The drinking of a *Salvia officinalis* infusion improves liver antioxidant status in mice and rats

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Abstract

In this study we evaluate the biosafety and bioactivity (antioxidant potential) of a traditional water infusion (tea) of common sage (*Salvia officinalis* L.) *in vivo* in mice and rats by quantification of plasma transaminase activities and liver GST and GR enzyme activities. The replacement of water by sage tea for 14 days in the diet of rodents did not affect the body weight and food consumption and did not induce liver toxicity. On the other hand, a significant increase of liver GST activity was observed in rats (24%) and mice (10%) of sage drinking groups. The antioxidant potential of sage tea drinking was also studied *in vitro* in a model using rat hepatocytes in primary culture. The replacement of drinking water with sage tea in the rats used as hepatocyte donors resulted in an improvement of the antioxidant status of rat hepatocytes in primary culture, namely a significant increase in GSH content and GST activity after 4 hours of culture. When these hepatocyte cultures were exposed to 0.75 or 1 mM of *tert*-butyl hydroperoxide for 1 hour, some protection against lipid peroxidation and GSH depletion was conferred by sage tea drinking. However, the cell death induced by *t*-BHP as shown by LDH leakage was not different from that observed in cultures from control animals. This study indicates that the compounds present in this sage preparation contain interesting bioactivities which improve the liver antioxidant potential.

Keywords: *Salvia officinalis* L. Infusion; Glutathione Status; Antioxidant Effects; Rat Hepatocytes; Mice; *tert*-Butyl Hydroperoxide.
1. Introduction

The oxidative damage of biological molecules is an important event in the development of a variety of human disorders that result from overwhelming the biological defense system against oxidative stress, drugs and carcinogens. The intake in the human diet of antioxidant compounds, or compounds that ameliorate or enhance the biological antioxidant mechanisms, can prevent and in some cases help in the treatment of some oxidative-related disorders and carcinogenic events (Havsteen, 2002).

Natural plant products have been used empirically for this purpose since ancient times and a tendency is emerging today for their increased use. *Salvia officinalis* L. (Lamiaceae) is a common aromatic and medicinal plant native from mediterranean countries that is in widespread use globally.

Experimental evidence already exists for a variety of bioactivities for different types of extracts of *S. officinalis* such as antioxidant, anti-inflammatory, hypoglycemic and anti-mutagenic activities (Cuvelier et al., 1994; Wang et al., 1998; Hohmann et al., 1999; Baricevic and Bartol, 2000; Zupko et al., 2001; Baricevic et al., 2001; Alarcon-Aguilar et al., 2002). However, the properties of sage infusion (hereafter referred to as tea), the most common form of consumption of this plant, have received little attention.

Many bioactivities have been researched and detected in tea and in infusions (or water extracts) of other plants. Among them, the phenolic content of different plants have been shown to have antioxidant activities and the capacity to modulate xenobiotic metabolizing enzymes involved in drug and carcinogen activation and detoxification (Triantaphyllou et al., 2001; Ferguson, 2001). Several studies showed that black and green tea (*Camellia sinensis*) enhance phase II enzymes (Khan et al., 1992; Yu et al., 1997; Bu-Abbas et al., 1998). A water-soluble extract of rosemary also induced both phase I and phase II enzymes (Debersac et al., 2001a; Debersac et al., 2001b). However,
the use of natural products may also result in toxic effects which underscore the need to understand the biological effects of natural compounds. Toxic effects to the liver, the main xenobiotic metabolizing organ, are particularly relevant.

In the present study we evaluate the biosafety and bioactivities of sage tea *in vivo* with mice and rats and *in vitro* using rat hepatocytes in primary culture. Toxic effects to the liver of sage tea drinking are tested *in vivo* on mice monitoring the plasma transaminase activities. The liver glutathione content and glutathione reductase and glutathione-s-transferase activities in the mouse livers and freshly isolated rat hepatocytes were also evaluated. In addition, primary cultures of hepatocytes isolated from sage tea and water drinking rats were challenged with the oxidant tert-butyl hydroperoxide and the antioxidant protection conferred by sage tea drinking evaluated.

2. Materials and methods

2.1. Chemicals

Collagenase (grade IV), tert-butyl hydroperoxide (t-BHP), glutathione reductase (EC 1.6.4.2.), 5,5’-dithio-bis-(2-nitrobenzoic acid) (DTNB), William’s Medium E (WME) and Bradford reagent were purchased from Sigma (St. Louis, MO, USA). L-Lactate dehydrogenase (EC 1.1.1.27) and L-malate dehydrogenase (EC 1.1.1.37) were purchased from Roche (Germany). All others reagents were of analytical grade.

2.2. Plant material, preparation of sage tea and analysis of its phenolic and volatile compounds

*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 lyophilized and kept a -20º C. Considering that sage is traditionally used as a tea, an
Infusion of sage was routinely prepared by pouring 150 ml of boiling water onto 2 g of the dried plant material and allowing to steep for 5 min. This produced an infusion of 3.5 ± 0.1 mg (mean ± SEM, n=6) of extract dry weight per ml of infusion (0.35 % (w/v)) and a yield of 26.3% (w/w) in terms of initial crude plant material dry weight.

Phenolic compounds were analysed by HPLC/DAD. Freeze-dried (Labconco Freeze Dry System) extract (0.01 g) was redissolved in 1 ml of ultrapure Milli Q water and aliquots of 20 microliters were injected in an HPLC/DAD system. Separation and identification of phenolic compounds by HPLC/DAD were performed as previously described (Santos-Gomes et al., 2002). The volatile constituents of the tea (150 ml) were extracted, at room temperature, with 5 ml of n-pentane containing 5a-cholestan (1 mg/ml). The volatile compounds were then identified by GC and GC-MS as previously described (Lima et al., 2004).

2.3. Animals

Female Balb/c mice (8-10 weeks) and male Wistar rats (150-200g) were purchased from Charles River Laboratories (Spain) and acclimated to our laboratory animal facilities for at least one week before the start of the experiments. During this period, the animals were maintained on a natural light/dark cycle at 20 ± 2 ºC and given food and tap water ad libitum. The animals used in the two experiments were kept and handled in accordance to our University regulations. In experiment 1, mice were used to evaluate in vivo the liver toxicity of sage tea drinking for 14 days and changes in the liver glutathione levels as well as in the activities of glutathione-related enzymes. In experiment 2, rats from two different drinking groups (water and sage tea) were used for hepatocyte isolation for establishment of primary cultures. The primary cultures of hepatocytes isolated from sage tea and water drinking rats were challenged with the
oxidant tert-butyl hydroperoxide and the antioxidant protection conferred by sage tea drinking evaluated.

2.4. Experiment 1

Ten female Balb/c mice were randomly divided into two groups, given food *ad libitum* and either drinking water (tap) or sage tea *ad libitum* for 14 days (beverage was renewed daily). On day 15 the animals were sacrificed by cervical dislocation and blood samples collected for measurement of plasma transaminase activities (ALT-alanine aminotransferase and AST-aspartate aminotransferase). The livers were also collected, frozen in liquid nitrogen and kept at -80°C for later analysis of glutathione content and activities of glutathione reductase (GR) and glutathione-s-transferase (GST).

2.5. Experiment 2

Eight male Wistar rats were randomly divided into two groups and given food *ad libitum* with either drinking water (tap) or sage tea *ad libitum* for 14 days (beverage was renewed daily). On day 15 hepatocytes were isolated and used to establish primary cultures.

Hepatocyte isolation was performed between 10:00 a.m. and 11:00 a.m. by collagenase perfusion as previously described by Moldeus (Moldeus et al., 1978) with some modifications (Lima et al., 2004). Cell viability was > 85% as estimated by the trypan blue exclusion test. Aliquots of the cell suspensions were kept at -80°C for measurement of GR and GST activities and quantification of glutathione levels at the start of the *in vitro* experiments, i.e., time zero of primary cultures. Then, cells were suspended in William’s medium E (WME) supplemented with 10 % fetal bovine serum (FBS), $10^{-9}$ M insulin and $10^{-9}$ M dexamethasone and seeded onto 6-well culture plates.
at a density of $1 \times 10^6$ cells/well. The culture plates were incubated at 37°C in a
humidified incubator gassed with 5 % CO$_2$/95 % air.

Three hours after plating, the culture medium was replaced with WME
supplemented with 10 % FBS and t-BHP 0, 0.75 or 1 mM for 1 hour to induce
cytotoxicity (Rush et al., 1985). To assess the protection conferred by sage tea drinking
culture medium and cells were collected and the activities of lactate dehydrogenase
(LDH), GR and GST determined. The levels of malondialdehyde and glutathione were
also measured.

2.6. Biochemical analysis
2.6.1. Enzyme activities

**ALT and AST:** The alanine aminotransferase and aspartate aminotransferase
activities were measured spectrophotometrically in plasma of mice following NADH
oxidation (at 30°C) at 340 nm on a plate reader (Spectra Max 340pc, Molecular
Devices). For ALT activity, the reaction mixture consisted of 200 mM L-alanine, 25
µM pyridoxalphosphate, 0.12 mM NADH, 12 Units/ml L-lactate dehydrogenase and
10.5 mM alpha-ketoglutarate in 50 mM imidazole (pH 7.4). For AST activity, the
reaction mixture consisted of 40 mM aspartate, 25 µM pyridoxalphosphate, 0.12 mM
NADH, 8 Units/ml L-malate dehydrogenase and 7 mM alpha-ketoglutarate in 50 mM
imidazole (pH 7.4). The activities are expressed as µmol of substrate oxidized per
minute per liter of plasma (U/L).

**GR and GST:** For measurement of mice liver glutathione reductase and
glutathione-s-transferase activities, the livers were homogenised individually in a
phosphate/glycerol buffer pH 7.4 (Na$_2$HPO$_4$ 20 mM; β-mercaptoethanol 5 mM; EDTA
0.5 mM; BSA 0.2% (w/v); aprotinine 10µg/ml and glycerol 50% (v/v)) and centrifuged
at 10000 g for 10 min at 4ºC and the supernatant collected. In the case of the cells
collected after exposure to t-BHP (primary cultures of hepatocytes) as well as the time
zero hepatocyte aliquots, the samples were homogenised by sonication in
phosphate/glycerol buffer pH 7.4, centrifuged at 10000 g for 10 min at 4ºC and the
supernatant collected.

The GR activity was measured spectrophotometrically at 340 nm following
NADPH oxidation at 30ºC. The reaction mixture consisted of 3 mM GSSG, 2.5 mM
EDTA and 0.12 mM NADPH in 50 mM Hepes (pH 7.4) and homogenized supernatant.
The activity is expressed as nmol of NADPH oxidized/min/mg protein (mU/mg).

The GST activity was measured spectrophotometrically at 340 nm following the
formation of GSH conjugate with 1-chloro-2,4-dinitrobenzene (CDNB) at 30ºC. The
reaction mixture consisted of 1 mM GSH and 1 mM CDNB (dissolved in ethanol) in 50
mM Hepes (pH 7.4). The activity was calculated using an extinction coefficient of 9.6
mM$^{-1}$ cm$^{-1}$ and expressed as nmol of conjugate/min/mg protein (mU/mg).

**LDH:** To assess the extend of cell death caused by t-BHP, the determination of
lactate dehydrogenase activity in the culture medium was used as indicator of plasma
membrane integrity of hepatocytes. The enzyme activity was measured at 30ºC by
quantification NADH (0.28 mM) consumption by continuous spectrophotometry (at 340
nm) on a plate reader using pyruvate (0.32 mM) as substrate in 50 mM phosphate buffer
(pH 7.4). The results are expressed as µmol of substrate oxidized per minute per mg
protein (U/mg).

2.6.2. Lipid peroxidation

The extent of hepatocyte lipid peroxidation was estimated by the levels of
malondialdehyde (MDA). The thiobarbituric acid reactive substances (TBARS) assay at
535 nm was used as described previously (Fernandes et al., 1995) but with some
modifications for cultured hepatocytes. Briefly, 360 µl of culture medium was
precipitated with 60 µl of 50% trichloroacetic acid. After centrifugation, 300 µl of the
supernatant were added to an equal volume of 1 % thiobarbituric acid and the mixture
was heated for 10 min in a boiling water bath, allowed to cool and the absorbance
measured at 535 nm. The results are expressed as nmol MDA/mg of protein using a
molar extinction coefficient of 1.56×10^5 M^-1 cm^-1.

2.6.3. Glutathione content

The glutathione content of mice livers, time zero hepatocyte aliquots and 4
hours of cultured rat hepatocytes were determined by the DTNB-GSSG reductase
recycling assay as previously described (Anderson, 1985), with some modifications
(Lima et al, 2004). 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. Statistical Analysis

Data are expressed as means ± SEM. The comparison between the means of
treatment (sage tea) and control group was performed using Student’s t-test. For
primary cultures of hepatocytes a two-way ANOVA followed by the Bonferroni post-
test were employed to compare the in vivo treatment (water vs sage tea) and in vitro
treatment (t-BHP concentrations). P values ≤ 0.05 were considered statistically
significant.

3. Results
3.1. Phenolic and volatile compounds in sage tea

The infusion is composed of the phenolic compounds rosmarinic acid and four luteolin glycosides - luteolin-7-glucoside being the most representative flavone (table 1) which constitute 0.05% of total wet weight. In this sage infusion we also identified 25 volatile compounds with 1,8-cineole, cis-thujone, trans-thujone, champhor and borneol being the most representative (85% of total volatile fraction). The most representative volatile compounds and their quantification are presented in table 1.

3.2. Experiment 1

Water replacement with sage tea for 14 days did not affected food consumption and body weights in mice groups during the experiment (data not shown). However drinking was slightly different between the two groups – water drinking group: 11.0 ± 0.4 ml/day/100 g; sage tea drinking group: 10.0 ± 0.5 ml/day/100 g of body weight. Plasma ALT and AST activities (table 2) were not different between water and sage drinking animals. Also the levels of reduced glutathione (GSH) and oxidized glutathione (GSSG) in the mice livers were not different between the two groups (table 2).

The activities of glutathione-related enzymes, GR and GST, were significantly higher (10%) in livers of sage tea drinking mice (table 2).

3.3. Experiment 2

The replacement of drinking water with the sage tea did not affect food and drink consumption as well as the body weight of rats (data not shown). Immediately after collagenase isolation glutathione levels of rat hepatocytes were similar in the two groups (table 3), water and sage tea drinking, and smaller than
those in the mice livers. GST activity was significantly enhanced in isolated rat hepatocytes from sage tea drinking animals (table 3) with an increase of 1.24 fold relative to the water drinking group. No differences were observed in GR activity.

There was a marked increase in GSH values from time zero hepatocyte aliquots to 4 hours cultured hepatocytes (table 3), both from water and sage tea drinking animals. However, comparing the values (t-BHP 0 mM) measured in the primary cultures, a significantly higher GSH content (1.35 fold) was observed (table 3) after 4 hours of culture (3 hours of pre-incubation plus 1 hour with 0 mM of t-BHP) in hepatocytes of sage drinking animals. After 4 hours in culture, the GST activity decreased somewhat but remained higher (1.25 fold) in the cells from sage drinking animals. The GR activity was also somewhat increased in the hepatocytes of sage drinking rats although not significantly.

Incubation of rat hepatocyte primary cultures with t-BHP at 0.75 mM or 1 mM for 1 hour resulted in significant cell damage as shown by a strong increase in LDH activity in the culture medium, higher cellular lipid peroxidation and GSSG levels, as well as the significant decrease in GSH levels (table 3). t-BHP did not affect GR activity and only at the concentration of 1 mM was the GST activity significantly reduced (table 3) when compared with the respective controls.

The extent of t-BHP-induced lipid peroxidation was lower in cells of sage tea drinking animals. This effect was only marginally non-significant (P = 0.051). The GSH levels of hepatocytes challenged with t-BHP remained significantly higher in the cultures of sage tea drinking rats (table 3). Following exposure to t-BHP the reduction of GSH in hepatocytes of the sage tea drinking group was not as dramatic as the one observed in hepatocytes from water drinking animals (figure 1) being significantly different at 1 mM of t-BHP. However, when exposed to 0.75 mM or 1 mM of t-BHP no
protective effect of sage tea drinking was observed in LDH leakage as well as in GSSG content (table 3).

4. Discussion

The present study shows that sage tea drinking had no toxicity to the liver and no adverse effects on growth parameters neither in mice nor in rats. It also shows that sage tea drinking positively affected the antioxidant status of the liver, mainly the GST and GR activities of the mice livers and GST activity in rats.

The positive effects of sage tea drinking were also present in cultured hepatocytes. Immediately after collagenase isolation GST activity was higher in cells isolated from sage tea drinking rats. At this point GSH levels were not different from those of control cells. After four hours in culture GSH content increased in both groups. However, this increase was dramatically higher in cells isolated from sage tea drinking animals indicating better recovery of this group of cells from the oxidative stress imposed by collagenase isolation.

Also following treatment with t-BHP, GSH content and GST activity remained significantly higher in the cells from tea drinking animals. This higher antioxidant status was probably the cause of the smaller extent of lipid peroxidation induced by t-BHP to these cells compared to those of water drinking animals. However, in spite of this, cell death, as indicated by LDH leakage, was not prevented in the cells of sage tea drinking animals. Although not done in this study cell recovery after the removal of the toxic might have been higher in cells of sage tea drinking animals.

An enhancement of GST activity and other phase II enzymes due to treatment with water extracts of plants, namely *Camellia sinensis* and *Rosmarinus officinalis* has been reported (Bu-Abbas et al., 1998; Debersac et al., 2001b), and related to cancer
chemoprevention (Saha and Das, 2003). In accordance with this, we also found an enhancement of GST activity in the livers of both mice and rats due to sage tea drinking for 14 days. The observed increase in liver GST activity after tea drinking was smaller in comparison with other studies, for example with the water-soluble extract of rosemary (Debersac et al., 2001a; Debersac et al., 2001b) and *Camellia sinensis* (Bu-Abbas et al., 1998). Apart from the differences in extract composition, this may be due to the fact that our water preparation was much more diluted, only about 0.35% (w/v), than that used in the above mentioned studies.

According to the work done by Debersac and collaborators (Debersac et al., 2001b), where individual compounds were administered orally to rats, rosmarinic acid (also the most abundant phenolic compound present in this sage tea) could not be responsible for the observed increase in GST activity. This effect could be due to the luteolin glycosides, since induction of GST activity has been reported as the result of dietary ingestion of certain antioxidant flavonoids (Siess et al., 1996; Birt et al., 2001; Ross and Kasum, 2002; Ren et al., 2003). There is also a possibility that components of the essential oil fraction present in sage tea could contribute to the increase in the GST activity, since monoterpenes (including camphor) have been reported to induce phase II enzymes such as GST and UGT (Elegbede et al., 1993; Banerjee et al., 1995). Unidentified compounds present in this water extract belonging to other classes of compounds, such as aminoacids, organic acids, sugars and other polar compounds could also contribute to the observed effects. It should also be kept in mind that due to the complexity of the mixture that plant extracts are, a synergistic interaction between the compounds could be the ultimate cause for the observed effects.

A higher content of glutathione as well as increased activity of GST and GR were present in the cells from sage tea drinking animals indicating a better recovery.
from collagenase treatment. Glutathione is the major cellular nucleophile and provides an efficient detoxification pathway for a variety of electrophilic reactive metabolites (Reed, 1990; Kedderis, 1996; Lu, 1999). The higher activity of GR could contribute to the maintenance of glutathione in the reduced form when challenged with t-BHP. In addition, an enhancement of de novo glutathione synthesis by the hepatocytes of sage drinking animals induced by a possible bioactive compound present in the sage water extract can not be ruled out. Some studies suggest that the enhancement of phase II enzymes by antioxidants, such as polyphenols present in plant water extracts, is achieved by upregulating the corresponding genes by interaction with antioxidant response elements (AREs) that transcriptionally regulate these genes (Ferguson, 2001). It has also been shown that the γ-glutamylcysteine synthetase (γ-GCS), a key enzyme in de novo glutathione synthesis, is also transcriptional regulated by AREs (Lu, 1999; Griffith, 1999; Myhrstad et al., 2002), and it is known that several treatments that induce expression of phase II detoxifying enzymes also result in elevated γ-GCS activity as well as increased intracellular GSH levels (Mulcahy et al., 1997). So, although not studied, there is the possibility that also in this case, the interaction of some compounds present in the water extract of sage with AREs in vivo, would result in a higher GST and γ-GCS activities and explain the significant increased GSH recovery after 4 hours in culture of hepatocytes of sage tea drinking rats.

Concluding, this study shows that the *S. officinalis* water extract obtained and consumed as the plant’s herbal tea positively affects the antioxidant status of the liver and may have hepatoprotective potential that justify further studies. Because failure to cope with oxidative stress is a common factor in the aetiology of many diseases salvia’s effects on the improvement of the antioxidant response could provide an explanation for the wide ranging medicinal properties attributed to salvia.
Acknowledgments

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References


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### Results (tables)

Table 1 – Phenolic and volatile compounds of sage tea.

<table>
<thead>
<tr>
<th>Component</th>
<th>%</th>
<th>µg/ml sage infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>99.65</td>
<td></td>
</tr>
<tr>
<td>Phenolic acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rosmarinic acid</td>
<td>0.04</td>
<td>362.0</td>
</tr>
<tr>
<td>Flavonoids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luteolin 7-glucoside</td>
<td>0.01</td>
<td>115.3</td>
</tr>
<tr>
<td>Others luteolin glycosides (3)</td>
<td>&lt; 0.01</td>
<td>48.5</td>
</tr>
<tr>
<td>Volatile components</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,8-Cineole</td>
<td>&lt;&lt; 0.01</td>
<td>4.8</td>
</tr>
<tr>
<td>cis-Thujone [= (-)-thujone]</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>trans-Thujone [= (+)-thujone]</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Camphor</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Borneol</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Others (20)</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>0.30</td>
<td>2972.0</td>
</tr>
</tbody>
</table>
Table 2 – Effect of sage tea on plasma transaminase activities, liver glutathione levels and liver glutathione-related enzyme activities after 14 days of treatment in mice.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>In vivo beverage</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>water</td>
<td>Sage tea</td>
<td></td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>36 ± 6</td>
<td>30 ± 6</td>
<td></td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>90 ± 11</td>
<td>89 ± 11</td>
<td></td>
</tr>
<tr>
<td>GR (mU/mg)</td>
<td>13.4 ± 0.1</td>
<td>14.7 ± 0.4 *</td>
<td></td>
</tr>
<tr>
<td>GST (mU/mg)</td>
<td>107 ± 3</td>
<td>119 ± 2 *</td>
<td></td>
</tr>
<tr>
<td>GSH (nmol/mg)</td>
<td>46.1 ± 0.9</td>
<td>47.4 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>GSSG (nmol GSHequiv/mg)</td>
<td>2.1 ± 0.1</td>
<td>2.0 ± 0.2</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SEM, n=5. * P≤ 0.05 when compared with the respective control.
Table 3 – Effect of sage tea consumption (*in vivo* for 14 days) on t-BHP-induced toxicity in primary culture of rat hepatocytes and on liver glutathione levels and liver glutathione-related enzymes activities of rat hepatocytes after collagenase isolation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>In vivo beverage</th>
<th>Rat hepatocytes (after isolation)</th>
<th>Primary cultures of rat hepatocytes - t-BHP (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>LDHextr (U/mg)</td>
<td>water</td>
<td>-</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>sage tea</td>
<td>-</td>
<td>0.09 ± 0.03</td>
</tr>
<tr>
<td>TBARS (nmol/mg)</td>
<td>water</td>
<td>-</td>
<td>0.10 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>sage tea</td>
<td>-</td>
<td>0.03 ± 0.02</td>
</tr>
<tr>
<td>GSH (nmol/mg)</td>
<td>water</td>
<td>21.9 ± 1.3</td>
<td>38.1 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>sage tea</td>
<td>20.4 ± 3.1</td>
<td>51.4 ± 3.6 **</td>
</tr>
<tr>
<td>GSSG (nmol GSH equiv/mg)</td>
<td>water</td>
<td>tr</td>
<td>0.9 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>sage tea</td>
<td>tr</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>GR (mU/mg)</td>
<td>water</td>
<td>21.4 ± 1.6</td>
<td>22.0 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>sage tea</td>
<td>21.5 ± 1.2</td>
<td>25.5 ± 2.9</td>
</tr>
<tr>
<td>GST (mU/mg)</td>
<td>water</td>
<td>209 ± 4</td>
<td>168 ± 9</td>
</tr>
<tr>
<td></td>
<td>sage tea</td>
<td>260 ± 18 *</td>
<td>210 ± 9 *</td>
</tr>
</tbody>
</table>

Values are means ± SEM, n=4 (except rat hepatocytes after isolation, n=3). *P ≤ 0.05, **P ≤ 0.01 and ***P ≤ 0.001 when compared with the respective control. # P ≤ 0.05, ## P ≤ 0.01 and ### P ≤ 0.001 between the water and sage tea in the same situation. tr – trace amounts.
**Results (figure)**

Figure 1 – Effect of sage tea consumption (*in vivo* for 14 days) on *t*-BHP-induced decrease in GSH content of primary hepatocyte cultures, presented as percentage from control. Absolute values presented in table 3. Values are means ± SEM, n=4. * $P \leq 0.05$, significantly different with Student’s *t*-test.
Lima et al. (figure 1)