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ABSTRACT BOOK

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NO radical scavenging and iNOS expression inhibition by *Cytisus multiflorus*

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INTRODUCTION

Cytisus multiflorus is used in folk medicine and it is claimed to have various health benefits, including anti-inflammatory properties [1]. Still, no scientific data regarding this ability has been described for this plant. The present work aims to clarify the antioxidant capacity and the anti-inflammatory mechanisms of *C. multiflorus*.

METHODS

- The ethanolic extract of flowers of *C. multiflorus* was obtained with an aqueous ethanolic solution (80%) and was further purified onto SPE C18-E cartridges [2]. The obtained extract was named as CME.
- Antioxidant abilities of CME were evaluated by DPPH scavenging and reducing power assays [3];
- Cytotoxicity of CME was assessed by the MTT colorimetric assay [4].
- The anti-inflammatory properties of CME were measured by nitric oxide (NO) scavenging ability, in a chemical and on LPS-stimulated Raw 264.7 macrophages models, and by the estimation of the intracellular levels of cyclooxygenase-2 (COX-2) and inducible NO synthase (iNOS), through Western Blot analysis [5].

RESULTS AND DISCUSSION

CME showed high antioxidant capacity and also efficiently scavenged the NO radical (Table 1) and inhibited the NO production, in the chemical and cellular models (Fig. 1), respectively. Furthermore, despite no changes on intracellular COX-2 levels were observed, iNOS expression was significantly diminished by the treatment with non-toxic concentrations of CME (Fig. 2).

ANTIOXIDANT ACTIVITY

Table 1. DPPH• scavenging, reducing power and NO scavenging activities of CME

EC_{50} DPPH• Scavenging (µg/mL)	EC_{50} Reducing Power (µg/mL)	EC_{50} NO Scavenging (µg/mL)
13.4 ± 1.0	11.4 ± 2.1	148.0 ± 9.1

Mean Values ± standard derivations of three replicate analyses
EC_{50} – Concentration for a 50% inhibition

ANTI-INFLAMMATORY ACTIVITY

Table 2. Effect of CME in Raw 264.7 macrophages viability

Condition	Cell Viability (% respect to control)
Control	100
LPS 1 µg/mL	87.60 ± 10.72
CME 325 µg/mL	90.67 ± 12.60
CME 325 µg/mL + LPS 1 µg/mL	86.93 ± 3.54
CME 160 µg/mL	105.50 ± 3.78
CME 160 µg/mL + LPS 1 µg/mL	94.11 ± 10.20

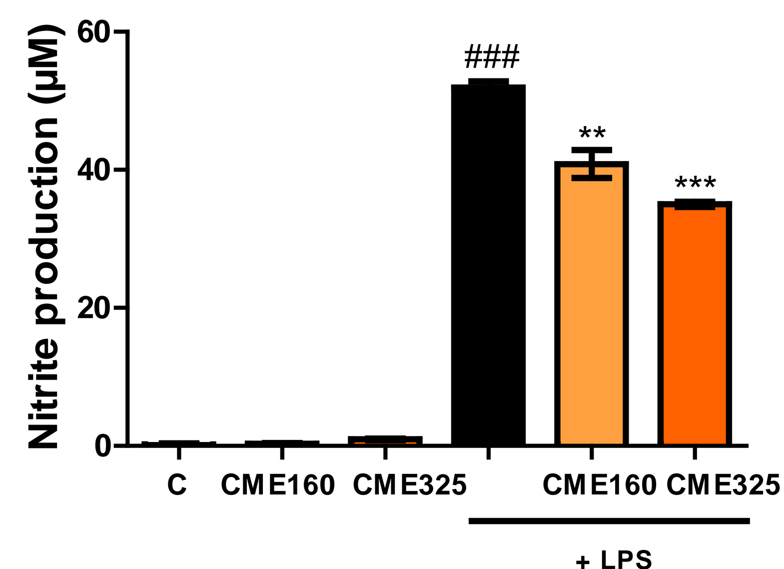

Figure 1. Effect of CME in the nitrite production of macrophages stimulated with LPS 1 µg/mL
**p < 0.01, ***p < 0.001 when compared to cells exposed to LPS, in the absence of extract; ###p < 0.001 when compared to untreated cells (control).

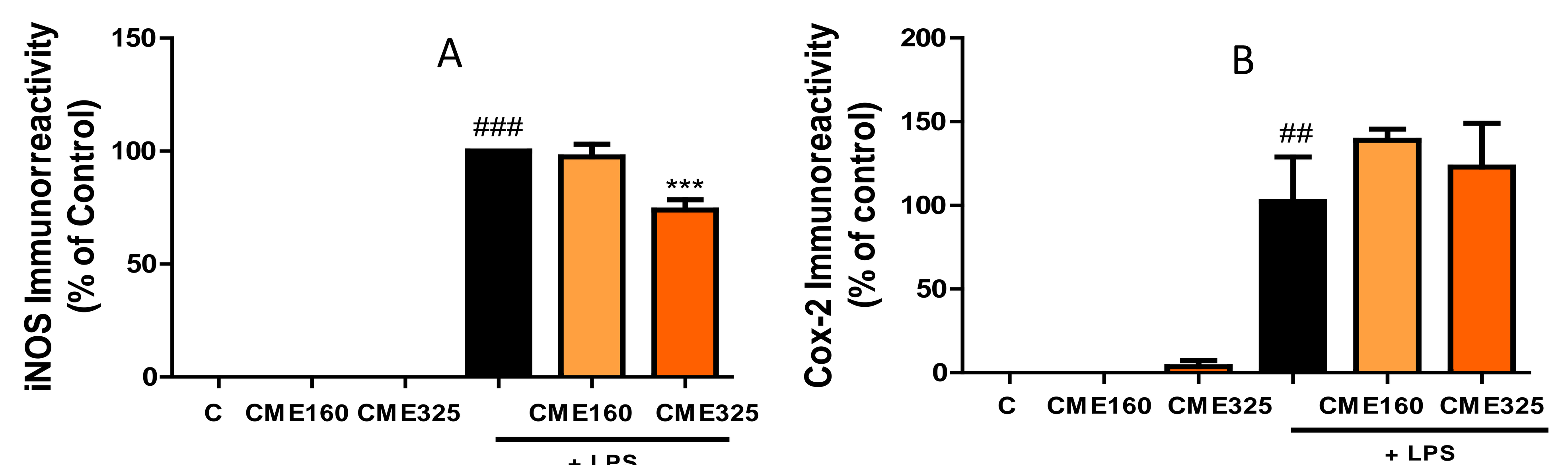

Figure 2. Effect of CME in the iNOS (A) and COX-2(B) of macrophages stimulated with LPS 1 µg/mL.
***p < 0.001 when compared to cells exposed to LPS, in the absence of extract; ###p < 0.001 when compared to untreated cells (control).

CONCLUSION

The present results suggest that *Cytisus multiflorus* is a good antioxidant and that it actually exerts an anti-inflammatory action by means of NO scavenging and iNOS inhibition expression.

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