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 - NFκB, TNFα and nitric oxide (NO).

- **NFκB** 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 NFκB, TNFα or NO activation will alter the immune response.

Results

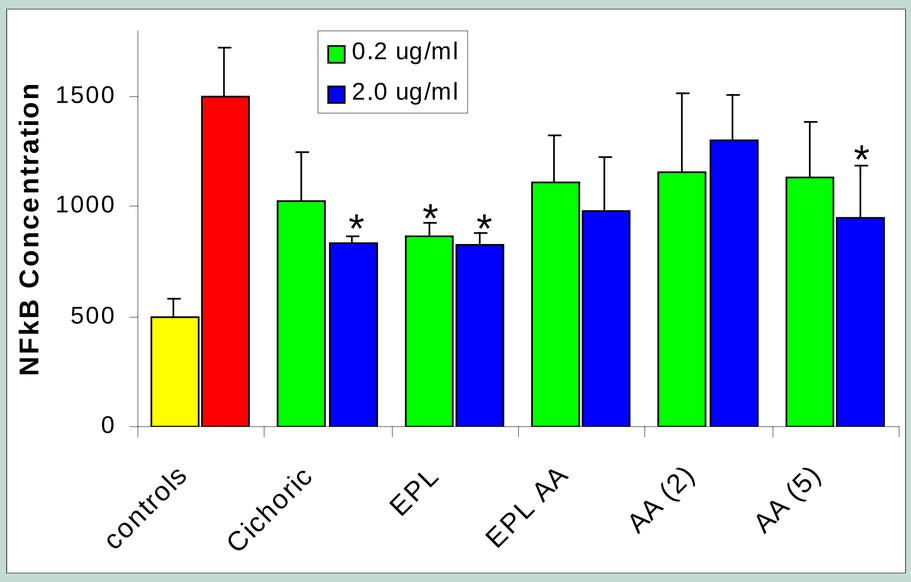


Figure 1: Effect of Echinacea compounds on LPS-stimulated NFκB production by macrophages.
Control levels (no addition) are shown in yellow. LPS alone is shown in red. Values are means ± SD for n = 3. * = $p \leq 0.05$

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

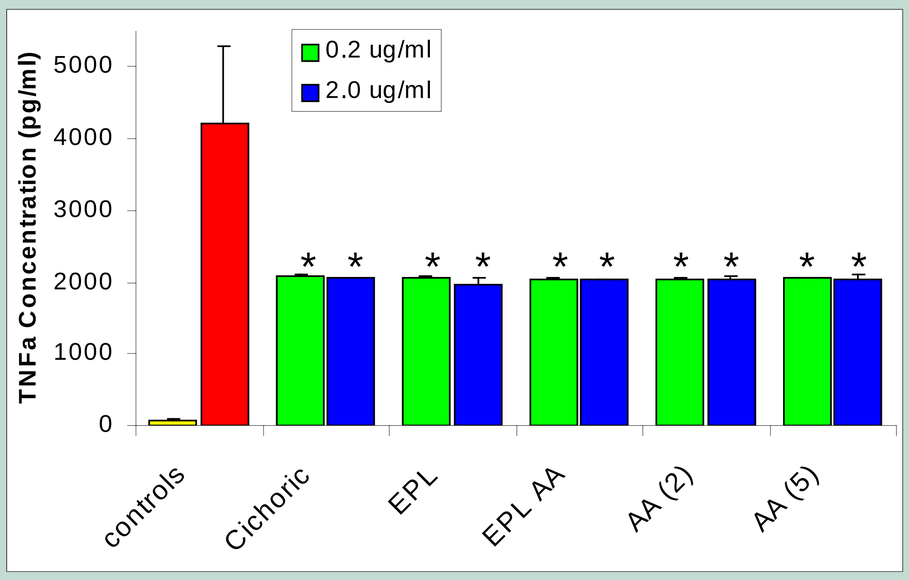


Figure 2: Effect of Echinacea compounds on LPS-stimulated TNFα production by macrophages.
Control levels (no addition) are shown in yellow. LPS alone is shown in red. Values are means ± SD for n = 3. * = $p \leq 0.05$

- **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.**

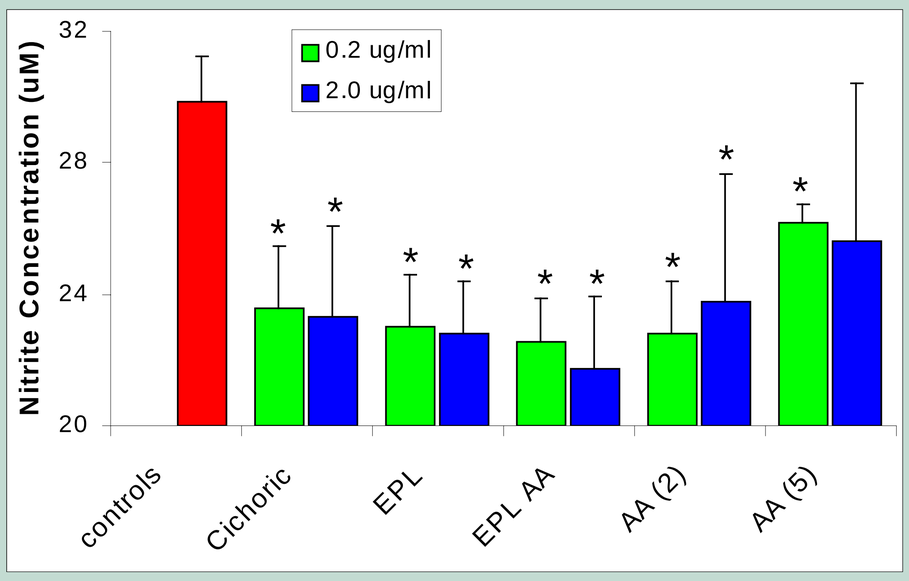


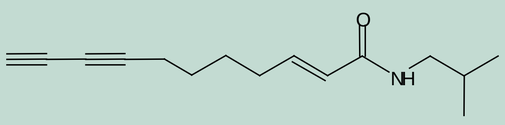
Figure 3: Effect of Echinacea compounds on LPS-stimulated NO production by macrophages.
Control levels (no addition) are shown in yellow. LPS alone is shown in red. Values are means ± SD for n = 3. * = $p \leq 0.05$

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

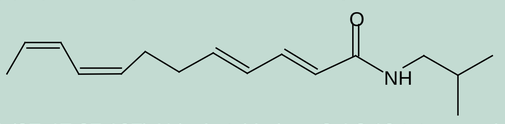
Methods

The macrophage cell line RAW 264 from the European Collection of Cell Cultures were cultivated in modified DMEM. Cells were maintained at 37°C in an atmosphere of 5% CO_2. 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 NFκB 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).



(2) (2E)-N-isobutylundeca-2-ene-8,10-diynamide m/z = 231



(5) (2E,4E,8Z,10Z)-N-isobutyldodeca-2,4,8,10-tetraenamide m/z = 247

Figure 4: Structures of synthetic isobutylamides.

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

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References:
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