



## CONTRIBUTIONS TO THE EVALUATION OF ANTIOXIDANT OF SOME POLYPHENOLIC COMPLEXES ISOLATED FROM *Vitis vinifera* SEEDS AND PEELS

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### INTRODUCTION

- There is a large pressure to extend the application of *in vitro* methods in pharmacological research, in order to replace as much as possible animal tests, to obtain more reproducible results using smaller amounts of active compound and to run a sufficient number of parallel experiments with relative low costs.
- Liver is the major organ for xenobiotic metabolism; the particularities of liver cells, which are rich in oxidative pathways, such as those derived of cytochrome P450, glutathione-peroxidase etc., may result in formation of free-radical species leading to cell injuries and cell death, especially when some imbalances in antioxidant protection occur. The decay of endogenous antioxidant and anti-free radical protection may be compensated by other natural antioxidants



### THE AIM

- To develop *in vitro* experimental models able to elicit reproducible responses, using hepatotoxic compounds to induce cell injuries and to measure protective effects exerted by some *Vitis vinifera* extracts (E1 and E2) with antioxidant activity
- The first model uses liver homogenates for a screening test
- The second model uses primary rat hepatocytes cultures and is a more sensitive test

### RESULTS AND DISCUCTIONS

#### MATERIALS AND METHODS

- Liver homogenates (in phosphate buffer saline) from Wistar rats
  - treated with CCl<sub>4</sub> (2ml/kg)
  - treated with acetaminophen (3,5 g/kg)
  - untreated animals (as control)
 Homogenate samples (2 ml) "per se" or treated with tested extracts E1 and E2 (25, respectively 50 g/ml) where processed for TBARS (thiobarbituric acid reactive species) determination (measured as malonedialdehyde MDA levels)
- Cell culture experimental system:
  - Whistar rat hepatocytes, isolated by non-enzymatic perfusion method
  - Cultivation: RPMI 1640 medium, 10% CFS in multiwell plates
  - Toxic agent:
    - acetaminophen 10 µg/ml
    - Markers for quantitative evaluations:
      - cell viability
      - glutathione
      - lipid peroxyde levels (measured as MDA levels)

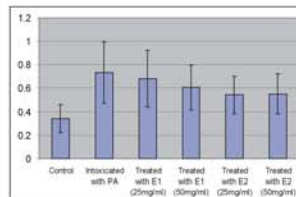


Fig 1: The antioxidant activity of E1 and E2 in acetaminophen intoxication (liver homogenate model)

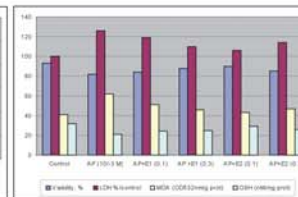


Fig.2 Effect of E1 and E2 on markers of viability and antioxidant status in cultivated hepatocytes intoxicated with acetaminophen

Table 1. The antioxidant activity of *Vitis vinifera* extracts in liver homogenate model (PA intoxication)

Group	% protection
Control	
Intoxicated with PA	
Treated with E1 (25mg/ml)	13
Treated with E1 (50mg/ml)	32.4
Treated with E2 (25mg/ml)	48
Treated with E2 (50mg/ml)	46

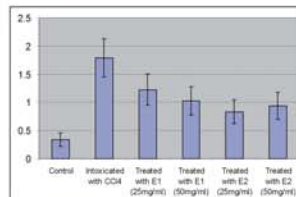


Fig 3 The antioxidant activity of E1 and E2 in Carbon tetrachloride intoxication (liver homogenate model)

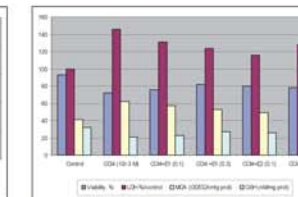
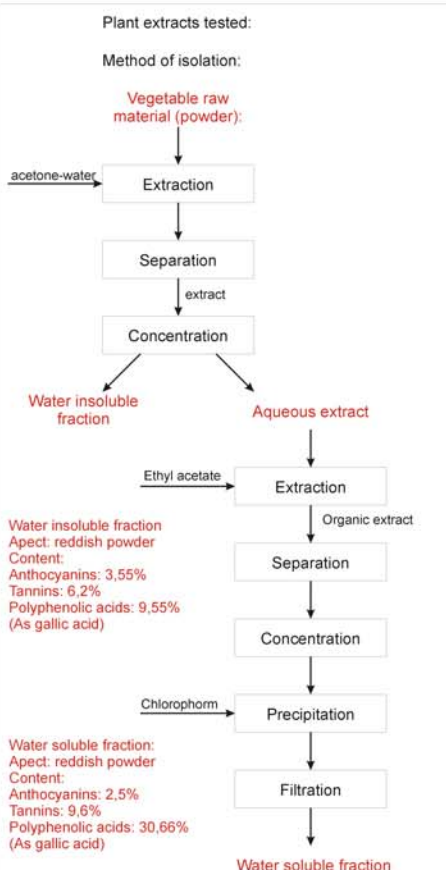


Fig 4 Effect of E1 and E2 on markers of viability and antioxidant status in cultivated hepatocytes intoxicated with carbon tetrachloride

Table 2. The antioxidant activity of *Vitis vinifera* extracts in liver homogenate model (CCl<sub>4</sub> intoxication)

Group	% protection
Control	
Intoxicated with CCl <sub>4</sub>	
Treated with E1 (25mg/ml)	38.7
Treated with E1 (50mg/ml)	52.3
Treated with E2 (25mg/ml)	65.8
Treated with E2 (50mg/ml)	58.9



### CONCLUSIONS

- Liver homogenates may represent a fast model in evaluation of oxidative effects and free radical formation.
- Also hepatocytes in culture represent a test system for the evaluation of anti-oxidant, anti-free-radical, and cell protective or pro-oxidative actions of natural extracts.
- Specific markers permit good dose-effect correlations. Such markers were general viability, leakage of L-lactate dehydrogenase, lipid peroxidation, and glutathione levels.
- Both models where applied in testing two extracts from *Vitis vinifera*, which exerted a good antioxidant activity.
- The models proved to be effective tools for primary screening, due to their reproducibility, accuracy and sensitivity
- One major benefit offered by *in vitro* models is that they may be applied prior to pharmaco-toxicological tests, and that they may provide also some preliminary information concerning toxicological properties of the tested compounds.

