Anti-inflammatory and hypolipidemic mechanisms of *Eucalyptus tereticornis* leaf extract

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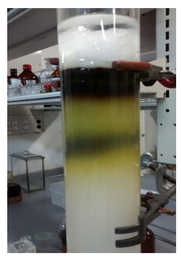
INTRODUCTION

Obesity is a complex condition with adipocyte hypertrophy as an important source for the development of adipose tissue inflammation that plays a central role in the pathogenesis of many obesity-associated complications. Ursolic acid (UA), Oleanolic acid (OA) and Ursolic acid lactone (UAL) are the main molecules (78%) in a *Eucalyptus tereticornis* (Eu) extract (OBE100). This enriched-triterpene fraction mixed with unknown minor metabolites has a synergistic effect providing superior anti-inflammatory and hypolipidemic effects than reconstituted triterpenoid mixtures (1).

METHODS

EXTRACTION

The dried leaves from *E. tereticornis (Eu)*, were extracted by a liquid-liquid separation with hexane: methanol: water 4:3:1(v/v); the organic phase was collected, and vacuum filtered. The precipitate formed was collected, stored, and called OBE100.



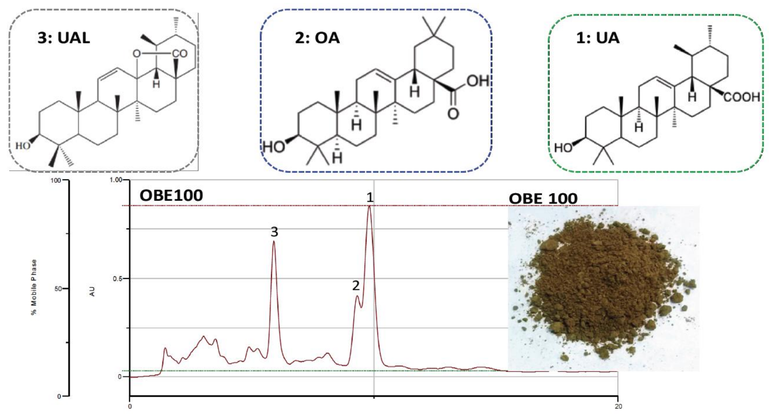
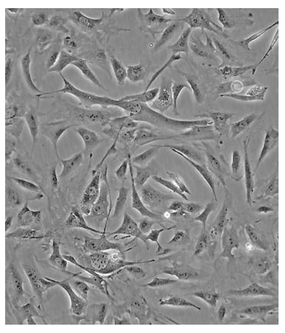


Figure 1. Eu extract composition. Ursolic acid (UA), Oleanolic acid (OA) and Ursolic acid lactone (UAL) are the major compounds of OBE100. They were identified by 1H NMR and 13C NMR. 1H and 13C-NMR and two-dimensional spectra were obtained in an AMX300 spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany). In addition to NMR, compounds were identified and quantified by HPLC. UA and OA were purchased from Sigma-Aldrich (St. Louis, MO) and UAL was purified by Sephadex column and preparative chromatography from OBE100.

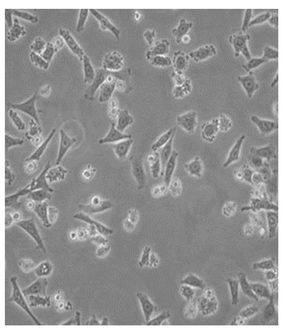
IN VITRO ANALYSIS



3T3-L1 pre-adipocytes were cultured in Growth Medium (GM). Differentiation was induced 2 days post-confluence by adding GM containing 0.5 mM IBMX, 0.25 µM dexamethasone, 2 µM Rosiglitazone and 1 µg/ml insulin. After 2 days of incubation, the medium was changed to GM medium containing 1 µg/ml insulin. Two days later, the medium was replaced by GM and incubated for another 7 days with the treatments: OBE100, M1 (mix of triterpenes present in OBE100), UA, OA, UAL

***Cell line viability was determined using methyl thiazol tetrazolium assay (MTT assay)**

***The ADP/ATP ratio was measured using a bioluminescent method**



Murine macrophage cell line J774A.1, activated with lipopolysaccharide (LPS) and INF-γ for 18 hours was used to evaluate the effect of OBE100, M1 (mix of triterpenes present in OBE100), UA, OA, UAL for 6 hours.

***p-AMPK and total AMPK protein expression was evaluated by Western blot**

***Flow cytometry was used to quantify oxidative burst using Dihydrorhodamine 123 (DHR) and mitochondrial membrane potential using MitoTracker**

RESULTS

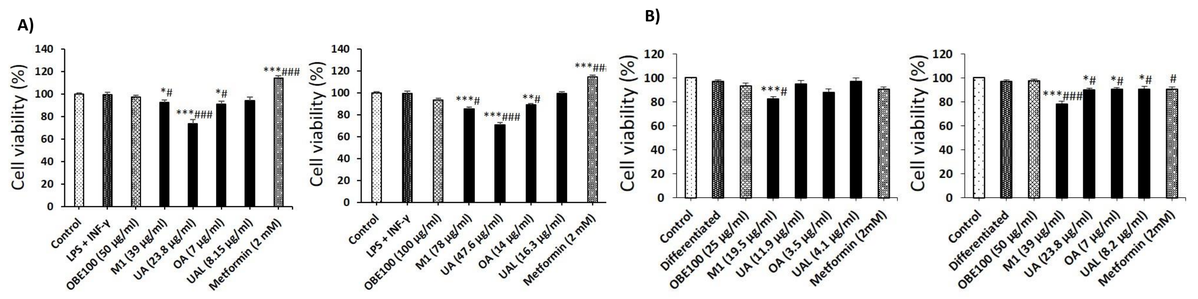


Figure 2. Effect of OBE100 and its major components on cell viability. **A)** Comparison of cell viability in J774.A1 and **B)** 3T3-L1 cells between OBE100 and M1, UA, OA and UAL treatments at the triterpenoid concentrations present in OBE100 extract. Cell viability was measured by the MTT assay after 6 hrs. of treatment. ∗ $P < 0.05$, ∗∗ $P < 0.01$, ∗∗∗ $P < 0.001$ compared with LPS+INF-γ (activated) or differentiated; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ compared with OBE100 (ANOVA with Dunnett´s post hoc test). Values are expressed as mean ± SEM of three independent experiments each one performed in triplicate.

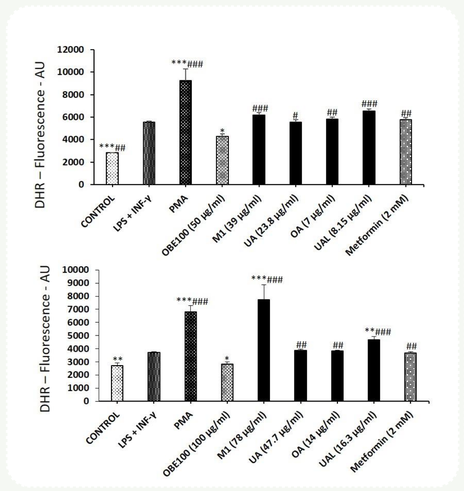


Figure 3: Effect of OBE100 and its major components on oxidative burst in activated J774A.1 macrophages. Comparison of oxidative burst between OBE100 and M1, UA, OA and UAL treatments at the same triterpenoid concentrations present in OBE100 extract. Oxidative burst was quantified by flow cytometry using DHR after 6 hrs. of treatment.

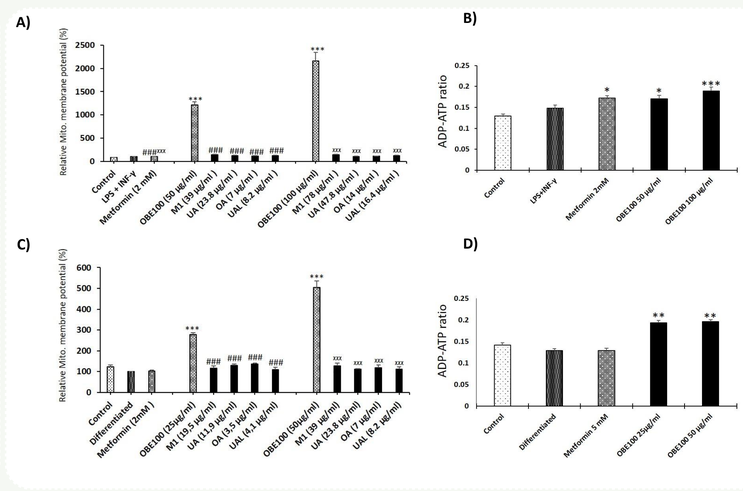


Figure 4. Effect of OBE100 and its major components on mitochondria. J774A.1 cells were activated for 18 hours and treated with OBE100, M1, UA, OA and UAL for 6 hours. **A)** Mitochondrial membrane potential, and **B)** ADP-ATP ratio. 3T3-L1 cells were differentiated and treated with OBE100, M1, UA, OA and UAL for 7 days. **C)** Mitochondrial membrane potential, and **D)** ADP-ATP ratio.

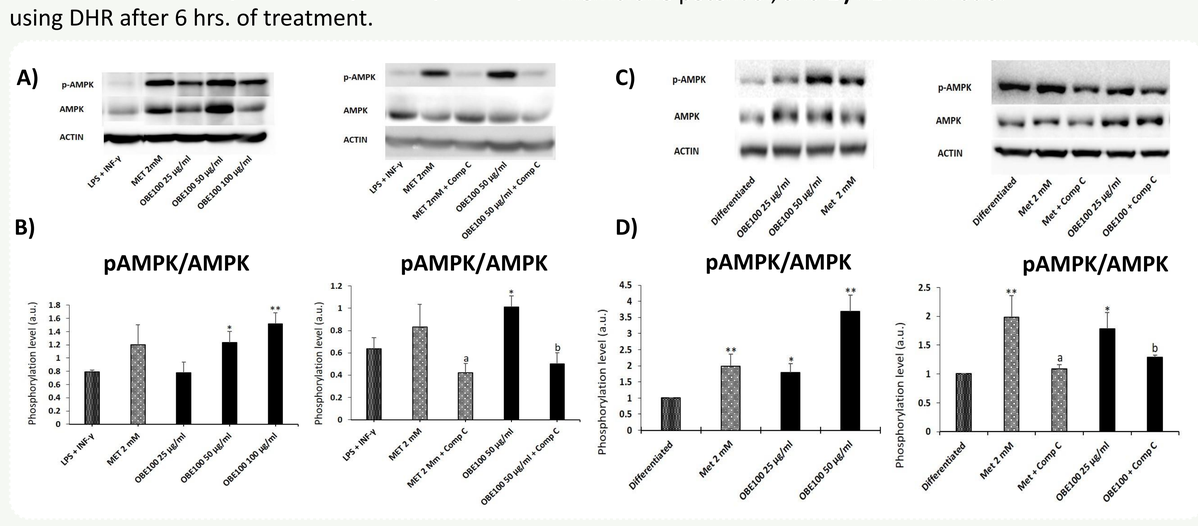


Figure 5. Effects of OBE100 and its major components on AMPK protein expression.

J774A.1 cells were activated for 18 hours and treated with OBE100 for 6 hours. **A)** Representative western blots of phosphorylated AMPK, and **B)** Quantitative analysis of the immunoblots of p-AMPK normalized with total AMPK. ∗ $P < 0.05$, ∗∗ $P < 0.01$ compared with LPS + INF-γ; a $P < 0.05$ compare with MET 2 mM; b $P < 0.05$ compare with OBE100 50 µg/ml (ANOVA with Dunnett´s post hoc test). 3T3-L1 cells were differentiated and treated with OBE100 for 2 days. **C)** Representative western blots of phosphorylated AMPK, and **D)** Quantitative analysis of the immunoblots of p-AMPK normalized with total AMPK. ∗ $P < 0.05$, ∗∗ $P < 0.01$ compared with Differentiated; a $P < 0.05$ compare with Met 2 mM; b $P < 0.05$ compare with OBE100 25 µg/ml (ANOVA with Dunnett´s post hoc test). Compound C (Comp C) was used as AMPK inhibitor. Values are expressed as mean ± SEM of three independent experiments.

CONCLUSIONS

- ✓**The treatment of macrophages and adipocytes with OBE100 was less toxic than those treated with triterpenes.**
- ✓**OBE100 extract reduced the oxidative burst in activated J774A.1 macrophages and significantly increased the mitochondrial membrane potential and ADP/ATP ratio in macrophages and adipocytes without affecting cell viability.**
- ✓**OBE100 treatment increased AMPK phosphorylation levels in macrophages and adipocytes.**
- ✓**The combination of triterpenes with other minor molecules present in the vegetal extract has a synergistic effect that may explain its superior anti-inflammatory and hypolipidemic effects.**

REFERENCES

(1) Betancur Laura, Muñoz Diana, Guillen Alis, Echeverri Fernando, Balcazar Norman, Acin Sergio. Major triterpenoids from Eucalyptus tereticornis have enhanced beneficial effects in cellular models when mixed with minor compounds present in raw extract. An Acad Bras Cienc (2021) 93(Suppl.3): e20201351 DOI 10.1590/0001-3765202120201351.

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