Biomedicine & Pharmacotherapy 101 (2018) 805–819

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

An in vitro comparative study of the antioxidant activity and SIRT1 modulation of natural compounds

Jonathan Fusi[a,1], Sara Bianchi[b,*,1], Simona Daniele[c], Silvia Pellegrini[a], Claudia Martini[c], Fabio Galetta[a], Luca Giovannini[b], Ferdinando Franzoni[a]

[a] Department of Clinical and Experimental Medicine, University of Pisa, Italy
[b] Department of Translational Research and New Technologies in Medicine and Surgery, Pharmacology, Medical School, University of Pisa, Italy
[c] Department of Pharmacy, University of Pisa, Italy

ARTICLE INFO

Keywords:
Oxidative stress
Antioxidants
Polyphenols
Berberine
SIRT1
AMPK

ABSTRACT

Oxidative stress arises from an imbalance between the production of free radicals and antioxidant defences. Several studies have suggested that dietary antioxidants (such as polyphenols and berberine) may counteract oxidative stress through the involvement of the Sirtuin 1/Adenosine Monophosphate-Activated Protein Kinase (SIRT1/AMPK) pathway. The aim of this study was to evaluate the direct and specific antioxidant activity of some natural compounds, as well as their ability to modulate the expression of SIRT1 and the activation of AMPK.

Quercetin, tyrosol, ferulic acid, catechin, berberine and curcumin were evaluated for their specific and direct antioxidant activity with TOSC assay. Their ability to modulate SIRT1 and AMPK was assessed by immunoblotting assay, while their cytotoxicity by CellTiter-Blue Cell Viability Assay.

No statistically significant decrease ($p > 0.05$) in the number of viable cells was found upon challenging with the natural compounds. Quercetin exhibited the highest antioxidant activity against peroxyl radical and peroxinitrate derivates, while curcumin showed the best anti-hydroxyl activity with respect to the other compounds and, most importantly, respect to the reference antioxidants. Finally, all the tested compounds significantly increased the SIRT1 expression and the activation of AMPK.

Our results clearly disclose the specific antioxidant activity of these natural compounds and their ability to increase SIRT1 expression and AMPK activation.

1. Introduction

Oxidative stress is commonly defined as an imbalance between the production of reactive oxygen species (ROS, free radicals) and antioxidant defences [1,2]. Reactive oxygen and nitrogen species (RONS) are ubiquitous reactive derivatives of O2 and nitrogen metabolism, responsible for numerous types of cell damage. At this purpose, there is a general agreement on the fact that a chronic imbalance between formation of RONS and antioxidant systems is a relevant determinant involved in the pathogenesis and development of a variety of chronic and degenerative diseases, including aging, cancer, cardiovascular disease and neurodegenerative disorders (Alzheimer's and Parkinson's Diseases) [2–5]. In support of this view, there has been growing evidence that oxidative stress and specific human diseases can be prevented by including in the diet plant foods that contain large amounts of antioxidants, such as vitamins C, E or natural antioxidants, such as polyphenols [6,7]. Dietary antioxidants act as free radical scavengers, radical chain reaction inhibitors, metal chelators, oxidative enzyme inhibitors and antioxidant enzyme cofactors [8–11].

Nowadays, a huge amount of studies support the beneficial role of antioxidants to counteract RONS both in sedentary subjects and in athletes. In this respect, many supplements, characterized by natural

Abbreviations: AMPK, adenosine monophosphate-activated protein kinase; APAB, 2,2′-azo-bisamidinopropane; BSA, bovine serum albumin; CCL_4, carbon tetrachloride; DMEM, Dulbecco's modified eagle's medium; DMSO, dimethyl sulfoxide; DTPA, diethylenetriaminepentaacetic acid; FBS, fetal bovine serum; GSH, reduced glutathione; GSSG, oxidised glutathione; HRP, horse radish protein; KMBA, α-cheto-γ-(methylthiol)butyric acid; mTOR, mechanistic target of rapamycin; NAD^+, nicotinamide adenine dinucleotide; NAMPT, nicotinamide phosphoribosyl transferase; PBS, phosphate buffered saline; PMSF, phenyl-methane-sulfonyl-fluoride; PVDF, polyvinylidene difluoride; RONS, reactive oxygen and nitrogen species; ROS, reactive oxygen species; SDS, sodium dodecyl sulfate; SIN-1, 3-morpholinosydnonimine *N*-ethylcarbamide; SIRT1, sirtuin 1; TOSC, total oxidant scavenging capacity; T-TBS, tween-tris buffered saline

* Corresponding author at: Via Roma 55, 56126, Pisa, Italy.
E-mail address: sara.bianchi1987@hotmail.it (S. Bianchi).
[1] These authors equally contributed to this work.

https://doi.org/10.1016/j.biopha.2018.03.006
Received 25 October 2017; Received in revised form 27 February 2018; Accepted 5 March 2018

compounds, are commercialized with the "therapeutic" purpose of improving the antioxidant defences. Some of these supplements are characterized by polyphenols, such as ferulic acid, quercetin, tyrosol, catechin and curcumin and natural compounds as berberine.

Polyphenols, such as ferulic acid, quercetin, tyrosol, catechin and curcumin, are secondary metabolites of plants and are usually classified based on their chemical structure, according to the number of phenol rings and on the basis of structural elements [12–14]. Research in animal and human models has shown that these compounds possess a wide range of biological protective effects, such as anti-inflammatory, antibacterial, anti-allergic and antioxidant ones. In addition, the protective role of polyphenols has been showed in cardiovascular, neurodegenerative and neoplastic diseases [15–17].

Berberine, a quaternary ammonium salt from the protoberberine group of benzylisoquinoline alkaloids, is the principal component for many popular medicinal plants (Coptidis chinensis, Phellodendron chinense and Mahonia bealei) [18]. The numerous pharmacological activities of berberine, in the last two decades, have been attracting high-level interests within the scientific community [19,20]. Indeed, berberine has been shown to have therapeutic effects on hypoglycemia, inflammation, cancer and it is also useful for prevention and treatment of Alzheimer's disease and cerebral ischemia [21,22]. Berberine is also able to ameliorate and alleviate oxidative stress both in vitro and in vivo models [23–26].

The fundamental mechanism of action underlying polyphenols' and berberine's impact on human health is probably represented by their action on Sirtuin 1 (SIRT1) and Adenosine Monophosphate-Activated Protein Kinase (AMPK), two important proteins involved in many pathophysiological processes, able to activate each other: AMPK activates SIRT1 (by increasing the Nicotinamide phosphoribosyltransferase (NAMPT)levels) and SIRT1 stimulates AMPK through LKB1 deacetylation [27–30].

SIRT1 is a NAD+ dependent histone/protein deacetylase able to deacetylate a lot of substrates, including p53, NF-kB, FOXO transcription factors, Ku-70, PPAR-γ, and PGC-1α, with roles in cellular processes ranging from energy metabolism to cell survival [31–36].

AMPK is a fuel-sensing enzyme activated by a decrease in a cell's energy state that inhibits anabolic processes and increases the catabolic ones with the aim of restoring ATP reserve [37]. Recent works suggest a relationship between SIRT1/AMPK and the oxidative stress, underlining how their activation could be crucial in this context and potentially protective against oxidative stress [38].

Based on this premise, the aim of this study was to evaluate the direct and specific antioxidant activity of some natural compounds (Fig. 1), such as quercetin, tyrosol, ferulic acid, catechin, berberine and curcumin. Moreover, to shed light on these compounds' molecular mechanisms, we evaluated the ability of these substances to increase the expression of SIRT1 and the activation of AMPK.

2. Materials and methods

2.1. Chemicals and antibodies

Ascorbic acid, KMBA (α-cheto-γ-(methylthiol)butyric acid)), 2,2′-azo-bisamidinopropane (ABAP), Diethylenetriaminepentaacetic acid (DTPA) and 3-morpholinosydnonimine N-ethylcarbamide (SIN-1) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cell culture reagents were purchased from Lonza (Basel, Switzerland) and Gibco-BRL (Grand Island, NY). General laboratory chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Carlo Erba (Milano, Italy). Tyrosol, (+)- Catechin, Berberine Chloride Hydrate, Curcumin, Quercetin Dihidrate and Ferulic Acid also were from Sigma-Aldrich (St. Louis, MO, USA). All substances were dissolved in DMSO (Sigma-Aldrich, St. Louis, MO, USA). The final concentrations of DMSO in culture were between 0.05–0.2%, which had no effect on cell viability (data not shown).

Ripa Lysis Buffer System, Protease Inhibitor Cocktail, Phosphatase Inhibitor Cocktail C, phenyl-methane-sulfonyl-fluoride (PMSF), Sodium Orthovanadate and Sodium Pyrophosphate were from Santa Cruz Biotechnology (California, USA). CellTiter-Blue Cell Viability Assay was purchased from Promega (Madison, USA). Reagents, protein markers and membrane for Western Blot were from Biorad Laboratories (California, USA), Rabbit polyclonal antibody against SIRT1 (H-300, sc-15404), mouse monoclonal antibody against Actin (C-2, sc-8432), goat anti-rabbit IgG-HRP antibody (sc-2004) and goat anti-mouse IgG-HRP antibody (sc-2005) were purchased from Santa Cruz Biotechnology (California, USA). Rabbit polyclonal antibody against Phosho-AMPKα (Thr172, #2531) and rabbit polyclonal antibody against Total AMPKα (#2532) were from Cell Signaling Technologies (Massachussets, USA). Luminata Crescendo Western HRP Substrate for chemiluminescent detection of bands were from Millipore (Massachussets, USA). Glutathione Fluorometric Assay Kit (GSH, GSSG and Total) was purchased from BioVision (California, USA).

2.2. TOSC assay

The total oxidant scavenging capacity (TOSC) assay was described in detail in our previous work [39]. Briefly, peroxyl radicals were generated by thermal homolysis of 20 mM ABAP at 35 °C in 100 mM potassium phosphate buffer, pH 7.4. Hydroxyl radicals were generated at 35 °C by the iron plus ascorbate-driven Fenton reaction (1.8 μM Fe3+, 3.6 μM EDTA, and 180 μM ascorbic acid in 100 mM potassium phosphate buffer, pH 7.4). Peroxynitrite was generated from the decomposition of SIN-1 in the presence of 0.2 mM KMBA, 100 mM potassium phosphate buffer, pH 7.4, and 0.1 mM DTPA (Diethylene Triamine Penta Acetic Acid), at 35 °C. The concentration of SIN-1 was varied to achieve an ethylene yield equivalent to the iron–ascorbate and ABAP systems. Reactions with 0.2 mM KMBA were carried out in 10 ml vials sealed with gas-tight Mininert® valves (Supelco, Bellefonte, PA) in a final volume of 1 ml.

Ethylene production was measured by gas-chromatographic analysis of 200 μl aliquots taken from the head space of vials at timed intervals during the course of the reaction. Analyses were performed with a Hewlett-Packard gas chromatograph (HP 6890 Series, Andoven, MA) equipped with a Supelco DB-1 (30 × 0.32 × 0.25 mm) capillary column and a flame ionization detector (FID). The oven, injection and FID temperatures were respectively, 35, 160 and 220 °C. Hydrogen was the carrier gas (at a flow rate of 1 ml/min); a split ratio of 20:1 was used. Total ethylene formation was quantified from the area under the kinetic curves that best define the experimental points obtained for control reactions and after addition of quercetin, tyrosol, ferulic acid, catechin, and berberine and curcumin during the reaction. TOSC values were quantified from the equation $TOSC = 100 - (SA/CA \times 100)$, where SA and CA are the integrated areas for sample and control reaction, respectively.

TOSC values were quantified from the equation $TOSC = 100 - (SA/CA \times 100)$, where SA and CA are respectively the area under the curve (AUC) for sample and control reaction. A TOSC value of 0 corresponds to a sample with no scavenging capacity. A TOSC value of 100 is attributed to a compound that entirely suppresses the ethylene formation whereas a pro-oxidant compound shows a negative TOSC value. Consequently, antioxidants and pro-oxidants molecules can be distinguished by the obtained results. The linearity of dose–response curve quercetin, tyrosol, ferulic acid, catechin, and berberine and curcumin concentration (μM) and the antioxidant (TOSC value) response was tested and good correlation coefficients (generally greater than 0.9) were obtained at the different doses used to test the validity of our experiments (Fig. 2). Each experiment was performed in duplicate to account for the intrinsic variability of the method. The results obtained with quercetin, tyrosol, ferulic acid, catechin, and berberine and curcumin were expressed in TOSC units, and compared to each other. In our hands, the coefficient of variation (CV) of the

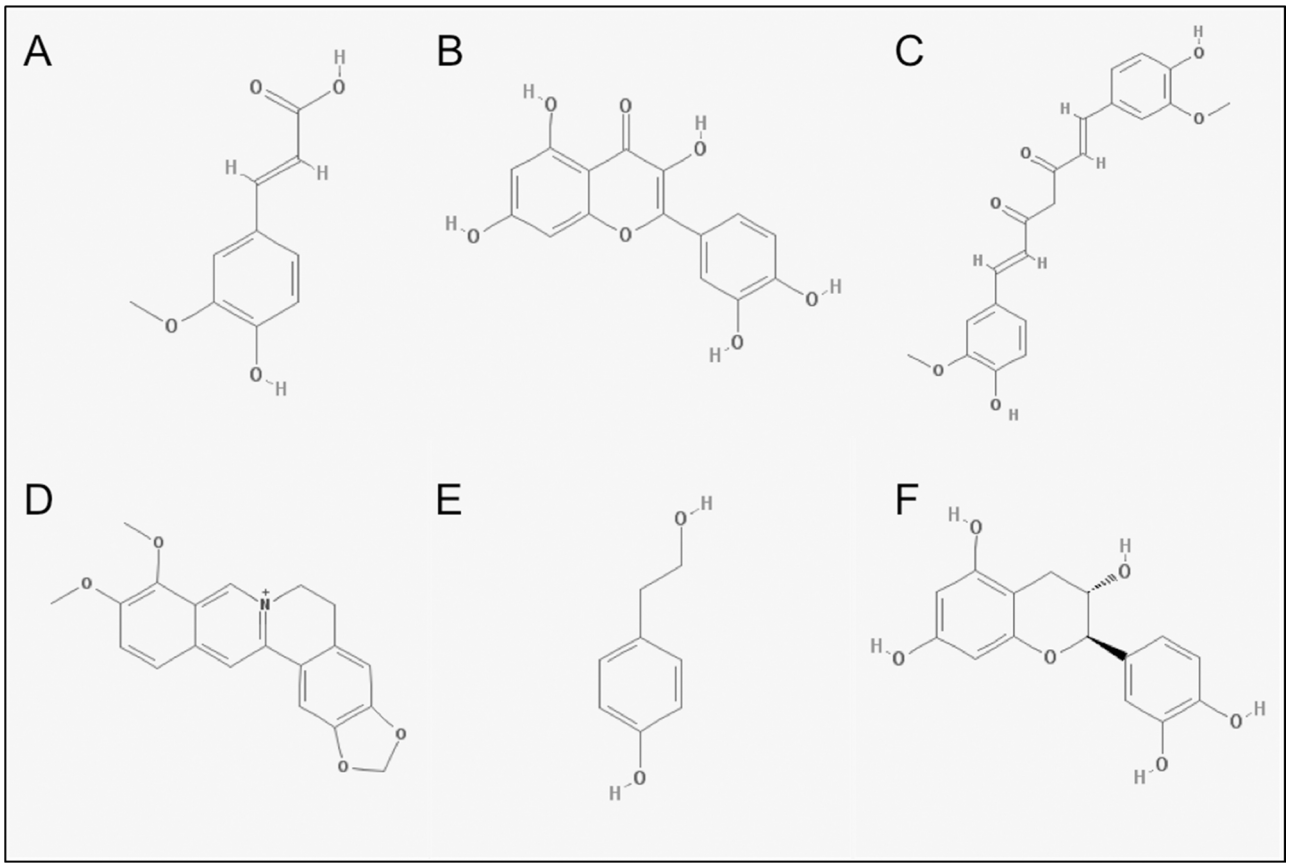

Fig. 1. Chemical structures of the analysed natural compounds.(A) Ferulic Acid; (B) Quercetin; (C) Curcumin; (D) Berberine; (E) Tyrosol; (F) Catechin. Source pubchem https://pubchem.ncbi.nlm.nih.gov/search/search.cgi#.

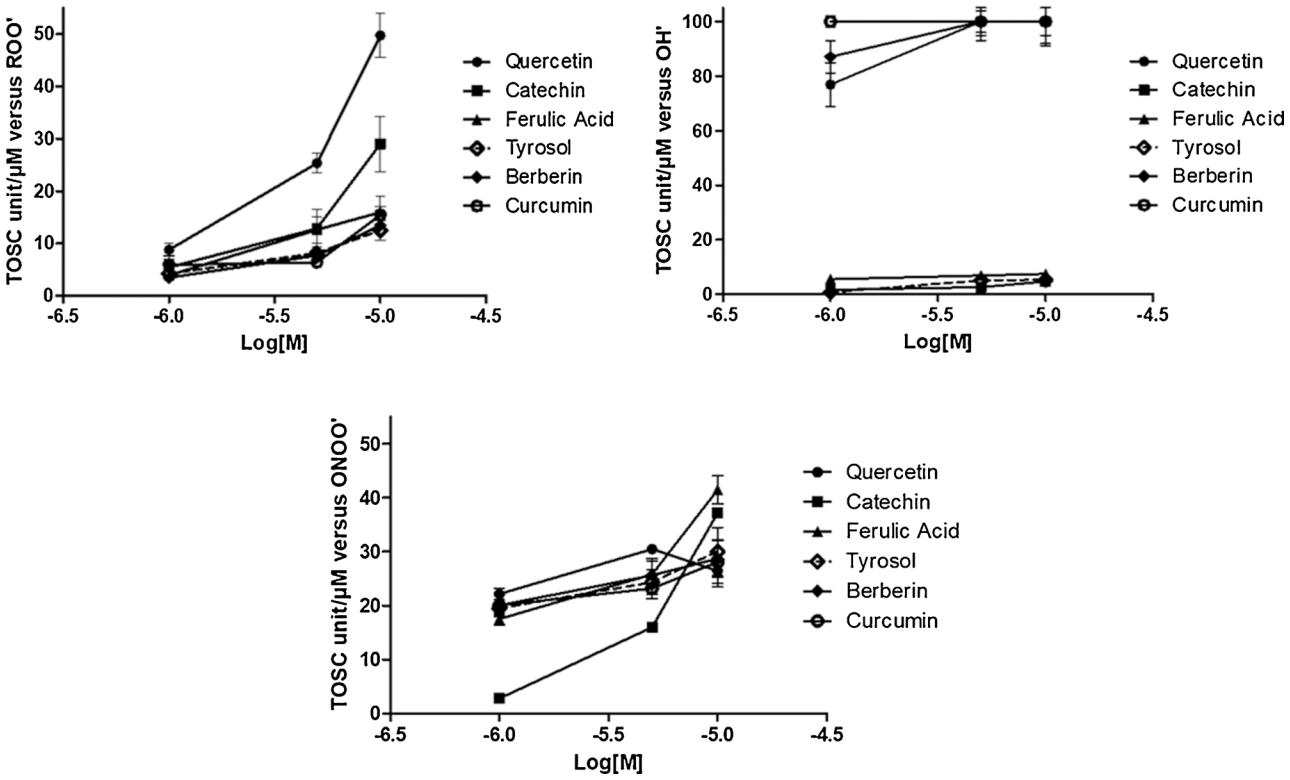

Fig. 2. Dose–response curve of natural tested compounds scavenging capacity towards peroxyl radicals (ROO·), hydroxyl radicals (OH·) and peroxynitrate (HOONO).

method ranged between 2% and 5%.

2.3. Cell culture

Human Cervical Carcinoma cells (HeLa) were cultured in DMEM supplemented with 10% (v/v) fetal bovine serum, 1% antibiotics (penicillin and streptomycin) and 1% L-glutamine at 37 °C in a humidified air atmosphere with 5% (v/v) CO_2.

The cells were grown to a confluence of 80%, were then trypsinized, counted with a Burker chamber and plated (100.000 cells/well). The day of the experiment, the cells were treated with the substances at the concentrations considered (1,5 and 10 μM) and left in incubation for 3, 6 and 24 h.

At the end of the incubation periods, the cells were washed with PBS (pH 7.4) and collected in a 1.5 ml microtube with 0.130 ml of lysis buffer (RIPA buffer) containing a protease inhibitor cocktail, a phosphatase inhibitor cocktail, PMSF, sodium orthovanadate and sodium pyrophosphate.

They were lysed by three freezing-thawing cycles and then the supernatants were recovered.

A part of the lysate was used to quantify the protein content with the spectrophotometer (Thermo Scientific Multiskan FC, Thermo Scientific), using a commercial kit based on the Bradford assay. The final concentrations are expressed in μg/μl.

2.4. Western blot analysis

The cell lysates (25 μg/μl) were combined with the appropriate amount of 1× SDS sample buffer (0.5 M Tris-HCl pH 6.8, 20% SDS, 10% [vol/vol] glycerol, 5% [vol/vol] β-mercaptoethanol, 0.2% bromophenol blue), heated at 95 °C for 5 min, separated by SDS-PAGE and then transferred to polyvinylidene difluoride membranes (PVDF).

The membranes were blocked with 5% (wt/vol) nonfat dry milk in Tris-buffered saline with Tween buffer (T-TBS) (20 mmol/l Tris-Hcl, pH 7.6, 0.138 mol/l NaCl, and 0.1% Tween 20) for SIRT1, β-actin and total AMPK, with 5% (wt/vol) albumin (BSA, bovine serum albumin) in T-TBS for phosho AMPKα.

The expression of SIRT1 and AMPK activation were analyzed incubating the membranes in the presence of specific primary antibodies and, subsequently, of secondary peroxidase-conjugated antibodies, suitable diluted in 5% BSA in T-TBS. Primary Antibodies were used overnight at 4 °C at the following conditions: anti-SIRT1, rabbit (1:1000); anti-phospho-AMPKα, rabbit (1:200); total-AMPK, rabbit (1:200); anti-β-actina, mouse (1:1000). Secondary antibodies were instead used at two different dilutions: goat anti-rabbit (1:10,000) for SIRT1, goat anti-mouse (1:10,000) for β-actin, goat anti-rabbit (1:2000) for total and phospho-AMPK.

Bound antibodies were visualized using Chemiluminescence Luminata Crescendo Western HRP Substrate and bands were detected and analyzed by the Kodak Image Station 440CF (Eastman Kodak, Rochester, NY, USA).

2.5. CellTiter-blue cell viability assay

The CellTiter-Blue Cell Viability Assay provides a homogeneous, fluorometric method for estimating the number of viable cells present in multiwell plates. It uses the indicator dye resazurin to measure the metabolic capacity of cells, an indicator of cell viability. Viable cells retain the ability to reduce resazurin into resorufin, which is highly fluorescent. Nonviable cells rapidly lose metabolic capacity, do not reduce the indicator dye, and thus do not generate a fluorescent signal.

For this purpose, were set up 96-well plates, containing medium, cells to the desired density and the substances to be analyzed, appropriately diluted in DMSO.

More precisely, were seeded 5000 cells per well, in a volume of medium such that the final volume was 100 μL per well. The cells were treated with the 6 substances and incubated for 3, 6 and 24 h.

At the expiry of treatment, the reagent (20 μL/well) was appropriately added and the plate was incubated in the dark for 4 h at 37 °C. Finally, the fluorescence ($560_{Ex}/590_{Em}$) was detected using the Cytofluor 2300 (Millipore). The fluorescence produced is directly proportional to the number of viable cells and, through the analysis, it was obtained a percentage value compared to the control (cells without treatment).

2.6. GSH/GSSG analysis

The level of reduced glutathione (GSH) was measured using a GSH assay kit, according to the manufacturer's instructions (BioVision, Mountain View, CA, USA). Briefly, cell samples were homogenized in ice cold GSH assay buffer (100 ml). The homogenate was added to a prechilled tube containing perchloric acid (10 ml) and vortexed for several seconds. It was kept on ice for 5 min and centrifuged for 2 min at 13,000*g*, and the supernatant collected. Potassium hydroxide (20 ml) was added to 40 ml of supernatant in order to neutralize the samples. After 5 min of centrifugation at 13,000*g*, the supernatant was taken for the assay. For GSH detection, the samples (10 ml) were diluted with 80 ml of assay buffer. For GSH/oxidized GSH (GSSG) detection, the samples (10 ml) were diluted with 60 ml of assay buffer, mixed with 10 ml of GSH quencher at room temperature (20_C) for 10 min and reduced with 10 ml of reducing agent. Finally, all samples were mixed with 10 ml of *o*-phthalaldehyde probe and agitated at room temperature for 40 min in the dark. The fluorescence intensity was measured using a microplate reader (Thermo Scientific Multiskan FC, Thermo Scientific) at Ex/Em of 340/450 nm. The ratio of GSH/ GSSG was calculated as described elsewhere [40,41].

2.7. Statistical analyses

Statistical analyses were performed using the statistical package Statview, version 5.0.1 (SAS Institute, Abacus Concept, Inc., Berkeley, California), the spreadsheet program Microsoft Excel 2010 and the statistical package GraphPad Prism 6 (GraphPad Software, Inc., La Jolla, California).

In detail, Western Blot data were analyzed by two-way analysis of variance (ANOVA) with Bonferroni post hoc test, while all TOSC, GSH/GSSG and cytotoxicity data significance was evaluated by applying the one-way ANOVA with Bonferroni post hoc test and, in the latter case, to perform a statistical analysis that was as complete as possible, the Pearson index correlation was calculated.

$P < 0.05$ was considered to be statistically significant. Data are expressed as means ± SE.

3. Results

3.1. CellTiter-blue cell viability assay (Promega) results

Hela cells were treated with the 6 compounds and incubated for 3, 6 and 24 h. Cell viability assay results show that no statistically significant decrease in the number of viable cells was obtained in any of the three experimental times. In detail, quercetin was tested at the following concentrations: 1 μM, 5 μM, 10 μM, 20 μM and 50 μM. Analysis of the data showed a no significant p-value and a negative linear correlation coefficient, index of a strong negative correlation between the two variables, both at 3h ($p = 0.81$, $r = -0.88$), that at 6 h ($p = 0.97$, $r = -0.95$) and 24 h ($p = 0.06$, $r = -0.88$), respectively (Fig. 3A). A no significant p-value and a negative linear correlation coefficient it was obtained also for ferulic acid, tyrosol, berberine, catechin and curcumin, tested at same concentrations of quercetin. (Fig. 3B–F).

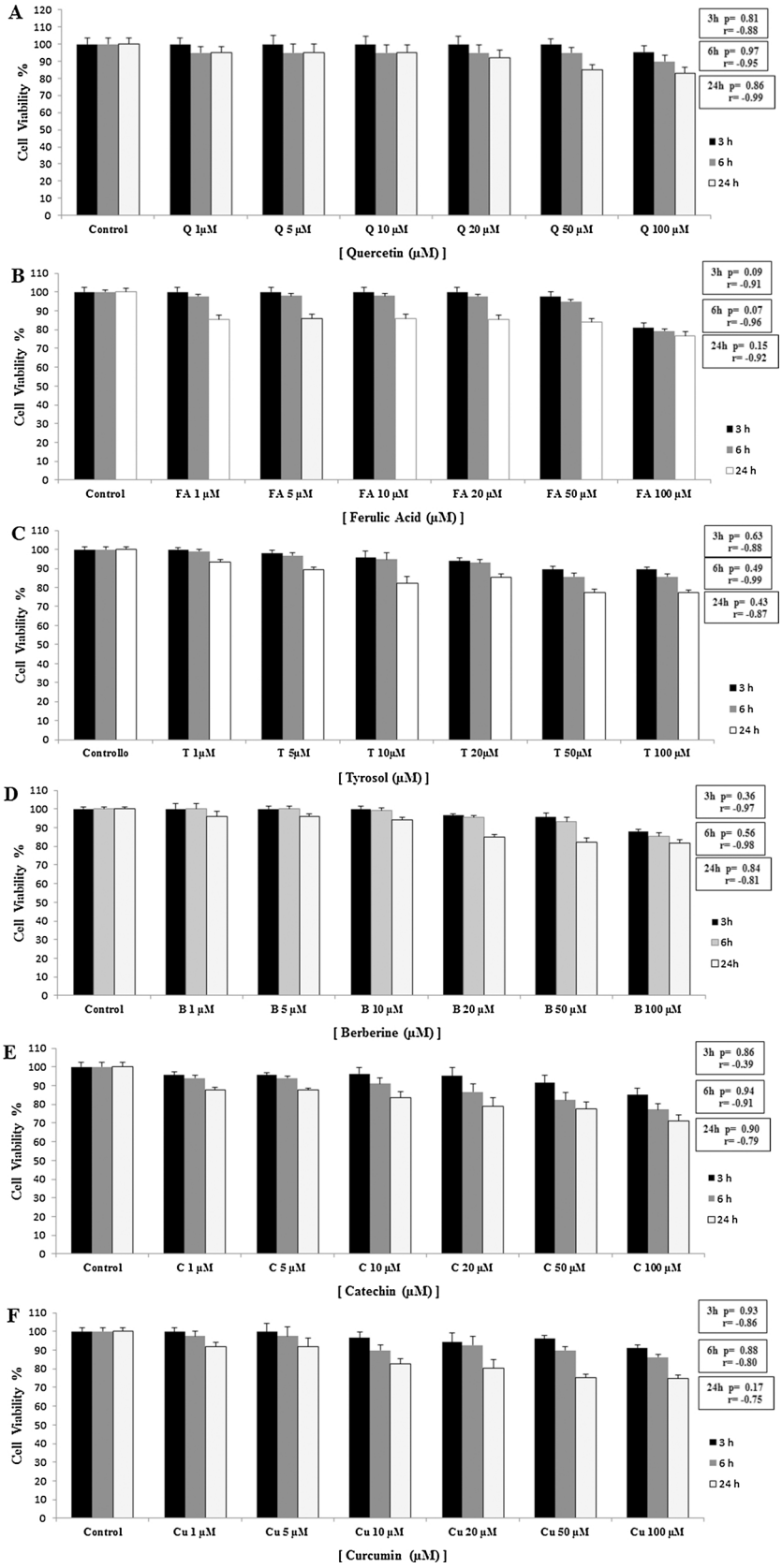

Fig. 3. CellTiter-Blue Cell Viability Assay (Promega) results: no statistically significant decrease in the number of viable cells was obtained in any of the experimental conditions. Hela cells were treated with the 6 compounds, tested at six different concentration (1 µM, 5 µM, 10 µM, 20 µM, 50 µM and 100 µM) and incubated for 3, 6 and 24 h. Indeed, analysis of the data (one-way ANOVA) showed a no significant p-value and a negative linear correlation coefficient, index of a strong negative correlation between the two variables, for all the 6 compounds. A) Quercetin (Q) : 3 h ($p = 0.81$, $r = -0.88$), 6 h ($p = 0.97$, $r = -0.95$) and 24 h ($p = 0.86$, $r = -0.99$), respectively. B) Ferulic Acid (FA) : 3 h ($p = 0.09$, $r = -0.91$), 6 h ($p = 0.07$, $r = -0.96$) and 24 h ($p = 0.15$, $r = -0.92$), respectively. C) Tyrosol (T) : 3 h ($p = 0.63$, $r = -0.98$), 6 h ($p = 0.49$, $r = -0.99$) and 24 h ($p = 0.43$, $r = -0.87$), respectively. D) Berberine (B) : 3 h ($p = 0.36$, $r = -0.97$), 6 h ($p = 0.56$, $r = -0.98$) and 24 h ($p = 0.84$, $r = -0.81$), respectively. E) Catechin (C) : 3 h ($p = 0.86$, $r = -0.39$), 6h ($p = 0.94$, $r = -0.91$) and 24 h ($p = 0.90$, $r = -0.79$), respectively. F) Curcumin (Cu) : 3 h ($p = 0.93$, $r = -0.86$), 6 h ($p = 0.88$, $r = -0.80$) and 24 h ($p = 0.17$, $r = -0.75$), respectively.

3.2. Total oxyradical scavenging capacity (TOSC) assay

Quercetin, ferulic acid, tyrosol, berberine, catechin and curcumin were tested at 1 µM to evaluate their Total Oxyradical Scavenging Capacity. Fig. 4 shows the specific TOSC values of natural compounds towards the various RONS, as compared to the reference antioxidant vs. peroxyl, hydrodyl radicals and peroxynitrite derivates according to Franzoni et al. [42]. Quercetin showed the best antioxidant activity

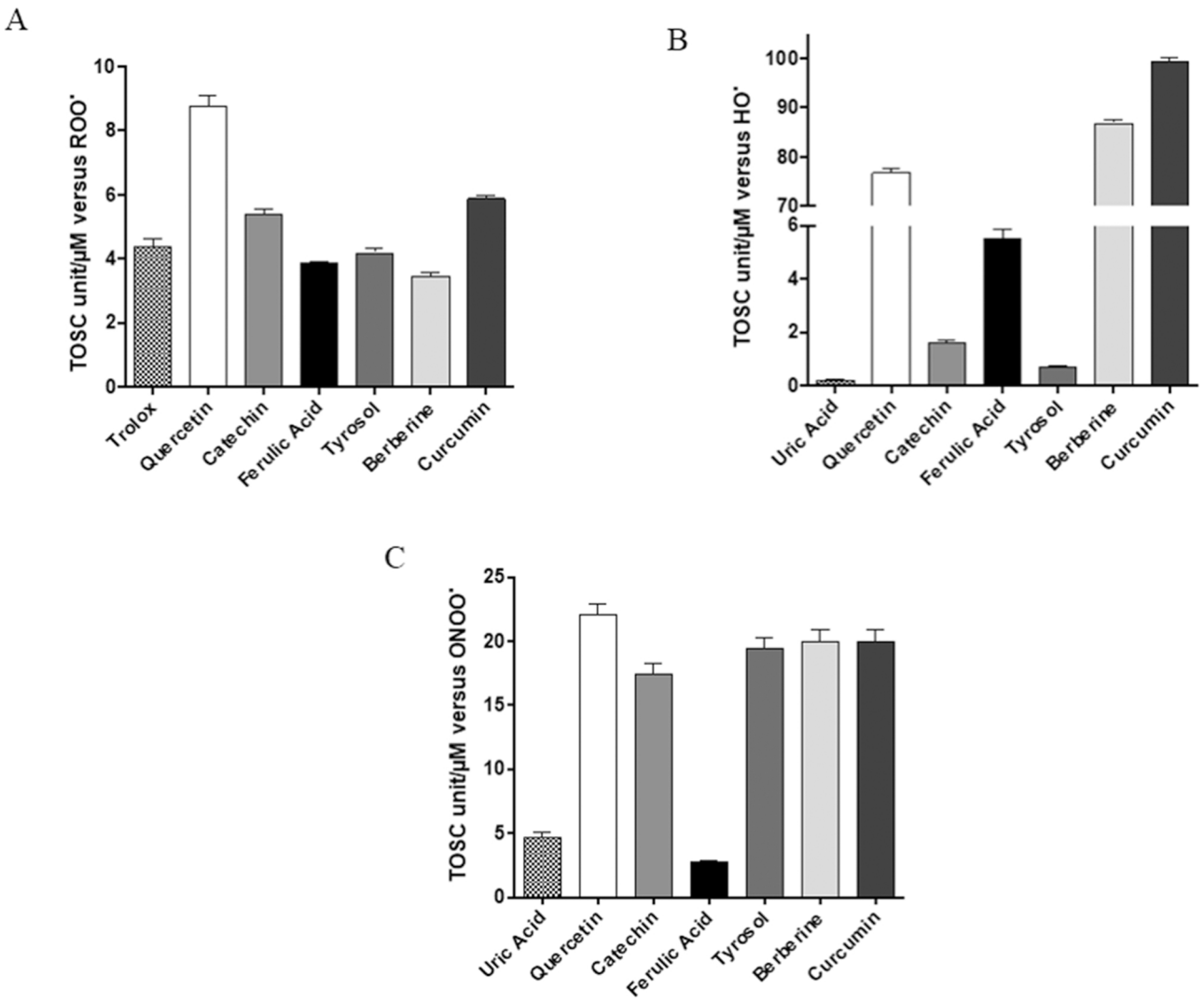

Fig. 4. Comparison between specific antioxidant activity of the analyzed natural compounds and the relative antioxidant reference. Quercetin, ferulic acid, tyrosol, berberine, catechin and curcumin were tested at 1 µM to evaluate their Total Oxyradical Scavenging Capacity. Quercetin has the higher antioxidant activity towards peroxyl radicals and peroxynitrate derivates than Trolox (***$p < 0.001$) and uric acid (***$p < 0.001$) rispectively. Curcumin has the higher antioxidant activity than Uric acid (****$p < 0.0001$). A) Analyzed natural compounds versus ROO. compared to TROLOX, the relative antioxidant reference ; B) Analyzed natural compounds versus HO. compared to uric acid, the relative antioxidant reference ; C) Analyzed natural compounds versus ONOO. compared to uric acid, the relative antioxidant reference.

against peroxyl radicals (8,76 ± 0,8 units) than the other compounds ($0.05 > p < 0.001$), and its activity was 99% higher than Trolox (4,4 ± 0,6 units, ***$p < 0.001$) the reference antioxidant against peroxyl radicals [40]. Catechins (5,41 ± 0,4 units) and curcumin (5,9 ± 0,18 units) exhibited an anti-peroxyl activity higher than Trolox (***$p < 0.001$) of 22,9% and 34,09 respectively, while ferulic acid (3,88 ± 011 units), tyrosol (4,2 ± 0,32 units) and berberine (3,47 ± 0,23 units) showed a lower anti-peroxyl activity than Trolox (***$p < 0.001$) of 11,8%, 4,55% and 21,13% respectively.

Curcumin exhibited the higher antioxidant activity (99,5 ± 0,5 units) against hydroxyl radicals than the other compounds and its activity was 4728,091% higher than uric acid (0,21 ± 004 units; $p < 0.001$) the reference antioxidant against hydroxyl radicals [40]. Compared to uric acid, quercetin (76,92 ± 1,61 units), catechins (1,62 ± 0,21 units), ferulic acid (5,56 ± 0,76 units), tyrosol (0,75 ± 0,02 units), and berberine (87,01 ± 152) showed and anti-hydroxyl activity higher of 3,652,857% (****$p < 0.0001$), 67,142% (***$p < 0.001$), 254,761% (****$p < 0.0001$), 25,714% (***$p < 0.001$) and 41333,34% (****$p < 0.0001$), respectively.

Quercetin presents the best antioxidant activity even against peroxynitrite derivates (22,16 ± 1,81 units). Indeed, compared to uric acid (4,7 ± 0,9 units), the reference anti-peroxynitrite derivates [40] its activity was 371,49% (***$p < 0.001$) higher. ferulic acid (17,52 ± 1,76 units), catechins (2,78 ± 0,21 units), tyrosol (19,49 ± 1,91 units), berberine (20,01 ± 212) and curcumin (20,04 ± 214) exhibited an antioxidant activity against peroxynitrite derivates higher than uric acid of 272,76%(***$p < 0.001$), 40,85% (**$p < 0.01$), 314,68% (***$p < 0.001$), 325,74%(***$p < 0.001$) and 326,38% (***$p < 0.001$) respectively.

3.3. The expression of SIRT1 and the activation of AMPK were significantly increased in all experimental groups compared with the control group

Quercetin, ferulic acid, tyrosol, berberine, catechin and curcumin were tested at three different concentrations (1, 5, and 10 µM) and at three different time of incubation (3, 6 and 24 h). The Western Blot analysis of the lysates of the cells incubated with these compounds showed that the expression of SIRT1 was significantly increased in all experimental groups compared with the control group (Fig. 5A–D). In detail, quercetin and berberine showed a statistically significant increase of SIRT1 expression, compared to the control, both at 3 h (1 and

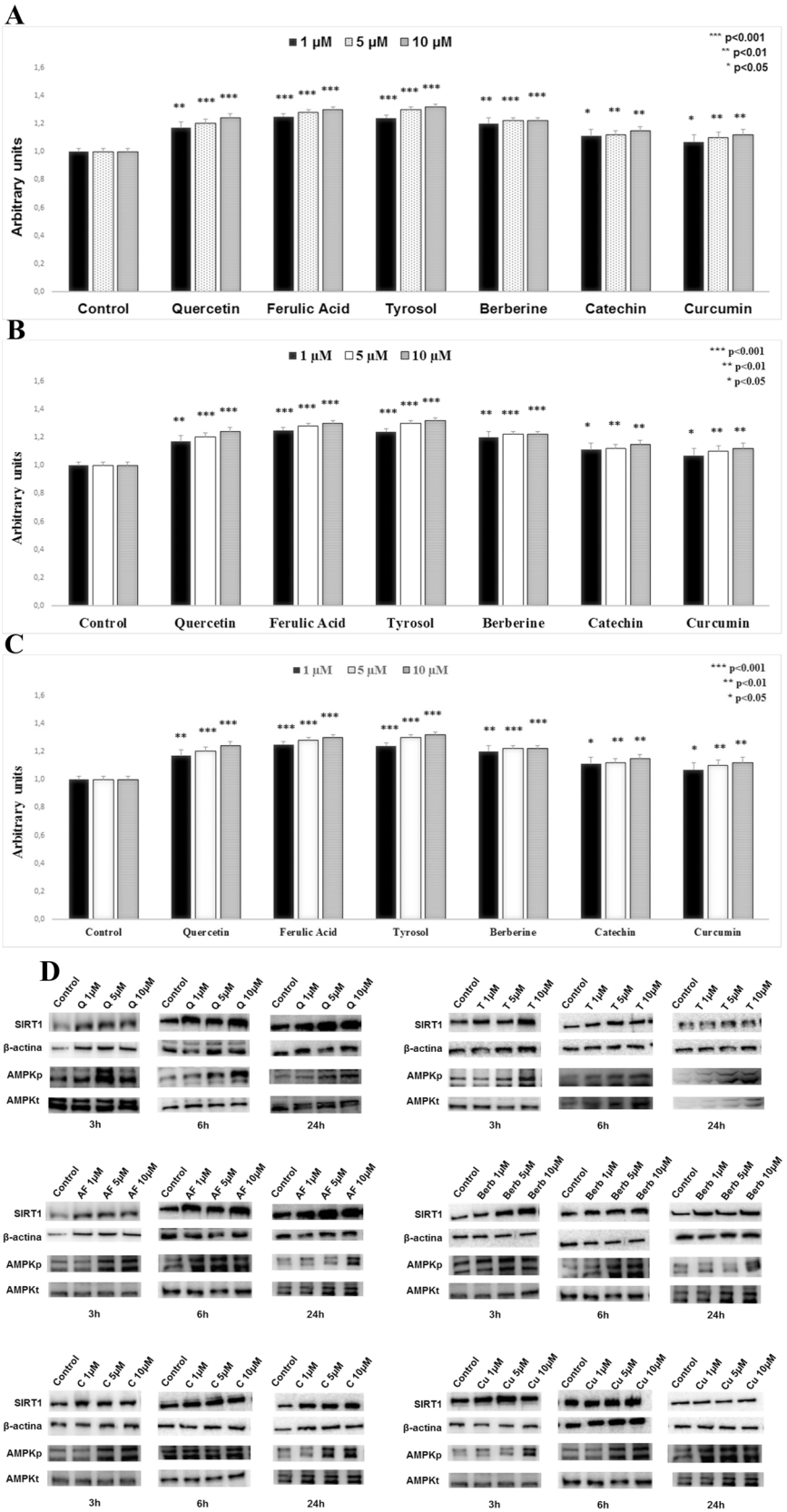

Fig. 5. Western blot analysis of the expression of SIRT1.The expression of SIRT1 was significantly increased in all experimental groups compared with the control group. A) 3 h; B) 6 h; C) 24 h. D) Representative Western blots. The expression of SIRT1 and the activation of AMPK was significantly increased in all experimental groups, at all the concentrations and all the experimental times. ***$p < 0.001$, **$p < 0.01$, * $p < 0.05$ vs control.

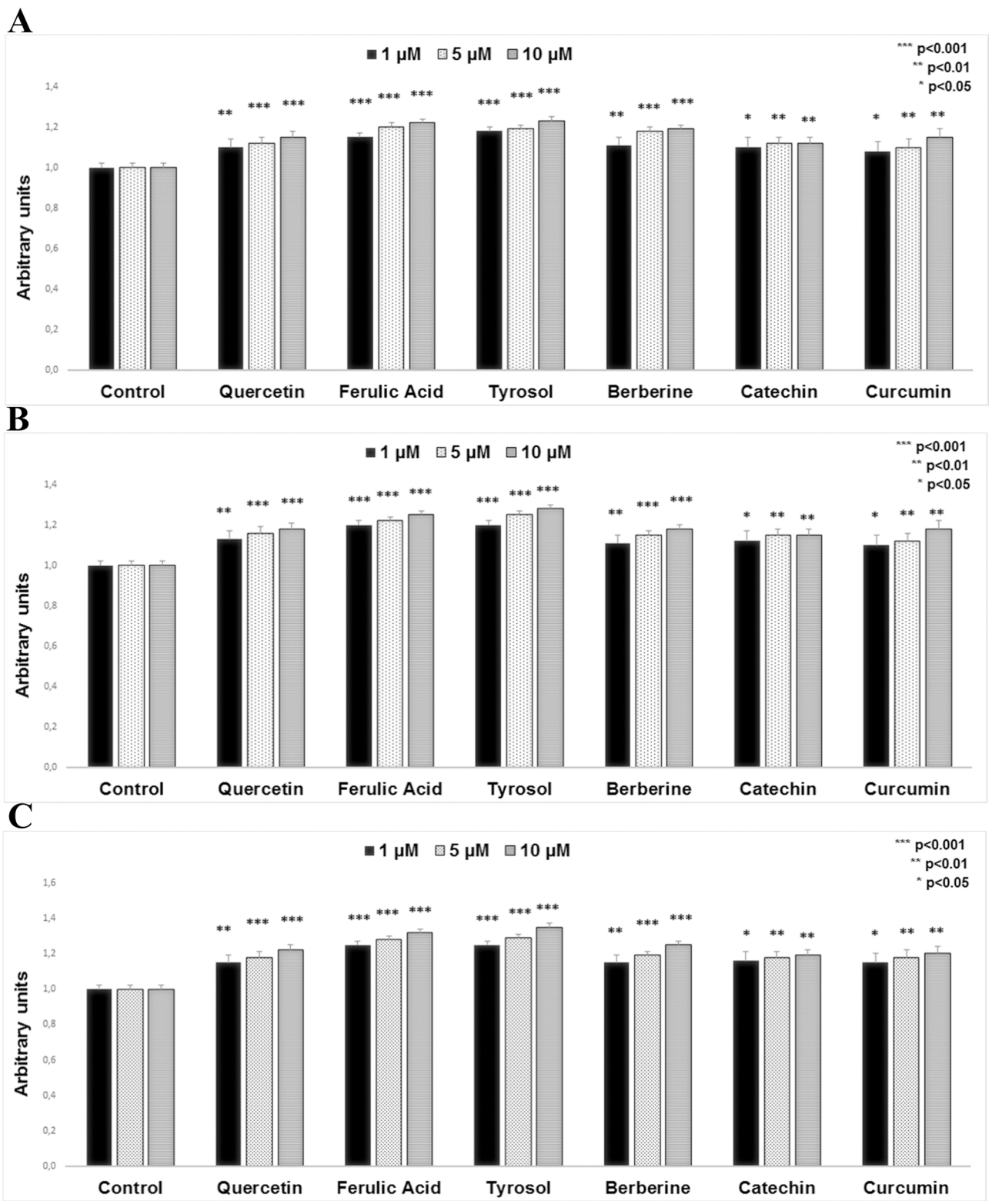

Fig. 6. Western blot analysis of the activation of AMPK. The activation of AMPK was significantly increased in all experimental groups compared with the control group. A) 3 h; B) 6 h; C) 24 h. ***p < 0.001, **p < 0.01, * p < 0.05 vs control.

5 µM: **p < 0.01, 10 µM: ***p < 0.001; Fig. 5A), that at 6 h (1 µM: **p < 0.01, 5 and 10 µM: ***p < 0.001; Fig. 5B) and 24 h (***p < 0.001; Fig. 5C,) respectively. Ferulic acid and tyrosol also showed a statistically significant increase of SIRT1 expression, compared to the control, both at 3 h (1 µM: **p < 0.01, 5 and 10 µM: ***p < 0.001; Fig. 5A), that at 6 h (1 µM: **p < 0.01, 5 and 10 µM: ***p < 0.001; Fig. 5B) and 24 h (***p < 0.001; Fig. 5C), respectively. Finally, catechin and curcumin showed a statistically significant increase of SIRT1 expression, compared to the control, both at 3 h (*p < 0.05; Fig. 5A), that at 6 h (1 and 5 µM: *p < 0.05, 10 µM: **p < 0.01; Fig. 5B) and 24 h (1 and 5 µM: *p < 0.05, 10 µM: **p < 0.01; Fig. 5C), respectively. It was subsequently verified if the

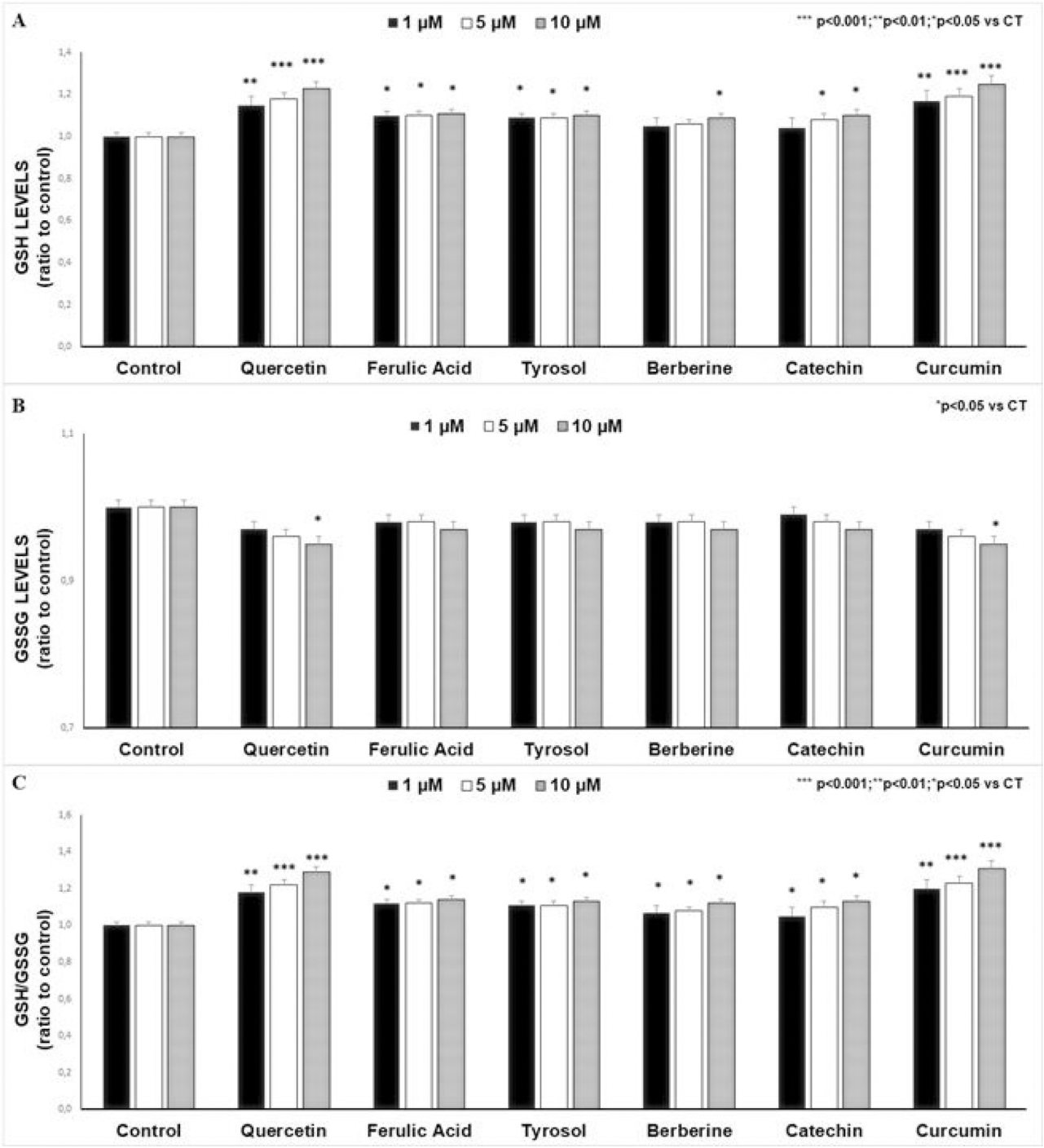

Fig. 7. GSH, GSSG, GSH/GSSG ratio levels, incubation time 3 h. Quercetin, ferulic acid, tyrosol, berberine, catechin and curcumin treatment induced a statistically significant increase of total GSH and GSH/GSSG ratio levels and a significant decrease of GSSG levels in all experimental groups compared with the control group. CT = control. A) GSH levels; B) GSSG levels; C) GSH/GSSG ratio levels. ***p < 0.001, **p < 0.01, * p < 0.05 vs CT.

compounds, proven to be SIRT1 activators, were also able to modulate and interact with the AMPK pathway, leading to a statistically significant increase of AMPK. Data analysis showed that the activation of AMPK is significantly increased in all experimental groups compared with the control group, at all the concentrations and all the experimental times (Figs. 6A–C).

3.4. GSH, GSSG and GSH/GSSG ratio levels were significantly modulates in all experimental groups compared with the control group

GSH is an important cellular antioxidant that protects cells against ROS-induced injury [43] damage by removing hydrogen peroxide (H2O2) and inhibiting lipid peroxidation [44]. The efficient transformation of GSH to GSSG has been suggested to be a marker of redox capacity to explain the cellular redox environment [45], while the GSH/GSSG ratio is considered to be a sensitive indicator of oxidative stress [46]. Thus, to evaluate the impact of the tested polyphenols on oxidative stress, the levels of GSH, GSSG and GSH/GSSG ratio were determined. As shown in Figs. 7–9 polyphenols and berberine treatment induced a statistically significant increase of total GSH and GSH/GSSG ratio levels, while a significant decrease of GSSG levels in all experimental groups compared with the control group (*p < 0.05;

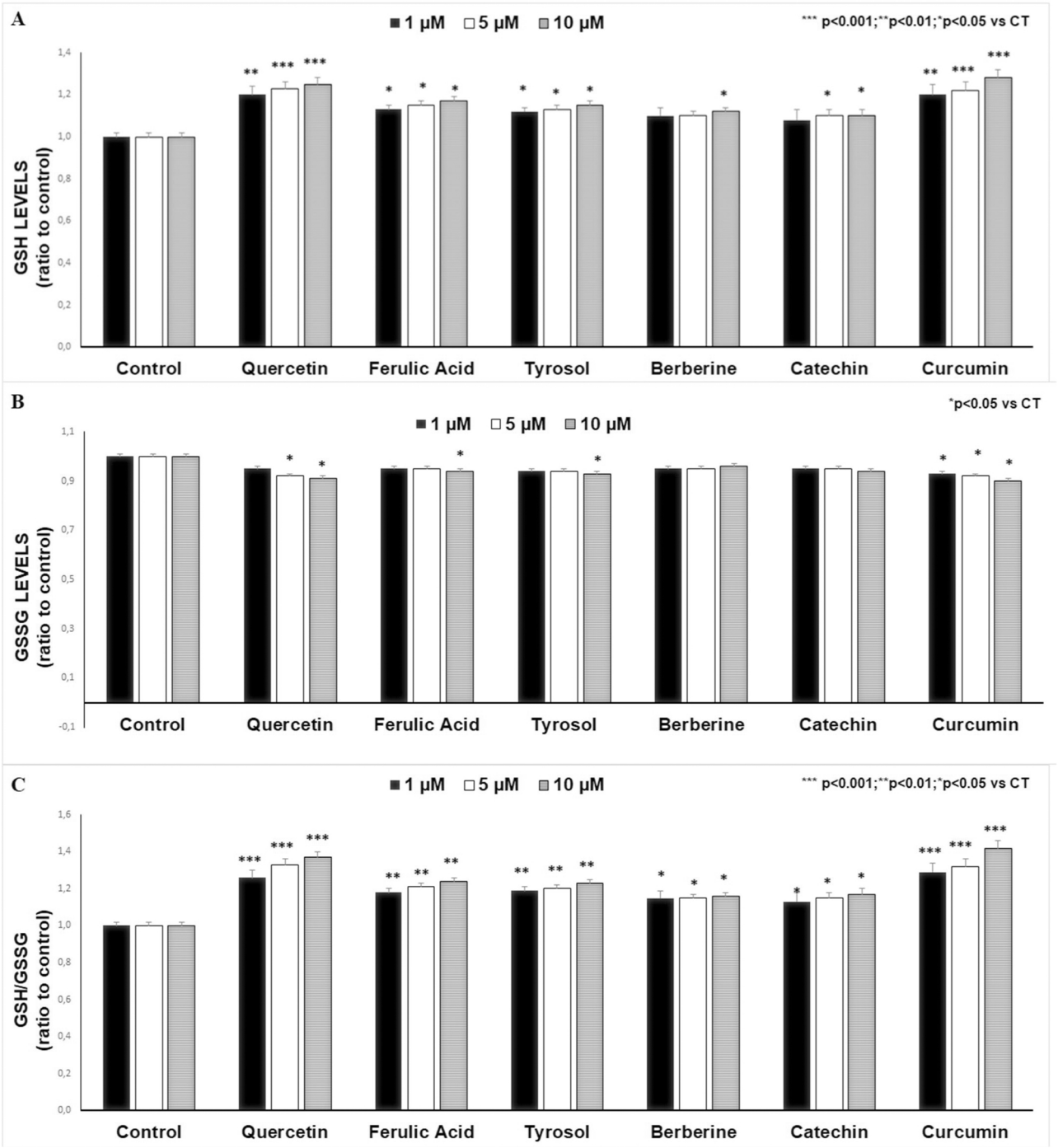

Fig. 8. GSH, GSSG, GSH/GSSG ratio levels, incubation time 6 h. Quercetin, ferulic acid, tyrosol, berberine, catechin and curcumin treatment induced a statistically significant increase of total GSH and GSH/GSSG ratio levels and a significant decrease of GSSG levels in all experimental groups compared with the control group. CT = control. A) GSH levels; B) GSSG levels; C) GSH/GSSG ratio levels. ***p < 0.001, **p < 0.01, * p < 0.05 vs CT.

**p < 0.01; ***p < 0.001). In detail, quercetin and curcumin showed the best antioxidant activity at all the concentrations and the experimental times (3, 6 and 24 h) followed by ferulic acid and tyrosol. Also berberine and catechin showed an antioxidant activity, even if lower than the others compounds.

4. Discussion

There has been growing evidence that dietary antioxidants act as free radical scavengers to counteract RONS [6–8]. The molecules mainly involved in the reduction of RONS induced damage are polyphenols [47–51] and berberine [52–60]. The beneficial effects of these natural compounds in human health and diseases seem to be related to their “direct” and “indirect” antioxidant activity [61–63]. The direct activity is related to in vitro metal chelation, inhibition of lipid peroxidation, RONS scavenging, reduction of hydroperoxide formation and quenching of electronically excited compounds [64–68]. On the other hand, the “indirect” effects are mainly related to the modulation of cell signalling pathways and gene expression, to the regulation of defence enzymes and changes in nuclear histone acetylation [68–72].

In support of this view, our results provide evidence that all the analysed molecules expressed a real and specific antioxidant activity.

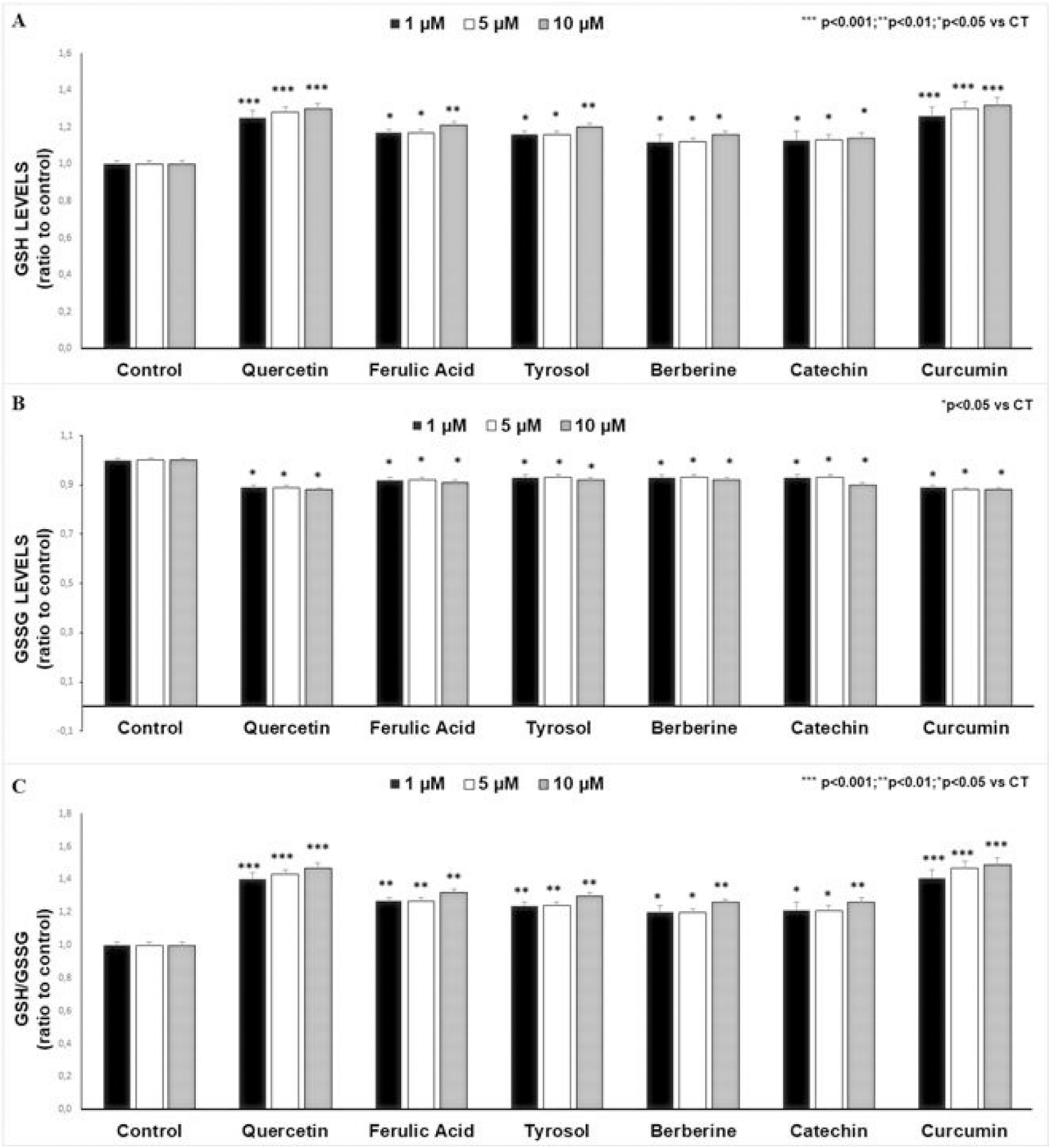

Fig. 9. GSH, GSSG, GSH/GSSG ratio levels, incubation time 24 h. Quercetin, ferulic acid, tyrosol, berberine, catechin and curcumin treatment induced a statistically significant increase of total GSH and GSH/GSSG ratio levels and a statistically significant decrease of GSSG levels in all experimental groups compared with the control group. CT = control. A) GSH levels; B) GSSG levels; C) GSH/GSSG ratio levels. ***p < 0.001, **p < 0.01, * p < 0.05 vs CT.

However, most interesting results were observed when the specific antioxidant capabilities were compared to each other.

Briefly, quercetin expressed the best antioxidant activity against peroxyl radicals and peroxynitrite derivates, while curcumin against hydroxyl radicals. In detail, against peroxyl radicals and peroxynitrite derivates quercetin showed a higher antioxidant activity, not only compared to the other molecules, but especially to Trolox and uric acid, the respective reference antioxidants. Even catechin and curcumin exhibited an antioxidant activity higher than Trolox and uric acid. Contrariwise, ferulic acid, tyrosol and berberine showed a lower antioxidant capability compared to Trolox and a higher antioxidant activity compared to uric acid. Thus, the antioxidant activity of quercetin is remarkable against peroxyl radicals and peroxynitrite derivates, but not against hydroxyl radicals. Several studies have showed the important role of quercetin in many physiopathological conditions, suggesting how its ability to scavenge highly reactive species, such as peroxynitrite and the peroxyl radicals, could be involved in its possible beneficial health effects [73,74]. Already in 1994, Hanasaki et al. [75], suggested that quercetin was the most potent scavenger of ROS, then confirmed by Cushnie et al. [76] and Saw et al. [77]. They indeed proved that quercetin at concentrations of 5–50 µM could directly scavenge ROS in vitro. Quercetin has been shown to be an excellent in vitro antioxidant,

able to counteract radical species, and this property seems to be related to the presence and location of the hydroxyl (OH) substitutions [78]. In this respect, Heijnen et al. [79] have demonstrated the antioxidative capacities of quercetin, attributing them to the presence of two antioxidant pharmacophores within the molecule that have the optimal configuration for free radical scavenging, i.e. the catechol group in the B ring and the OH group at position 3 of the AC ring. Moreover, Arts et al. [80] suggested that quercetin empower the endogenous antioxidant shield due to its contribution to the total plasma antioxidant capacity, which is 6.24 times higher than the reference antioxidant Trolox, whereas, for example, the contribution of both vitamin C and uric acid virtually equals that of Trolox. In human metabolism, quercetin seems to inhibit glucose-, ribose-, and MGO-mediated AGE formation, with trapping reactive oxygen species [81,82].

Instead, as for the hydroxyl radicals, we found that curcumin exhibited the best anti-hydroxyl activity compared to the other compounds and its ability was higher than that of uric acid, the reference antioxidant. In this case, also the others tested compounds showed an antioxidant capability higher than uric acid. Curcumin has traditionally been regarded as an antioxidant for thousands of years. Shortly, the protective effects of curcumin against oxidative stress can be summarized as follows: upregulating endogenous antioxidant pathways, including SOD, glutathione (GSH), catalase; inhibiting ROS production and eliminating it; preserving mitochondrial function [83–85]. Curcumin seems to act as a superoxide radical scavenger and as a singlet oxygen quencher. Already in 1994, Reddy et al. described this natural compound as a potent scavenger of a variety of reactive oxygen species, especially hydroxyl radicals [86]. Ahsan et al. reported that curcumin, Bisdemethoxycurcumin and Demethoxycurcumin (derivates of curcumin) are able to degrade DNA in the presence of Cu(II), inhibiting damage to super coiled plasmid DNA by hydroxyl radicals [87]. Ak et al. confirmed all the previous studies demonstrating that curcumin is an effective antioxidant in different in vitro assays, including reducing power, DPPH, ABTS+, O2− and DMPD+ radical scavenging, hydrogen peroxide scavenging and metal chelating activities, when compared to standard antioxidant compounds, such as BHA, BHT, α-tocopherol, a natural antioxidant, and Trolox [88]. Finally, Borra et al. [89], based on various in vitro and ex vivo assays, hypothesized that strong antioxidant activity exhibited by curcumin could be related with the presence of phenolic components [89,90]. However, many studies have instead focused on the ability of polyphenols and berberine to modulate the GSH profiles, thus confirming their protective effect against oxidative stress [91–95]. Gordillo et al. observed a protective action of curcumin against acute liver damage by inhibiting oxidative stress in rats, in which acute hepatotoxicity was induced by oral administration of CCL_4 (Carbon Tetrachloride) [96]. In detail, they demonstrated that the GSH significantly decrease in CCl4-treated rats could be prevented by curcumin administration. Indeed, the group receiving both curcumin and CCl4 showed significantly higher levels of GSH than control. Moreover, the GSH/GSSG ratio decreased following CCl4 administration, while curcumin preserved it at normal levels [96]. Total glutathione (GSH + GSSG) decreased significantly in CCl4-treated rats, but increased significantly in the group that received both curcumin and CCl4, and in rats administered with curcumin alone [96]. In 2009, Lavoie et al. proved curcumin and quercetin modulation of glutathione levels in astrocytes and neurons [97]. These data was also confirmed by Li et al. in human aortic endothelial cells [98] and by Lee et al. in CCl_4-induced acute hepatic stress rats [99]. According with these studies, also our data showed a statistically significant increase of total GSH and GSH/GSSG ratio levels, while a significant decrease of GSSG levels in all experimental groups compared with the control group. These results confirm what has already been observed by the TOSC assay or rather, the best antioxidant activity is that obtained by treating the cells with quercetin and curcumin. Ferulic acid and tyrosol showed an intermediate antioxidant activity compared to all the other compounds, while berberine and catechin showed the lower antioxidant activity

The fundamental mechanism of action underlying polyphenols' and berberine's impact on human health is probably their action on SIRT1 and AMPK. Consistent with this hypothesis, we proved that all the tested substances stimulated both SIRT1 and AMPK.

Many recent studies have highlighted the importance of SIRT1 and AMPK in many pathophysiological processes, showing how their activation could be crucial in this context and potentially protective against oxidative stress. SIRT1 can influence either directly or indirectly the redox property of cells and regulates a variety of processes that alter cell response to genotoxicity, including the detoxification of ROS by up-regulation of MnSOD. Elliott et al. proved that SIRT1 reduces cellular oxidative stress indirectly through deacetylation of FOXO3 and it leads to up-regulation of catalase and MnSOD [100]. Consistent with these data, Tanno et al. have showed how SIRT1 also regulates aging and oxidative stress in the cardiomyocytes [101]. Previously, Alcendor et al. revealed the relationship between the SIRT1 expression and the level of oxidative stress in mouse cardiac muscle [102]. Using the over-expression technique, they demonstrated that the moderate over-expression of SIRT1 could protect against oxidative stress by inducing the expression of catalase. In contrast, the high level of SIRT1 expression (12.5-fold increase) clearly increased oxidative stress and evoked pathological changes in the heart. Furthermore, SIRT1 promotes mitochondrial biogenesis by activating PGC-1α (peroxisome proliferator-activated receptor co-activator 1-α) and inactivates the p65 subunit of NF-κB through direct deacetylation [103]. Finally, SIRT1 can also inhibit some other transcription factors, which are involved in the regulation of cellular redox balance. Kawai et al. [104] demonstrated that SIRT1-mediated deacetylation of NRF2 protein terminated the transcription of antioxidant genes e.g., driving the expression of glutathione peroxidase 2, peroxiredoxin 4 and thioredoxin reductase [104,105].

Recently, other studies have shown that AMPK may be an oxidative stress sensor and redox regulator in addition to its traditional role as an energy sensor and regulator [106–109]. Clearly, AMPK is functional as a redox sensor that can be quickly activated by increased intracellular ROS/RNS [110–112]. Activated AMPK appears to be essential in maintaining intracellular redox status by inhibiting oxidant production by NADPH oxidases, mitochondria or by increasing the expression of antioxidant enzymes, such as SOD2 [113–115]. In 2004, Alba et al. showed that activation of AMPK by AICAR suppresses O2− by reducing the translocation and phosphorylation of p47phox in human neutrophils [116]. Similarly, Hwang et al. proved that rosiglitazone reduces high-glucose-induced NADPH-oxidase-mediated oxidative stress in a manner dependent on AMPK activation in HUVECs (human umbilical vein endothelial cells) [117]. Moreover, Piwkowska et al. revealed metformin ability to suppress high-glucose-induced oxidative stress in cultured podocytes by an AMPK-mediated reduction of NADPH oxidase activity [118]. In addition, emerging evidence suggests that AMPK also plays an important role in the regulation of cellular antioxidant defenses.

Therefore, activation of SIRT1 and AMPK by polyphenols and berberine seems to be beneficial in the oxidative stress regulation and in counteracting the chronic imbalance between formation of RONS and antioxidant systems.

5. Conclusion

Many studies, in recent years suggested a relationship between dietary antioxidants, such as polyphenols and berberine, SIRT1/AMPK and the oxidative stress, underlining how their activation could be crucial in this context and potentially protective against oxidative stress.

Our results clearly disclose the specific antioxidant activity of these natural compounds and their ability to increase the expression of SIRT1 and the activation of AMPK. Thus, they may potentially find a preventive or curative pharmacological application in the pathogenesis

and development of a variety of chronic and degenerative diseases, including aging, cancer, cardiovascular disease and neurodegenerative disorders (Alzheimer's and Parkinson's Diseases), in which RONS are a relevant determinant. Nevertheless, the problems related to their bioavailability can't be disregarded. About this, some researches consider their poor bioavaibility in the human body the main drawback to using polyphenols [119]. However, on the other hand, some studies confine this poor bioavaibility when used alone, highlighting how an approach to counteracting this effect may be a combination treatment with several polyphenols or with polyphenols and other drugs, as shown in our previous work [120].

In addition, much of the evidence on the beneficial effects of polyphenols on diseases are derived from in vitro or animal models, which are often performed with doses/concentrations much higher than those to which humans are exposed through the diet [121–123]. A better clarification and understanding of the mechanisms presumably involved in the protective role of polyphenols against oxidative stress will help to more precisely define the clinical situations where polyphenol consumption will prove to be beneficial.

Therefore, further in vitro and in vivo studies are required to overcome the mentioned issues limits, to elucidate on the mechanism of the in vivo effects of polyphenols and to development of specifically pharmacological SIRT1/AMPK activators, a crucial step in understanding their potentially clinical application.

Author contributions

"J.F., S.B. conducted the experiments.; S.B. bought the substances; J.F., S.B., S.D. and F.F. analysed the data; J.F., S.B. and F.F. wrote the manuscript; J.F., S.B., L.G., F.G., S.P. and F.F. designed the study and provided overall supervision for the project; all authors contributed to the drafting and critical revision of the manuscript and have given final approval of the version to be published.

Conflicts of interest

"The authors declare no conflict of interest."

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgements

The authors acknowledge the laboratory staff and the trainees for their participation and their continued commitment to some experimental phases and especially in the development of some western blot.

References

[1] S. Saeidnia, M. Abdollahi, Toxicological and pharmacological concerns on oxidative stress and related diseases, Toxicol. Appl. Pharmacol. 273 (2013) 442–455, http://dx.doi.org/10.1016/j.taap.2013.09.031.
[2] L.A. Pham-Huy, H. He, C. Pham-Huy, Free radicals, antioxidants in disease and health, Int. J. Biomed. Sci. 4 (2008) 89–96.
[3] Z. Duracková, Some current insights into oxidative stress, Physiol. Res. 59 (2010) 459–469 Review.
[4] V.J. Thannickal, B.L. Fanburg, Reactive oxygen species in cell signaling, Am. J. Physiol. Lung Cell. Mol. Physiol. 279 (2000) L1005–L1028.
[5] S.P. Hussain, L.J. Hofseth, C.C. Harris, Radical causes of cancer, Nat. Rev. Cancer 3 (2003) 276–285 Review.
[6] B. Poljsak, Strategies for reducing or preventing the generation of oxidative stress, Oxid. Med. Cell. Longev. 2011 (2011) 194586, http://dx.doi.org/10.1155/2011/194586 Review.
[7] A. Karadag, B. Ozcelik, S. Saner, Review of methods to determine antioxidant capacities, Food Anal. Methods 2 (2009) 41–60.
[8] J.W. Finley, A.N. Kong, K.J. Hintze, E.H. Jeffery, L.L. Ji, X.G. Lei, Antioxidants in foods: state of the science important to the food industry, J. Agric. Food Chem. 59 (2011) 6837–6846, http://dx.doi.org/10.1021/jf2013875 Review.
[9] S. Kumar, A.K. Pandey, Free radicals: health implications and their mitigation by herbals, Br. J. Med. Med. Res. 7 (2015) 438–457.
[10] M.J. Kim, J.N. Hyun, J.A. Kim, et al., Relationship between phenolic compounds, anthocyanins content and antioxidant activity in colored barley germplasm, J. Agric. Food Chem. 55 (2007) 4802–4809.
[11] M. Omwamba, F. Li, G. Sun, et al., Antioxidant effect of roasted barley (Hordeum vulgare L.) grain extract towards oxidative stress in vitro and in vivo, Food Nutr. Sci. 4 (2013) 139–146, http://dx.doi.org/10.4236/fns.2013.48A017.
[12] A. Bruckbauer, M.B. Zemel, Synergistic effects of polyphenols and methylxanthines with leucine on AMPK/Sirtuin-mediated metabolism in muscle cells and adipocytes, PLoS One (2014), http://dx.doi.org/10.1371/journal.pone.0089166.
[13] S. Wang, N. Moustaid-Moussa, L. Chen, et al., Novel insights of dietary polyphenols and obesity, J. Nutr. Biochem. 25 (2014) 1–18, http://dx.doi.org/10.1016/j.jnutbio.2013.09.001.
[14] S. Kumar, A.K. Pandey, Chemistry and biological activities of flavonoids: an overview, Sci. World J. 2013 (2013) 162750, http://dx.doi.org/10.1155/2013/162750.
[15] V. Neveu, J. Perez-Jiménez, F. Vos, et al., Phenol-explorer: an online comprehensive database on polyphenol contents in foods, Database (Oxf.) 2010 (2010), http://dx.doi.org/10.1093/database/bap024 bap024.
[16] C. Manach, G. Williamson, C. Morand, et al., Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies, Am. J. Clin. Nutr. 81 (2005) 230S–242S Review.
[17] F. Malongane, L.J. McGaw, F.N. Mudau, The synergistic potential of various teas, herbs and therapeutic drugs in health improvement: a review, J. Sci. Food Agric. (2017), http://dx.doi.org/10.1002/jsfa.8472.
[18] A. Shirwaikar, A. Shirwaikar, K. Rajendran, I.S.R. Punitha, In vitro antioxidant studies on the benzyl tetra isoquinoline alkaloid berberine, Biol. Pharm. Bull. 29 (2006) 1906–1910.
[19] W. Kong, J. Wei, P. Abidi, et al., Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins, Nat. Med. 10 (2004) 1344–1351, http://dx.doi.org/10.1038/nm1135.
[20] Y. Jin, D.B. Khadka, W.J. Cho, Pharmacological effects of berberine and its derivatives: a patent update, Expert Opin. Ther. Pat. 26 (2016) 229–243, http://dx.doi.org/10.1517/13543776.2016.1118060.
[21] S.Q. Wen, P. Jeyakkumar, S.R. Avula, et al., Discovery of novel berberine imidazoles as safe antimicrobial agents by down regulating ROS generation, Bioorg. Med. Chem. Lett. 26 (2016) 2768–2773, http://dx.doi.org/10.1016/j.bmcl.2016.04.070.
[22] The anti-inflammatory potential of berberine in vitro and in vivo, Cancer Lett. 203 (2004) 127–137.
[23] H.R. Sadeghnia, M. Kolangikhah, E. Asadpour, et al., Berberine protects against glutamate-induced oxidative stress and apoptosis in PC12 and N2a cells, Iran. J. Basic Med. Sci. 20 (2017) 594–603, http://dx.doi.org/10.22038/IJBMS.2017.8847.
[24] L.C. Zhang, Y. Wang, L.C. Tong, et al., Berberine alleviates dextran sodium sulfate-induced colitis by improving intestinal barrier function and reducing inflammation and oxidative stress, Exp. Ther. Med. 13 (2017) 3374–3382, http://dx.doi.org/10.3892/etm.2017.4402.
[25] D.L. Chen, K.Y. Yang, Berberine alleviates oxidative stress in islets of diabetic mice by inhibiting miR-106b expression and up-regulating SIRT1, J. Cell. Biochem. (2017), http://dx.doi.org/10.1002/jcb.26089.
[26] W. Li, Y. Liu, B. Wang, et al., Protective effect of berberine against oxidative stress-induced apoptosis in rat bone marrow-derived mesenchymal stem cells, Exp. Ther. Med. 12 (2016) 4041–4048, http://dx.doi.org/10.3892/etm.2016.3866.
[27] S.R. Narala, R.C. Allsopp, T.B. Wells, et al., SIRT1 acts as a nutrient-sensitive growth suppressor and its loss is associated with increased AMPK and telomerase activity, Mol. Biol. Cell. 19 (2008) 1210–1219, http://dx.doi.org/10.1091/mbc.E07-09-0965.
[28] D.G. Hardie, AMPK: positive and negative regulation, and its role in whole-body energy homeostasis, Curr. Opin. Cell Biol. 33 (2015) 1–7, http://dx.doi.org/10.1016/j.ceb.2014.09.004.
[29] N.A. Shirwany, M.H. Zou, AMPK: a cellular metabolic and redox sensor. A minireview, Front. Biosci. 19 (2014) 447–474 Review.
[30] Y. Sun, J. Li, N. Xiao, et al., Pharmacological activation of AMPK ameliorates perivascular adipose/endothelial dysfunction in a manner interdependent on AMPK and SIRT1, Pharmacol. Res. 89 (2014) 19–28, http://dx.doi.org/10.1016/j.phrs.2014.07.006.
[31] S. Naiman, H.Y. Cohen, The contentious history of sirtuin debates, Rambam Maimonides Med. J. 3 (2012), http://dx.doi.org/10.5041/RMMJ.10093.
[32] R.H. Houtkooper, E. Pirinen, J. Auwerx, Sirtuins as regulators of metabolism and healthspan, Nat. Rev. Mol. Cell Biol. 13 (2012) 225–238, http://dx.doi.org/10.1038/nrm3293.
[33] H.C. Chang, L. Guarente, SIRT1 and other sirtuins in metabolism, Trends Endocrinol. Metab. 25 (2014) 138–145, http://dx.doi.org/10.1016/j.tem.2013.12.001.
[34] A.M. Davenport, F.M. Huber, A. Hoelz, Structural and functional analysis of human SIRT1, J. Mol. Biol. 426 (2014) 526–541, http://dx.doi.org/10.1016/j.jmb.2013.10.009.
[35] H. Yakamoto, L. Schoonjans, J. Auwerx, Sirtuin functions in health and disease, Mol. Endocrinol. 21 (2007) 1745–1755.
[36] H.S. Kwon, M. Ott, The ups and downs of SIRT1, Trends Biochem. Sci. 33 (2008) 517–525, http://dx.doi.org/10.1016/j.tibs.2008.08.001.
[37] C. Cantó, Z. Gerhart-Hines, J.N. Feige, et al., AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity, Nature 458 (2009) 1050–1060, http://dx.doi.org/10.1038/nature07813.

[38] F. Lan, J.M. Cacicedo, N. Ruderman, et al., SIRT1 modulation of the acetylation status, cytosolic localization, and activity of LKB1. Possible role in AMP-activated protein kinase activation, J. Biol. Chem. 283 (2008) 27628–27635, http://dx.doi.org/10.1074/jbc.M805711200.
[39] S. Bianchi, J. Fusi, F. Franzoni, et al., Effects of recombinant human erythropoietin high mimicking abuse doses on oxidative stress processes in rats, Biomed. Pharmacother. 82 (2016) 355–363, http://dx.doi.org/10.1016/j.biopha.2016.05.022.
[40] Y.J. Huh, J.M. Kim, H. Kim, et al., Regulation of osteoclast differentiation by the redox-dependent modulation of nuclear import of transcription factors, Cell Death Differ. 13 (2006) 1138–1146.
[41] E. Odkhyy, N. Koidel, B. Tsolmongyn, et al., Involvement of redox balance in in vitro osteoclast formation of RAW 264.7 macrophage cells in response to LPS, Innate Immun. 21 (2015) 194–202, http://dx.doi.org/10.1177/1753425914524242.
[42] S. Srivastava, D. Sinha, P.P. Saha, et al., Magmas functions as a ROS regulator and provides cytoprotection against oxidative stress-mediated damages, Cell Death Dis. 5 (2014) 1–15, http://dx.doi.org/10.1038/cddis.2014.355.
[43] E. Nur, M. Verwijs, D.R. de Waart, et al., Increased efflux of oxidized glutathione (GSSG) causes glutathione depletion and potentially diminishes antioxidant defense in sickle erythrocytes, Biochim. Biophys. Acta 1812 (2011) 1412–1417.
[44] M. Mizuashi, T. Ohtani, S. Nakagawa, et al., Redox imbalance induced by contact sensitizers triggers the maturation of dendritic cells, J. Invest. Dermatol. 124 (2005) 579–586.
[45] O. Zitka, S. Skalickova, J. Gumulec, et al., Redox status expressed as GSH:GSSG ratio as a marker for oxidative stress in paediatric tumour patients, Oncol. Lett. 4 (2012) 1247–1253.
[46] F. Franzoni, R. Colognato, F. Galetta, et al., An in vitro study on the free radical scavenging capacity of ergothioneine: comparison with reduced glutathione, uric acid and trolox, Biomed. Pharmacother. 60 (September) (2006) 453–457 Epub 2006 Aug 14. PubMed PMID: 16930933.
[47] Z. Ungvari, Z. Orosz, A. Rivera, et al., Resveratrol increases vascular oxidative stress resistance, Am. J. Physiol. Heart Circ. Physiol. 292 (2007) H2417–H2424.
[48] G. Spanier, H. Xu, N. Xia, et al., Resveratrol reduces endothelial oxidative stress by modulating the gene expression of superoxide dismutase 1 (SOD1), glutathione peroxidase 1 (GPx1) and NADPH oxidase subunit (Nox4), J. Physiol. Pharmacol. 60 (2009) 111–116.
[49] A.S. Strimpakos, R.A. Sharma, Curcumin: preventive and therapeutic properties in laboratory studies and clinical trials, Antioxid. Redox Signal. 10 (2008) 511–545, http://dx.doi.org/10.1089/ars.2007.1769 Review.
[50] T. Hussain, B. Tan, Y. Yin, et al., Oxidative stress and inflammation: what polyphenols can do for us? Oxid. Med. Cell. Longev. 2016 (2016) 1–9.
[51] M.T. Mitjavila, J.J. Moreno, The effects of polyphenols on oxidative stress and the arachidonic acid cascade. Implications for the prevention/treatment of high prevalence diseases, Biochem. Pharmacol. 84 (November) (2012) 1113–1122, http://dx.doi.org/10.1016/j.bcp.2012.07.017.
[52] Z. Li, Y.N. Geng, J.D. Jiang, et al., Antioxidant and anti-inflammatory activities of berberine in the treatment of diabetes mellitus, Evid. Based Complement. Altern. Med. 2014 (2014) 1–22, http://dx.doi.org/10.1155/2014/289264.
[53] T. Ahmed, A.U. Gilani, M. Abdollahi, et al., Berberine and neurodegeneration: a review of literature, Pharmacol. Rep. 67 (2015) 970–979, http://dx.doi.org/10.1016/j.pharep.2015.03.002.
[54] M.H. Jang, H.Y. Kim, K.S. Kang, et al., Hydroxyl radical scaveing activities of isoquinoline alkaloids isolated from coptis chinensis, Arch. Pharm. Res. 32 (2009) 341–345, http://dx.doi.org/10.1007/s12272-009-1305-z.
[55] J.F. Jiang, F. Lei, Z.Y. Yuan, et al., Mechanism underlying berberine's effects on HSP70/TNFα under heat stress: correlation with the TATA boxes, Chin. J. Nat. Med. 15 (2017) 178–191, http://dx.doi.org/10.1016/S1875-5364(17)30034-1.
[56] Y.L. Siow, L. Sarna, Redox regulation in health and disease – therapeutic potential of berberine, Food Res. Int. 44 (2011) 2409–2417.
[57] W.J. Shan, L. Huang, Q. Zhou, F.C. Meng, X.S. Li, Synthesis, biological evaluation of 9-N-substituted berberine derivatives as multi-functional agents of antioxidant, inhibitors of acetylcholinesterase, butyrylcholinesterase and amyloid-β aggregation, Eur. J. Med. Chem. 46 (2011) 5885–5893, http://dx.doi.org/10.1016/j.ejmech.2011.09.051.
[58] J.M. Hur, M.S. Hyun, S.Y. Lim, et al., The combination of berberine and irradiation enhances anti-cancer effects via activation of p38 MAPK pathway and ROS generation in human hepatoma cells, J. Cell. Biochem. 107 (2009) 955–964, http://dx.doi.org/10.1002/jcb.22198.
[59] L.K. Sarna, N. Wu, S.Y. Hwang, et al., Berberine inhibits NADPH oxidase mediated superoxide anion production in macrophages, Can. J. Physiol. Pharmacol. 88 (2010) 369–378, http://dx.doi.org/10.1139/Y09-136.
[60] D.U. Lee, Y.J. Kang, M.K. Park, et al., Effects of 13-alkylsubstituted berberine alkaloids on the expression of COX-II, TNF-a, iNOS, and IL-12 production in LPS-stimulated macrophages, Life Sci. 73 (2003) 1401–1412.
[61] A. Salminen, K. Kaarniranta, A. Kauppinen, Crosstalk between oxidative stress and SIRT1: impact on the aging process, Int. J. Mol. Sci. 14 (2013) 3834–3859, http://dx.doi.org/10.3390/ijms14023834.
[62] F. Giampieri, J.M. Alvarez-Suarez, M.D. Cordero, et al., Strawberry consumption improves aging-associated impairments, mitochondrial biogenesis and functionality through the AMP-activated protein kinase signaling cascade, Food Chem. 234 (2017) 464–471, http://dx.doi.org/10.1016/j.foodchem.2017.05.017.
[63] J.C. Serrano, H. Gonzalo-Benito, M. Jové, et al., Dietary intake of green tea polyphenols regulates insulin sensitivity with an increase in AMP-activated protein kinase α content and changes in mitochondrial respiratory complexes, Mol. Nutr. Food Res. 57 (2013) 459–470, http://dx.doi.org/10.1002/mnfr.201200513.
[64] M. Ferrali, C. Signorini, B. Caciotti, et al., Protection against oxidative damage of erythrocyte membrane by the flavonoid quercetin and its relation to iron chelating activity, FEBS Lett. 416 (1997) 123–129.
[65] E.N. Frankel, A.L. Waterhouse, J.E. Kinsella, Inhibition of human LDL oxidation by resveratrol, Lancet 341 (1993) 1103–1104.
[66] W.M. Loke, J.M. Proudfoot, J.M. Hodgson, et al., Specific dietary polyphenols attenuate atherosclerosis in apolipoprotein E-knockout mice by alleviating inflammation and endothelial dysfunction, Arterioscler. Thromb. Vasc. Biol. 30 (2010) 749–757, http://dx.doi.org/10.1161/ATVBAHA.109.199687.
[67] P.C. Hollman, A. Cassidy, B. Comte, et al., The biological relevance of direct antioxidant effects of polyphenols for cardiovascular health in humans is not established, J. Nutr. 141 (2011) 989S–1009S, http://dx.doi.org/10.3945/jn.110.131490.
[68] J.J. Haddad, Antioxidant and prooxidant mechanisms in the regulation of redox (y)-sensitive transcription factors, Cell. Signal. 14 (2002) 879–897.
[69] G. Duthie, A. Crozier, Plant-derived phenolic antioxidants, Curr. Opin. Lipidol. 11 (2000) 43–47.
[70] C.A. Rice-Evans, N.J. Miller, G. Paganga, Structure-antioxidant activity relationshipsof flavonoids and phenolic acids, Free Radic. Biol. Med. 20 (1996) 933–956.
[71] D. Kluth, A. Banning, I. Paur, et al., Modulation of pregnane X receptor-and electrophile responsive element-mediated gene expression by dietary polyphenolic compounds, Free Radic. Biol. Med. 42 (2007) 315–325.
[72] R. Patel, G. Maru, Polymeric black tea polyphenols induce phase II enzymes via Nrf2 in mouse liver and lungs, Free Radic. Biol. Med. 44 (2008) 1897–1911, http://dx.doi.org/10.1016/j.freeradbiomed.2008.02.006.
[73] M. Belviranli, N. Okudan, Well-known antioxidants and newcomers in sport nutrition: coenzyme Q10, quercetin, resveratrol, pterostilbene, pycnogenol and astaxanthin, in: M. Lamprecht (Ed.), Source Antioxidants in Sport Nutrition, CRC Press/Taylor & Francis, Boca Raton (FL), 2015Chapter 5.
[74] A.W. Boots, G.R. Haenen, A. Bast, Health effects of quercetin: from antioxidant to nutraceutical, Eur. J. Pharmacol. 585 (2008) 325–337, http://dx.doi.org/10.1016/j.ejphar.2008.03.008.
[75] Y. Hanasaki, S. Ogawa, S. Fukui, The correlation between active oxygens scavenging and antioxidative effects of flavonoids, Free Radic. Biol. Med. 16 (1994) 845–850.
[76] T.P. Cushnie, A.J. Lamb, Antimicrobial activity of flavonoids, Int. J. Antimicrob. Agents 26 (2005) 343–356 Review. Erratum in: Int J Antimicrob Agents. 2006;27:181.
[77] C.L.L. Saw, Y. Guo, A.Y. Yang, et al., The berry constituents quercetin, kaempferol, and pterostilbene synergistically attenuate reactive oxygen species: involvement of the Nrf2-ARE signaling pathway, Food Chem. Toxicol. 72 (2014) 303–311, http://dx.doi.org/10.1016/j.fct.2014.07.038.
[78] C.G. Heijnen, G.R. Haenen, F.A. van Acker, et al., Flavonoids as peroxynitrite scavengers: the role of the hydroxyl groups, Toxicol. In Vitro 15 (2001) 3–6.
[79] C.G. Heijnen, G.R. Haenen, R.M. Oostveen, et al., Protection of flavonoids against lipid peroxidation: the structure activity relationship revisited, Free Radic. Res. 36 (2002) 575–581.
[80] I.C. Arts, A.L. Sesink, M. Faassen-Peters, et al., The type of sugar moiety is a major determinant of the small intestinal uptake and subsequent biliary excretion of dietary quercetin glycosides, Br. J. Nutr. 91 (June) (2004) 841–847.
[81] S. Wolffram, M. Blöck, P. Ader, Quercetin-3-glucoside is transported by the glucose carrier SGLT1 across the brush border membrane of rat small intestine, J. Nutr. 132 (2002) 630–635.
[82] M.N. Bhuiyan, S. Mitsuhashi, K. Sigetomi, et al., Quercetin inhibits advanced glycation end product formation via chelating metal ions, trapping methylglyoxal, and trapping reactive oxygen species, Biosci. Biotechnol. Biochem. 81 (2017) 882–890, http://dx.doi.org/10.1080/09168451.2017.1282805.
[83] U. Kukongviriyapan, K. Apaijit, V. Kukongviriyapan, Oxidative stress and cardiovascular dysfunction associated with cadmium exposure: beneficial effects of curcumin and tetrahydrocurcumin, Tohoku J. Exp. Med. 239 (2016) 25–38, http://dx.doi.org/10.1620/tjem.239.25 Review.
[84] L.R. Shen, L.D. Parnell, J.M. Ordovas, et al., Curcumin and aging, Biofactors 39 (2013) 133–140, http://dx.doi.org/10.1002/biof.1086.
[85] A.C. Reddy, B.R. Lokesh, Effect of dietary turmeric (Curcuma longa) on iron-induced lipid peroxidation in the rat liver, Food Chem. Toxicol. 32 (1994) 279–283.
[86] A.C. Reddy, B.R. Lokesh, Studies on the inhibitory effects of curcumin and eugenol on the formation of reactive oxygen species and the oxidation of ferrous iron, Mol. Cell. Biochem. 137 (1994) 1–8.
[87] H. Ahsan, N. Parveen, N.U. Khan, et al., Pro-oxidant, anti-oxidant and cleavage activities on DNA of curcumin and its derivatives demethoxycurcumin and bis-demethoxycurcumin, Chem. Biol. Interact. 121 (1999) 161–175.
[88] T. Ak, I. Gülçin, Antioxidant and radical scavenging properties of curcumin, Chem. Biol. Interact. 174 (2008) 27–37, http://dx.doi.org/10.1016/j.cbi.2008.05.003.
[89] S.K. Borra, J. Mahendra, P. Gurumurthy, et al., Effect of curcumin against oxidation of biomolecules by hydroxyl radicals, J. Clin. Diagn. Res. 8 (2014) CC01–CC05, http://dx.doi.org/10.7860/JCDR/2014/8517.4967.
[90] S. Fujisawa, T. Atsumi, M. Ishihara, et al., Cytotoxicity, ros-generation activity and radical-scavenging activity of curcumin and related compounds, Anticancer Res. 24 (2B) (2004) 563–569.
[91] M. Iio, H. Kawaguchi, Y. Sakota, et al., Rffects of polyphenols, including flavonoids, on glutathione S-transferase and glutathione reductase, Biosci. Biotechnol. Biochem. 57 (1993) 1678–1680, http://dx.doi.org/10.1271/bbb.57.1678.
[92] J.Ø Moskaug, H. Carlsen, M.C. Myhrstad, et al., Polyphenols and glutathione synthesis regulation, Am. J. Clin. Nutr. 81 (2005) 277S–283S Review.
[93] T. Lao-ong, W. Chatuphonprasert, N. Nemoto, et al., Alteration of hepatic glutathione peroxidase and superoxide dismutase expression in streptozotocin-

induced diabetic mice by berberine, Pharm. Biol. 50 (2012) 1007–1012, http://dx.doi.org/10.3109/13880209.2012.655377.
[94] M. Bacanlı, S. Aydın, G. Taner, et al., The protective role of ferulic acid on sepsis-induced oxidative damage in Wistar albino rats, Environ. Toxicol. Pharmacol 38 (2014) 774–782, http://dx.doi.org/10.1016/j.etap.2014.08.018.
[95] C. St-Laurent-Thibault, M. Arseneault, F. Longpré, et al., Tyrosol and hydroxytyrosol, two main components of olive oil, protect N2a cells against amyloid-β-induced toxicity. involvement of the NF-κB signaling, Curr. Alzheimer Res. 8 (2011) 543–551.
[96] K. Reyes-Gordillo, J. Segovia, M. Shibayama, et al., Curcumin protects against acute liver damage in the rat by inhibiting NF-kappaB, proinflammatory cytokines production and oxidative stress, Biochim. Biophys. Acta 1770 (2007) 989–996.
[97] S. Lavoie, Y. Chen, T.P. Dalton, et al., Curcumin, quercetin, and tBHQ modulate glutathione levels in astrocytes and neurons: importance of the glutamate cysteine ligase modifier subunit, J. Neurochem. 108 (2009) 1410–1422, http://dx.doi.org/10.1111/j.1471-4159.2009.05908.
[98] C. Li, W.J. Zhang, J. Choi, et al., Quercetin affects glutathione levels and redox ratio in human aortic endothelial cells not through oxidation but formation and cellular export of quercetin-glutathione conjugates and upregulation of glutamate-cysteine ligase, Redox Biol. 9 (2016) 220–228, http://dx.doi.org/10.1016/j.redox.2016.08.012.
[99] G.H. Lee, H.Y. Lee, M.K. Choi, et al., Protective effect of Curcuma longa L. extract on CCl(4)-induced acute hepatic stress, BMC Res. Notes 10 (2017) 77, http://dx.doi.org/10.1186/s13104-017-2409-z.
[100] P.J. Elliott, M. Jirousek, Sirtuins: novel targets for metabolic disease, Curr. Opin. Investig. Drugs 9 (2008) 371–378 Review.
[101] M. Tanno, A. Kuno, T. Yano, et al., Induction of manganese superoxide dismutase by nuclear translocation and activation of SIRT1 promotes cell survival in chronic heart failure, J. Biol. Chem. 285 (2010) 8375–8382, http://dx.doi.org/10.1074/jbc.M109.090266.
[102] R.R. Alcendor, S. Gao, P. Zhai, et al., Sirt1 regulates aging and resistance to oxidative stress in the heart, Circ. Res. 100 (2007) 1512–1521.
[103] A. Brunet, L.B. Sweeney, J.F. Sturgill, et al., Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase, Science 303 (2004) 2011–2015.
[104] Y. Kawai, L. Garduño, M. Theodore, et al., Acetylation-deacetylation of the transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2) regulates its transcriptional activity and nucleocytoplasmic localization, J. Biol. Chem. 286 (2011) 7629–7640, http://dx.doi.org/10.1074/jbc.M110.208173.
[105] M. Tanito, M.P. Agbaga, R.E. Anderson, Upregulation of thioredoxin system via Nrf2-antioxidant responsive element pathway in adaptive-retinal neuroprotection in vivo and in vitro, Free Radic. Biol. Med. 42 (2007) 1838–1850.
[106] R.R. Lamberts, G. Onderwater, N. Hamdani, et al., Reactive oxygen species-induced stimulation of 5′AMP-activated protein kinase mediates sevoflurane-induced cardioprotection, Circulation 120 (2009) S10–S15.
[107] T. Toyoda, T. Hayashi, L. Miyamoto, et al., Possible involvement of the α1 isoform of 5′AMP-activated protein kinase in oxidative stress-stimulated glucose transport in skeletal muscle, Am. J. Physiol. Endocrinol. Metab. 287 (2004) E166–E173.
[108] B.M. Emerling, F. Weinberg, C. Snyder, et al., Hypoxic activation of AMPK is dependent on mitochondrial ROS but independent of an increase in AMP/ATP ratio, Free Radic. Biol. Med. 46 (2009) 1386–1391, http://dx.doi.org/10.1016/j.freeradbiomed.2009.02.019.
[109] S.L. Choi, S.J. Kim, K.T. Lee, et al., The regulation of AMPactivated protein kinase by H2O2, Biochem. Biophys. Res. Commun. 287 (2001) 92–97, http://dx.doi.org/10.1006/bbrc.2001.5544.
[110] J.W. Zmijewski, S. Banerjee, H. Bae, A. Friggeri, E.R. Lazarowski, E. Abraham, Exposure tohydrogen peroxide induces oxidation and activation of AMP-activated protein kinase, J. Biol. Chem. 285 (2010) 33154–33164, http://dx.doi.org/10.1074/jbc.M110.143685.
[111] Z. Xie, Y. Dong, M. Zhang, et al., Activation of protein kinase Cζ by peroxynitrite regulates LKB1-dependent AMP-activated protein kinase in cultured endothelial cells, J. Biol. Chem. 281 (2006) 6366–6375.
[112] B. Lassègue, K.K. Griendling, NADPH oxidases: functions and pathologies in the vasculature, Arterioscler. Thromb. Vasc. Biol. 30 (2010) 653–661, http://dx.doi.org/10.1161/ATVBAHA.108.181610.
[113] T.J. Guzik, S. Mussa, D. Gastaldi, J. Sadowski, C. Ratnatunga, R. Pillai, K.M. Channon, Mechanisms of increased vascular superoxide production in human diabetes mellitus: role of NAD(P)H oxidase and endothelial nitric oxide synthase, Circulation 105 (2002) 1656–1662.
[114] L. Gao, G.E. Mann, Vascular NAD(P)H oxidase activation in diabetes: a double-edged sword in redox signalling, Cardiovasc. Res. 82 (2009) 9–20, http://dx.doi.org/10.1093/cvr/cvp031 Review.
[115] R.P. Brandes, N. Weissmann, K. Schröder, NADPH oxidases in cardiovascular disease, Free Radic. Biol. Med. 49 (5 (September 1)) (2010) 687–706, http://dx.doi.org/10.1016/j.freeradbiomed.2010.04.030.
[116] G. Alba, R. El Bekay, M. Alvarez-Maqueda, et al., Stimulators of AMP-activated protein kinase inhibit the respiratory burst in human neutrophils, FEBS Lett. 573 (2004) 219–225.
[117] J. Hwang, D.J. Kleinhenz, H.L. Rupnow, et al., The PPARgamma ligand, rosiglitazone, reduces vascular oxidative stress and NADPH oxidase expression in diabetic mice, Vasc. Pharmacol. 46 (2007) 456–462.
[118] A. Piwkowska, D. Rogacka, M. Jankowski, et al., Metformin induces suppression of NAD(P)H oxidase activity in podocytes, Biochem. Biophys. Res. Commun. 393 (2010) 268–273, http://dx.doi.org/10.1016/j.bbrc.2010.01.119.
[119] M. D'Archivio, C. Filesi, R. Varì, et al., Bioavailability of the polyphenols: status and controversies, Int. J. Mol. Sci. 11 (2010) 1321–1342, http://dx.doi.org/10.3390/ijms11041321.
[120] L. Giovannini, S. Bianchi, Role of nutraceutical SIRT1 modulators in AMPK and mTOR pathway: evidence of a synergistic effect, Nutrition 34 (2017) 82–96, http://dx.doi.org/10.1016/j.nut.2016.09.008.
[121] C. Morand, H. Sies, Polyphenols and health, Arch. Biochem. Biophys. 599 (2016) 1–2, http://dx.doi.org/10.1016/j.abb.2016.03.015.
[122] J. Teixeira, D. Chavarria, F. Borges, et al., Dietary polyphenols and mitochondrial function: role in health and disease, Curr. Med. Chem. (2017), http://dx.doi.org/10.2174/0929867324666170529101810.
[123] K. Ganesan, B. Xu, A critical review on polyphenols and health benefits of black soybeans, Nutrients (2017), http://dx.doi.org/10.3390/nu9050455.