The Reduction of the Combined Effects of Aflatoxin and Ochratoxin A in Piglet Livers and Kidneys by Dietary Antioxidants

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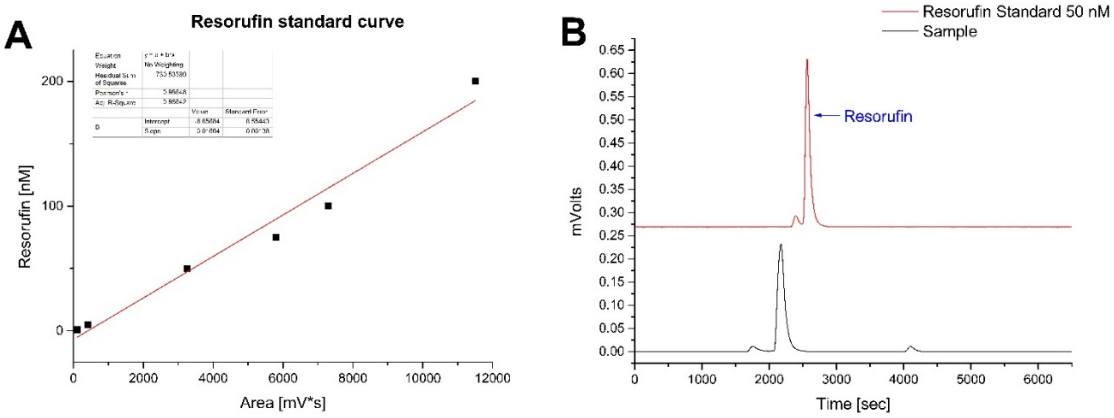

Figure S1. Quantification and separation of resorufin using reversed-phase HPLC (C18). Linear regression with a coefficient of determination of 0.97 was obtained for quantitative determination by integration of the peak area of six resorufin samples with known concentration levels: 1, 5, 50, 75, 100 and 200 nM (**A**). The fluorescence spectra with excitation and emission wavelengths of 560 and 586 nm were recorded using a Varian HPLC system. Mobile phase was 20 mM K_2HPO_4/KH_2PO_4 buffer (pH 6.8), methanol and acetonitrile (30:35:35 v/v) with a flow rate of 0.5 mL /min at 40 °C, the stationary phase was C18, column dimensions: 150 ×4.6 mm; 3 µm particle size. Sample volume was 20 µL of 50 nM resorufin standard, respective sample of enzymatic reactions. Resorufin elutes at 3.66 minutes and is clearly separated from other similar /impurity molecules (**B**). Obtained data were analyzed using OriginPro 9 software.

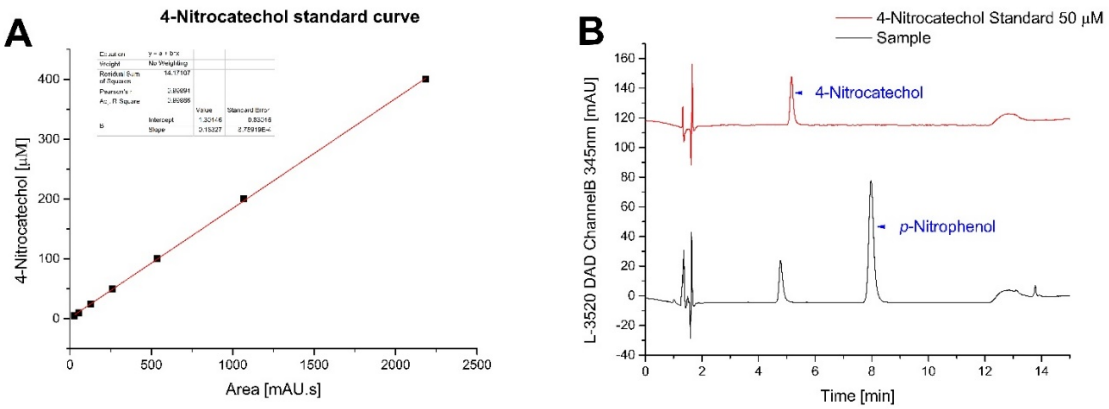

Figure S2. Quantification and separation of 4-nitrocatechol using reversed-phase HPLC (C18). Linear regression with a coefficient of determination of 0.99 was obtained for quantitative determination by integration of the peak area of seven p-nitrocatechol samples with known concentration levels: 5, 10, 25, 50, 100, 200 and 400 µM (**A**). The light absorption spectra at 345 nm were recorded using a High-

Performance Liquid Chromatography Systems L-3000 (RIGOL Technologies, Inc.). Mobile phase was 0.1% trifluoroacetic acid in water as solvent A and 0.1% trifluoroacetic acid in acetonitrile as solvent B. The gradient profile was as follows: 0-10 min 85% solvent A; 10-12 min 85% solvent B; 12-15 min 100% solvent A, with a flow rate of 1 mL /min at 30 °C, the stationary phase was C18, column dimensions: 150 ×4.6 mm; 5 μm particle size. Sample volume was 20 μL of 50 μM p-nitrocatechol standard, respective sample of enzymatic reactions. P-Nitrocatechol elutes at 5.16 minutes and is clearly separated from other similar /impurity molecules (**B**). Obtained data were analyzed using OriginPro 9 software.

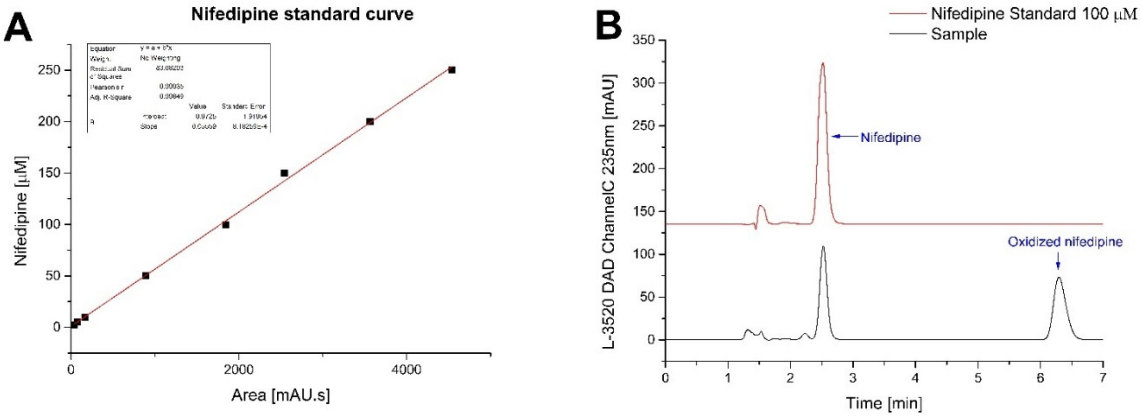

Figure S3. Quantification and separation of nifedipine using reversed-phase HPLC (C18). Linear regression with a coefficient of determination of 0.99 was obtained for quantitative determination by integration of the peak area of six nifedipine samples with known concentration levels: 2.5, 5, 10, 50, 200 and 250 μM (**A**). The light absorption spectra at 235 nm were recorded using a High-Performance Liquid Chromatography Systems L-3000 (RIGOL Technologies, Inc.). Mobile phase was 0.1% trifluoroacetic acid in water /acetonitrile /methanol (40:30:30) with a flow rate of 1 mL /min at 30 °C, the stationary phase was C18, column dimensions: 150 ×4.6 mm; 5 μm particle size. Sample volume was 20 μL of 100 μM nifedipine standard, respective sample of enzymatic reactions. Nifedipine elutes at 2.56 minutes and is clearly separated from other similar /impurity molecules (**B**). Obtained data were analyzed using OriginPro 9 software.