



MilkQua

<http://www.milkqua.eu>

Milk Quality along the Dairy
Chain for a Safe and
Sustainable MILK

MILKQUA SC Meeting September 27th, 2021

In vitro evaluation of bioactive
molecules and extracts

David M. Pereira, Renato B. Pereira



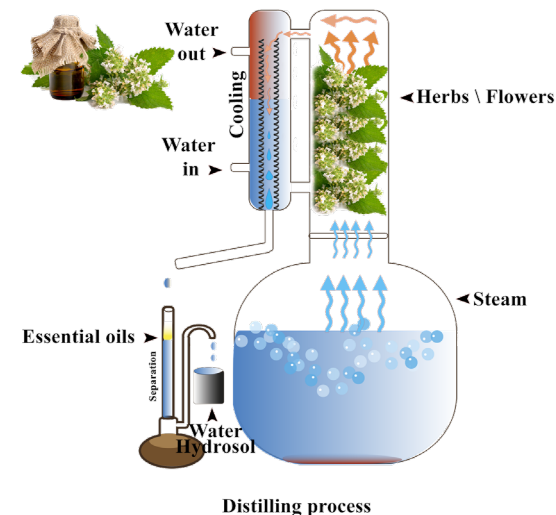
The PRIMA programme is supported under Horizon 2020, the European Union's Framework Programme for Research and Innovation



Essential oils (EOs)



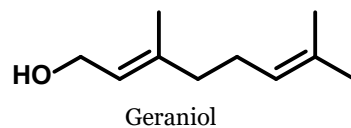
- Volatile
- Lipid soluble
- Density - generally lower than water
- Mostly obtain by steam distillation
- Chemical variation even for the same plant species



Terpene type compounds

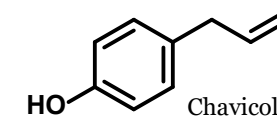
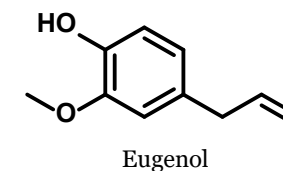
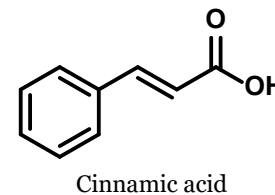
Monoterpenes

- Acyclic
- Cyclic



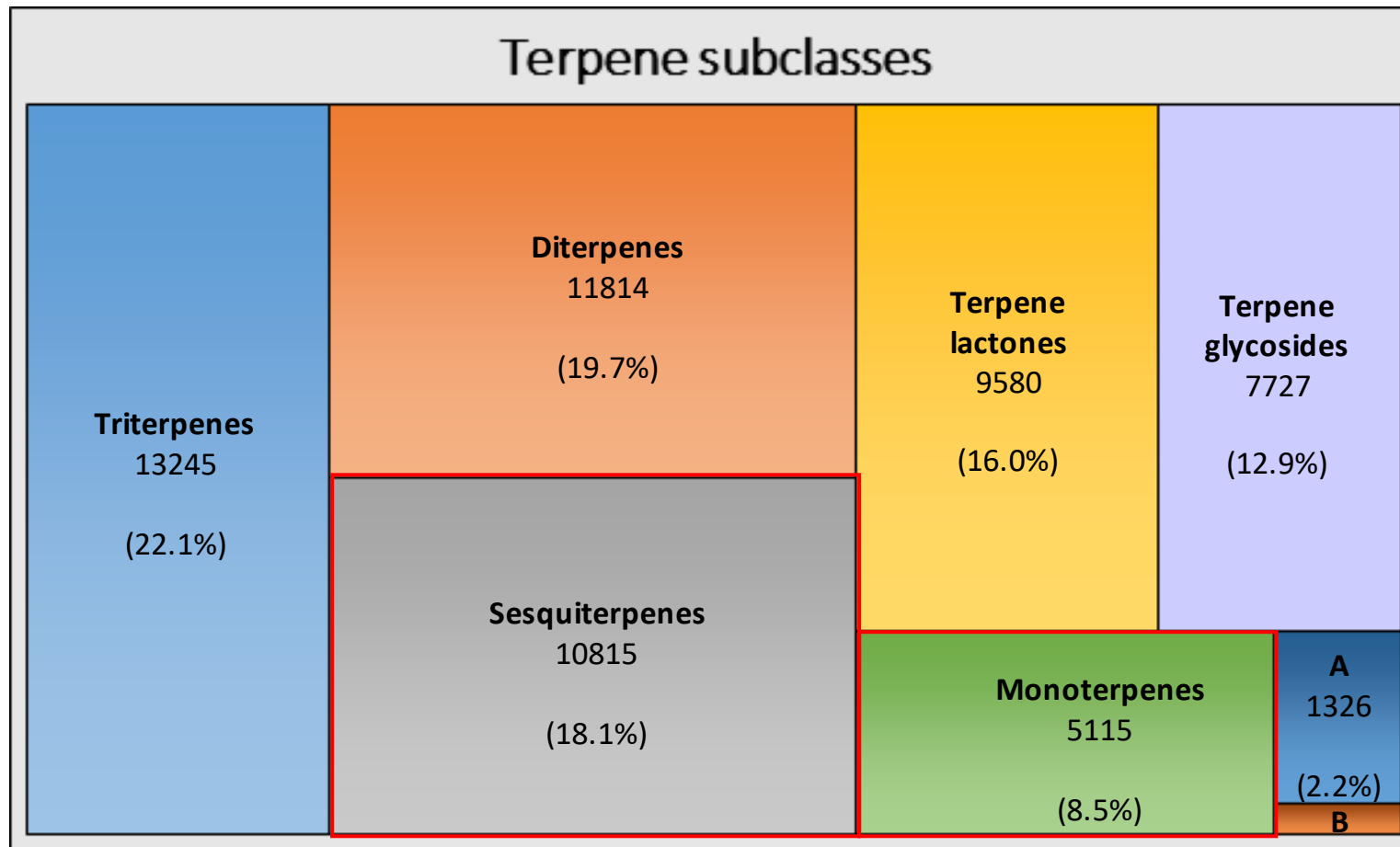
Aromatic type compounds

Phenylpropanoids

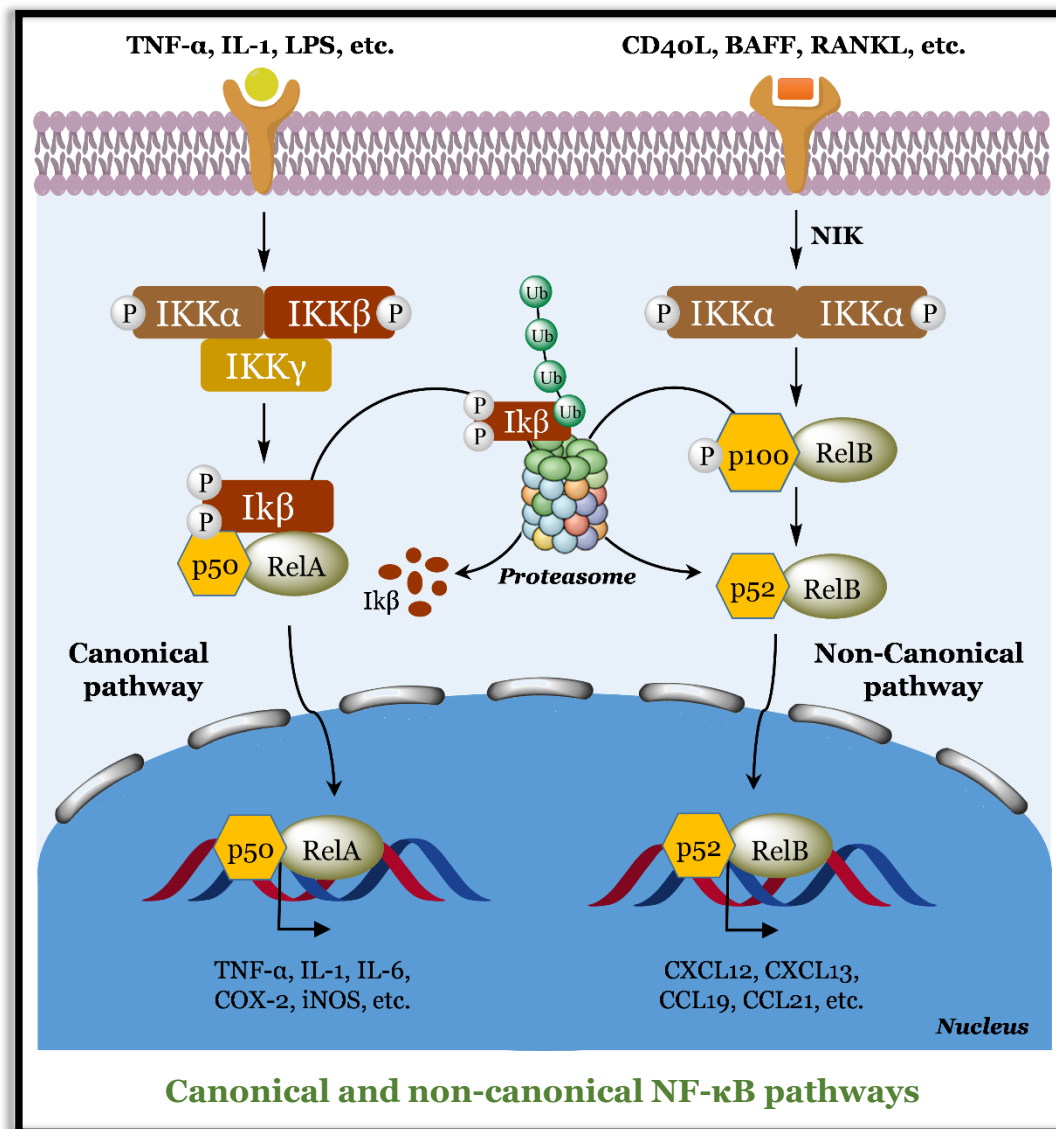




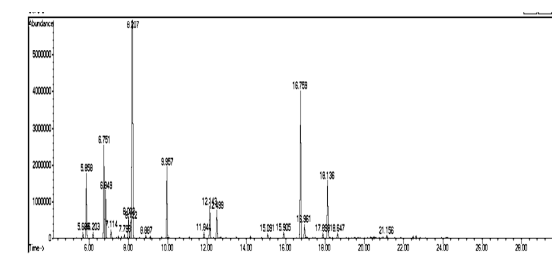
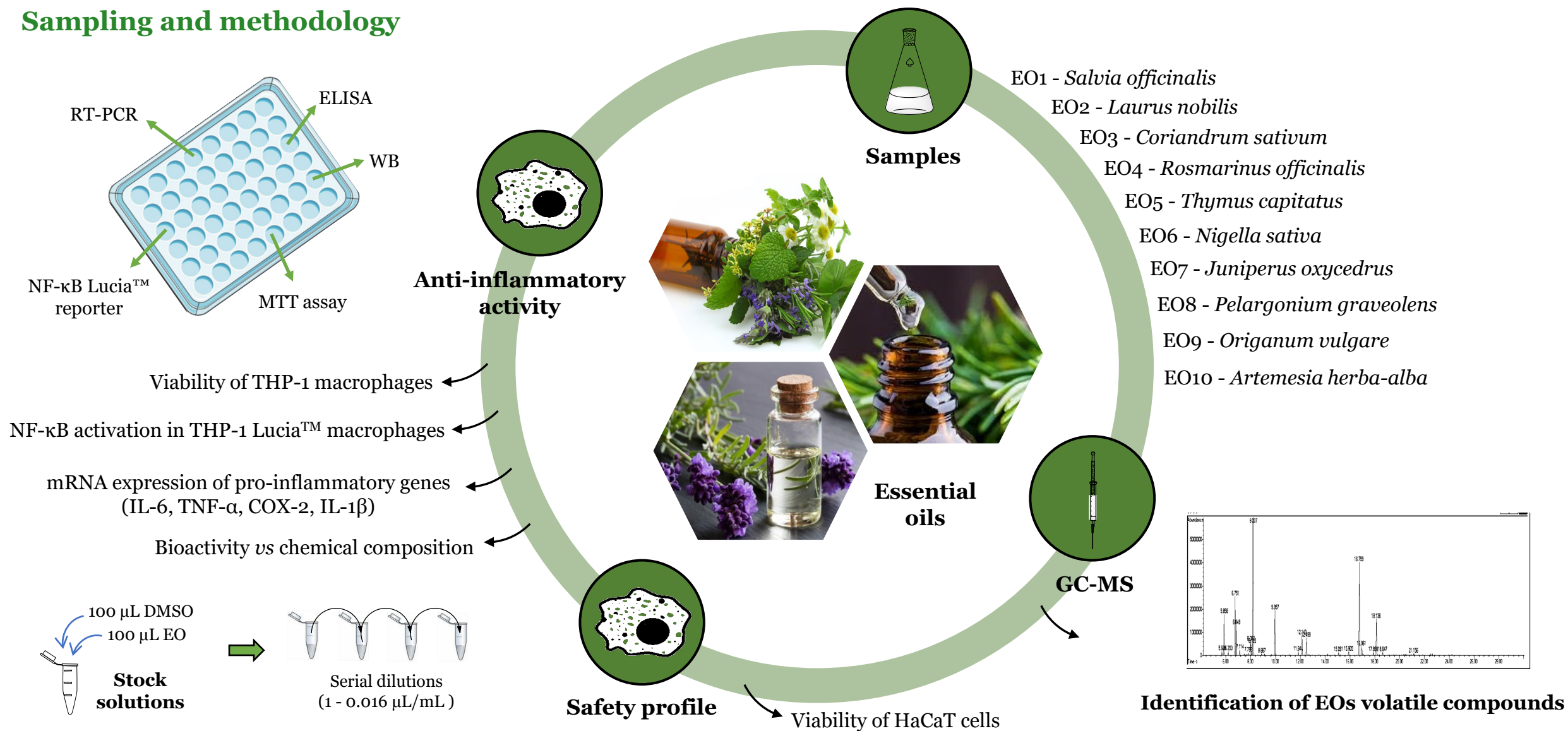
Essential oils (EOs)



NF- κ B pathway



Sampling and methodology



Preliminary screening – Cytotoxic effects to human keratinocytes (HaCaT cells)

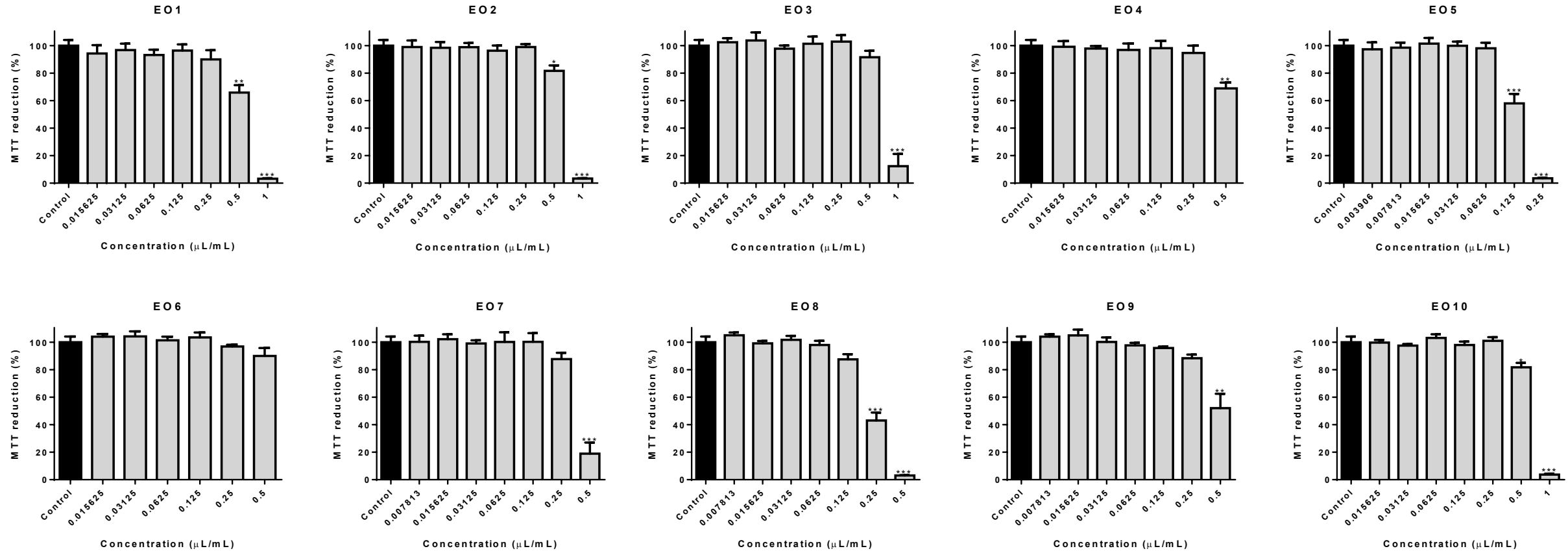


Fig. 1: Viability of HaCaT cells exposed to EOs 1-10 for 24h based on MTT reduction.

Cytotoxic effects to THP-1 macrophages

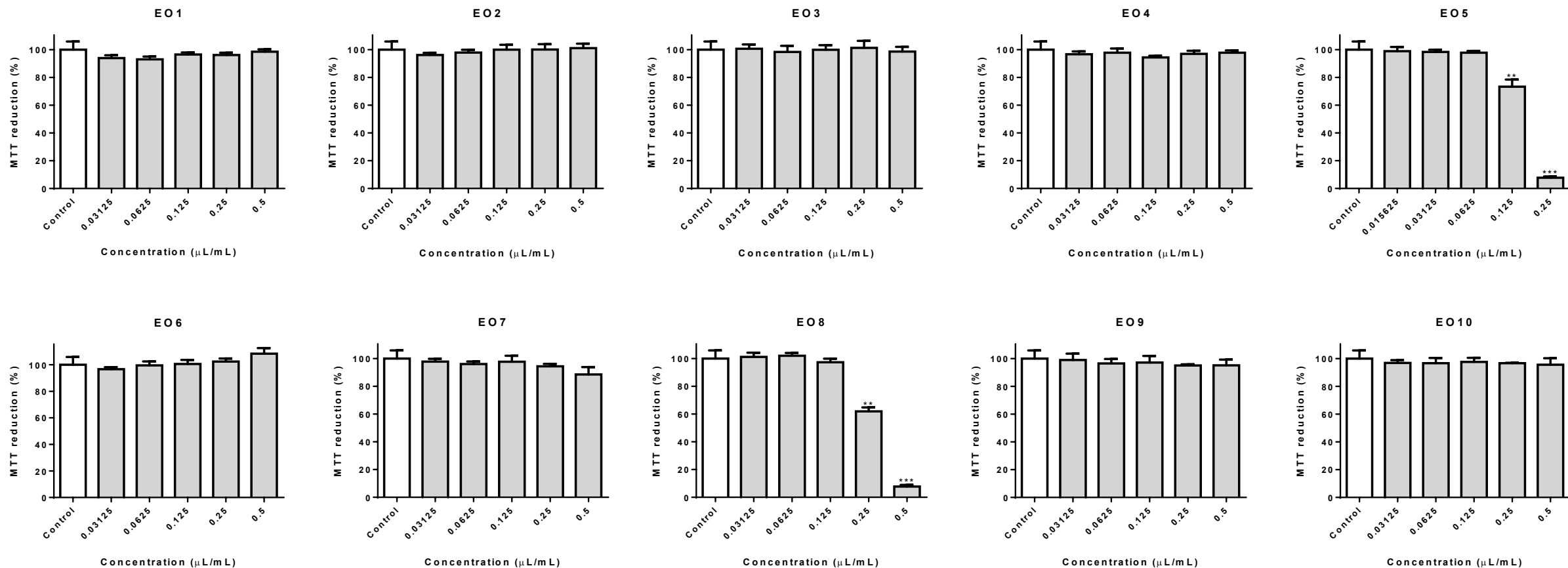


Fig. 2: Viability of THP-1 cells exposed to EOs 1-10 for 24h based on MTT reduction.

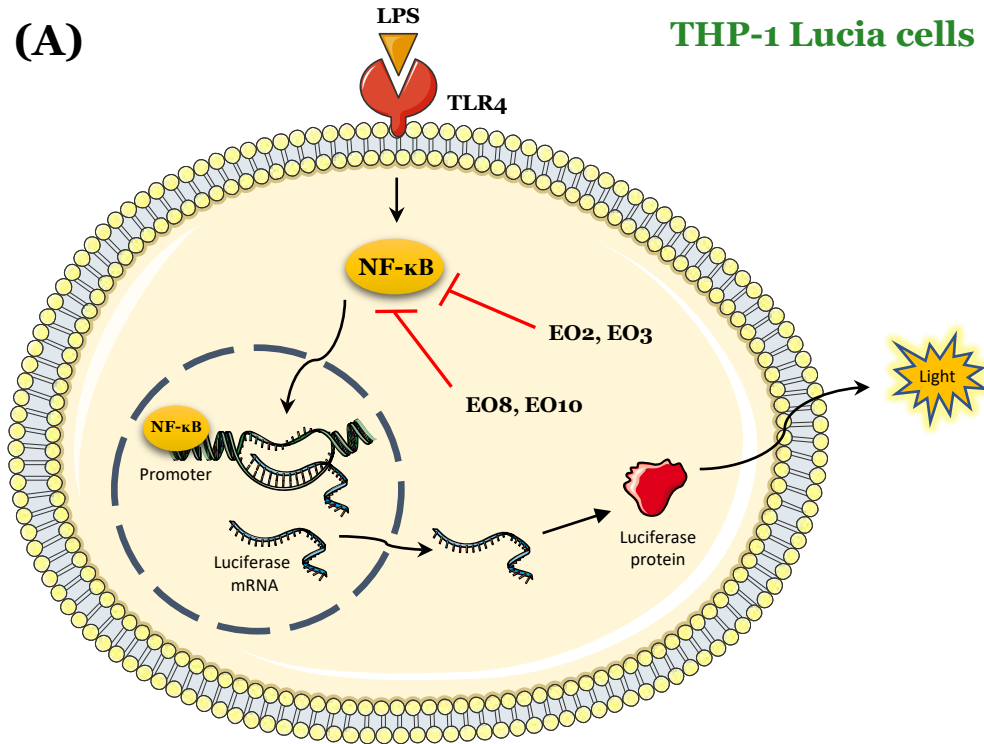


Fig. 3A: Experimental model used for the assessment of NF-κB.

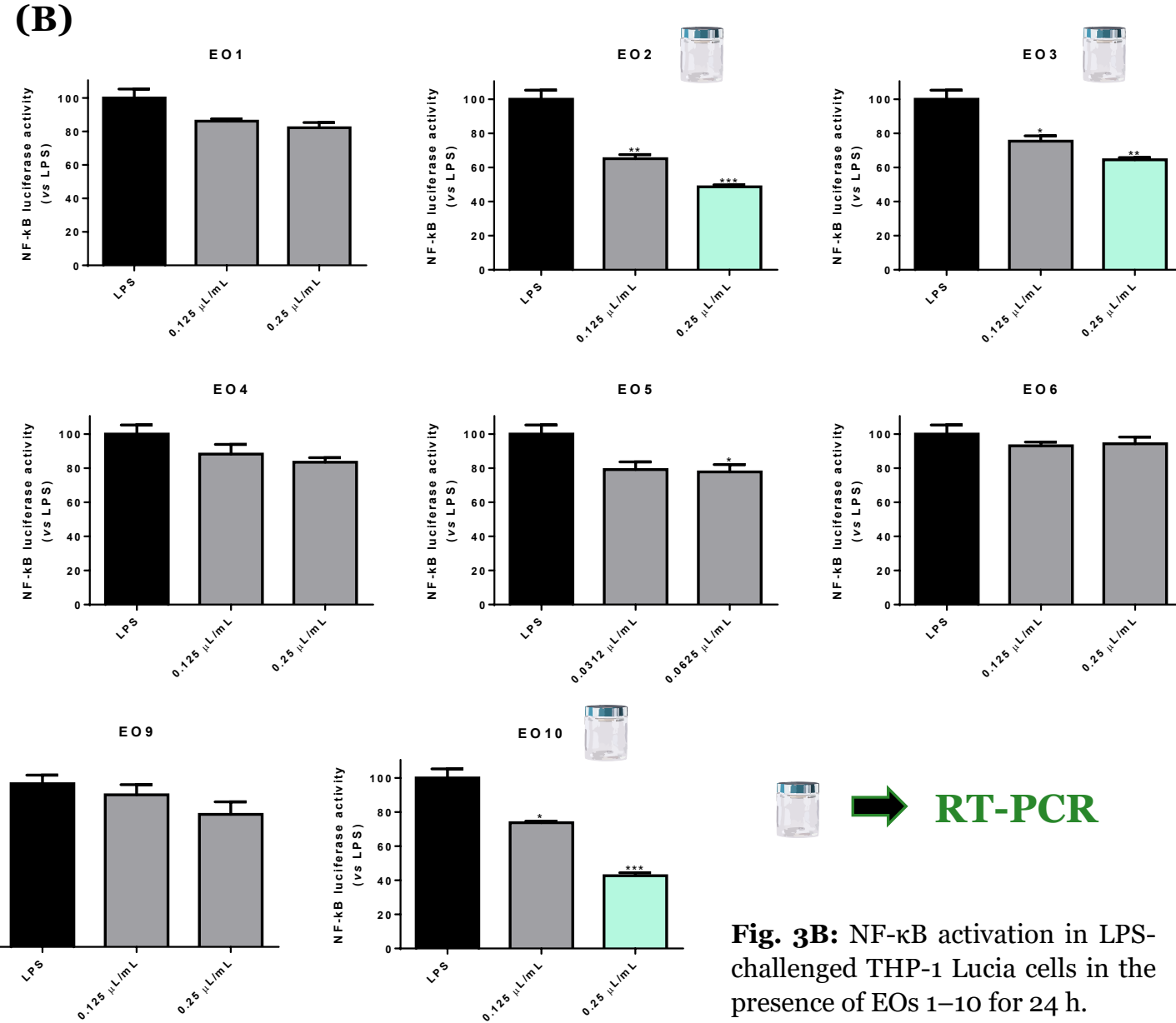
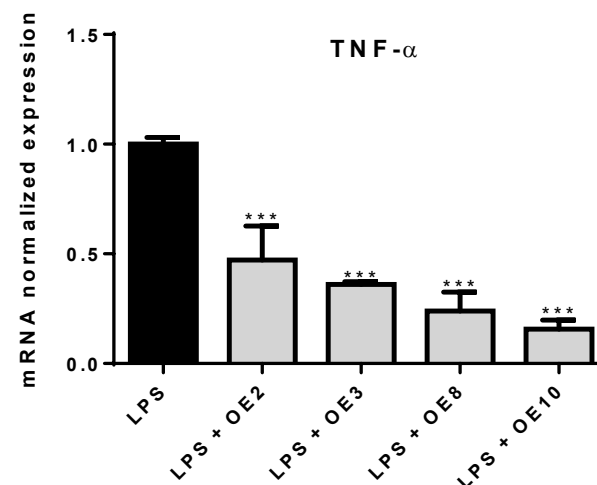
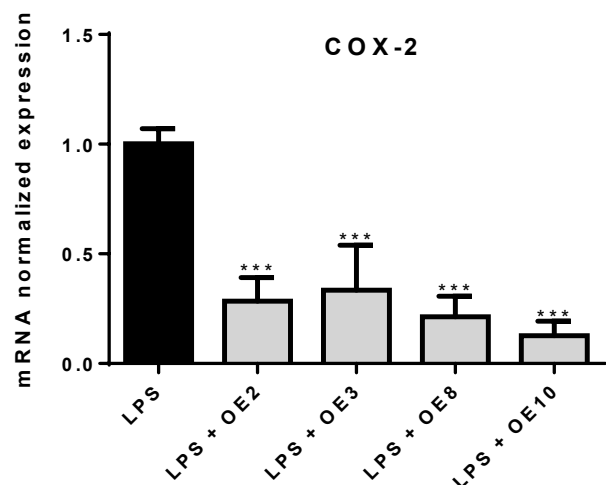
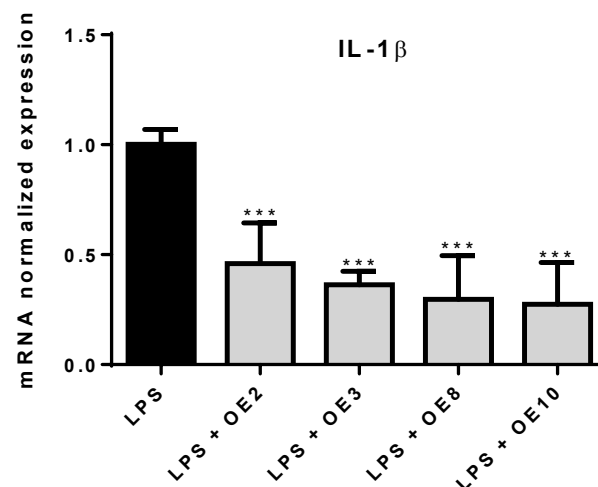
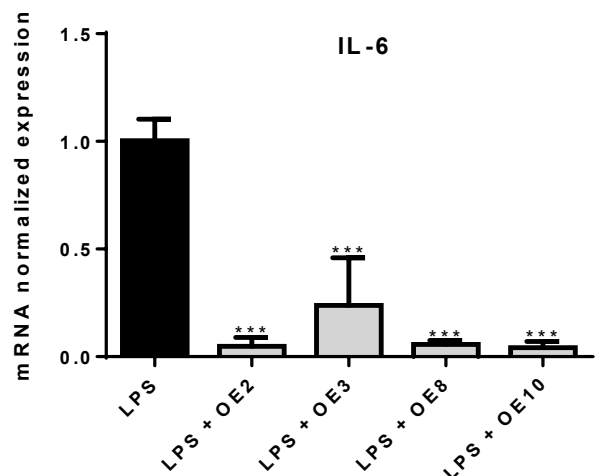


Fig. 3B: NF-κB activation in LPS-challenged THP-1 Lucia cells in the presence of EOs 1–10 for 24 h.

THP-1 macrophages: qPCR analysis of selected EOs



OE2 – *Laurus nobilis*

1,8-cineole (43.85%)

Camphene (13.82%)

Sabinene (7.03%)

□ Inhibits NF-κB activity in U373 and HeLa cells.

□ Inhibits TNF-α, IL-1β, IL-6, IL-8 production in LPS-stimulated monocytes and ionomycin/PMA stimulated lymphocytes.

OE3 – *Coriandrum sativum*

Linalool (64.10%)

α-Pinene (7.26%)

o-Cymene (5.91%)

Camphor (5.50%)

□ Inhibits endotoxin-induced TNF-α and IL-1β in RAW 264.7 macrophages and in BV2 cells.

□ Inhibits NF-κB translocation and promote IκB-α accumulation in LPS-stimulated THP-1 cells.

OE8 – *Pelargonium graveolens*

β-citronellol (36.88%)

Geraniol (15.02%)

Citronellyl formate (8.74%)

□ Reduces iNOS, COX-2 mRNA and protein expression induced by LPS and reverses the cytoplasmic degradation of IκB and the upregulation of NF-κB p65 in the nucleus in RAW 264.7 cells.

EO10 - *Artemisia herba-alba*

β-thujone (38.27%)

Camphor (11.88%)

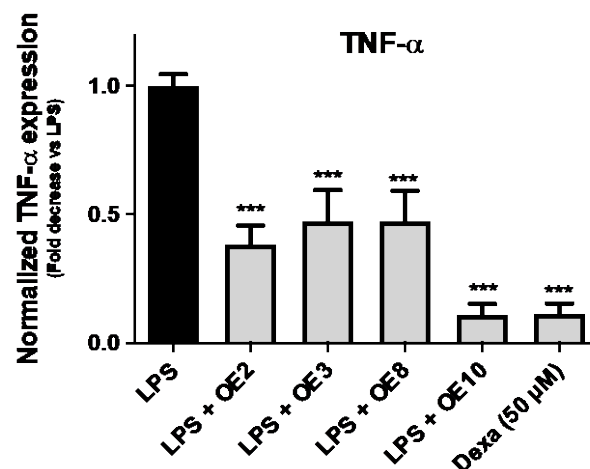
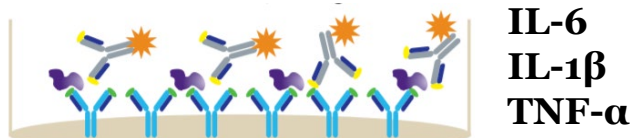
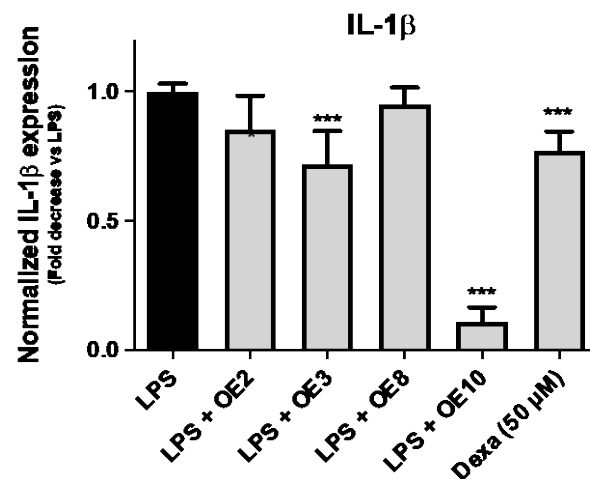
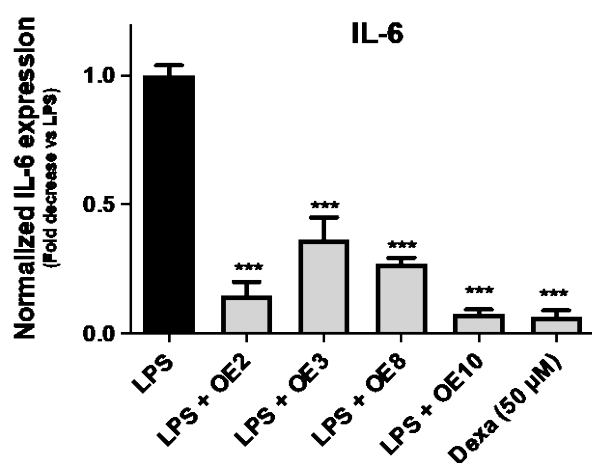
α-thujone (11.58%)

□ Decreases the release of IL-6 and IL-8 in PMA/I-stimulated HGF-1 cells.

Fig. 4: IL-6, IL-1β, COX-2 and TNF-α mRNA expression in THP-1 cells, pre-treated for 2h with EOs 1-10 followed by 22h co-treatment with LPS (1 μg/mL).

mRNA expression was determined by qPCR after normalization with GAPDH reference gene. EO2, EO3, EO10 – 0.25 μL/mL; EO8 – 0.125 μL/mL.

NEW RESULTS - THP-1 macrophages: ELISA analysis of selected EOs



OE2 – *Laurus nobilis*

1,8-cineole (43.85%)

Camphene (13.82%)

Sabinene (7.03%)

OE3 – *Coriandrum sativum*

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EO10 - *Artemisia herba-alba*

β-thujone (38.27%)

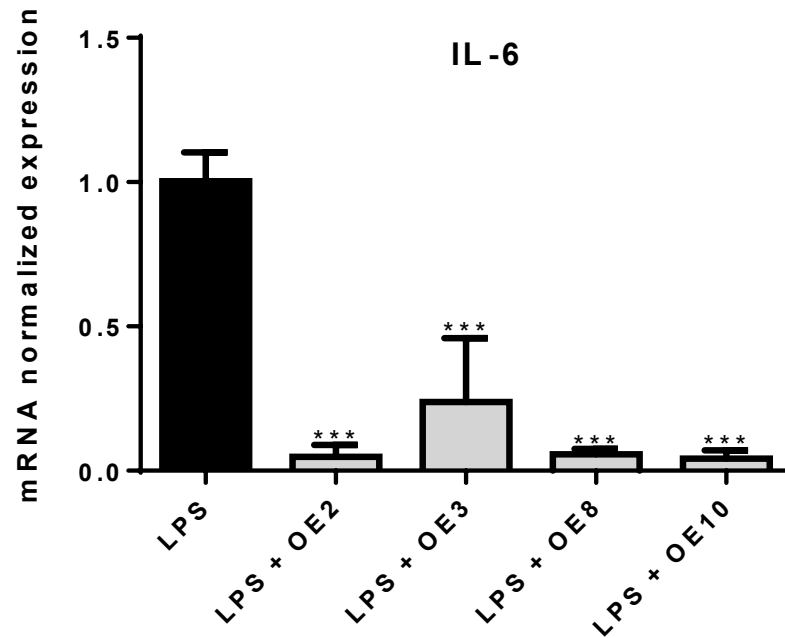
Camphor (11.88%)

α-thujone (11.58%)

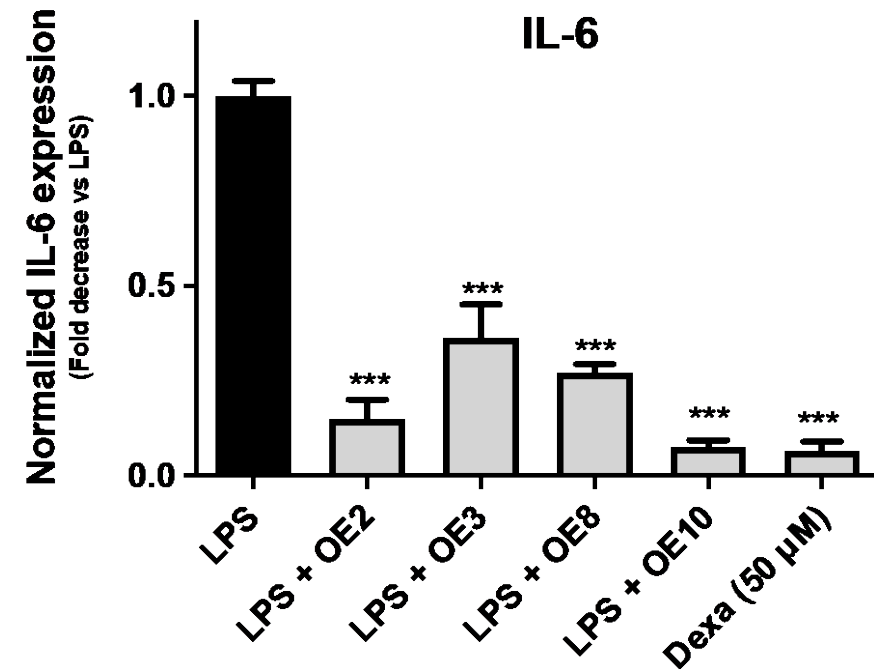
Fig. 5: IL-6, IL-1β and TNF-α protein levels in THP-1 cells, pre-treated for 2h with EOs 1-10 followed by 22h co-treatment with LPS (1 µg/mL).

PCR vs ELISA

PCR

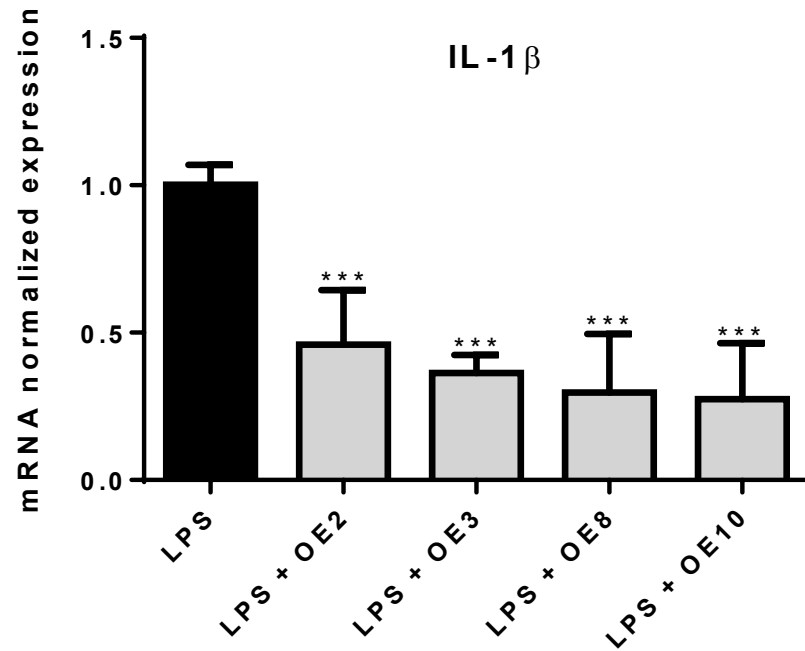


Protein

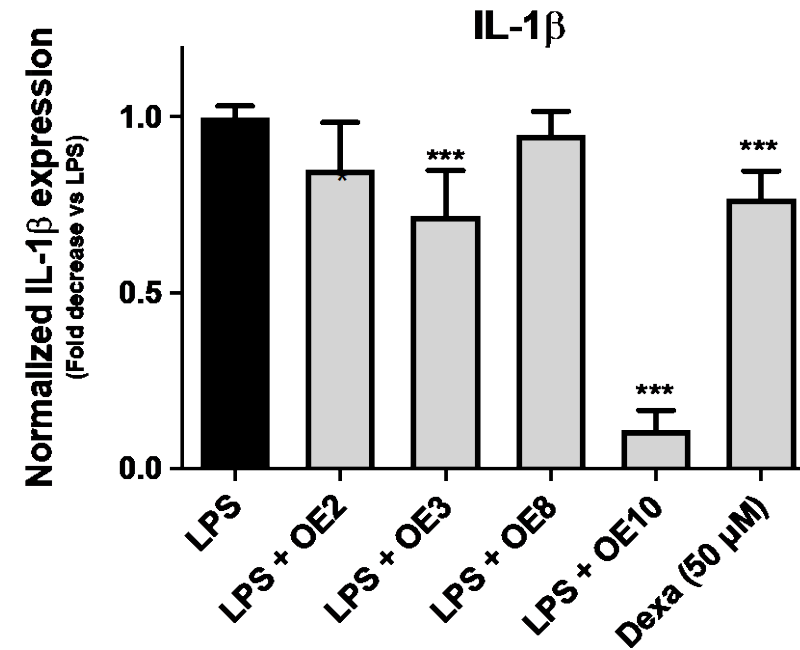


PCR vs ELISA

PCR

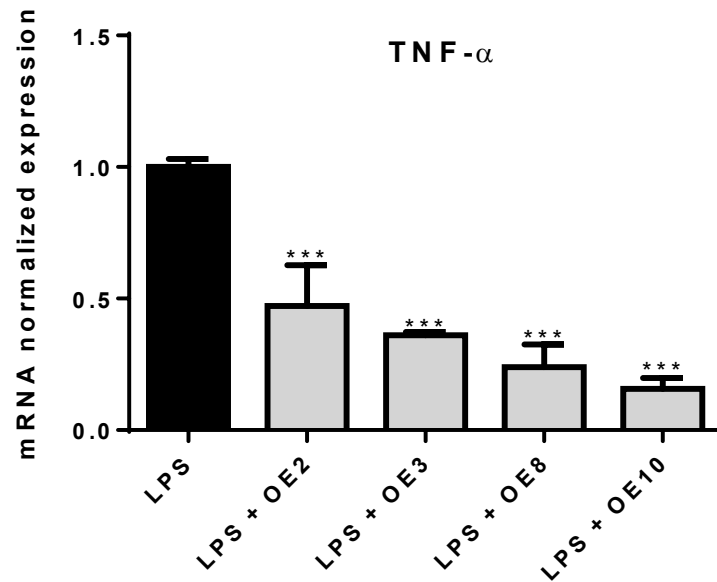


Protein

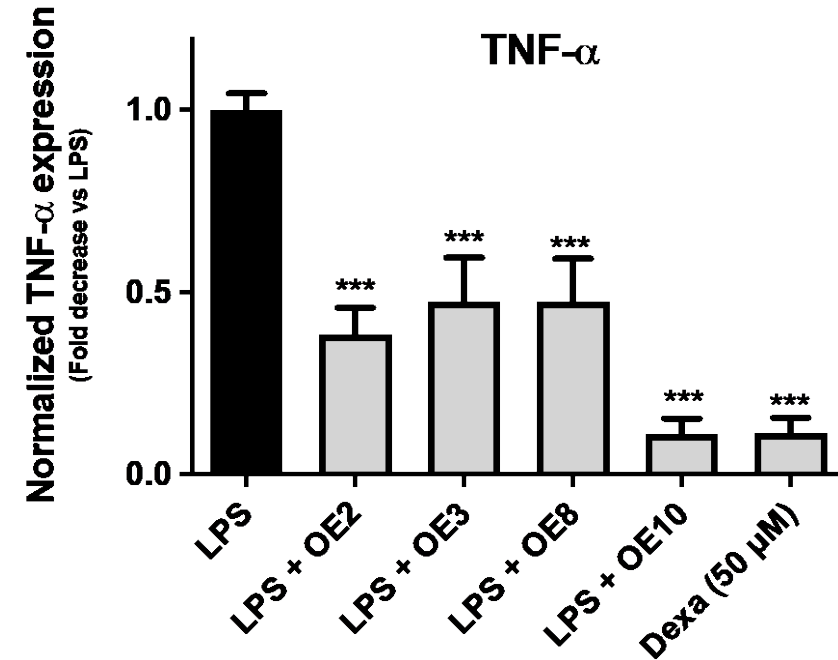


PCR vs ELISA

PCR



Protein



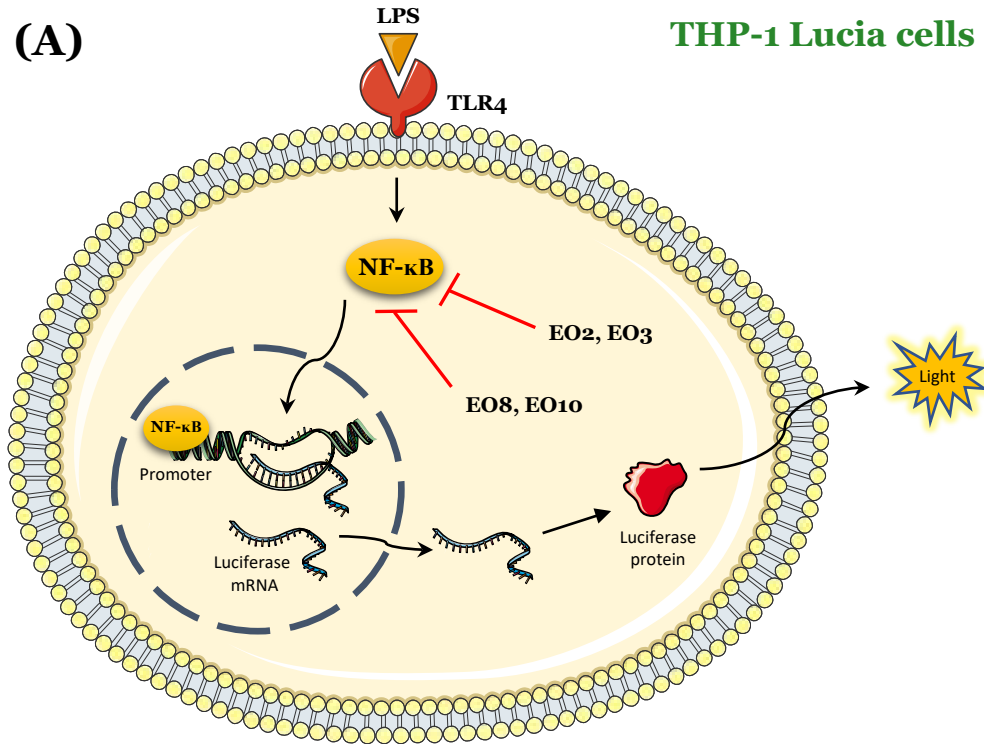


Fig. 3A: Experimental model used for the assessment of NF-κB.

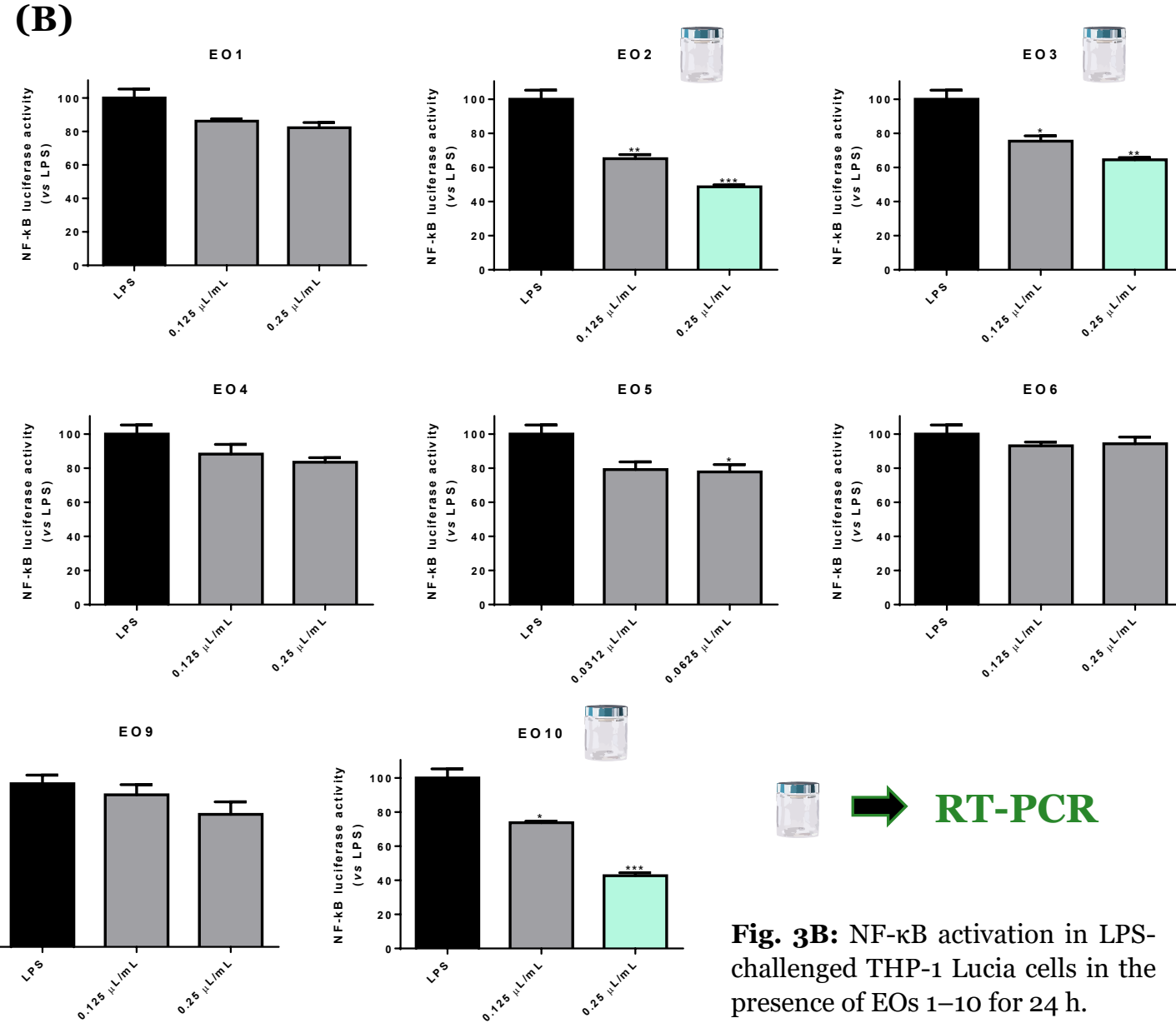
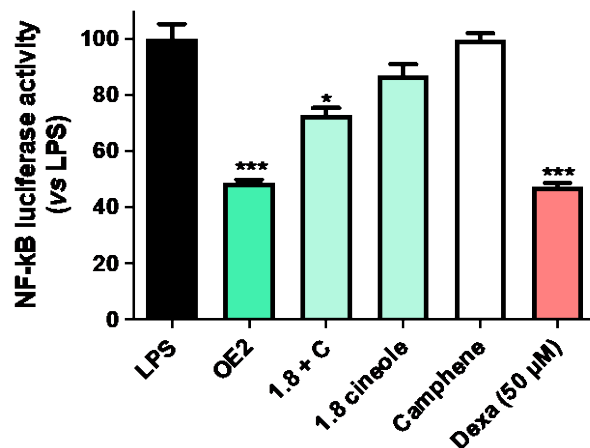


Fig. 3B: NF-κB activation in LPS-challenged THP-1 Lucia cells in the presence of EOs 1–10 for 24 h.

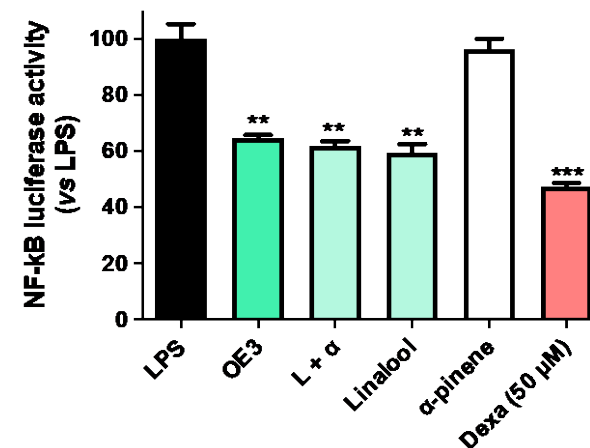


New results

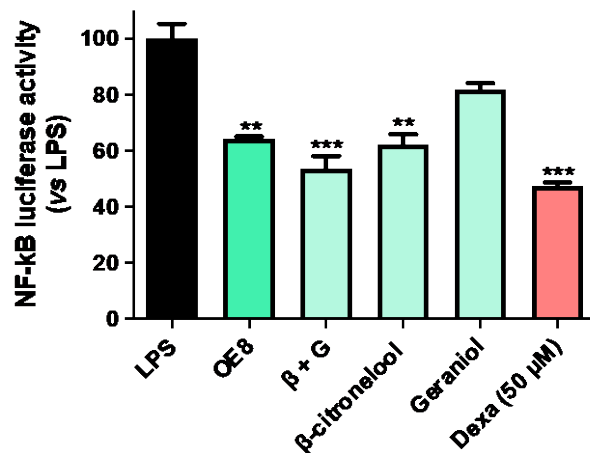
Major components



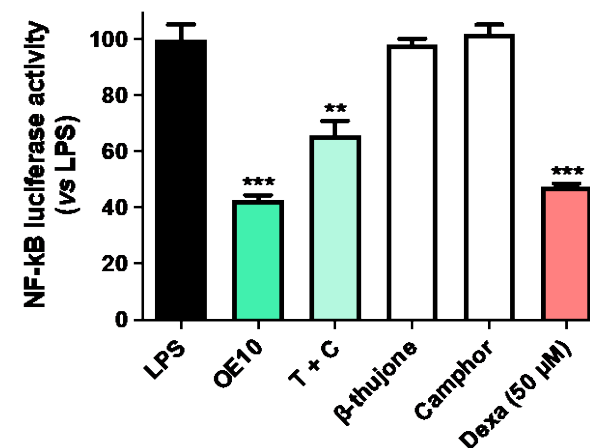
Major components



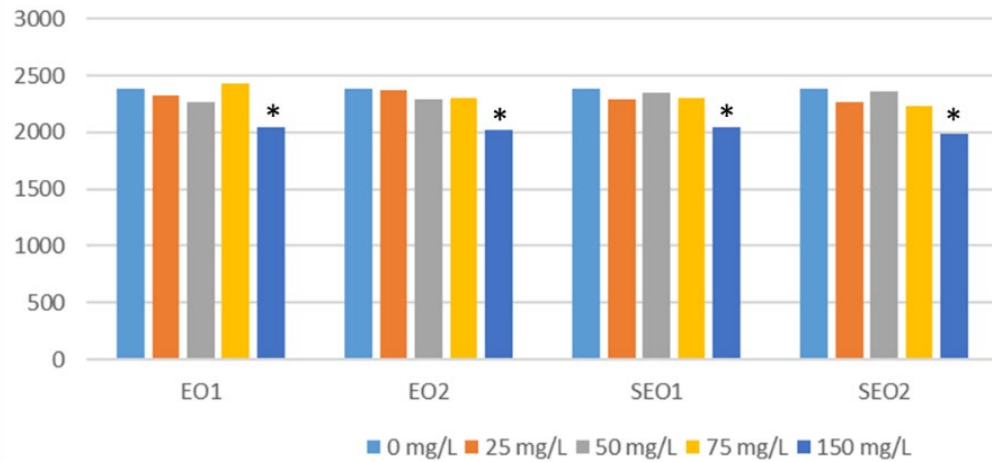
Major components



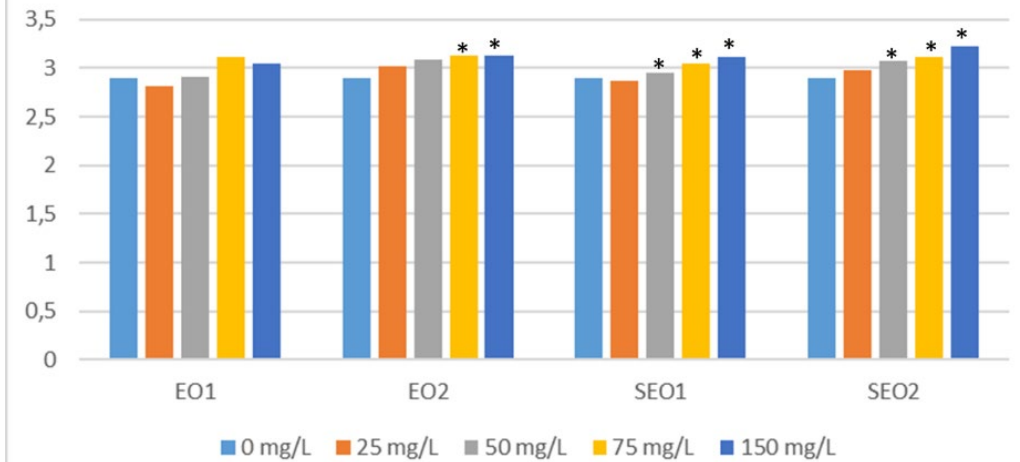
Major components



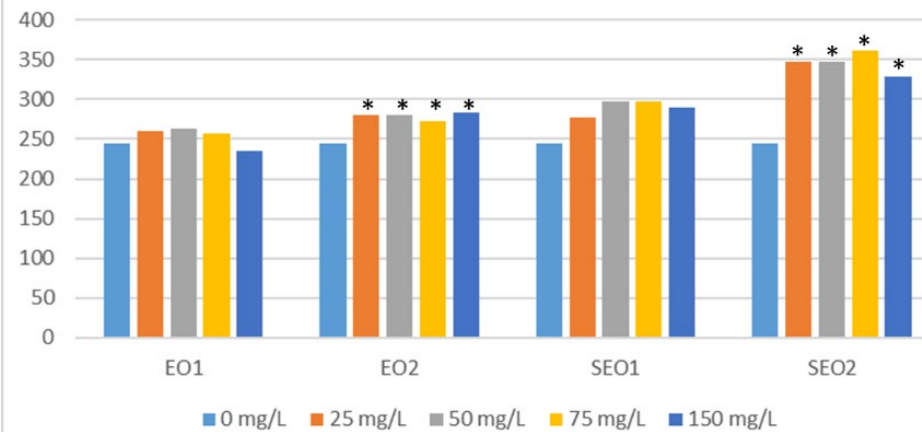
Total Volatile Fatty Acids ($\mu\text{mol/L}$)



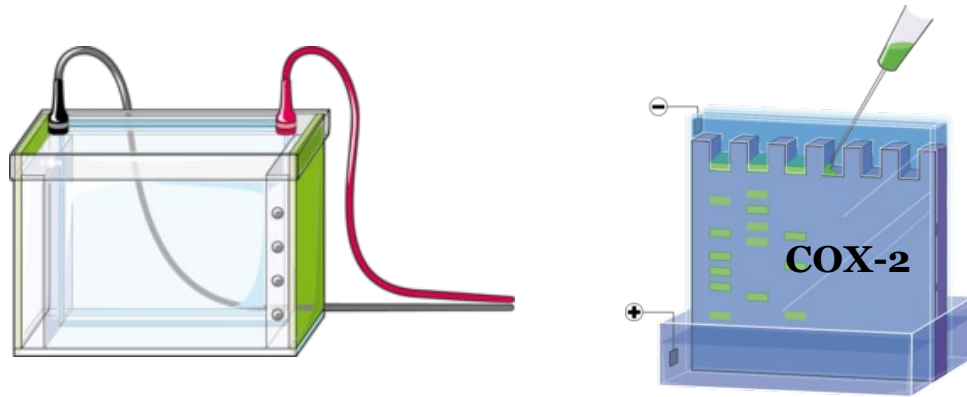
Acetate:Propionate ratio



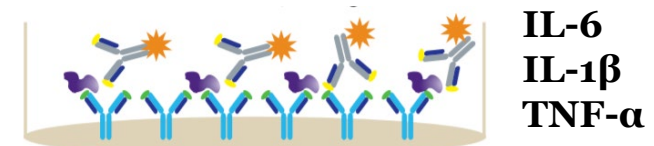
Methane (μmol)



Expected work & valorization

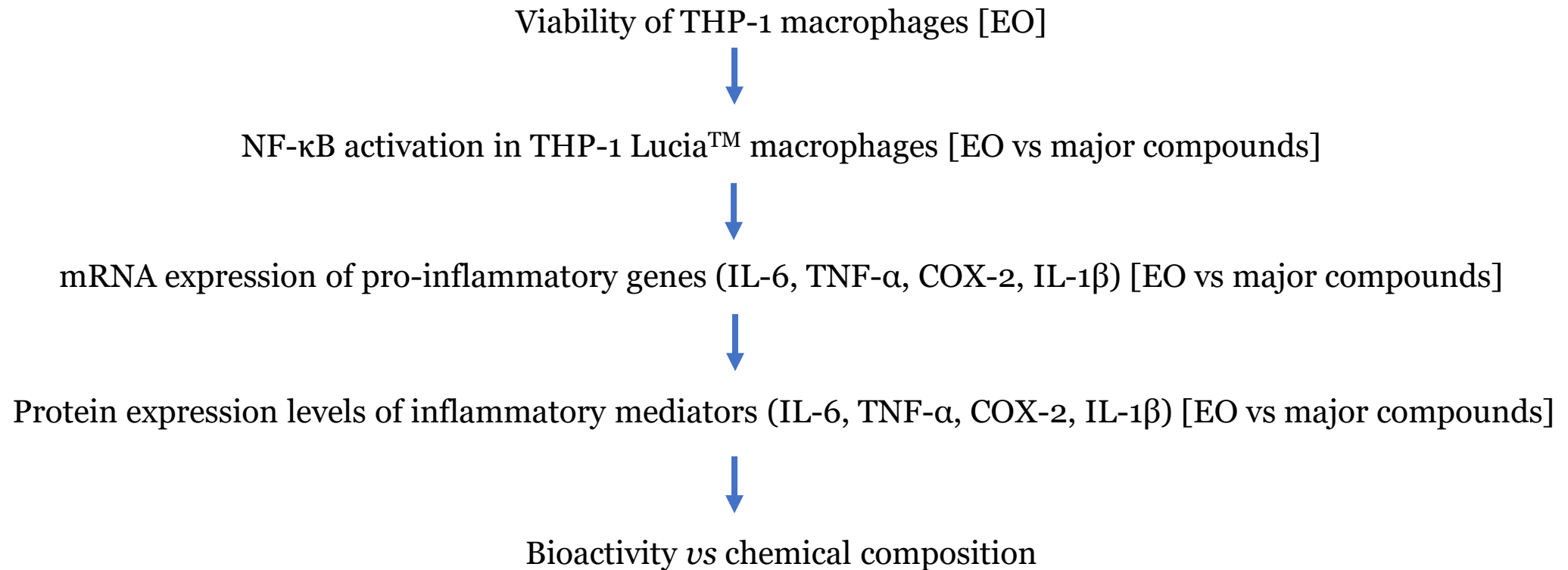


Western-blot



PCR & ELISA for major components

Paper outline





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Chain for a Safe and
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In vitro evaluation of bioactive molecules and extracts

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