Unsaponifiable fraction of extra virgin olive oil inhibits the induction of inducible nitric oxide synthase and cyclooxygenase-2 in murine peritoneal macrophages

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Background: Extra virgin olive oil (EVOO) has demonstrated a great functional versatility related to its antioxidant and anti-inflammatory properties. Nowadays, we know that many of the beneficial effects of EVOO may be due to its minor components present in the unsaponifiable fraction (UF). On the other hand, macrophages play a key role in the immunopathogenesis of inflammatory-based diseases by expressing several mediators, including cyclooxygenase (COX)-2 and inducible nitric oxide synthase (iNOS) and nitrogen intermediates. Aim: We investigated the anti-inflammatory activity of UF from EVOO in a lipopolysaccharide (LPS)-stimulated murine peritoneal macrophage model. Methods: The isolation of UF fraction was carried out by conventional procedures and the quantitative/qualitative characterizations were done according to the European Regulation. Cell viability was assayed by sulforhodamine (SRB) test and nitrite accumulation was measured in 24 h supernatants. Moreover, COX-2, iNOS and mitogen-activated protein kinases (MAPKs) protein expression changes were detected by western blotting. Results: Sterols were majority detected compounds (1351 mg/kg EVOO), being the mainly β-sitosterol (95.04%) followed by triterpenic dialcohols (542 mg/kg EVOO), aliphatic alcohols (160 mg/kg EVOO) and α-tocopherol (85 mg/kg EVOO). The treatment with UF from EVOO at µg/mL range did not produce any changes in cell viability whereas the generation of nitrites was significantly prevented after 24 h in UF-treated cells. Similarly, UF treatment induced a down-regulation of pro-inflammatory COX-2 and iNOS protein expression. However, UF-treated macrophages did not show significant changes in phosphorylation of pp38, JNK, ERK and MAPKs. Conclusion: These novel findings suggest that UF of EVOO could exert a protective/preventive role in inflammatory-based diseases.