

## INTRODUCTION

The production of reactive oxygen species and an imbalance of the cellular oxidative status have been related to the initiation and progression of inflammatory processes. To counteract this mechanism, include or supplement diet with nutraceutical constituents with anti-inflammatory properties are often beneficial and necessary to improve this condition. Coffee is one of the most well documented foods with antioxidant effectiveness, rich in phenolic compounds, such as chlorogenic acids who act as a potent antioxidant and subsequently in the reduction of inflammation (1).

The carrageenan-induced paw edema of the rat hindpaw, is a suitable model to study acute local inflammation and widely considered to be one of the most useful models in the evaluation of anti-inflammatory activity of investigational compounds (2, 3).

Based on this we designed the study with the aim to investigate the potential of chlorogenic acid (CLA) as an effective compound in inflammatory condition.

## METHODS

### Sample

Twenty nine male Wistar rats (100-150g) were randomly allocated into three groups as described: (1) Control group: animals subjected to subplantar injection into the rat left hind paw of saline (100µL) and administered with 10% DMSO p.o. (10 mL/kg) 30 minutes prior to subplantar injection (n=4); (2) Carrageenan group: animals subjected to subplantar injection of carrageenan and administered with 10% DMSO p.o. (10 mL/kg) 30 minutes prior to edema induction (n=10); (3) RA10: animals subjected to subplantar injection of carrageenan and administered with RA (10 mg/kg p.o.) 30 minutes prior to edema induction (n=8); (4) RA25 group: animals subjected to subplantar injection of carrageenan and administered with RA (25 mg/kg p.o.) 30 minutes prior to edema induction (n=8); (5) RA50 group: animals subjected to subplantar injection of carrageenan and administered with RA (50 mg/kg p.o.) 30 minutes prior to edema induction (n=8); (6) E25 group: animals subjected to subplantar injection of carrageenan and administered with *R. officinalis* extract (dosed as 25 mg/kg of rosmarinic acid p.o.) 30 minutes prior to edema induction (n=8); (7) E50 group: animals subjected to subplantar injection of carrageenan and administered with *R. officinalis* extract (dosed as 50 mg/kg of rosmarinic acid p.o.) 30 minutes prior to edema induction (n=8); (8) Indomethacin group: animals subjected to subplantar injection of carrageenan and administered with indomethacin (10 mg/kg p.o.) 30 minutes prior to edema induction (n=4).

### Carrageenan-induced paw edema study

Paw edema was induced by a single subplantar injection into the rat left hind paw of 0.1 ml of a 1% λ-carrageenan sterile saline solution.

Paw volume was measured by means of a volume displacement method using a plethysmometer. Paw volume was measured immediately after injection of carrageenan (V0 or basal volume) and 6 hours later (V6h). Paw edema was expressed as percentage of increase in paw volume 6 hours after carrageenan injection relative to the basal values according to the equation:  $\% = (V6h - V0) / V0 \times 100$ .



## Results

The *in vivo* results (mean ± standard error of mean) were compared using a one-factorial ANOVA test, followed by a Bonferroni's post-test. A *P* value less than 0.05 was considered to be statistically significant.

The chromatograms obtained in the analysis of the *R. officinalis* extract prepared show some of the compounds detected at 280 nm are also detected in the chromatogram obtained with the electrochemical detector. Compounds as rosmarinic acid, ellagic acid, ferulic acid, *p*-coumaric acid and caffeic acid were detected. Rosmarinic acid is undoubtedly the majority compound.

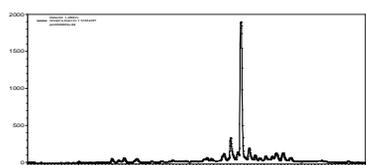


Figure 1 – Chromatogram of the *R. officinalis* extract at 280 nm. The peak at the retention time of 63-64 min. Is related to rosmarinic acid

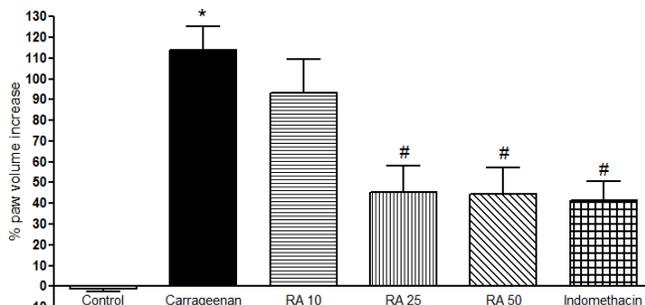


Figure 2 – Effect of rosmarinic acid administration (at 10, 25 and 50 mg/kg p.o.) on the percent of paw volume increase induced by subplantar administration of carrageenan. \**P*<0.05 vs. Control; #*P*<0.05 vs. Carrageenan

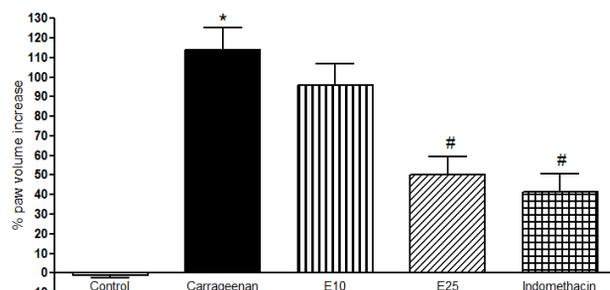


Figure 3 – Effect of *R. officinalis* extract (at 25 and 50 mg/kg p.o. - dosed as rosmarinic acid) on the percent of paw volume increase induced by subplantar administration of carrageenan. \**P*<0.05 vs. Control; #*P*<0.05 vs. Carrageenan

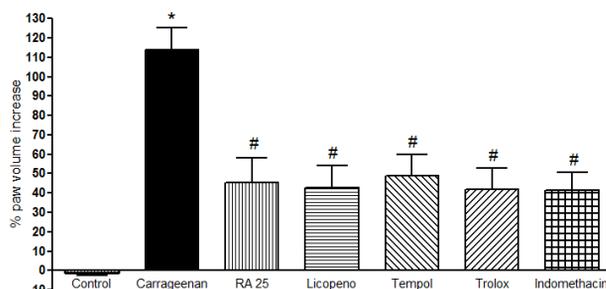


Figure 4 – Comparison of the effect of rosmarinic acid (25 mg/kg p.o.) and *R. officinalis* extract (at 25 mg/kg p.o. - dosed as rosmarinic acid) with known anti-oxidant properties (tempol - 30 mg/kg and trolox - 30 mg/kg) on the percent of paw volume increase induced by subplantar administration of carrageenan. \**P*<0.05 vs. Control; #*P*<0.05 vs. Carrageenan

## Conclusions

We demonstrate here that administration of rosmarinic acid as well as *R. officinalis* extract reduce the increase of paw volume induced by carrageenan administration. The percent of reduction was approximately 60% for the groups treated with the dose of 25 mg/kg.

Comparison of this effect with a known non-steroidal anti-inflammatory drug (indomethacin) and two known anti-oxidant substances (tempol and trolox) reveal that rosmarinic acid exhibits the same magnitude of edema reduction.

We propose that polyphenolic compounds, such as rosmarinic acid, may be useful in the therapy of inflammatory associated conditions.

## References

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