

## Background:

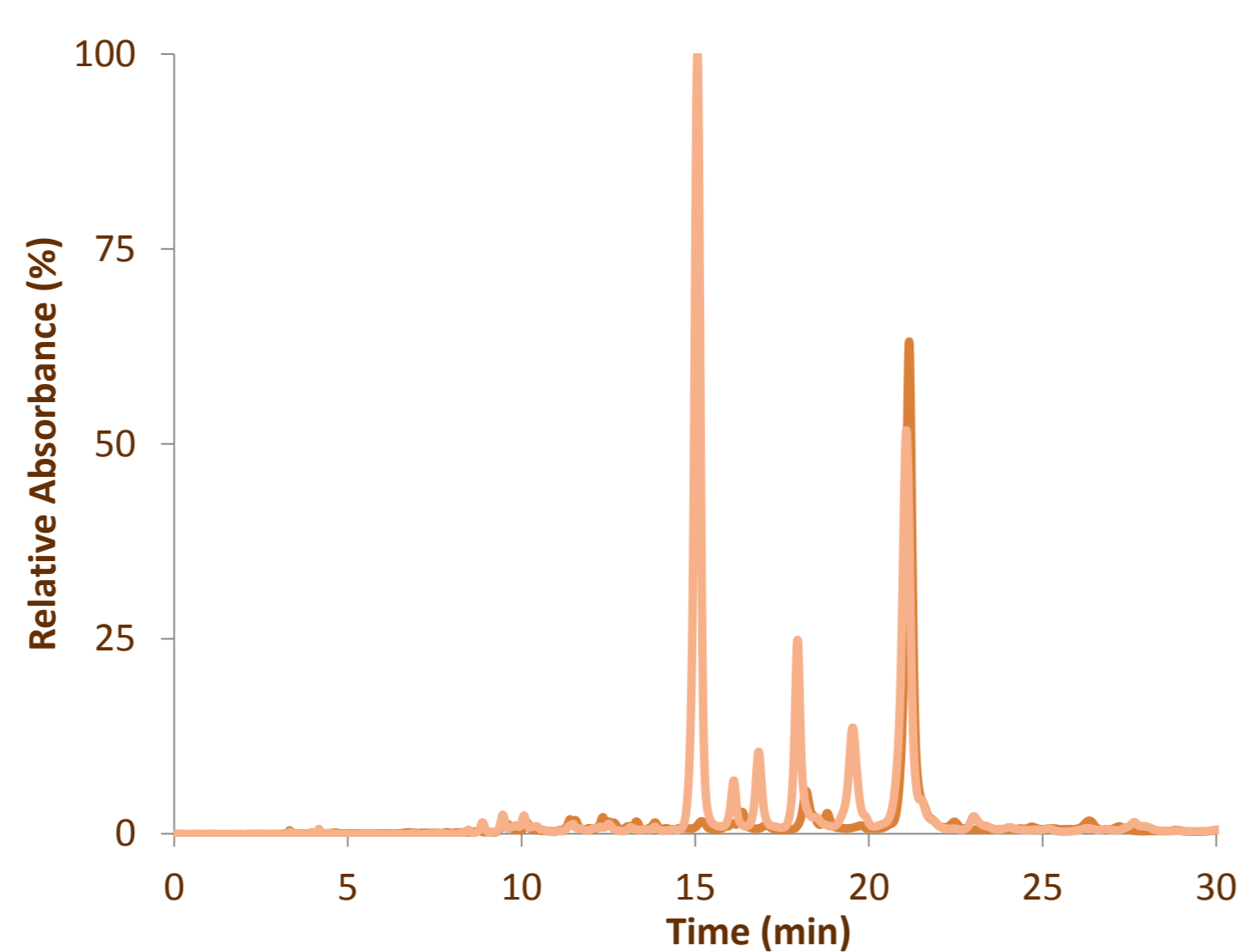
- ✓ *Mentha aquatica* L. (water mint), a Lamiaceae, is a perennial herb common in marshes and damp places from the South-western Cape to tropical Africa, where is used as a sedative and anticonvulsant;
- ✓ due to its strong odor and particular taste, *Mentha aquatica* L. is used in food processing, to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

## Major Goal:

Evaluate the potential toxicity of *Mentha aquatica*, using isolated liver mitochondria.

## Results

### Polyphenols characterization



Peak	RT (min)	$\lambda_{max}$	[M-H] <sup>-</sup>	Main fragment ESI-MS <sup>n</sup>	Compound	mg/g of extract <sup>a</sup>
1	15.0	283, 325	595	MS <sup>1</sup> [595]: 287; MS <sup>2</sup> [287]: 269(3%), 151(100%), 125(2%), 107(11%); MS <sup>3</sup> [151]: 107	Eriodictyol- <i>O</i> -rutinoside	144.6 ± 22.4 (48%)
2	16.8	254, 267, 345	609	MS <sup>1</sup> [609]: 285 (100%), 267(10%), 241(3%); MS <sup>2</sup> [285]: 241 (100%), 217 (67%), 199 (65%), 175 (62%), 151(20%)	Luteolin-7- <i>O</i> -rutinoside	43.3 ± 10.0 (14%)
3	17.9	254, 267, 350	461	MS <sup>1</sup> [609]: 343(5%), 301(100%), 255 (2%), 271 (2%), 179 (2%); MS <sup>2</sup> [301]: 273 (10%), 257 (10%), 179 (100%), 151 (60%)	Rutin	
				MS <sup>2</sup> [449]: 287; MS <sup>3</sup> [287]: 151	Eriodictyol- <i>O</i> -glucoside	
				MS <sup>1</sup> [447]: 285; MS <sup>2</sup> [285]: 243(50%), 241(100%), 217(90%), 201(7%), 199(85%), 175(75%)	Luteolin-7- <i>O</i> -glucoside	
4	19.5	283, 325	595	MS <sup>1</sup> [579]: 271; MS <sup>2</sup> [271]: 177(5%), 151(100%)	Naringenin-7- <i>O</i> -rutinoside	24.4 ± 3.7 (8%)
				MS <sup>1</sup> [577]: 269; MS <sup>2</sup> [269]: 241(10%), 225(100%), 224(60%), 203(30%), 183(10%), 182(20%), 151(35%)	Apigenin-7- <i>O</i> -rutinoside	
				MS <sup>1</sup> [609]: 301(100%), 286(c1), 242(c1); MS <sup>2</sup> [301]: 286(100%), 283(40%), 257(25%), 242(40%), 233(3%), 199(5%), 125(10%); MS <sup>3</sup> [286]: 268(5%), 258(75%), 242(100%), 199(5%), 174(5%); MS <sup>4</sup> [241]: 227(100%), 199(60%)	Hesperetin-7- <i>O</i> -rutinoside	25.9 ± 3.6 (9%)
5	21.1	290, 328	461	MS <sup>1</sup> [461]: 285; MS <sup>2</sup> [285]: 243(20%), 241(90%), 217(45%), 199(10%), 175 (100%), 133 (14%)	Luteolin-7- <i>O</i> -glucuronide	64.2 ± 8.8 (21%)
				MS <sup>1</sup> [359]: 315(2%), 224(10%), 197(15%), 179(20%), 161(100%), 133(11%); MS <sup>2</sup> [179]: 135	Rosmarinic acid	
Total						302.5 ± 28.7

Identification and quantification of phenolic compounds. The identification of the phenolic constituents of the purified ethanolic plant extracts was carried out by the combination of HPLC-DAD and ESI-MSn data.

✉ The main polyphenols present in *Mentha aquatica* extract are:

- eriodictyol-*O*-rutinoside (peak 1, [M-H]<sup>-</sup> ion at  $m/z$  595) was identified as the major flavanone in *M. aquatica* accounting for almost 50% of the total quantified phenolics (16.4 ± 2.5 mg/g of dry plant).
- flavone luteolin-7-*O*-rutinoside (4.9 ± 1.1 mg/g of dry plant);
- \* flavanone naringenin-7-*O*-rutinoside;
- and
- \* bioflavonol hesperetin-7-*O*-rutinoside

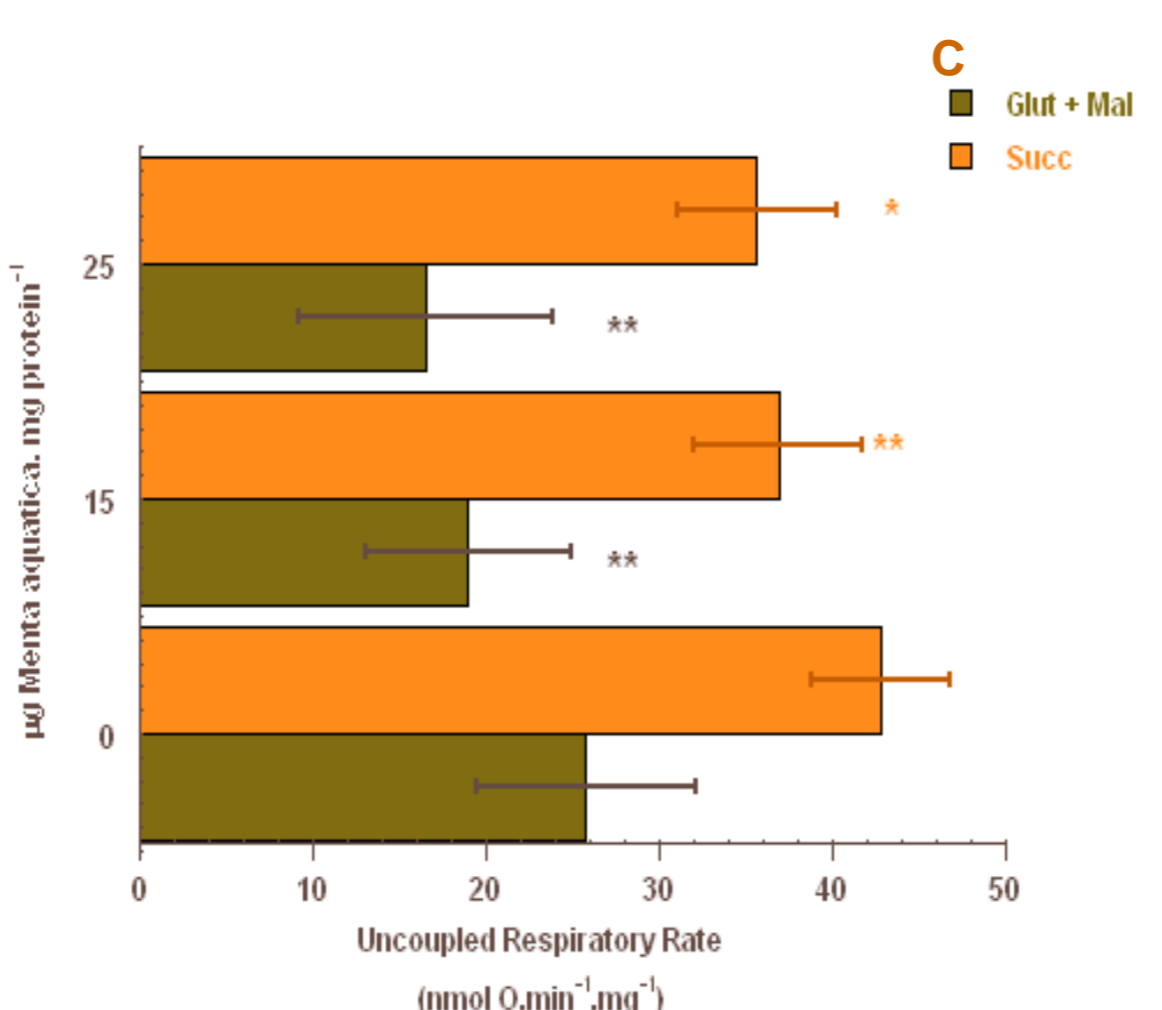
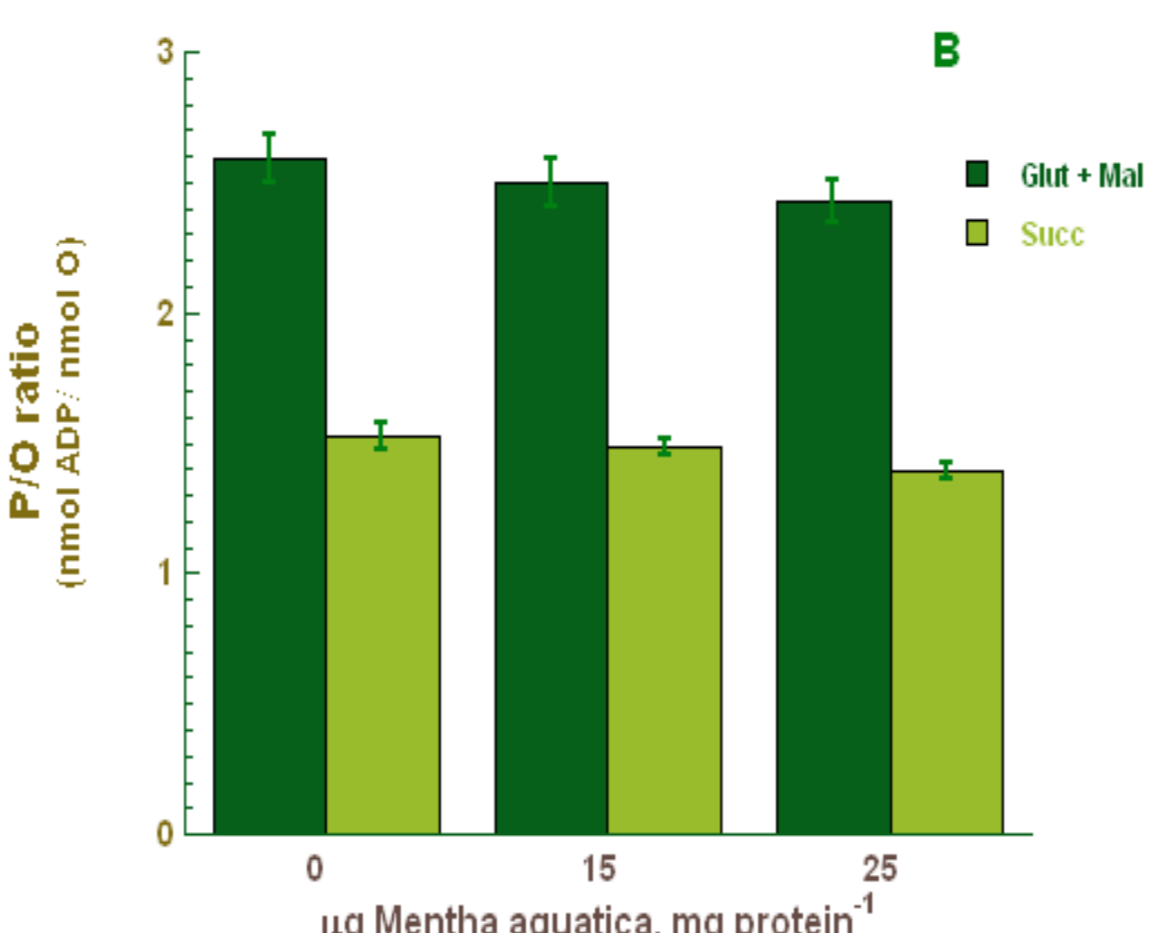
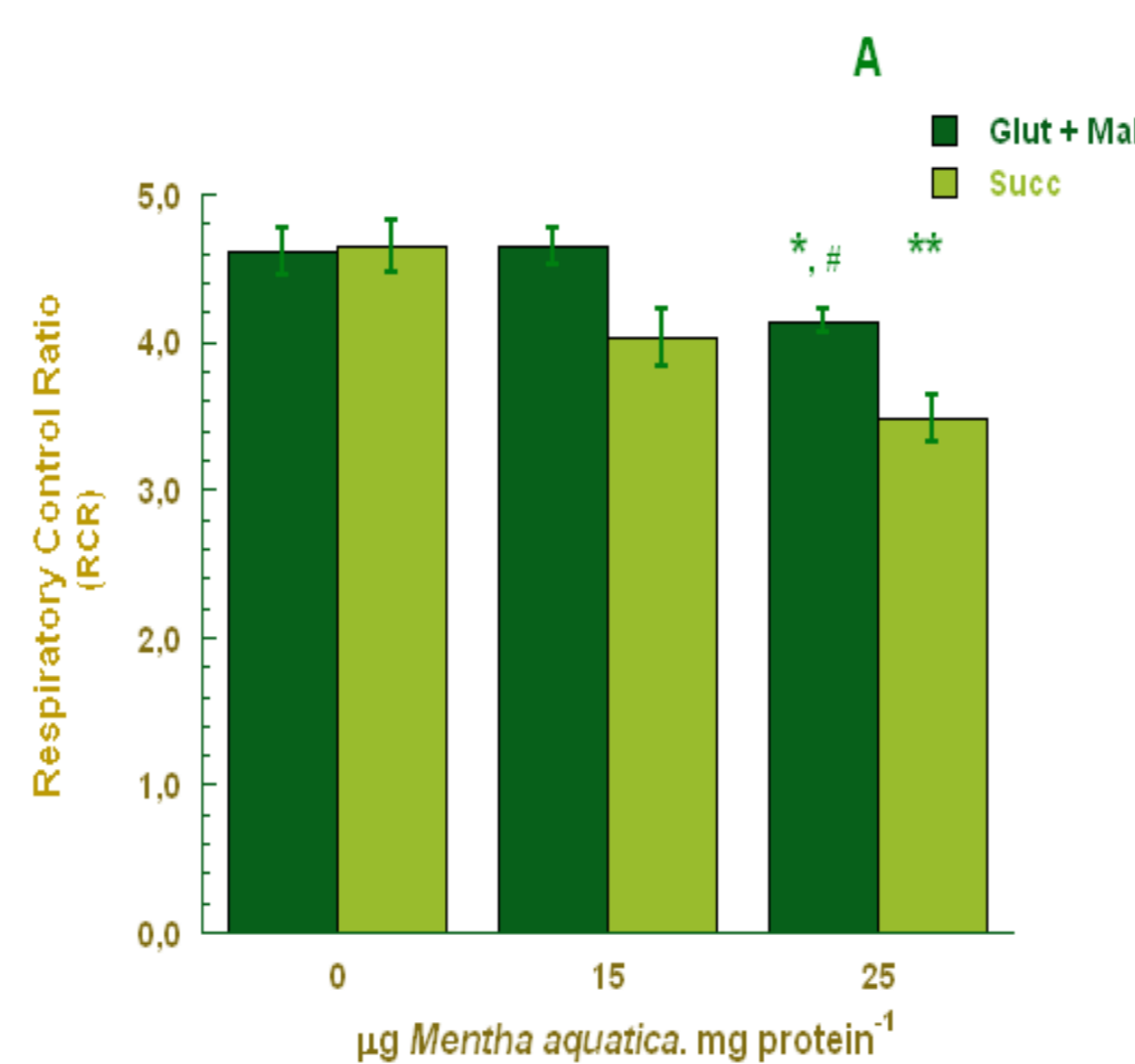
## Membrane potential



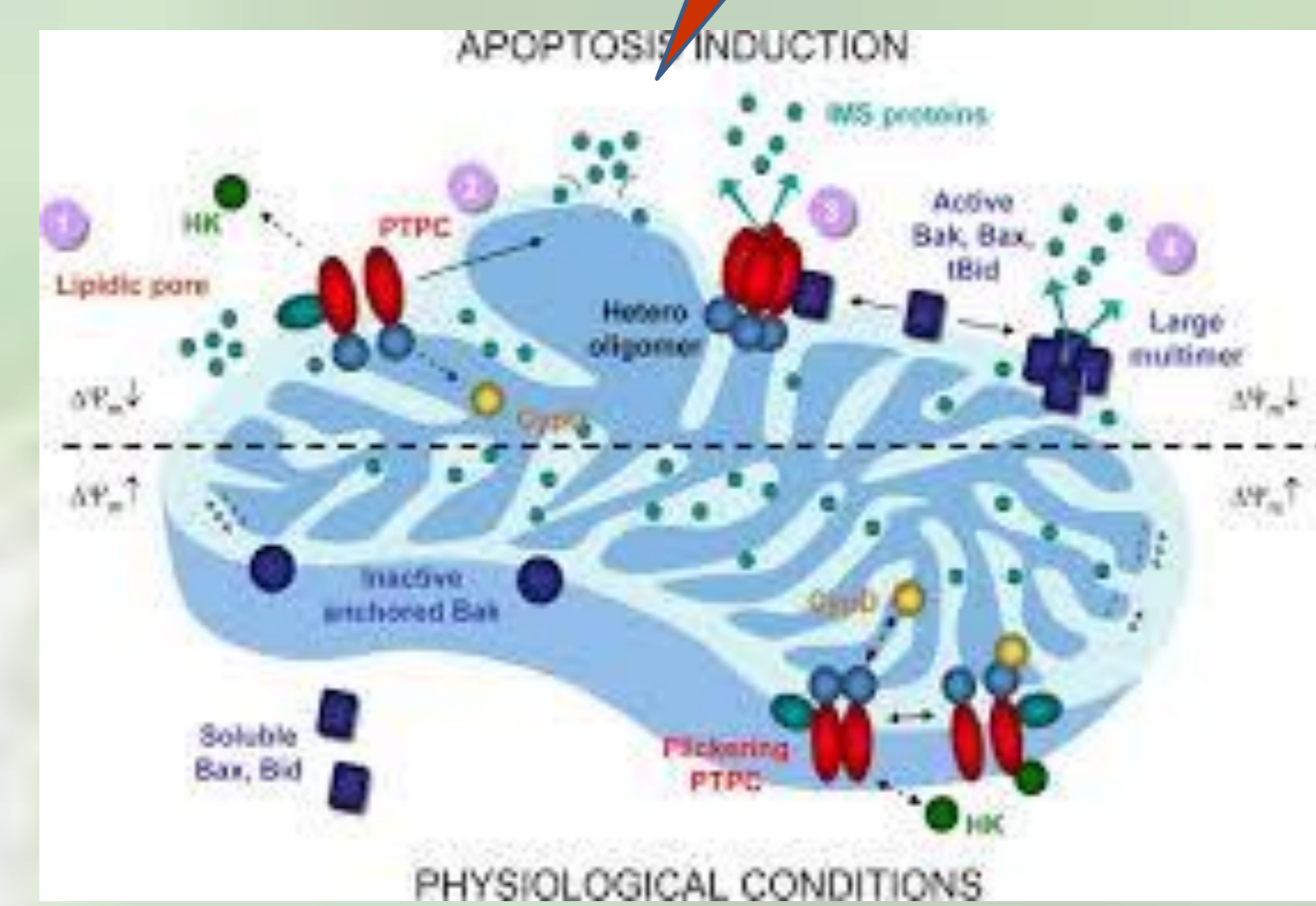
## & Respiratory indexes

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

Condition	Energization (mV)	$\Delta$ ADP (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 ± 6.0	46.7 ± 1.2
PEE	202.9 ± 2.3	27.1 ± 2.1	200.3 ± 2.4 *	84.8 ± 5.0	55.5 ± 1.0 <sup>b</sup>
15 $\mu$ g protein <sup>-1</sup>					
PEE	200.8 ± 2.4 <sup>a</sup>	25.8 ± 1.4	199.9 ± 1.3 *	75.5 ± 5.2 *	65.5 ± 3.2 <sup>ab</sup>
25 $\mu$ g protein <sup>-1</sup>					
Succinate					
Control	218.8 ± 1.0	29.0 ± 1.1	218.4 ± 1.1	100.0 ± 5.7	66.6 ± 5.1
PEE	218.3 ± 0.9	28.5 ± 2.4	216.7 ± 1.6	91.4 ± 4.3	64.0 ± 6.6
15 $\mu$ g protein <sup>-1</sup>					
PEE	215.0 ± 1.2 <sup>a</sup>	27.9 ± 1.3	214.1 ± 1.4 *	75.7 ± 3.4 **	76.6 ± 5.0 <sup>a</sup>
25 $\mu$ g protein <sup>-1</sup>					



Higher doses (25  $\mu$ g.mg protein<sup>-1</sup> or higher) increase MPTP induction



✉ mitochondrial parameters, RCR and uncoupled respiration are significantly decreased by *Mentha aquatica* ethanolic extract, either in the presence of glutamate + malate or succinate, as respiratory substrates, in the range of concentrations used (up to 25  $\mu$ g plant extract per mg protein) → mitochondrial dysfunction, affecting respiratory chain

In conclusion, the present study suggests that a high daily consumption of ethanolic extract of *M. aquatica* leaves should be regarded as hazardous.

Nevertheless, the mild mitochondrial stress induced by the polyphenols present in *M. aquatica* extract, at lower dosages act as hormetic stimuli and can account for the antioxidant and anti-inflammatory properties of *M. aquatica* observed in vivo [1] and contribute also to a higher mitochondrial flexibility [2].

## References:

- 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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