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ABSTRACT BOOK

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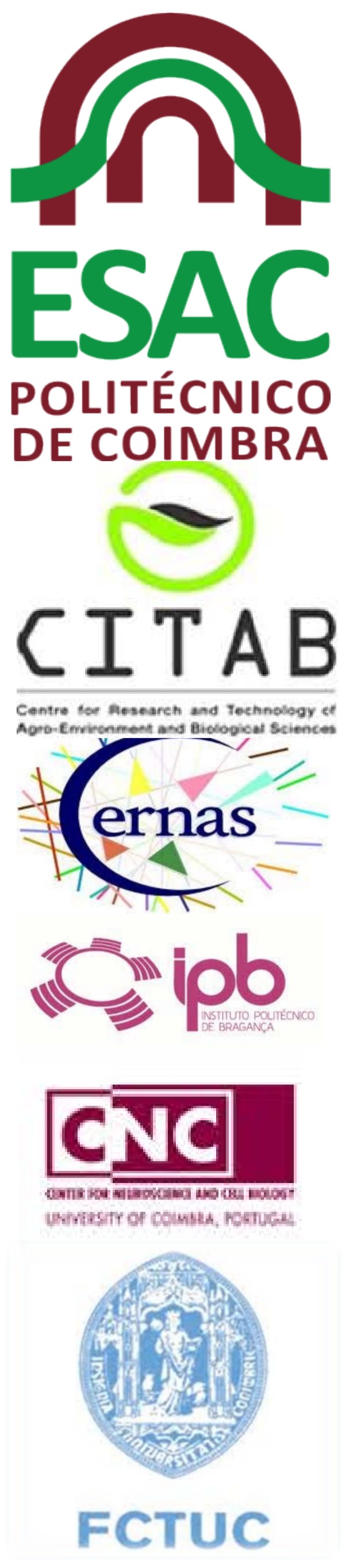
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Mentha aquatica L. extract affects mitochondrial bioenergetics

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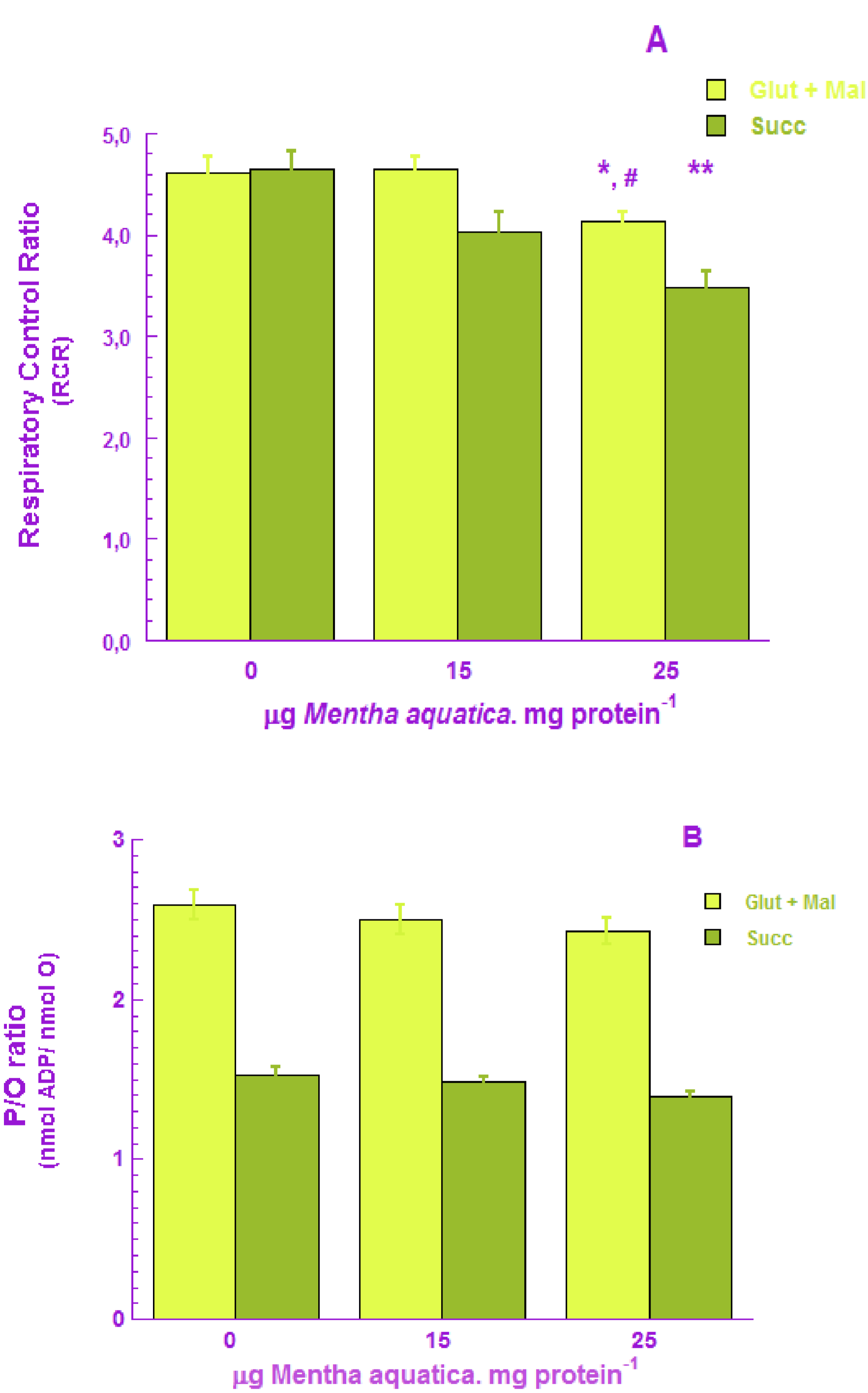


Background:

- ✓ *Mentha aquatica* L. (water mint), a Lamiaceae, is a perennial herb common in marshes and damp places from the South-western Cape to tropical Africa, where is used as a sedative and anticonvulsant
- ✓ due to its strong odor and particular taste, *Mentha aquatica* L. is used in food processing to flavor beverages, ice-cream and candies
- ✓ despite its wide traditional use, no toxicological assessment of this plant has been performed

Results

Respiratory indexes



Mitochondrial membrane potential variations induced by the purified ethanolic extract of *M. aquatica*:

Condition	Energization (mV)	ΔADP (mV)	Repolarization (mV)	Repolarization rate (% of mean control)	Lag Phase (s)
Glutamate + Malate					
Control	209.3 ± 1.5	26.9 ± 2.0	205.7 ± 1.2	100.0 ± 6.	46.7 ± 1.2
PEE 15 µg.mg protein ⁻¹	202.9 ± 2.3	27.1 ± 2.1	200.3 ± 2.4 *	84.8 ± 5.0	55.5 ± 1.0*
PEE 25 µg.mg protein ⁻¹	200.8 ± 2.4*	25.8 ± 1.4	199.9 ± 1.3 *	75.5 ± 5.2 *	65.5 ± 3.2**
Succinate					
Control	218.8 ± 1.0	29.0 ± 1.1	218.4 ± 1.1	100.0 ± 5.7	66.6 ± 5.1
PEE 15 µg.mg protein ⁻¹	218.3 ± 0.9	28.5 ± 2.4	216.7 ± 1.6	91.4 ± 4.3	64.0 ± 6.6
PEE 25 µg.mg protein ⁻¹	215.0 ± 1.2*	27.9 ± 1.3	214.1 ± 1.4 *	75.7 ± 3.4 **	76.6 ± 5.0*

Effects of the purified ethanolic extract of *M. aquatica* (PEE) on rat liver mitochondria membrane potential. Mitochondria (1 mg) were incubated with plant extract for 5 min in 1 ml of the standard respiration medium supplemented with 3 µM TPP⁺ at 30 °C, accordingly to Materials and Methods section. Statistics: * p < 0.05 and ** p < 0.001 as compared to control.

ROS formation in mitochondria occurs at high membrane potentials and increases exponentially above 140 mV (negative inside) [1-2] but is absent below this value [2-4]. Hence, due to the interaction of the flavonoids with mitochondrial inner membrane, the protonic electrochemical gradient decreases and thus membrane potential is lower also decreasing the amount of ROS produced by phosphorylative oxidation.

Figure 1. Effects of the purified ethanolic extract of *M. aquatica* on liver mitochondria respiratory indexes: respiratory control ratio (RCR) (A) and P/O ratio (B). Mitochondria (1 mg protein) were incubated in 1 mL respiratory standard medium containing glutamate + malate (5 mM + 5 mM) or succinate (5 mM) + rotenone (1 µM). State 3 respiration was initiated by the addition of 125 nmol ADP. Values are the means ± SEM of triplicates performed with 4 different mitochondrial preparations. Statistics: * p < 0.05; ** p < 0.01 as compared to control; # p < 0.05, compared to 15 µg.mg⁻¹ protein.

RCR is significantly decreased by PEE leaf extract, either in the presence of glutamate + malate or succinate as respiratory substrates, in the range of concentrations used (up to 25 µg plant extract per mg protein), suggesting that the extract seemed to induce mitochondrial dysfunction.

P/O ratio was not affected, either for glutamate + malate or succinate, suggesting that despite the ATP synthesis rate was decreased, as less ATP was produced per period of time, all external ADP added was phosphorylated.

In conclusion:

our results show that *M. aquatica* ethanolic extract accounts for a partial energetic uncoupling, decreasing membrane potential that induce a decrease in ROS production. Hence, the mild mitochondrial stress induced by the polyphenols present in *M. aquatica* extract, that act as hormetic stimuli, can account for the antioxidant and anti-inflammatory properties of *M. aquatica* observed in vivo [5] and contribute also to a higher mitochondrial flexibility [6]. These hormetic stimuli can also be important in the prevention of other chronic pathologies, related with oxidative stress and the human modern nutritional milieu [6].



References:

- Hansford, R. G., Hogue, B. A., and Mildaziene, V. (1997) *J.Bioenerg. Biomemb.* 29, 89-95.
- Liu, Y., Fiskum, G., and Schubert, A. (2002) *J. Neurochem.* 80, 780-787.
- Korshunov, S. S., Skulachev, V. P., and Starkov, A. A. (1997) *FEBS Lett.* 416, 15-18.
- Lee, I., Bender, E., and Kadenbach, B. (2002) *Mol. Cell.Biochem.* 234-235, 63-70.
- Conforti, F., Sosa, S., Marrelli, M., Menichini, F., Statti, G. A., Uzunov, D., Tubaro, A., Menichini, F., and Loggia, R. D. (2008) *J. Ethnopharmacol.* 116, 144-151.
- Nunn, A., Bell, J., and Guy, G. (2009) *Nutr. Metabol.* 6, 16.

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