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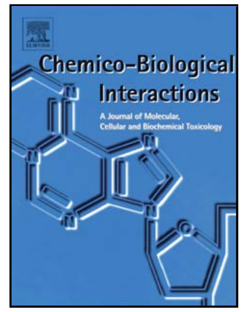

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Water and methanolic extracts of *Salvia officinalis* protect HepG2 cells from *t*-BHP induced oxidative damage

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Abstract

Common sage (*Salvia officinalis* L., *Lamiaceae*) is an aromatic and medicinal plant well known for its antioxidant properties. Some in vivo studies have shown the biological antioxidant effects of sage. However, the intracellular antioxidant mechanisms of action are still poorly understood. In this study, we evaluated the cytoprotective effects of two sage extracts (a water and a methanolic extract) against *tert*-butyl hydroperoxide (*t*-BHP)-induced toxicity in HepG2 cells. The most abundant phenolic compounds present in the extracts were rosmarinic acid and luteolin-7-glucoside. Both extracts, when co-incubated with the toxicant, protected significantly HepG2 cells against cell death. The methanolic extract, with a higher content of phenolic compounds than the water extract, conferred better protection in this in vitro model of oxidative stress with liver cells. Both extracts, tested in a concentration that protects 80% against cell death (IC_{80}), significantly prevented *t*-BHP-induced lipid peroxidation and GSH depletion, but not DNA damage assessed by the comet assay. The ability of sage extracts to reduce *t*-BHP-induced GSH depletion by 62% was probably the most relevant contributor to the observed cytoprotection. A good correlation between the above cellular effects of sage and the effects of their main phenolic compounds was found. When incubated alone for 5 hours, sage extracts induced an increase in basal GSH levels of HepG2 cells, which indicates an improvement of the antioxidant potential of the cells. Compounds present in sage extracts other than phenolics may also contribute to this latter effect. Based in these results, it would be of interest to investigate whether sage has protective effects in suitable in vivo models of liver diseases, where it is known that oxidative stress is involved.

Keywords: *Salvia officinalis L.* / Phenolic Compounds / Antioxidant Effects / HepG2 cells / *tert*-Butyl Hydroperoxide.

1. Introduction

Reactive oxygen species (ROS) and other free radicals are produced during the normal cell metabolism and they are a necessary and normal process that provides important physiological functions [1,2]. The production of ROS and other free radicals is normally compensated by an elaborate endogenous antioxidant system. However, due to many environmental, lifestyle and pathological factors, an excess of radicals can be accumulated in cells resulting in oxidative stress. Because of their high reactivity, accumulation of radicals above cells' defenses may affect cellular functionality and integrity by damaging critical molecules, such as the DNA, proteins, carbohydrates and lipids, which ultimately can cause cell death. In fact, oxidative stress has been recognized to be involved in the etiology of several diseases, including liver diseases [3,4]. The liver, because of its high metabolic activity and its anatomical positioning to receive blood from the gastrointestinal tract, is vulnerable to toxicity from a variety of drugs and environmental contaminants. Consequently, mechanisms of cytoprotection relevant to the liver are of particular interest. Natural antioxidants have been proposed and utilized as therapeutic agents to counteract liver damage [3,4].

Salvia officinalis L. (*Lamiaceae*) is an aromatic and medicinal plant of Mediterranean origin well known for its antioxidant properties, mainly due to its composition in phenolic compounds [5]. Sage extracts revealed strong antioxidant activity in several assays: by increasing the stability of food oils [6-10], in an assay based on the disappearance of methyl linoleate in a lipophilic solvent under strong oxidizing conditions [11,12], by the ability to scavenge $DPPH^{\cdot}$ [13] and $ABTS^{\cdot}$ free radicals [14] as well as by having oxygen radical absorbance capacity (ORAC assay) [15]. In addition, the reported superoxide and hydroxyl radicals scavenging activities using the electron spin resonance technique [16] and the protective effects against enzyme-dependent and enzyme-independent lipid peroxidation [17,18] of sage extracts also showed its antioxidant potential. More recently, results from in vivo studies suggest a biological antioxidant effect of sage. The drinking of a sage infusion (tea) for 14 days was reported to improve liver antioxidant status in mice and rats [19]. Also, the treatment of rats with a water extract of sage for 5 weeks was shown to protect against the hepatotoxicity of azathioprine [20]. However, little is known about the active compounds and cellular mechanisms action. Only in a small experiment using fibroblasts, performed by Masaki et al. (1995), sage antioxidant effects were related with cytoprotective effects. In their study, a sage extract protected significantly against cell death induced by a superoxide-generating system [16]. Very recently, a hydro alcoholic extract of sage was reported to possess neuroprotective effects against amyloid β (Aβ)-induced toxicity in PC12 cells, and the effect was attributed, at least in part, to rosmarinic acid [21].

In this study we propose to evaluate the potential antioxidant/cytoprotective effects of two sage extracts (a water and a methanolic crude extracts) against *tert*-butyl hydroperoxide (*t*-BHP)-induced oxidative damages in HepG2 cells. This hepatoma cell line is considered a good tool to study the toxic/cytoprotective and genotoxic/ antigenotoxic effects of compounds to liver cells [22]. Furthermore, this model of in vitro hepatotoxicity (*t*-BHP and HepG2 cells) was recently used to evaluate the cytoprotective effects of individual phenolic compounds, which included the two most representative ones of the above sage extracts – rosmarinic acid and luteolin-7-

glucoside [22]. Here, the concentration of sage extracts that protected 50% (IC_{50}) against *t*-BHP-induced cell death were determined in order to establish their cytoprotective potential. Subsequently, IC_{80} values, a concentration that effectively protects against cell death, were used to evaluate the effects of each extract on three markers of oxidative damage: lipid peroxidation, intracellular glutathione levels and DNA damage. The importance of modulation of these parameters by sage extracts in the protection against *t*-BHP-induced cell death is discussed. Throughout the experiment quercetin was used as a positive control.

2. Materials and methods

2.1. Chemicals

Minimum Essential Medium Eagle (MEM), *tert*-butyl hydroperoxide, quercetin and Bradford reagent were purchased from Sigma (St. Louis, MO, USA). Fetal Bovine Serum (FBS) was obtained from Biochrom KG (Germany). All others reagents were of analytical grade.

2.2. Plant material, preparation of sage extracts and analysis of their phenolic composition

Salvia officinalis L. plants were cultivated in an experimental farm located in Arouca, Portugal, and were collected in April, 2001. The aerial parts of plants were lyophilised and kept at -20º C. Voucher specimen is kept in an active bank under the responsibility of the DRAEDM (Direcção Regional de Agricultura de Entre Douro e Minho) from the Portuguese Ministry of Agricultural.

The dried and powdered aerial plant material (4 g) was extracted with 2 × 100 ml of 90% methanol in water at room temperature, using an ultrasonic bath (15 min).

The filtered extract (SOME) was evaporated to dryness under reduced pressure at 40ºC and a yield of 26.2% (w/w) was obtained.

Considering that sage is traditionally consumed as a tea, an infusion of sage (SOI) was also prepared following a previous methodology [19]. In brief, 300 ml of ultrapure Milli Q boiling water were poured over 4 g of lyophilised aerial plant material and allowed to steep for 5 min. The filtered extract was lyophilised to dryness and a yield of 25.8% (w/w) was obtained.

Phenolic compounds present on SOME and SOI extracts were identified and quantified by HPLC/DAD as described in Santos-Gomes et al. (2002) [23] and Lima et al. (2005) [19] for each extract, respectively.

2.3. Antiradical activity

The free radical scavenging (antiradical) activity of sage extracts was studied against two radicals: the stable free radical 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$) and the superoxide radical.

For DPPH scavenging activity, after addition of different concentrations of extract to DPPH (90µM), the percentage of remaining DPPH was determined at different times from the absorbance at 515 nm using a plate reader spectrophotometer. At steady state, the percentage of remaining DPPH was plotted against the concentration of the extract and the amount of antioxidant necessary to decrease by 50% the initial DPPH concentration (IC_{50}) calculated. We also present the parameter antiradical efficiency (AE) [24] using the estimated T_{IC50} – time needed to reach the steady state at the corresponding IC_{50} concentration, where $AE = 1/(IC_{50} \times T_{IC50})$.

The superoxide radical scavenging activity was determined using the phenazine methosulphate-NADH nonenzymatic assay as previously described [25].

2.4. Cell culture

HepG2 cells (hepatocellular carcinoma cell line) were obtained from the American Type Culture Collection (ATCC) and maintained in culture in 75 cm^2 polystyrene flasks (Falcon) with MEM containing 10% FBS, 1% antibiotic-antimycotic solution, 1 mM sodium pyruvate, 10 mM Hepes and 1.5 g/l sodium bicarbonate under an atmosphere of 5% CO_2 at 37ºC.

2.5. Experimental outline

2.5.1. Assay for protection against t-BHP-induced toxicity in HepG2 cells

In order to determine the concentration of sage extract/quercetin that protects the cells 50% from the oxidative damage (IC_{50}), cells were incubated with 2 mM of *t*-BHP for 5 h to induce significant cell death as previously described [22]. HepG2 cells were plated in 24-multiwell culture plates at 2.5×10^5 cells per well. The prevention of LDH leakage (cell death) was measured in co-incubations with sage extract/quercetin dissolved in DMSO (1% v/v final concentration, controls with DMSO only) at several concentrations. The IC_{50} and the Hillslope – slope from the plotted sage extract/quercetin's concentrations (in logarithm) versus cell death protection relative to the control (2 mM *t*-BHP, 5 h) – were calculated graphically using a computer program (GraphPad Prism, version 4.00, GraphPad Software Inc.). Based on the dose-response curves of protection against cell death by sage extract/quercetin, the IC_{80} concentrations were estimated and used in the following experiments to evaluate the protective potential of the compounds on several cellular parameters as previously described [22]. Briefly:

2.5.2. Evaluation of the effects of sage extract/quercetin at the IC_{80} concentration against t-BHP-induced lipid peroxidation and GSH depletion in HepG2 cells.

In order to evaluate the potential protective effect of sage extract/quercetin at IC_{80} concentration against *t*-BHP-induced lipid peroxidation and GSH depletion, cells were incubated with 2 mM *t*-BHP for 5 h. HepG2 cells were plated in 6-multiwell culture plates at 7.5×10^5 cells per well. Forty hours after plating, the medium was discarded and fresh medium containing 2 mM *t*-BHP and/or the IC_{80} concentration of sage extract/quercetin was added. Both sage extracts and quercetin did not change significantly the pH of the culture medium at their IC_{80} concentration. Five hours later, cell culture medium and cell scrapings were harvested and kept at -80ºC for following quantification of lipid peroxidation and glutathione levels.

2.5.3. Evaluation of the effects of sage extract/quercetin at the IC_{80} concentration against t-BHP-induced DNA damage in HepG2 cells

In order to evaluate the potential protective effect of sage extract/quercetin at IC_{80} concentration against *t*-BHP-induced DNA damage, cells were incubated with 200 μM *t*-BHP for 1 h. HepG2 cells were plated in 6-multiwell culture plates at 5×10^5 cells per well. Sixteen hours after plating, the medium was discarded and fresh medium containing 200 μM *t*-BHP and/or the IC_{80} concentration of sage extract/quercetin was added to the cells. After 1 h incubation, cells were rinsed with warm PBS and then incubated for 5 min with 0.125% (w/v) trypsin in PBS. The cells were then harvested in PBS to be used in the alkaline version of the comet assay for evaluation of DNA damage.

2.6. Biochemical analysis

2.6.1. LDH

To assess the extend of cell death caused by *t*-BHP, the determination of lactate dehydrogenase leakage to the culture medium was used as indicator of plasma membrane integrity of HepG2 cells. LDH activity was measured spectrophotometrically at 30ºC as previously described [19].

2.6.2. Lipid peroxidation

The extent of lipid peroxidation was estimated by the levels of malondialdehyde measured using the thiobarbituric acid reactive substances (TBARS) assay at 535 nm following a methodology previously described [26] with some modifications [19]. The results are expressed as nmol/mg of protein using a molar extinction coefficient of 1.56×10^5 $M^{-1}cm^{-1}$.

2.6.3. Glutathione content

The glutathione levels of HepG2 cells were determined by the DTNB-GSSG reductase recycling assay as previously described [27], with some modifications [28]. The results are expressed as nmol GSH/mg of protein.

2.6.4. Protein

Protein content was measured with a Bradford Reagent purchased from Sigma using bovine serum albumin as a standard.

2.7. Comet assay

The alkaline version of the single cell gel electrophoresis (comet) assay was performed based in previous descriptions [29-31] with slight modifications [22]. The comet images were analysed using the semiquantitative method of visual scoring [32]. Each cell was classified in five classes according to the intensity of fluorescence in the comet tail, attributing a value of 0, 1, 2, 3 or 4 from undamaged to maximal damage. In this way, the total score for 100 images can range from 0 (all undamaged) to 400 (all maximally damaged), the overall DNA damage of the cell population expressed in arbitrary units.

2.8. Statistical analysis

Data are expressed as means ± SEM. Statistical significances were determined using a one-way ANOVA followed by the Student-Newman-Keuls post-hoc test. *P* values ≤ 0.05 were considered statistically significant.

3. Results

3.1. Phenolic composition of sage extracts and their antiradical activity

A methanolic (SOME) and a water (SOI) extract were prepared from aerial parts of *Salvia officinalis* and analysed for phenolic compounds by HPLC/DAD (Table 1). Eight phenolic compounds were identified, 5 phenolic acids and 3 flavonoids, SOME having the highest content. SOME's main phenolic compound was rosmarinic acid whereas SOI's were rosmarinic acid and luteolin-7-glucoside.

The antiradical activity of both extracts was then evaluated against DPPH and superoxide radicals (Table 2). SOME, with higher content in phenolic compounds, had higher antiradical activity against DPPH presenting a lower IC_{50} and a higher antiradical efficiency than SOI extract. The activity of both extracts was smaller than the positive control quercetin. Regarding the scavenging of superoxide radical, SOI extract showed a higher antiradical activity than SOME.

3.2. Potential cytoprotective effects of sage extracts

The potential cytoprotective effects of both sage extracts against the cell death induced by *t*-BHP were evaluated in HepG2 cells (Table 3, Fig. 1). *t*-BHP 2 mM for 5 hours was previously shown to induce oxidative damage to HepG2 cells causing about 40-50% of cell death [22]. As shown in Fig. 1, both extracts protected against cell death in a dose-dependent manner. SOME had, however, higher cytoprotective activity (lower IC_{50}) than SOI (Table 3). The Hillslope was also higher in SOME than SOI (Table 3), which indicates a narrower concentration (in logarithm) range from 0 to 100% of cytoprotective activity of SOME (Fig 1).

3.3. Effects of sage extracts on lipid peroxidation, glutathione levels and DNA damage

To study the effects of sage extracts against lipid peroxidation, GSH depletion and DNA damage induced by *t*-BHP, concentrations that effectively protect against cell death (IC_{80}) were used. IC_{80} concentrations were used to determine if the same level of cytoprotection for each extract correlate with similar effects on the above mentioned parameters. IC_{80} concentration for each extract (Table 3) was estimated based on the curves presented in Fig. 1 and, as can be seen in Fig. 2, *t*-BHP-induced cell death was prevented by around 80% by both sage extracts as well as quercetin. No significant cell death was observed in incubations of HepG2 cells with sage extracts or quercetin alone (Fig. 2).

As shown in Fig. 3, *t*-BHP-induced lipid peroxidation was significantly decreased by around 25% by both extracts. Quercetin also significantly protected against lipid peroxidation by 30%. None of the extracts, when incubated alone with HepG2 cells, induced significant lipid peroxidation.

t-BHP-induced GSH (reduced glutathione) depletion was also significantly inhibited by both extracts by around 62% while quercetin inhibited GSH depletion by only 40% (Fig. 4). The increase in GSSG levels induced by *t*-BHP was slightly decreased by both sage extracts and quercetin, although the effect was not statistically significant (data not shown). When the cells were incubated with the extracts alone, a significant increase in the basal GSH levels (Fig. 4) was observed for SOME (15%). On the other hand, quercetin induced a decrease in the basal levels of GSH.

The incubation of HepG2 cells for 1 h with 200 µM of *t*-BHP induced significant DNA damages without cell death [22], conditions that can be used to assess effects of compounds or extracts against DNA damage by the comet assay. As shown in Fig. 5, contrarily to what happened with quercetin, both sage extracts did not protect HepG2 cells against DNA damage induced by *t*-BHP. None of the tested extracts induced DNA damages at IC_{80} concentration when incubated alone with HepG2 cells.

4. Discussion and conclusions

Since oxidative stress has been recognized to be involved in the etiology of several liver diseases [3,4] and because the liver is very susceptible to toxic effects, natural antioxidants and plant extracts have been proposed as therapeutic agents to counteract liver damage. *Salvia officinalis* is well known for its antioxidant activity, mainly based on results from several subcellular and noncellular in vitro studies [5]. Previous work in our laboratory has shown the ability of sage tea drinking to improve liver antioxidant status in mice and rats [19]. That was, however, not enough to protect against CCl_4-induced hepatotoxicity in mice and, instead, a herb-toxicant interaction was observed [33]. On the other hand, in an in vivo experiment, Amin and Hamza (2005) have shown that the treatment of rats with a water extract of sage for 5 weeks protected against the hepatotoxicity of azathioprine [20]. However, despite all these effects, little is known about the active compounds and mechanisms of antioxidant protection of sage extracts at cellular level.

Here, the potential antioxidant and cytoprotective effects of sage crude extracts, a methanolic (SOME) and a water extract (SOI), were tested against *t*-BHP-induced toxicity in HepG2 cells. Both sage extracts, in co-incubations with the toxicant, showed protective effects against *t*-BHP-induced cell death. SOME revealed higher cytoprotective activity than SOI, as shown by the lower IC_{50} obtained for this extract against *t*-BHP-induced cell death compared to that of SOI extract. This biological activity is in agreement with the literature where sage's antioxidant activity has been attributed to its phenolic compounds, more abundant in the methanolic extract.

In this model of cytoprotection, because effects were tested in co-incubations with the toxicant, the antioxidant protection may reflect mainly direct actions on *t*-BHP toxicity [22]. These direct effects would include, besides the antiradical scavenging or hydrogen-donating activity measured in this study, the compounds' ability to chelate metal ions [34]. Since ROS [35], *t*-BHP radicals [36,37] and intracellular iron ions [38] are involved in the toxicity of *t*-BHP, direct effects on these parameters would tend to reduce the level of damage. Antiradical activity of sage is well known from previous studies [11,13-16] and was also shown here against DPPH and superoxide radicals. Considering the composition of the extracts in phenolic compounds, they most likely also possess the ability to chelate metal ions [34].

Irrespective of their antiradical and metal chelating ability of extracts, they will act as intracellular antioxidants if only the compounds permeate cell membranes. Our previous results underscored the importance of the compound's lipophilicity, in addition to its antioxidant potential, for biological activity [22]. Incubation of HepG2 cells with *t*-BHP induced significant lipid peroxidation, GSH depletion and DNA damage. At IC_{80}, both sage extracts significantly prevented lipid peroxidation and GSH depletion, but failed to prevent DNA damage. In general, there seems to be a good correlation between the many biological effects of sage extracts and those of their main phenolic constituents, rosmarinic acid and luteolin-7-glucoside. These compounds have previously shown in this experimental model to possess cytoprotective activities (IC_{50}'s of 69 μM and 78 μM, respectively) [22]. Although both these compounds have lower lipophilicity than quercetin, they too were able to protect against *t*-BHP-induced toxicity in HepG2 cells (albeit with a 3 times higher IC_{50} than quercetin). In our previous study, rosmarinic acid and luteolin-7-glucoside also protected significantly against *t*-BHP-induced lipid peroxidation and intracellular GSH depletion, as was the case here for the sage extracts. They seem, therefore, to permeate cell membrane, at least in some extent, and in the case of luteolin-7-glucoside, the removal of the glucoside moiety would probably increase bioavailability.

The fact that sage extracts did not prevent DNA damage may be explained by the low lipophilicity of the compounds present. In our previous study, the main phenolic compounds present in this sage extracts, rosmarinic acid and luteolin-7-glucoside, showed poor ability to prevent DNA damage induced by *t*-BHP [22]. In that study, the lipophilicity of phenolic compounds appeared to be of even greater importance for DNA protection than for cytoprotective effects. Only antioxidant compounds with hydrophobicities similar to quercetin were able to protect against DNA damage induced by *t*-BHP in HepG2 cells.

Based on previous studies, lipid peroxidation and DNA damage seem not to be as relevant for the *t*-BHP-induced cell death as GSH depletion [22,35]. GSH depletion has been suggested as primary mechanism of *t*-BHP-induced toxicity in liver cells

[35,39,40]. GSH plays an important role in hepatocyte defence against ROS, free radicals and electrophilic metabolites [41,42]. A severe GSH depletion leaves cells more vulnerable to oxidative damage and is normally associated with calcium homeostasis disruption, which ultimately causes cell death [42]. The prevention of *t*-BHP-induced GSH depletion in about 40% has previously been suggested as a major contribution to cytoprotective effects in a same experimental model [22]. Thus, the 62% protection against GSH depletion was probably the most relevant effect of the extracts used in this study. In agreement with this in vitro data, Amin and Hamza (2005) [20] showed the ability of sage to protect in vivo against the hepatotoxicity of azathioprine, a drug that acts by depleting GSH levels.

Although rosmarinic acid and luteolin-7-glucoside present in the extracts may contribute to the observed prevention of GSH depletion induced by *t*-BHP, they cannot be the sole explanation for the effects of sage extracts on the GSH levels. In the same experimental model, both phenolic compounds were shown to have some pro-oxidant effects decreasing slightly GSH levels when incubated alone with HepG2 cells for 5 hours [22], an effect similar to what was observed in this study with quercetin – the positive control. For some phenolic compounds and, in particular, quercetin, the formation of quinone metabolites are thought to mediate the formation of conjugates with GSH, decreasing its basal levels [43,44]. Contrarily to the effects of incubations with the individual phenolic compounds present in the extracts, when sage extracts were incubated alone with HepG2 cells for 5 hours, a slight increase in GSH levels was observed, which was significant for the SOME extract. Compounds other than phenolics present in the extracts appear, therefore, to be important for this effect of sage extracts in HepG2 cells. Since the increase in GSH levels was accompanied by an increase in the total glutathione levels (and not to a reduction in GSSG levels), sage extracts seem to have an ability to increase the *de novo* synthesis of glutathione. In a previous study, after a stress-induced GSH depletion, SOI given in vivo to rats restored GSH levels of subsequent hepatocyte cultures to a higher value than controls [19], which also suggested an increase in the *de novo* glutathione synthesis.

In conclusion, this study showed clearly the antioxidant effects at cellular level of sage, namely preventing cell death, lipid peroxidation and GSH depletion induced by *t*-BHP in HepG2 cells. The protection of cell viability conferred by sage extracts seemed to be due mainly to their ability to prevent GSH depletion (by about 60%). This work also showed a good correlation of the above cellular effects of sage with the effects of their main phenolic compounds, rosmarinic acid and luteolin-7-glucoside. Nevertheless, unknown compounds other than phenolics also seem to contribute to the antioxidant effects of sage on basal GSH levels. In fact, this work showed for the first time the ability of sage (mainly the methanolic extract) to increase basal GSH levels, probably by the induction of glutathione synthesis, an effect that may be relevant in the face of oxidative stress. Based on these results, it would be of interest to investigate whether sage has protective effects in suitable in vivo models of liver diseases, where it is known that oxidative stress is involved.

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Results (tables)

Table 1 – Composition (µg/mg extract) in phenolic compounds of *S. officinalis* methanolic extract (SOME) and *S. officinalis* infusion (SOI).

Compound	SOME	SOI
Phenolic acids		
Rosmarinic acid	132.2	52.0
Caffeic acid	tr	0.8
Ferulic acid	tr	0.5
3-Caffeoylquinic acid	tr	tr
5-Caffeoylquinic acid	tr	tr
Flavonoids		
Luteolin-7-glucoside	1.2	19.7
4´,5,7,8-Tetrahydroxyflavone	0.1	0.9
Apigenin-7-glucoside	tr	0.4

tr – trace amounts

Table 2 – Antiradical activity of the sage extracts and quercetin against DPPH and superoxide radical.

Extract/compound	DPPH[a]		Superoxide radical[b]
	IC_{50} (μg/ml)	AE ($\times 10^{-3}$)[c]	IC_{50} (μg/ml)
SOME	13.5 ± 0.5	6.12	162 ± 39
SOI	14.9 ± 0.3	5.14	14.4 ± 1.4
Quercetin	3.43 ± 0.07	13.2	10.6 ± 1.0

[a] Values represent mean ± SD of 5 replicates.

[b] Values represent mean ± SD of 3 independent experiments with 3 replicates each.

[c] AE – antiradical efficiency: AE = $1/(IC_{50} \times T_{IC50})$, where T_{IC50} is the time needed to reach the steady state at the corresponding IC_{50} concentration.

Table 3 – Potential cytoprotective effects[a] of the sage extracts against *t*-BHP-induced toxicity in HepG2 cells.

Extract/Compound	IC_{50} (μg/ml)	Hillslope	IC_{80} (μg/ml)
SOME	7.6 ± 0.5	1.89 ± 0.23	16
SOI	101.4 ± 11.3	1.02 ± 0.13	~250
Quercetin	6.5 ± 0.5	1.95 ± 0.28	13

[a] Tested in co-incubations with 2 mM of *t*-BHP (5 h) in HepG2 cells. IC_{50} and the Hillslope were taken form the plotted dose-response curve (Fig. 1). IC_{80} concentration was estimated from the same dose-response curve. Values are mean ± SEM of at least 4 independent experiments.

Results (figures)

Fig. 1 – Dose-response effect of the sage extracts against *t*-BHP-induced toxicity in HepG2 cells. After incubating HepG2 cells with 2 mM of *t*-BHP and sage extracts/quercetin for 5 h, protection against cell death (as measured by LDH leakage) versus sage extract/quercetin concentrations (in logarithm) were plotted in order to take the IC_{50} and Hillslope of each compound (Table 3). Values are mean ± SEM of at least 4 independent experiments.

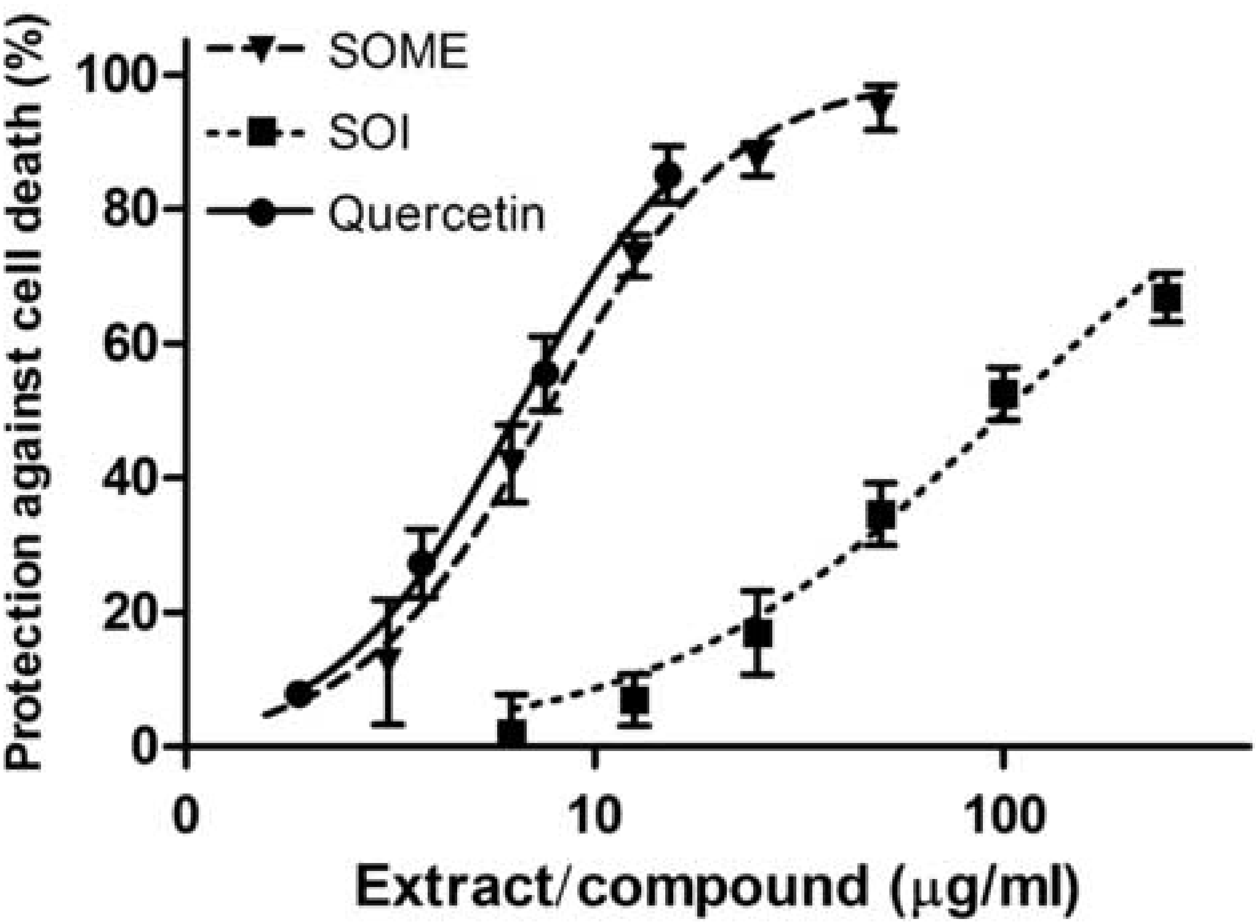
Fig. 2 – Effects of sage extracts at IC_{80} concentration against *t*-BHP-induced cell death. HepG2 cells were incubated with *t*-BHP 2 mM (5 h) and/or with sage extract/quercetin at IC_{80} concentration and cell viability measured by LDH leakage. Values are mean ± SEM, n = 5. *** P≤0.001 when compared with the negative control. ### P≤0.001 when compared with the *t*-BHP control.

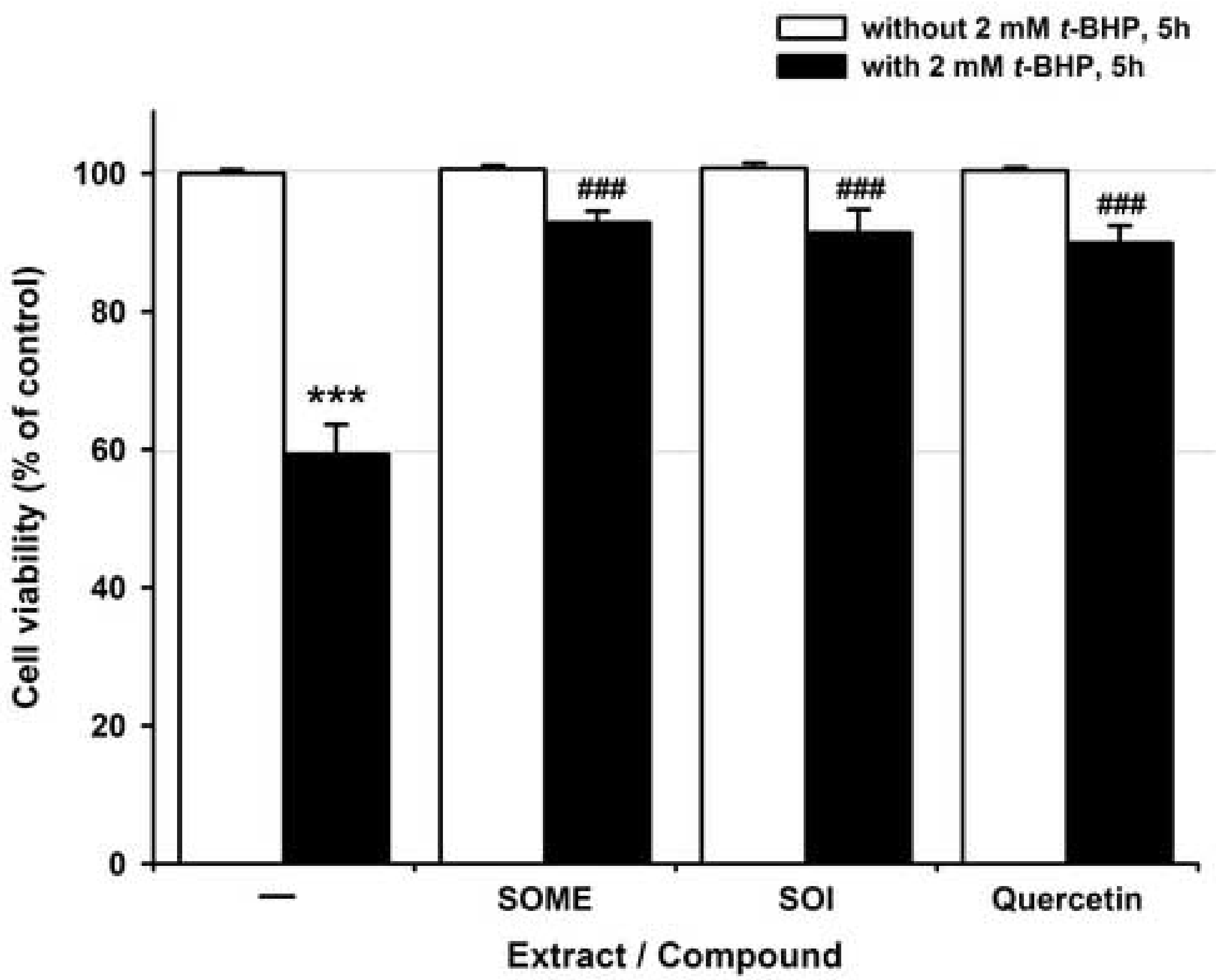
Fig. 3 – Effects of sage extracts at IC_{80} concentration against *t*-BHP-induced lipid peroxidation in HepG2 cells. HepG2 cells were incubated with *t*-BHP 2 mM (5 h) and/or with sage extract/quercetin at IC_{80} concentration and lipid peroxidation measured by TBARS assay. Values are mean ± SEM, n = 5 (100% = 2.25 nmol/mg). *** P≤0.001 when compared with the negative control. ## P≤0.01 and ### P≤0.001 when compared with the *t*-BHP control.

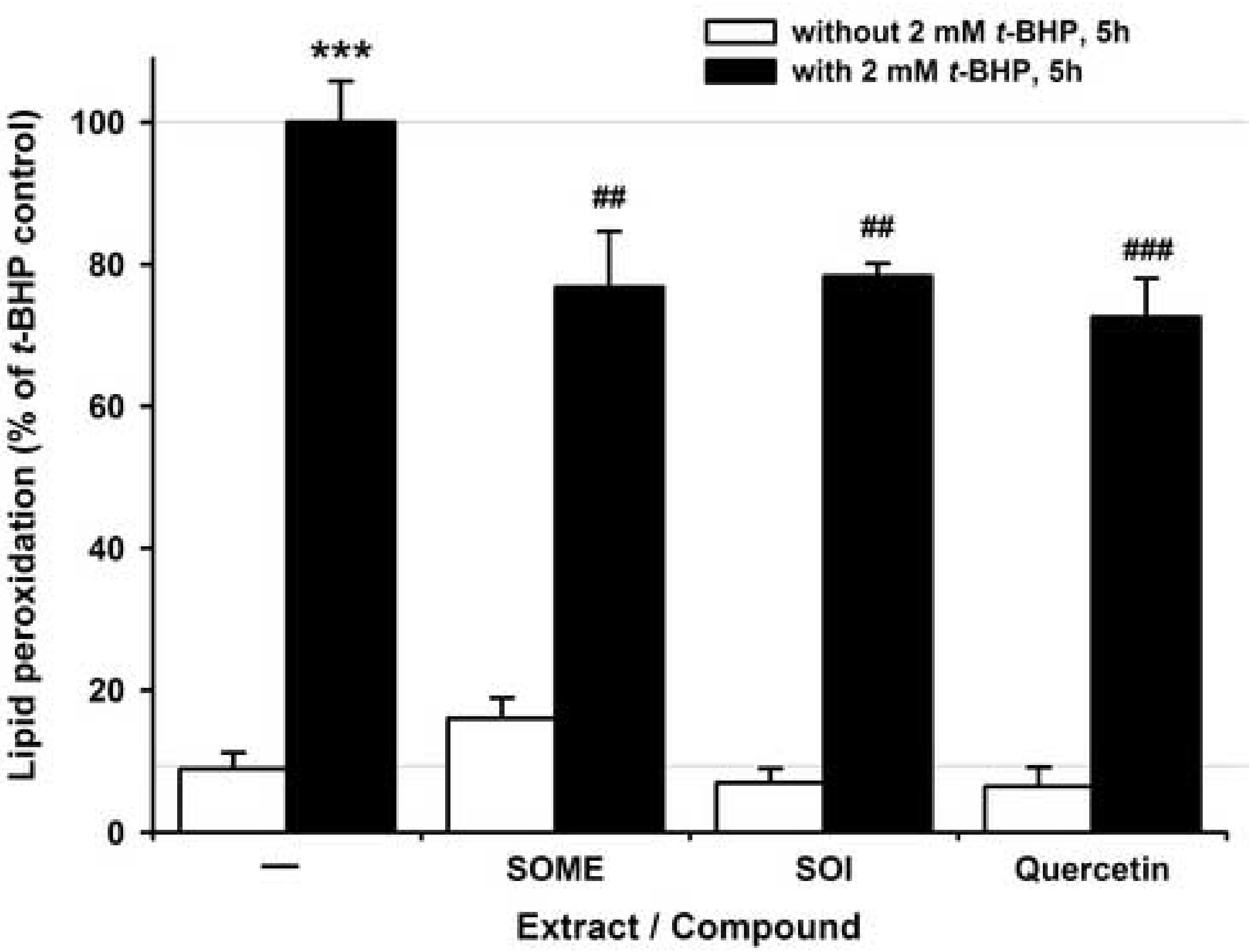
Fig. 4 – Effects of sage extracts at IC_{80} concentration against *t*-BHP-induced decrease in GSH levels in HepG2 cells. HepG2 cells were incubated with *t*-BHP 2 mM (5 h) and/or with sage extract/quercetin at IC_{80} concentration and GSH levels determined by the DTNB-GSSG reductase recycling assay. Values are mean ± SEM, n = 5 (100% = 72.4

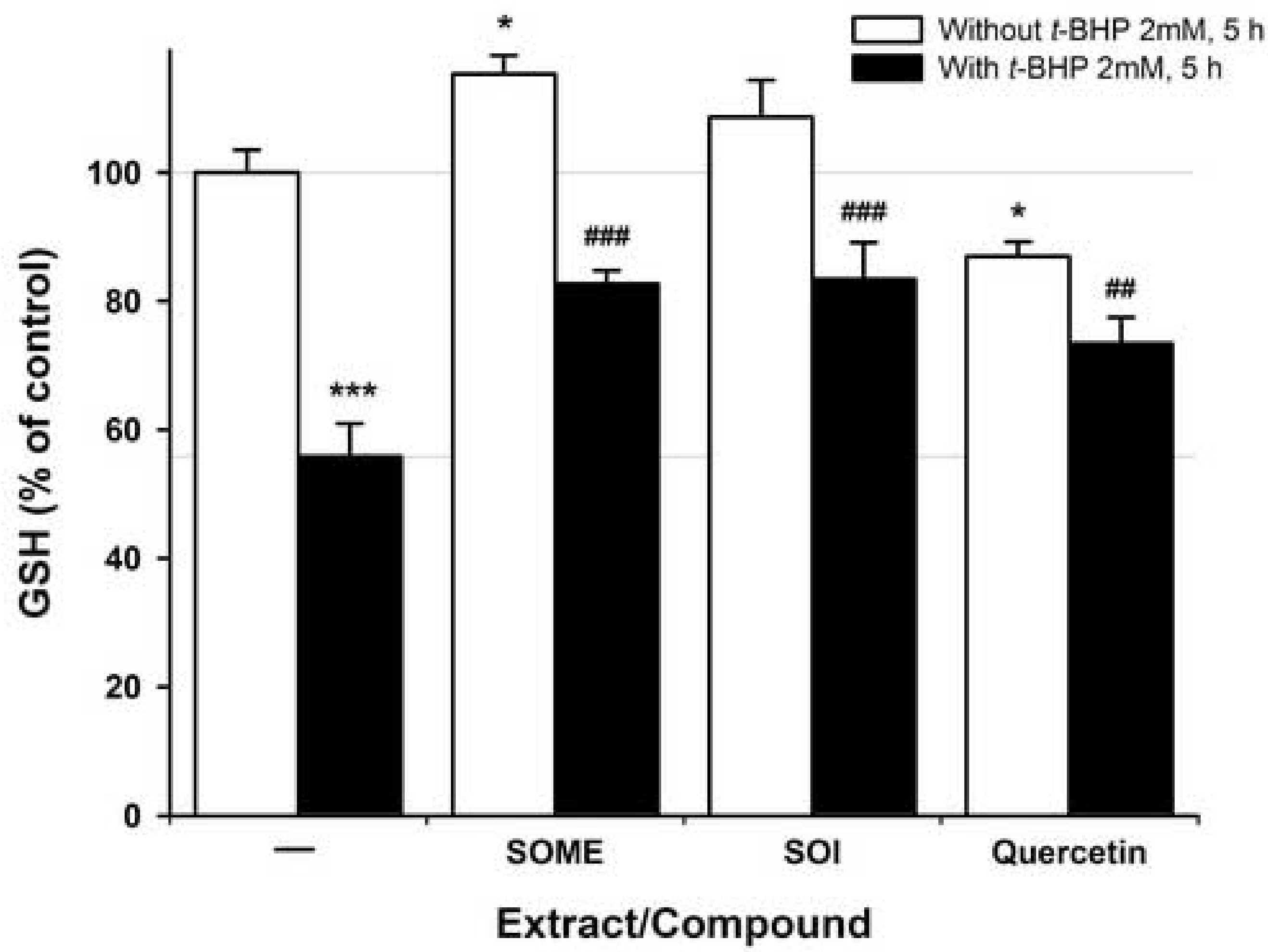
nmol/mg). * P≤0.05 and *** P≤0.001 when compared with the negative control. ## P≤0.01 and ### P≤0.001 when compared with the *t*-BHP control.

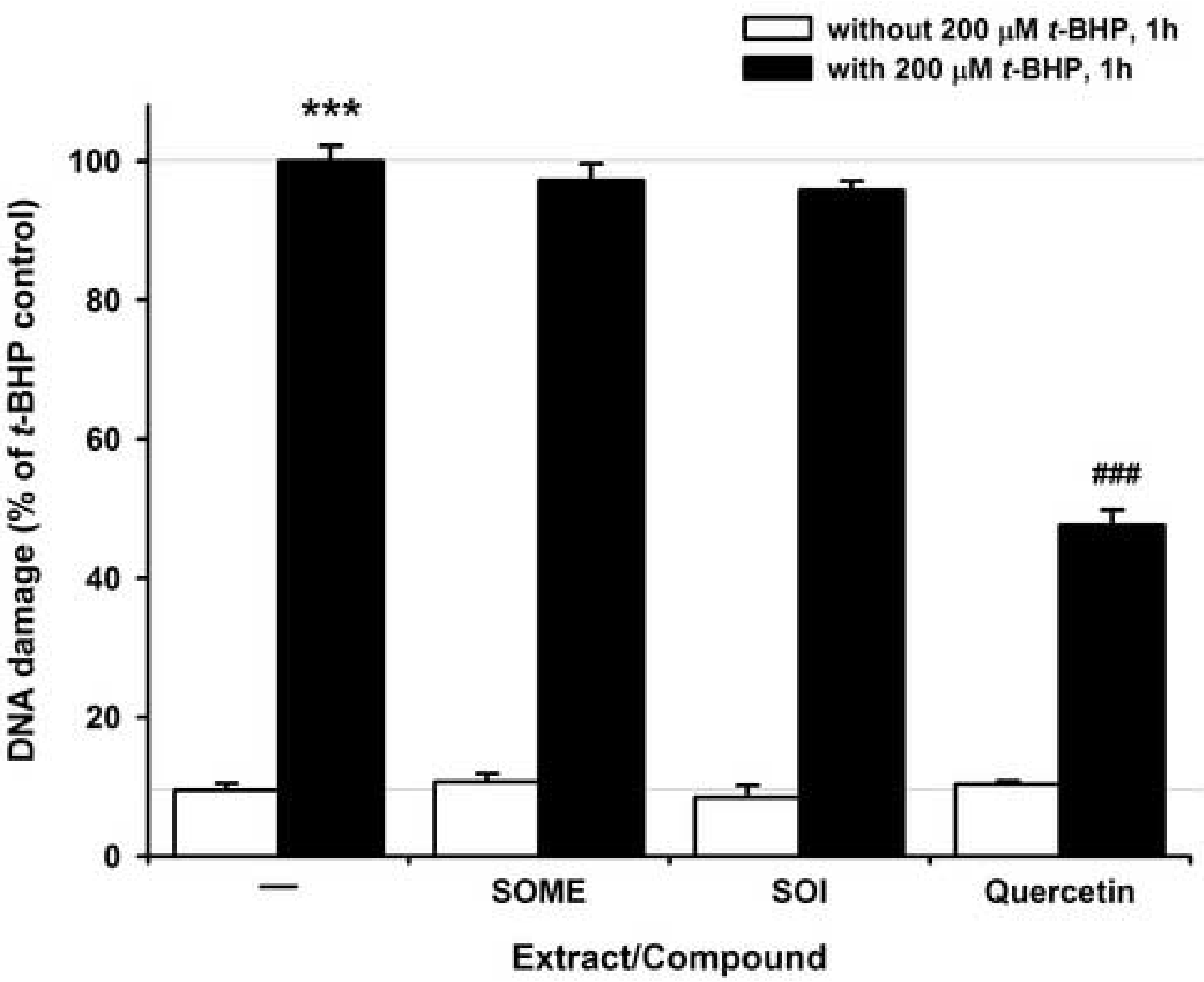
Fig. 5 – Effects of sage extracts at IC_{80} concentration against *t*-BHP-induced DNA damage in HepG2 cells. HepG2 cells were incubated with *t*-BHP 200 µM (1 h) and/or with sage extract/quercetin at IC_{80} concentration and DNA damage evaluated by the comet assay. DNA damage was assessed by the semiquantitative method of visual scoring. Values are mean ± SEM, n = 4 (100% = 187.1 arbitrary units). *** P≤0.001 when compared with the negative control. ### P≤0.001 when compared with the *t*-BHP control.


SOME
SOI
Quercetin
Protection against cell death (%)
100
80
60
40
20
0
0
10
100
Extract/compound (μg/ml)


without 2 mM t-BHP, 5h
with 2 mM t-BHP, 5h
Cell viability (% of control)
100
80
60
40
20
0
***
###
###
###
—
SOME
SOI
Quercetin
Extract / Compound


without 2 mM t-BHP, 5h
with 2 mM t-BHP, 5h
***
##
##
###
Lipid peroxidation (% of t-BHP control)
100
80
60
40
20
0
—
SOME
SOI
Quercetin
Extract / Compound


Without t-BHP 2mM, 5 h
With t-BHP 2mM, 5 h
GSH (% of control)
0
20
40
60
80
100
***
*
###
###
*
##
—
SOME
SOI
Quercetin
Extract/Compound


without 200 μM t-BHP, 1h
with 200 μM t-BHP, 1h
***
###
100
80
60
40
20
0
DNA damage (% of t-BHP control)
—
SOME
SOI
Quercetin
Extract/Compound