Is *Cytisus multiflorus* an anti-inflammatory plant?

Susana C. Saraiva[a], Olívia R. Pereira[a,b], Joana Liberal[d,e], Maria T. Batista[c,d], Maria T. Cruz[c,e], Susana M. Cardoso[a]

[a]CERNAS, Escola Superior Agrária de Coimbra, Instituto Politécnico de Coimbra, Coimbra, Portugal
[b]Departamento de Tecnologias de Diagnóstico e Terapêutica, Escola Superior de Saúde, Instituto Politécnico de Bragança, Bragança, Portugal
[c]Faculdade de Farmácia, Universidade de Coimbra, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal
[d]Centro de Estudos Farmacêuticos, Azinhaga de santa Comba, 3000-548 Coimbra, Portugal
[e]Centro de Neurociências e Biologia Celular, Departamento de Zoologia da Universidade de Coimbra, 3004-517 Coimbra, Portugal

INTRODUCTION

Cytisus multiflorus is a leguminous shrub native from Iberian Peninsula that is distributed in the south-west Mediterranean region. This plant is used in folk medicine and it is claimed to have various health benefits, including anti-inflammatory properties [1]. Yet, the anti-inflammatory usage of C. *multiflorus* is totally based on the available ethnopharmacological information while no scientific data on this capacity and on molecular targets has been reported for the plant. Hence, the present work aims to clarify the possible anti-inflammatory mechanisms of *C. multiflorus*.

Fig. 1- *Cytisus multiflorus*

METHODS

A purified ethanolic extract was prepared [1], and its antioxidant capacity was evaluated though the DPPH radical scavenging [2] and reducing power [3] assays. Moreover, to test the anti-inflammatory properties of the *C. multiflorus* extract, the nitric oxide (NO) production scavenging activity and cytotoxicity of the distinct concentrations of the extract were assessed on a lipopolysaccharide-stimulated Raw 264.7 macrophages model, and measured by the Griess and the MTT reduction colorimetric assays[3], respectively. Furthermore, the effects on two proteins that are potential targets to prevent or treat chronic inflammation, namely cyclooxygenase-2 (COX-2) and inducible NO synthase (iNOS), were estimated by Western Blot analysis.

RESULTS AND DISCUTION

Tab.1- Antioxidant activity of *C. multiflorus* ethanolic extract

[a] DPPH (EC_{50}) (µg/ml)	[b] Reducing Power (EC50) (µg/ml)
13.4 ± 1.0	11.4 ± 2.1

Mean Values ± standard derivations of three replicate analyses

[a]EC_{50} – concentration for a 50 % inhibitionof the 60 µM radical 2,2-diphenyl-1-picrylhydrazyl (DPPH); [b]Amount of extract able to providing 0.5 of absorbance by reducing of 3.5 µM Fe^{3+} to Fe^{2+}

Tab.2- Effect of C. *multiflorus* ethanolic extracts in Raw 264.7 macrophages viability

Condition	Cell Viability
Control	100
LPS (1µg/mL)	82.25 ± 1.14
C. multiflorus (325 µg/mL)	90.67 ± 14
C. multiflorus (325 µg(mL) + LPS (1µg/mL)	86.93 ± 4.1
C. multiflorus (160 µg/mL)	104.78 ± 4.1
C. multiflorus (160µg/mL) + LPS (1µg/mL)	94.01 ± 13

As indicated by its low EC_{50} values (13.4±1.0 and 11.4±2.1 µg/mL for DPPH scavenging potential and reducing power, respectively), the *C. multiflorus* ethanolic extract has a high antioxidant capacity. Moreover, the extract did not cause cytotoxicity against RAW 264.7 macrophages for concentrations up to 325 µg/mL .

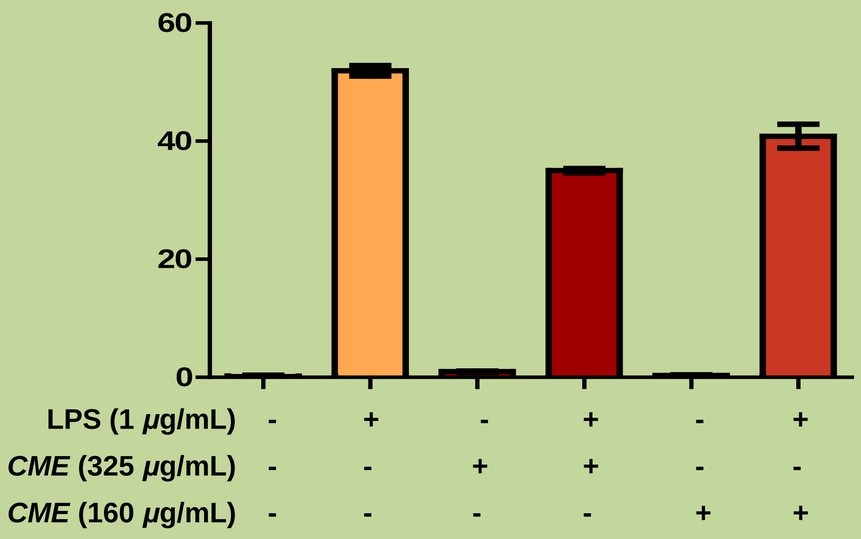

Fig.2- Effect of *C. multiflorus* extract (CME) in the nitrite production of macrophages stimulated with LPS 1 µg/mL (* relative to LPS)

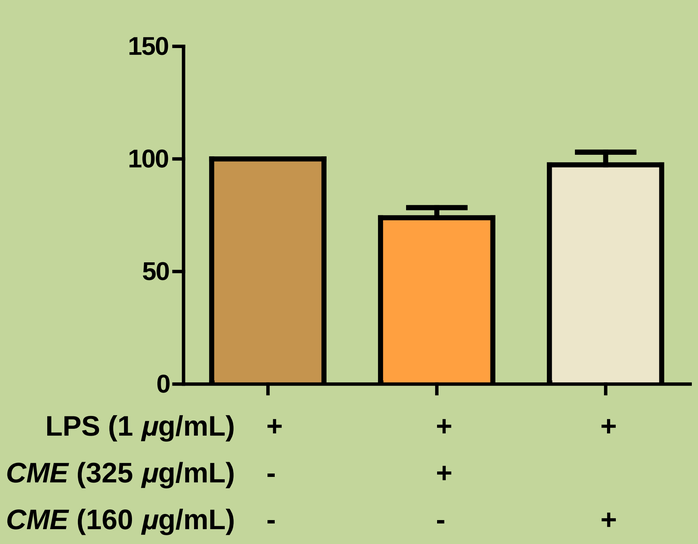

Fig.4- Effect of *C. multiflorus* extract (CME) in the iNOS of macrophages stimulated with LPS 1 µg/mL (* relative to LPS).

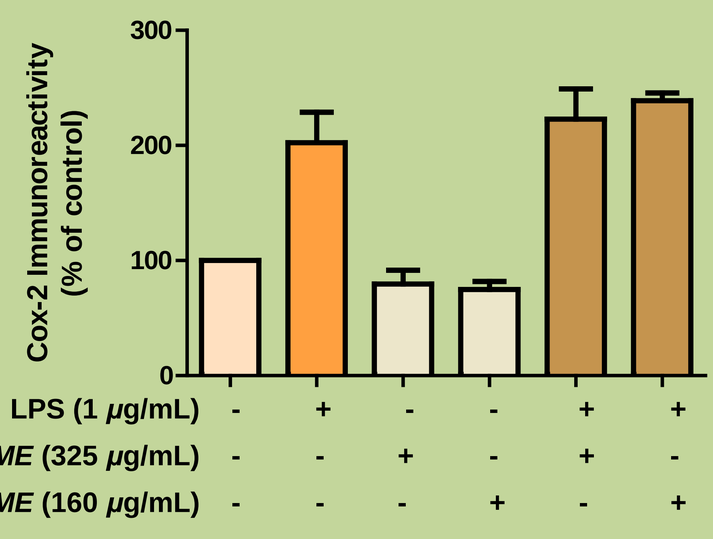

Fig.3- Effect of *C. multiflorus* extract (CME) in the Cox-2 of macrophages stimulated with LPS 1 µg/mL.

The treatment of this cell line with 160 µg/mL and 325 µg/mL of the purified extract induced a decrease in the levels of NO of 24% and 32%, respectively. Furthermore, despite no statistically significant changes on COX-2 levels were observed, iNOS expression was significantly diminished by the treatment with the highest concentration of the extract.

CONCLUSION

Overall, the present results suggest that C. multiflorus actually exerts an anti-inflammatory action which is, at least partially, mediated through the inhibition of iNOS expression.

REFERENCES

1- Pereira, O. R.; Silva, A.M.S.; Domingues, M.R.M.; Cardoso, S.M.. *Food Chem.* **2012**, *131*, 652.
2- Ferreira, A.; Proença, C.; Serralheiro, M.L.M.; Araújo, M.E.M. *J. Ethnopharmacol*, **2006**, *108*, 31.
3- Oyaizu, M. *Jpn J Nutr* **1986,** *44*, 307.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support provided by the FCT to CERNAS (project PEst-OE/AGR/UI0681/CNC/CEF/2011). Olívia R. Pereira was supported by a PhD grant (SFRH/PROTEC/49600/2009).