFα and nitric oxide (NO).

- **NFkB** stimulates the expression of several genes including key components of the inflammatory response such as TNFα, IL-1, chemokines, adhesion molecules and COX-2. Together these mediators help mount an immune response.
- The inflammatory cytokine TNFα is involved in the immune response and this includes the induction of iNOS, the inducible form of nitric oxide synthase, that initiates the generation of nitric oxide (NO).
- **NO** exerts multiple modulating effects on inflammation and plays a key role in the regulation of the immune response.

Therefore, inhibition of NFkB, TNFα or NO activation will alter the immune response.

**Results**

**Figure 1:** Effect of Echinacea compounds on LPS-stimulated NFkB production by macrophages.

Control levels (no addition) are shown in yellow. LPS alone is shown in red. Values are means ± SD for n = 3. * = p < 0.05

- Echinacea does not elicit an immune response in the absence of other immunological stimuli.
- LPS but not PMA increased NFkB levels in macrophage cells.
- In LPS-stimulated cells, all compounds decreased NFkB concentrations.

**Figure 2:** Effect of Echinacea compounds on LPS-stimulated TNFα production by macrophages.

Control levels (no addition) are shown in yellow. LPS alone is shown in red. Values are means ± SD for n = 3. * = p < 0.05

- All compounds decreased basal TNFα levels.
- LPS and PMA stimulated TNFα levels in macrophage cells.
- All compounds significantly decreased TNFα production in LPS-stimulated cells.

**Figure 3:** Effect of Echinacea compounds on LPS-stimulated NO production by macrophages.

Control levels (no addition) are shown in yellow. LPS alone is shown in red. Values are means ± SD for n = 3. * = p < 0.05

- LPS but not PMA increased macrophage NO production.
- All compounds decreased NO production in LPS-stimulated cells.

**Summary**

The following preparations and compounds have altered an induced immune response in macrophages in three measures of *in vitro* immune function:

- Echinacea Premium Liquid (EPL)
- Echinacea alkylamides (EPL AA)
- Cichoric acid
- Alkylamides (2) and (5)

Alkylamides are therefore suitable quality markers for Echinacea

**Methods**

The macrophage cell line RAW 264.7 from the European Collection of Cell Cultures were cultivated in modified DMEM. Cells were maintained at 37°C in an atmosphere of 5% CO2. Cells were preincubated for 1 hour with test compounds before addition of either lipopolysaccharide (LPS) or phorbol 12-myristate 13-acetate (PMA) to initiate an immune response. Nitrite and TNFα levels were examined after an 18 hour incubation and NFkB after 2 hours.

EPL = MediHerb Echinacea Premium Liquid; EPL AA = MediHerb Echinacea Premium Liquid alkylamide fraction; AA(2) = synthetic alkylamide m/z=231; AA(5) = synthetic alkylamide m/z=247 (see Fig. 4).

**Figure 4:** Structures of synthetic isobutylamides.

**References:**


**Acknowledgments:** This work was funded by AUSINDUSTRY through a Biotechnology Innovation Fund Grant (No. BIF02651) and MediHerb Pty. Ltd.