Anti-inflammatory effects of different Phaeodactylum tricornutum extracts on human peripheral blood mononuclear cells

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Introduction

Phaeodactylum tricornutum Bohlin:
- Diatom, 3 morphotypes
- Complete genome known (Bowler, C. et al., 2008)
- Source of eicosapentanoic acid and carotenoids

Peripheral blood mononuclear cells (PBMCs)
- Lymphocytes and monocytes
- Primary cells
- Involved in inflammation secrete cytokines, e.g. interleukins, tumor necrosis factor, leukotrienes, prostaglandins

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Source: Qin et al. 2016
**Method:** Pressurized Liquid Extraction of *P. tricornutum*

- **Pilot Scale Outdoor Cultivation**
  - at Fraunhofer CBP, Leuna, Germany
  - 112 days in 25 FPA-reactors
  - (180 L each), > 5 % (w/w) EPA,
  - > 2 % (w/w) Fucoxanthin

- **Mech. Cell Disruption**
  - Stirred ball milling

- **Pressurized Liquid Extraction**
  - Solvents: Ethanol, n-Hexane, Water
  - $P = 100$ bar; $T = 100 \, ^\circ C$; $t = 20 \, \text{min}$
  - $Y_{\text{EPA}} = 91,4 \, \%$; $Y_{\text{FX}} = 70,8 \, \%$

**Harvesting**

- Centrifugation

**Extracts**

*Traces of EPA and Zeaxanthin*
Method: Anti-inflammatory effects on human PBMCs

Isolation of human PBMCs by density gradient centrifugation

Incubation with extracts (f.c. 0.01 - 1%) for 24 hrs

Cell Viability: MTT Assay

mRNA: Stimulation with LPS (f.c. 1µg / ml) for 6 hrs, primer: IL-1β, IL-6, TNFα, COX-2, GAPDH

Western Blot: Stimulation with LPS (f.c. 1µg / ml) for 15 min
Results: Cytotoxicity of *P. tricornutum* extracts on human PBMCs

Fig. 1: Data are expressed as percent, compared to the control (Mean ± SD; n=5). Asterisks (*) indicate a significant difference to control (t-test, *** p < 0.001, ** p < 0.01, * p < 0.05).
anti-inflammatory effect of different *P. tricornutum* extracts on PBMCs

![Graphs showing mRNA expression of IL-1, IL-6, TNFα, and COX-2 relative to LPS concentration and extract types.](image)
anti-inflammatory effect of different *P. tricornutum* extracts on PBMCs

![Graphs showing relative mRNA-expression of IL-1, IL-6, TNF-α, and COX-2](image)

Control: 0.1% DMSO, hexanolic extract, ethanolic extract, aqueous extract

Concentration: 1, 0.01, 0.1, 1
anti-inflammatory effect of different *P. tricornutum* extracts on PBMCs

Fig. 2.: Data are expressed as percent, compared to the stimulated control. Values were normalized to GAPDH as a reference gene and are expressed as mean ± SD (A-C n=5-6, D n=4-5). Asterisks (*) indicate a significant difference to stimulated control (t-test, *** p < 0.001, ** p < 0.01, * p < 0.05).
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Source: Qin et al. 2016
Effect on LPS-induced phosphorylation of ERK 1/2

Fig. 3: Representative pictures and densitometric analyses in % of stimulated control for Western blots of phosphorylated ERK1/2 and β-Actin (Mean ± SEM; n=4-5). Asterisks (⋆) indicate a significant difference to control (t-test, ⋆ p < 0.05, ⋆⋆ p < 0.01).

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Effect on LPS-induced phosphorylation of IκBα

Fig. 4: Representative pictures and densitometric analyses in % of stimulated control for Western blots of phosphorylated IκBα (Ser32) and β-Actin (Mean ± SEM; n=5-6). Asterisks (*) indicate a significant difference to control (t-test, * p < 0.05).

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Summary

- Pressurized liquid extracts of *P. tricornutum* contain carotenoids and fatty acids (mainly EPA and fucoxanthin)

- Ethanolic and aqueous extracts exhibit a dose-dependent anti-inflammatory effect on human PBMCs

- This effect is caused by the inhibition of phosphorylation of IκBα and ERK 1/2
Thank you for your attention!

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