SUPPLEMENTARY FIGURES

Lagoa et al. (2011)

Figure S1 – Determination of mitochondrial SOD activity and effect of flavonoids.

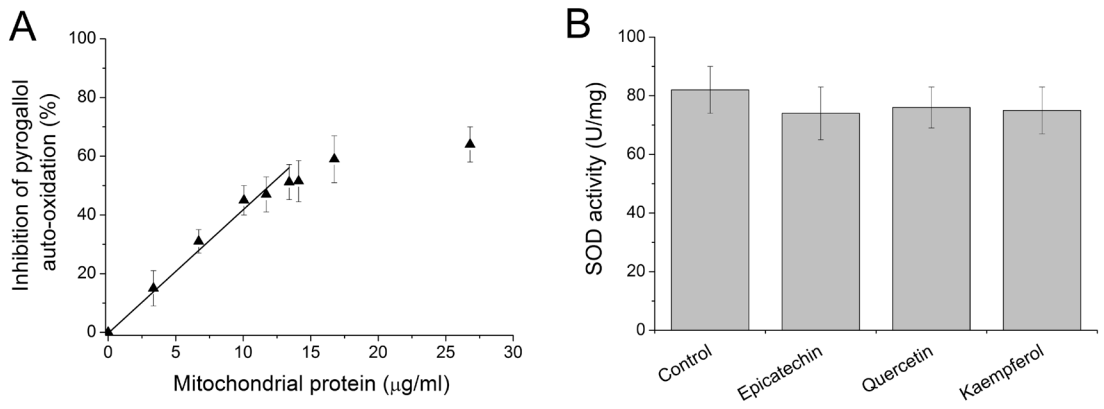

Figure S1. Effect of flavonoids on mitochondrial SOD activity. (A) Inhibition of the rate of pyrogallol auto-oxidation by different concentrations of brain sub-mitochondrial particles. The line was obtained by linear regression analysis from the experimental points up to a concentration of 14 μg/ml (~55% inhibition). The correlation coefficient for this experiment was 0.984. Above 55-60% inhibition, the relationship is no longer linear, as observed by other authors [57]. Using the linear fit, the amount of mitochondria that inhibits 50% the auto-oxidation of pyrogallol was calculated, i.e., the amount of mitochondrial protein needed to have 1 U of SOD activity. (B) Effect of flavonoids on SOD activity of rat brain sub-mitochondrial particles. The results shown are the mean ± standard error of measurements done with three preparations of mitochondria.

Figure S2 – Response of the Amplex Red method in the presence of flavonoids.

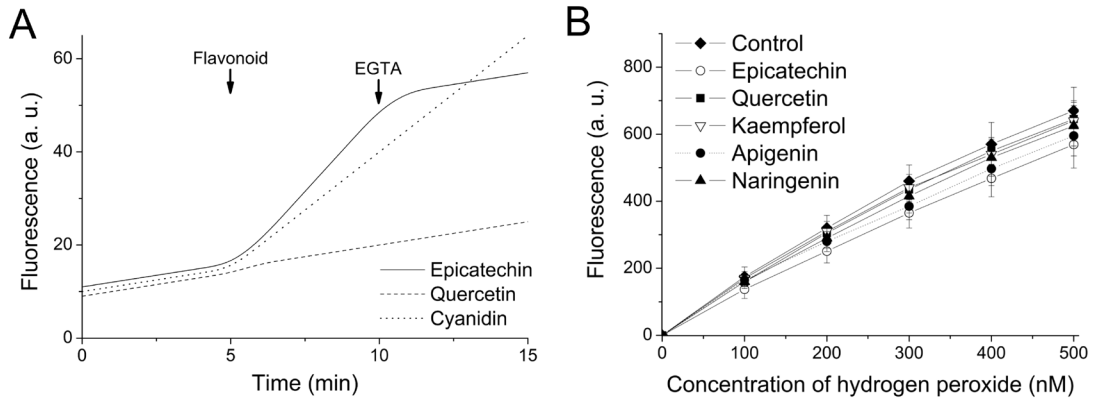

Figure S2. Response of the Amplex Red method for detection of H_2O_2 in the presence of flavonoids. (A) The experimental traces shown are representative of the three types of results obtained when flavonoids (10 μM) were added to the assay medium of the Amplex Red method in the absence of mitochondria. Quercetin, kaempferol, apigenin and naringenin had no significant effect on the basal rate of Amplex Red fluorescence increase, but myricetin, cyanidin and malvidin amplified the probe signal. Epicatechin at 10 μM concentration also caused an increase in the basal rise of Amplex Red fluorescence, but this effect was completely abolished in the presence of 2 mM EGTA. The curves shown are representative of experiments done by triplicate. (B) Calibration curves of Amplex Red signal response to known amounts of hydrogen peroxide, in the absence and in the presence of different flavonoids at 10 μM concentration. The results shown are the mean ± standard error of the data obtained in triplicate experiments.

Figure S3 - Effects of flavonoids on mitochondrial oxygen consumption.

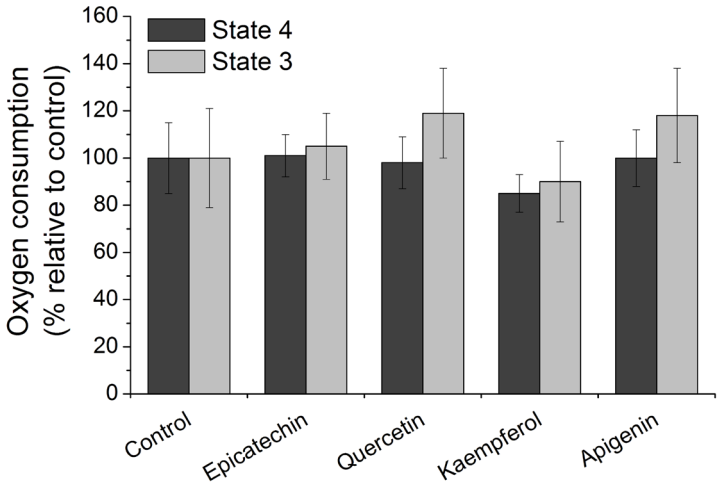

Figure S3. Effects of flavonoids (10 μM) on mitochondrial oxygen consumption. Mitochondria (1 mg/ml) were incubated with each flavonoid in respiration buffer for 10 minutes at 37 ºC, before addition of substrates malate/pyruvate and measurement of oxygen consumption rate in state 4. Respiration rate in state 3 was measured in the presence of 0.2 mM ADP. Results are shown as percentage of oxygen consumption rate relative to the rate observed with mitochondria incubated in the absence of flavonoid (control value 100%). The data shown are mean ± standard error from assays with 3 preparations of mitochondria.

Figure S4 - Effect of flavonoids on the activity of complex II and the coupled activity of complex II plus III.

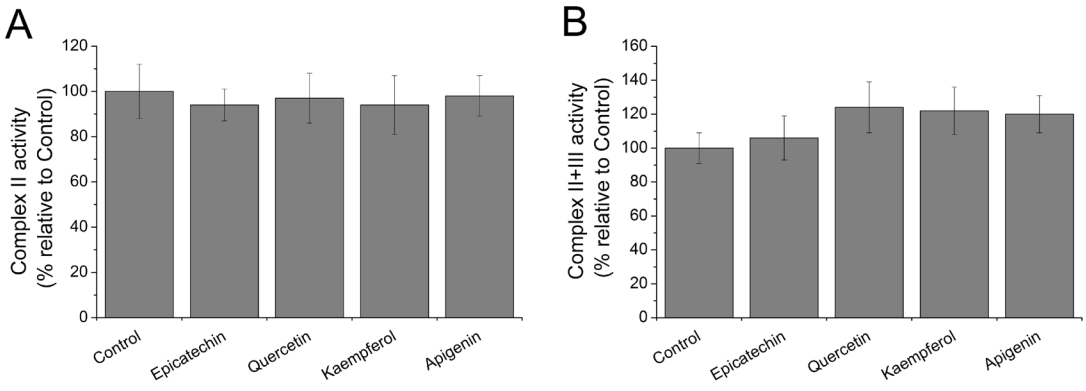

Figure S4. Effect of flavonoids (10 μM) on the activity of complex II of sub-mitochondrial particles (A) and the coupled activity of complex II plus III of mitochondria from rat brain (B). Results are presented as percentage of activity relative to the activity of the same preparation observed in absence of flavonoid and presence of the volume of DMSO added with the flavonoid (control values, taken as 100%). The control specific activities (in nmol/min/mg) were 318±48 (complex II) and 157±14 (complex II+III). Results shown are the mean ± standard error from measurements done with 6 preparations of mitochondria.