



# APImondia 2009

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## **Effect of chrysin detected in honey on melanoma cells**

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# Chemico-physical parameters

Water content	HMF	Diastase	pH	Fructose	Glucose	Sucrose
16 g/100g	5 mg/kg	10 S. Unit	4.13	44.5 g/100g	27.1 g/100g	0.02 g/100g

Flavonoid (mg QE/Kg)	Phenolic acid (mg GAE/Kg)	FRAP (mmol Fe <sup>2+</sup> /Kg)	DPPH IC <sub>50</sub> (mg/ml)
67.3 ± 3.4	113.5 ± 5.6	1.38 ± 0.7	21.6 ± 1.2

# Characterisation of the phenolic and flavonoid fractions and antioxidant power of Italian honeys of different botanical origin

Elena Pichichero, Lorena Canuti and Antonella Canini\*

**Table 1.** Phenolics and flavonoids identified by HPLC-UV in different honeys<sup>a</sup>

Peak	Phenolic/flavonoid component	Type of honey								
		Acacia	Ailanthus	Chestnut	Orange	Savory	Sulla	Thymus	Honeydew	Multifloral
1	Gallic acid	0.295 ± 0.015	ND	0.351 ± 0.018	0.195 ± 0.009	1.770 ± 0.095	ND	0.265 ± 0.013	1.043 ± 0.052	0.309 ± 0.015
2	Chlorogenic acid	0.229 ± 0.018	0.181 ± 0.011	0.552 ± 0.022	0.166 ± 0.007	1.172 ± 0.059	0.229 ± 0.011	0.967 ± 0.048	0.764 ± 0.022	0.295 ± 0.015
3	<i>p</i> -Coumaric acid	0.084 ± 0.012	0.298 ± 0.021	0.223 ± 0.011	ND	0.216 ± 0.013	0.084 ± 0.005	ND	0.253 ± 0.009	0.098 ± 0.004
4	Caffeic acid	0.098 ± 0.008	0.056 ± 0.002	0.106 ± 0.005	ND	ND	0.098 ± 0.004	0.106 ± 0.005	0.187 ± 0.007	0.108 ± 0.006
5	Myricetin	ND	0.072 ± 0.003	1.389 ± 0.072	0.101 ± 0.007	ND	0.065 ± 0.005	0.567 ± 0.028	0.097 ± 0.005	ND
6	Quercetin	0.044 ± 0.007	0.043 ± 0.006	0.046 ± 0.004	ND	0.057 ± 0.005	0.044 ± 0.002	0.067 ± 0.004	0.093 ± 0.004	0.087 ± 0.004
7	Genistein	0.183 ± 0.015	0.142 ± 0.016	0.101 ± 0.007	ND	0.175 ± 0.017	ND	0.101 ± 0.007	0.131 ± 0.006	ND
8	Kaempferol	0.094 ± 0.014	0.093 ± 0.007	0.095 ± 0.008	0.070 ± 0.006	0.114 ± 0.005	0.094 ± 0.005	ND	0.079 ± 0.005	0.143 ± 0.006
9	Apigenin	0.048 ± 0.004	0.042 ± 0.004	0.036 ± 0.002	0.023 ± 0.001	0.067 ± 0.006	0.048 ± 0.004	0.136 ± 0.002	0.076 ± 0.003	0.067 ± 0.003
10	Chrysin	0.467 ± 0.022	0.236 ± 0.024	0.081 ± 0.004	0.318 ± 0.016	0.360 ± 0.018	ND	0.081 ± 0.004	0.230 ± 0.018	0.383 ± 0.015
11	Galangin	0.506 ± 0.024	0.320 ± 0.013	0.149 ± 0.006	0.380 ± 0.015	0.609 ± 0.024	ND	0.185 ± 0.006	0.322 ± 0.016	0.426 ± 0.017
	Total identified phenolics	2.048 ± 0.095	1.480 ± 0.074	3.099 ± 0.15	1.253 ± 0.062	4.540 ± 0.227	0.662 ± 0.046	2.475 ± 0.120	3.275 ± 0.188	2.325 ± 0.007

<sup>a</sup> Values are expressed as mean (mg kg<sup>-1</sup> honey) ± standard deviation of three independent analyses. ND, compound not detected.

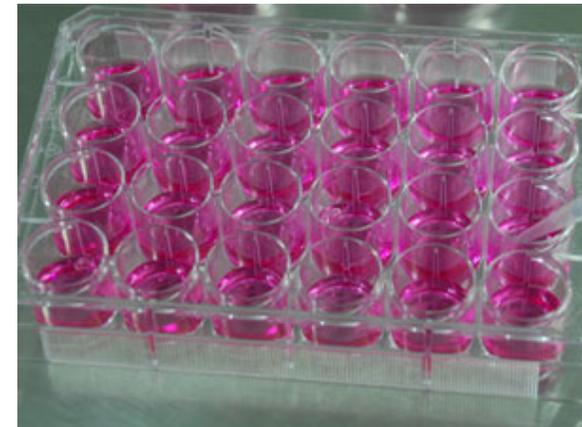
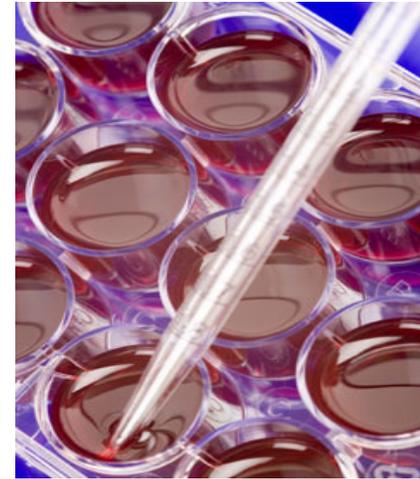


# Experimental plain



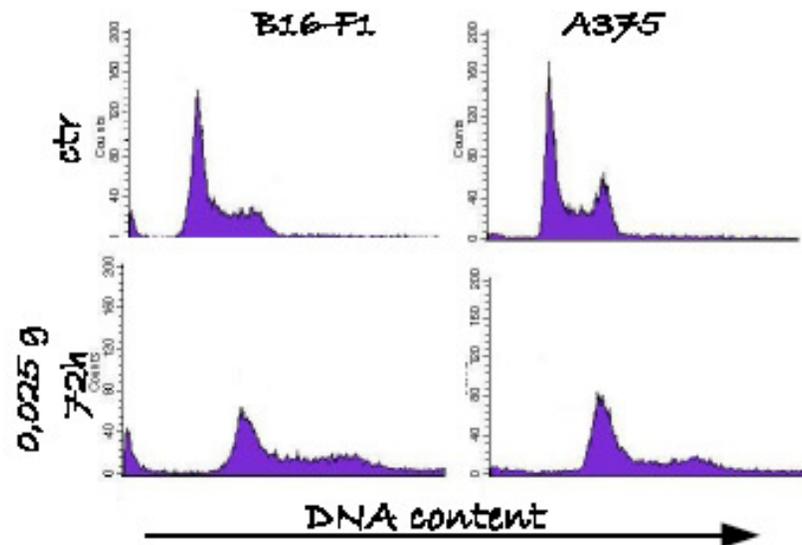
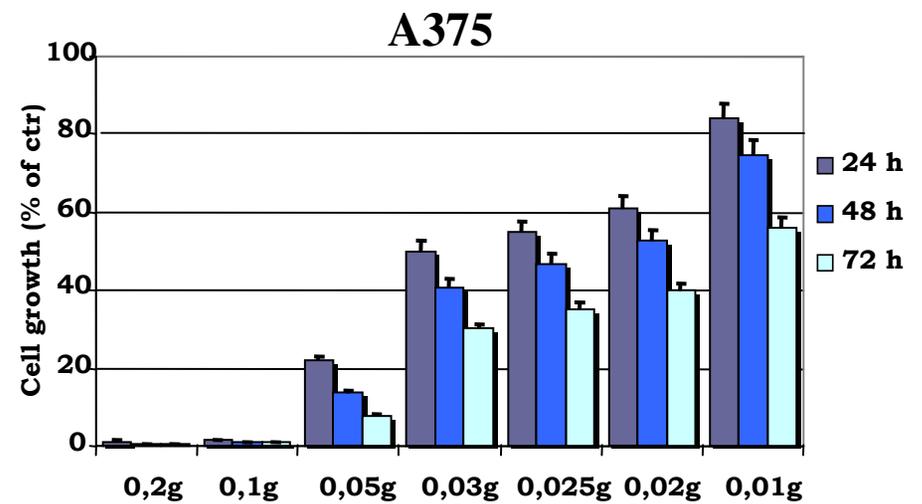
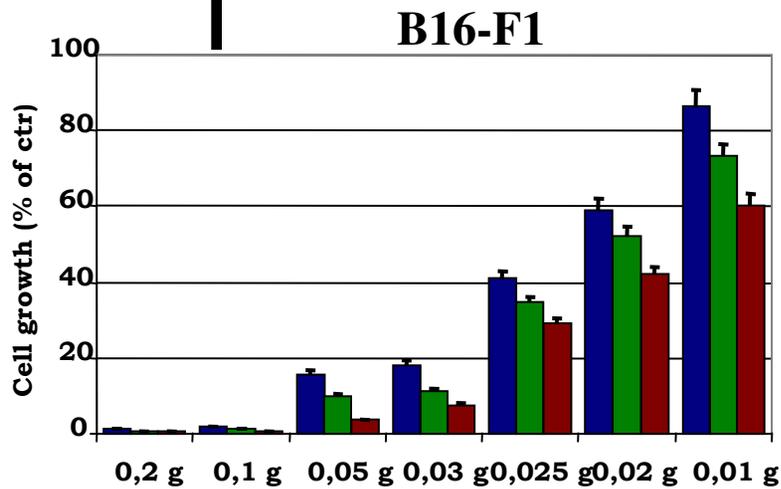
A375 human melanoma

B16-F1 murine melanoma



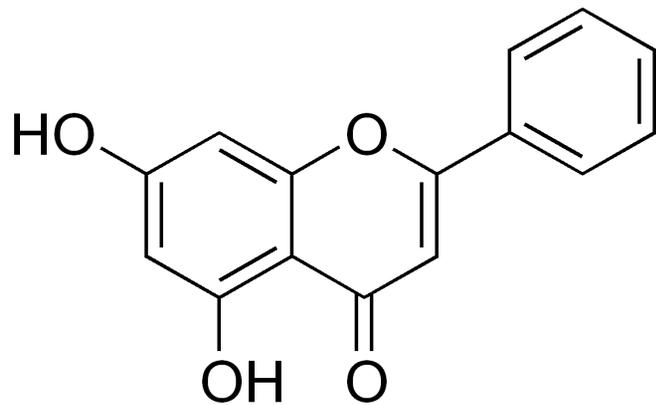


# Biological effect of honey on melanoma cells





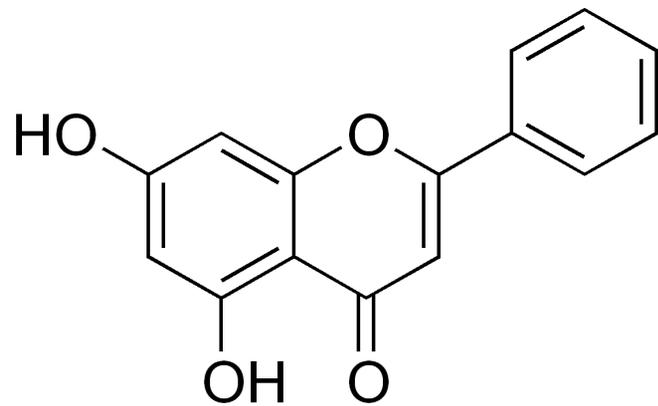
# Chrysin



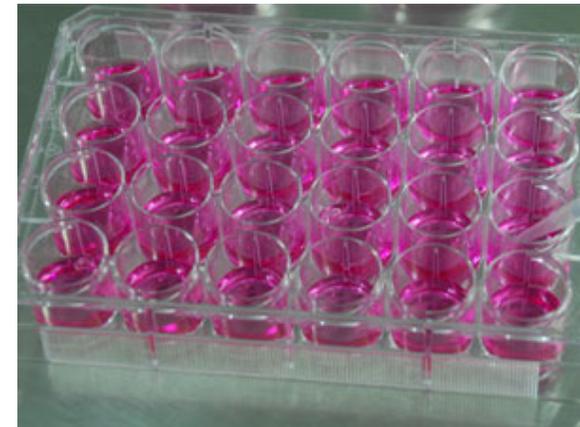
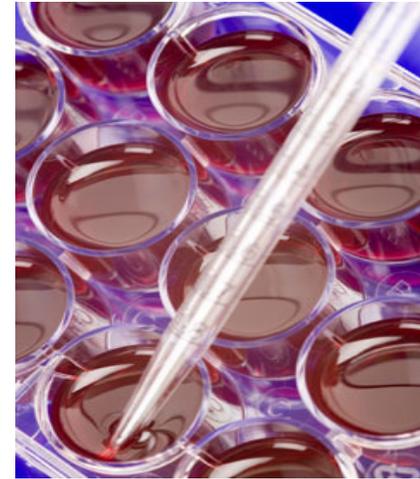
- ✓ Chrysin is a secondary metabolite of the class of flavones.
- ✓ Was extracted for the first time from the blue passion flower.
- ✓ Has multiple biological activity, such as anti-inflammation, anti-cancer and anti-oxidation



# Experimental plain



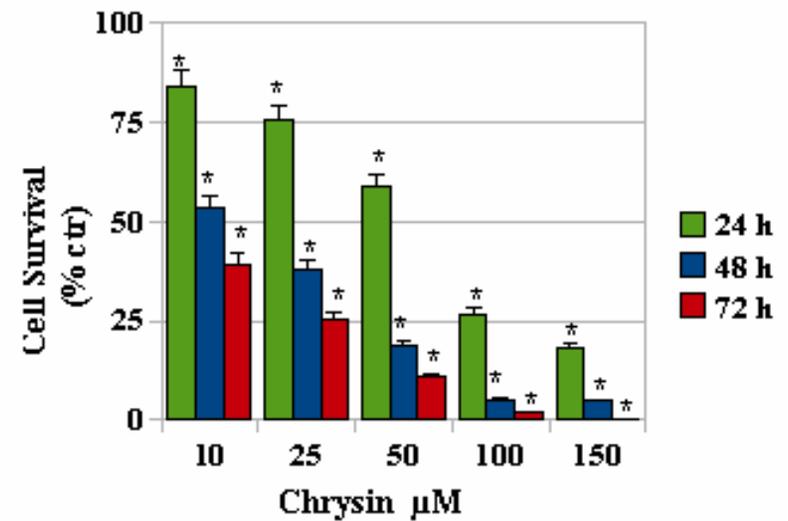
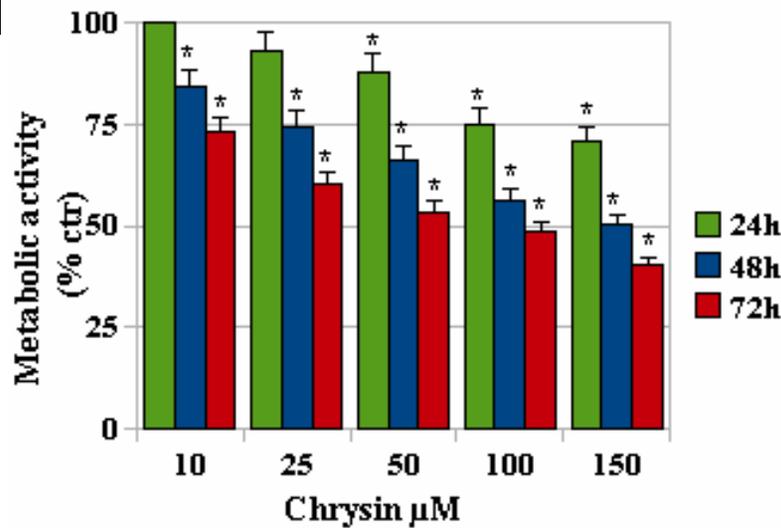
A375 human melanoma  
B16-F1 murine melanoma



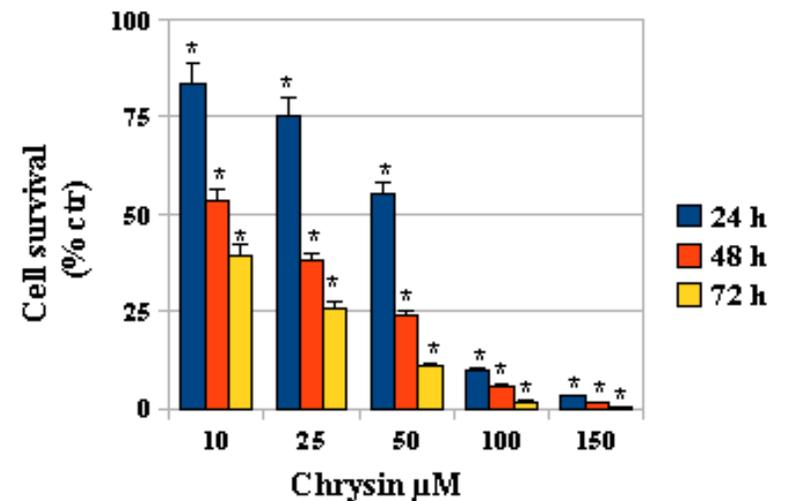
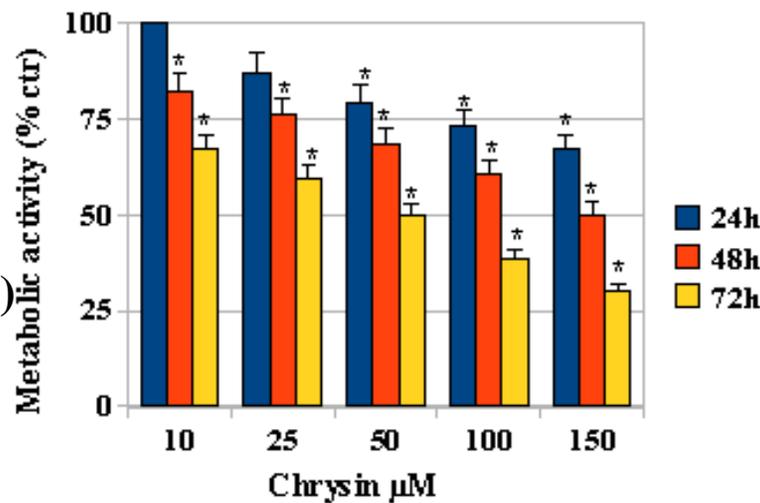


# Chrysin inhibited cell proliferation

**B16-F1  
(murine)**



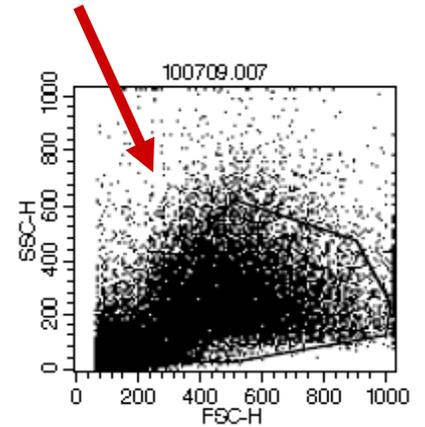
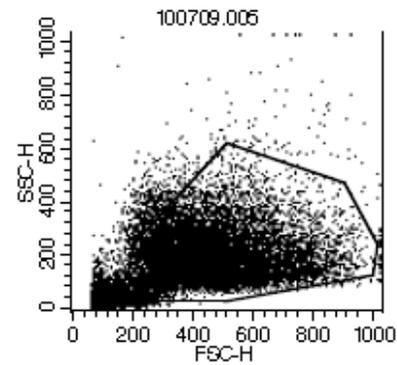
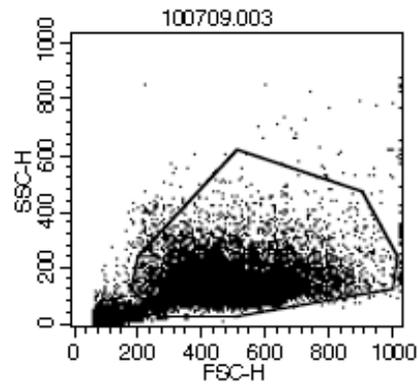
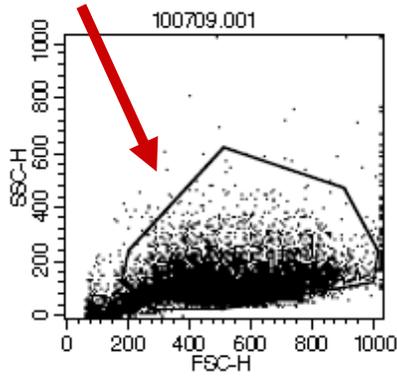
**A375  
(human)**





# Chrysin induced phenotypic alteration

**B16-F1**



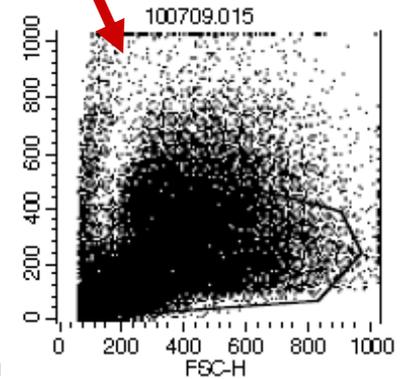
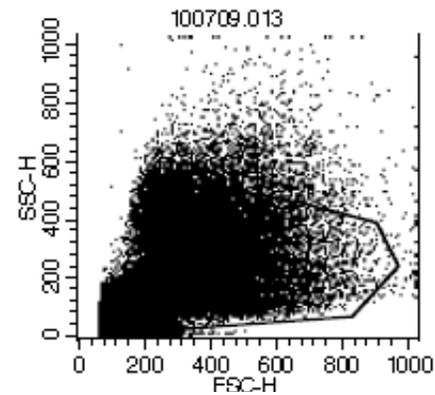
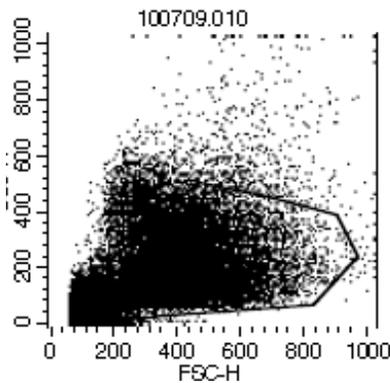
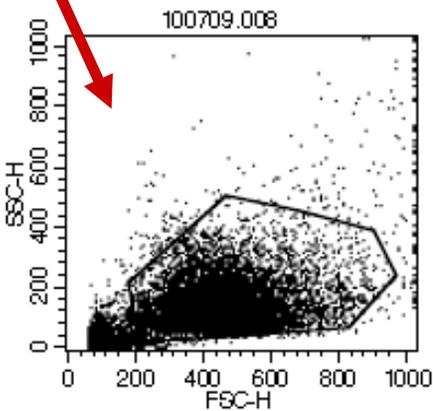
**Ctrl**

**Chrysin 10  $\mu$ M**

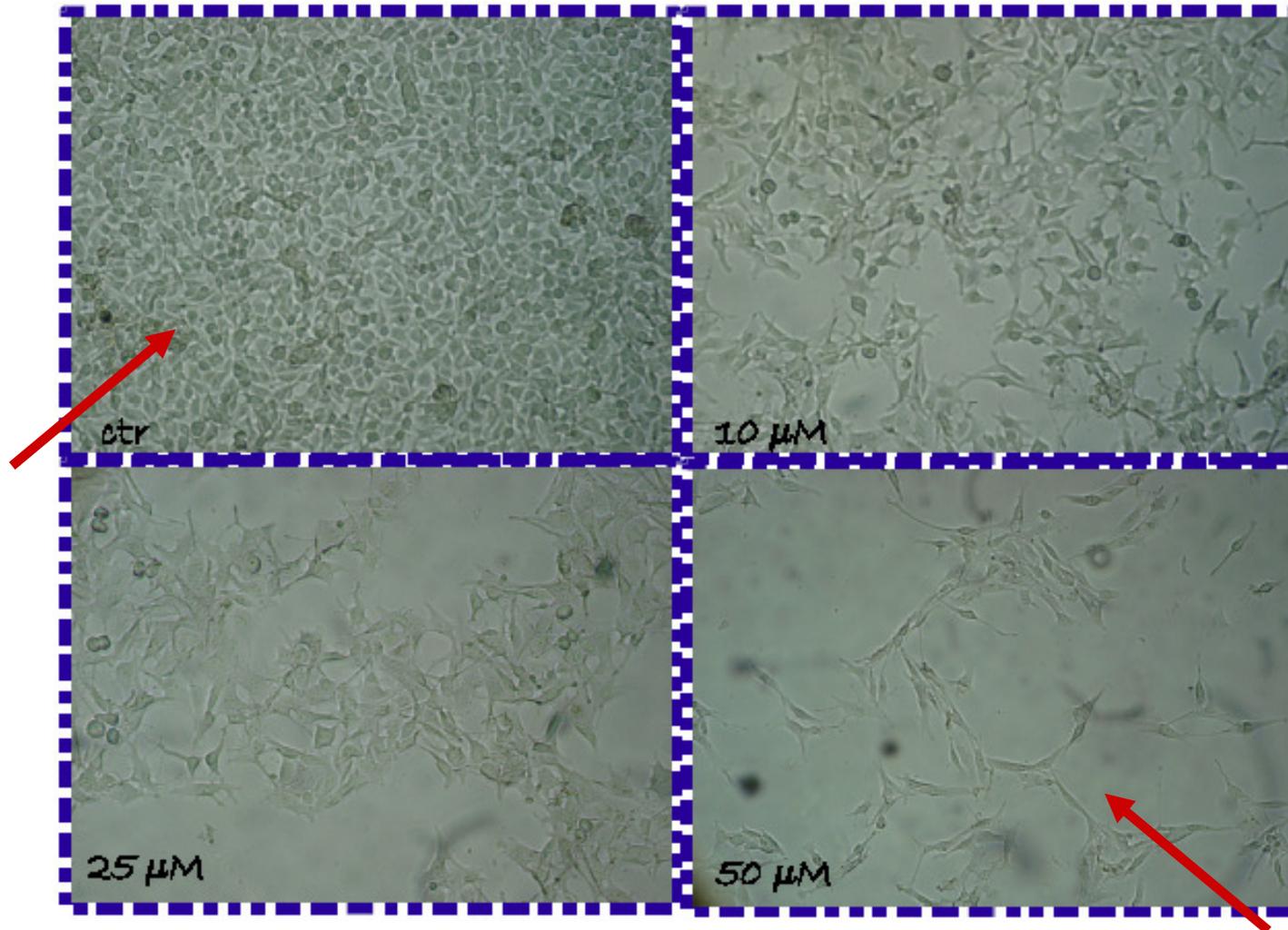
**Chrysin 25  $\mu$ M**

**Chrysin 50  $\mu$ M**

**A375**



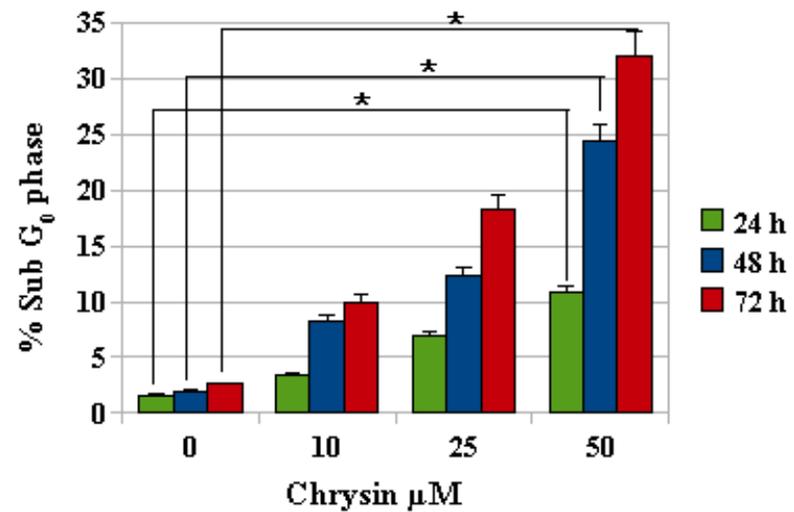
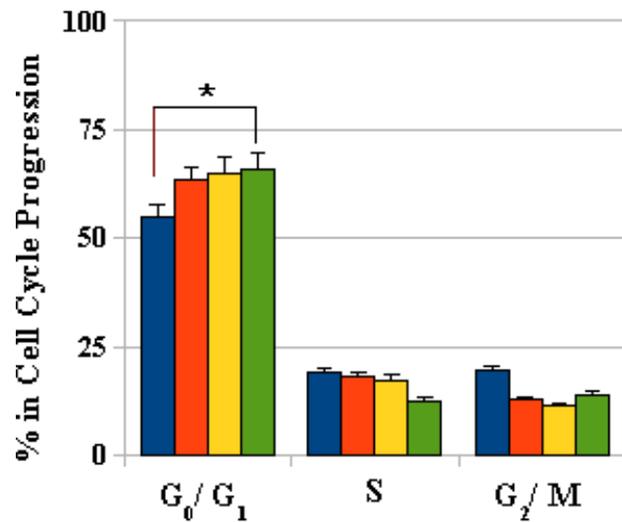
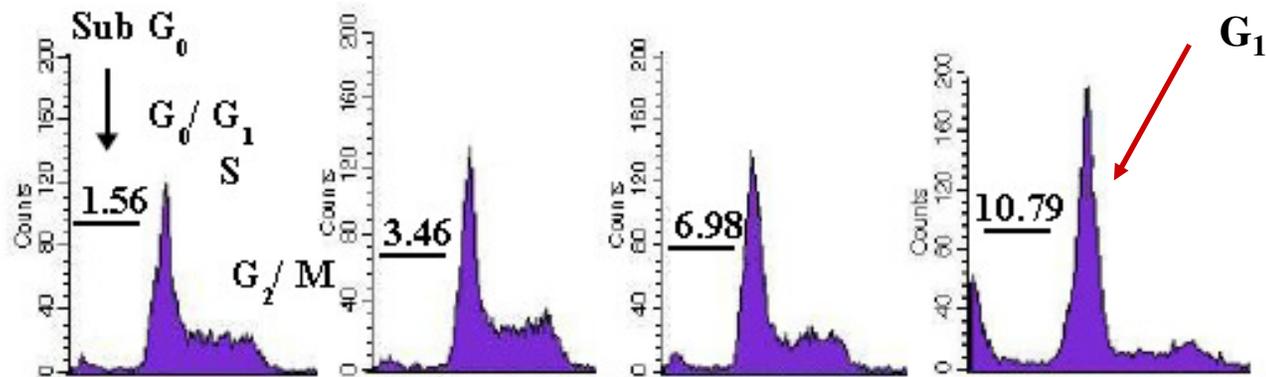
# Morphological alteration after chrysin treatment



**B16-F1 cells exposed for 72 h to chrysin change their morphology into the shape of neural type cells**

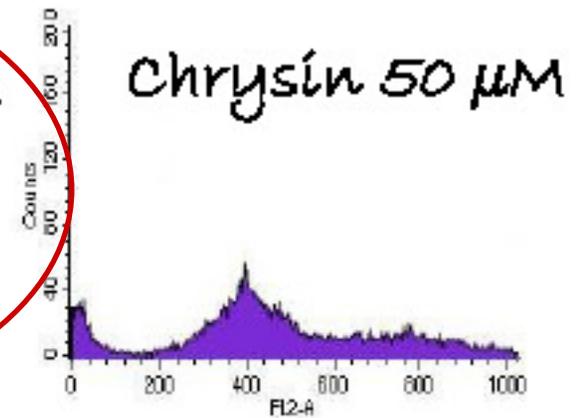
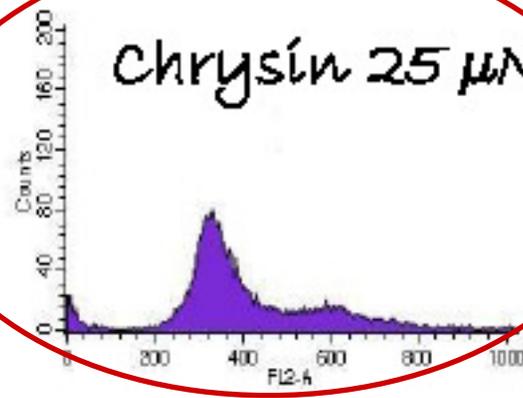
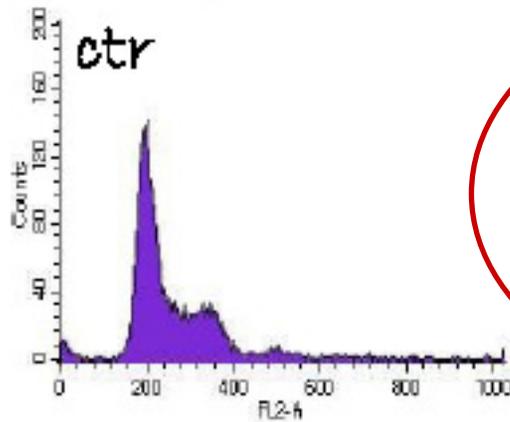
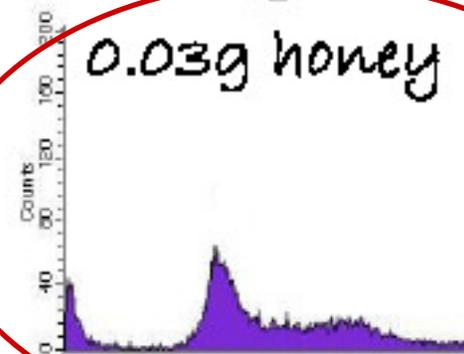
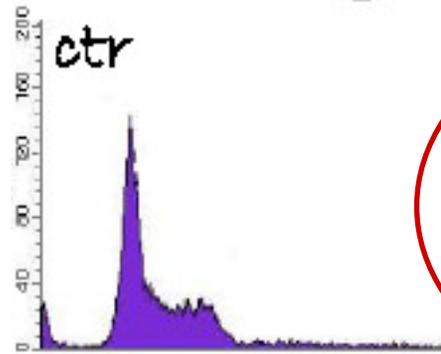


# Chrysin change the cell cycle progression



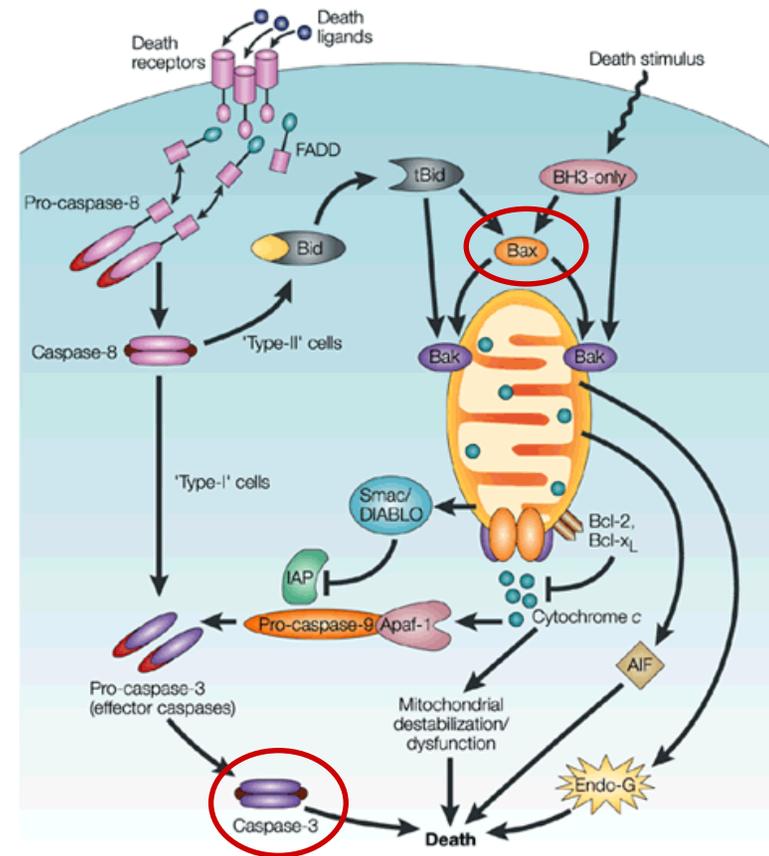
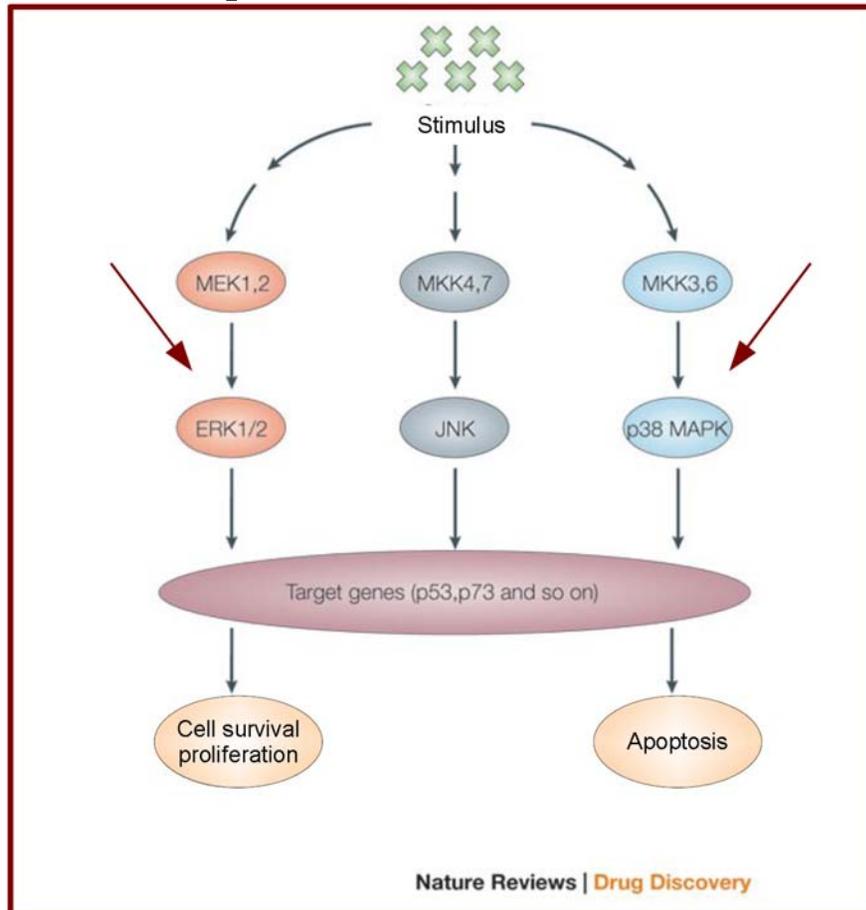


# Chrysin induced polyploidy in melanoma cells



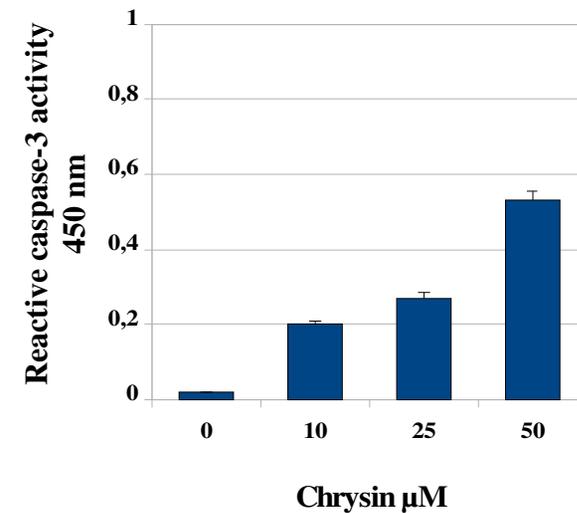
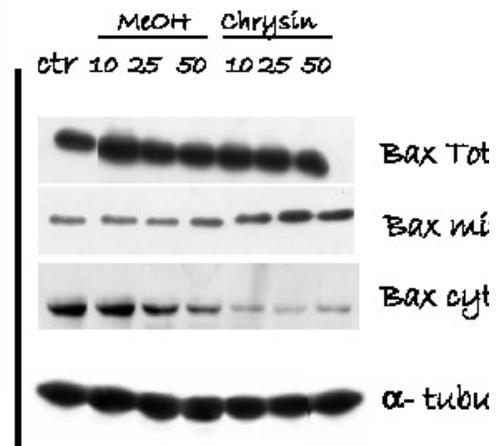
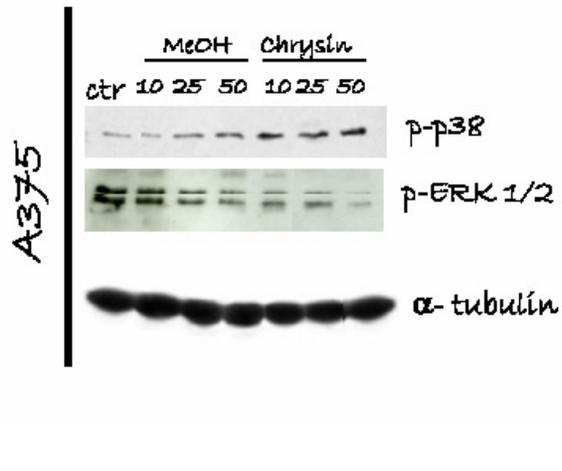
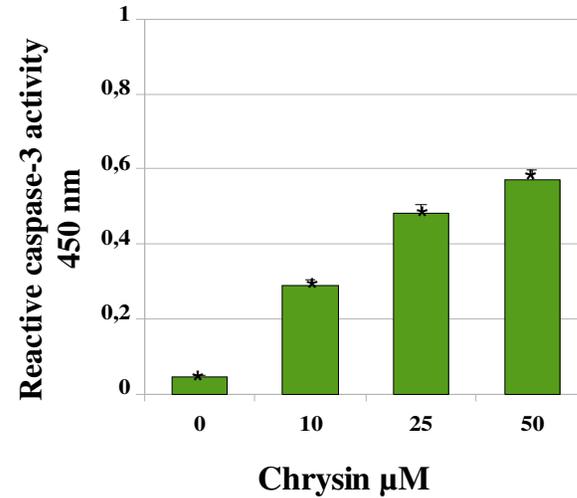
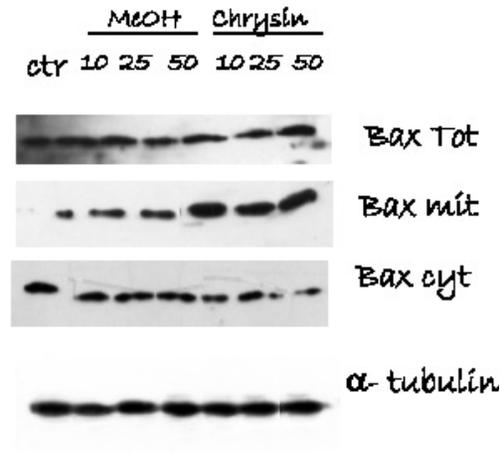
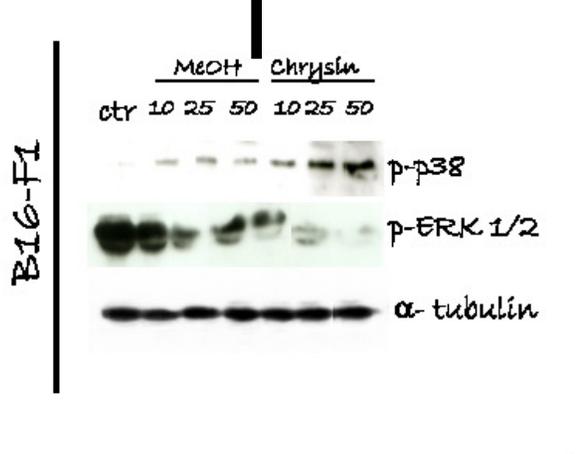


# Cell molecular target





# Molecular pattern activated in chrysin cells treated





# Conclusion

- ✓ Robinia honey inhibited cell proliferation and altered the cell cycle of melanoma cells
- ✓ Chrysin is implicated in the effect observed in honey treatment
- ✓ Chrysin induced cell differentiation by the inhibition of ERK  $\frac{1}{2}$  and activation of p38 MAPKs
- ✓ Chrysin induced apoptosis by the activation of p38 MAP kinase that might mediate Bax translocation to mitochondria and subsequent activation of the apoptotic cascade
- ✓ These results are starting point for the apitherapy in Italy.