# 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-cholestane (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 hapatocyte 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 -80ºC 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 a -80ºC 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

126 at a density of $1\times10^6$ cells/well. The culture plates were incubated at 37ºC in a
127 humidified incubator gassed with 5 % $CO_2$/95 % air.
128 Three hours after plating, the culture medium was replaced with WME
129 supplemented with 10 % FBS and *t*-BHP 0, 0.75 or 1 mM for 1 hour to induce
130 cytotoxicity (Rush et al., 1985). To assess the protection conferred by sage tea drinking
131 culture medium and cells were collected and the activities of lactate dehydrogenase
132 (LDH), GR and GST determined. The levels of malondialdehyde and glutathione were
133 also measured.
134
135 *2.6. Biochemical analysis*
136 *2.6.1. Enzyme activities*
137 *ALT and AST:* The alanine aminotransferase and aspartate aminotransferase
138 activities were measured spectrophotometrically in plasma of mice following NADH
139 oxidation (at 30ºC) at 340 nm on a plate reader (Spectra Max 340pc, Molecular
140 Devices). For ALT activity, the reaction mixture consisted of 200 mM L-alanine, 25
141 μM pyridoxalphosphate, 0.12 mM NADH, 12 Units/ml L-lactate dehydrogenase and
142 10.5 mM alpha-ketoglutarate in 50 mM imidazole (pH 7.4). For AST activity, the
143 reaction mixture consisted of 40 mM aspartate, 25 μM pyridoxalphosphate, 0.12 mM
144 NADH, 8 Units/ml L-malate dehydrogenase and 7 mM alpha-ketoglutarate in 50 mM
145 imidazole (pH 7.4). The activities are expressed as μmol of substrate oxidized per
146 minute per liter of plasma (U/L).
147 *GR and GST:* For measurement of mice liver glutathione reductase and
148 glutathione-s-transferase activities, the livers were homogenised individually in a
149 phosphate/glycerol buffer pH 7.4 ($Na_2HPO_4$ 20 mM; β-mercaptoethanol 5 mM; EDTA
150 0,5 mM; BSA 0,2% (w/v); aprotinine 10μg/ml and glycerol 50% (v/v)) and centrifuged

151 at 10000 g for 10 min at 4ºC and the supernatant collected. In the case of the cells
152 collected after exposure to *t*-BHP (primary cultures of hepatocytes) as well as the time
153 zero hepatocyte aliquots, the samples were homogenised by sonication in
154 phosphate/glycerol buffer pH 7.4, centrifuged at 10000 g for 10 min at 4ºC and the
155 supernatant collected.

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

160 The GST activity was measured spectrophotometrically at 340 nm following the
161 formation of GSH conjugate with 1-chloro-2,4-dinitrobenzene (CDNB) at 30ºC. The
162 reaction mixture consisted of 1 mM GSH and 1 mM CDNB (dissolved in ethanol) in 50
163 mM Hepes (pH 7.4). The activity was calculated using an extinction coefficient of 9.6
164 $mM^{-1}$ $cm^{-1}$ and expressed as nmol of conjugate/min/mg protein (mU/mg).

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

172

173 *2.6.2. Lipid peroxidation*

174 The extent of hepatocyte lipid peroxidation was estimated by the levels of
175 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 $1.56\times10^5$ $M^{-1}$ $cm^{-1}$.

*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 $\leq 0.05$ 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

226 those in the mice livers. GST activity was significantly enhanced in isolated rat
227 hepatocytes from sage tea drinking animals (table 3) with an increase of 1.24 fold
228 relative to the water drinking group. No differences were observed in GR activity.
229 There was a marked increase in GSH values from time zero hepatocyte aliquots
230 to 4 hours cultured hepatocytes (table 3), both from water and sage tea drinking
231 animals. However, comparing the values (*t*-BHP 0 mM) measured in the primary
232 cultures, a significantly higher GSH content (1.35 fold) was observed (table 3) after 4
233 hours of culture (3 hours of pre-incubation plus 1 hour with 0 mM of *t*-BHP) in
234 hepatocytes of sage drinking animals. After 4 hours in culture, the GST activity
235 decreased somewhat but remained higher (1.25 fold) in the cells from sage drinking
236 animals. The GR activity was also somewhat increased in the hepatocytes of sage
237 drinking rats although not significantly.
238 Incubation of rat hepatocyte primary cultures with *t*-BHP at 0.75 mM or 1 mM
239 for 1 hour resulted in significant cell damage as shown by a strong increase in LDH
240 activity in the culture medium, higher cellular lipid peroxidation and GSSG levels, as
241 well as the significant decrease in GSH levels (table 3). *t*-BHP did not affect GR
242 activity and only at the concentration of 1 mM was the GST activity significantly
243 reduced (table 3) when compared with the respective controls.
244 The extent of *t*-BHP-induced lipid peroxidation was lower in cells of sage tea
245 drinking animals. This effect was only marginally non-significant ($P = 0.051$). The GSH
246 levels of hepatocytes challenged with *t*-BHP remained significantly higher in the
247 cultures of sage tea drinking rats (table 3). Following exposure to *t*-BHP the reduction
248 of GSH in hepatocytes of the sage tea drinking group was not as dramatic as the one
249 observed in hepatocytes from water drinking animals (figure 1) being significantly
250 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 colagenase 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

276 chemoprevention (Saha and Das, 2003). In accordance with this, we also found an
277 enhancement of GST activity in the livers of both mice and rats due to sage tea drinking
278 for 14 days. The observed increase in liver GST activity after tea drinking was smaller
279 in comparison with other studies, for example with the water-soluble extract of
280 rosemary (Debersac et al., 2001a; Debersac et al., 2001b) and *Camellia sinensis* (Bu-
281 Abbas et al., 1998). Apart from the differences in extract composition, this may be due
282 to the fact that our water preparation was much more diluted, only about 0.35% (w/v),
283 than that used in the above mentioned studies.

284 According to the work done by Debersac and collaborators (Debersac et al.,
285 2001b), where individual compounds were administered orally to rats, rosmarinic acid
286 (also the most abundant phenolic compound present in this sage tea) could not be
287 responsible for the observed increase in GST activity. This effect could be due to the
288 luteolin glycosides, since induction of GST activity has been reported as the result of
289 dietary ingestion of certain antioxidant flavonoids (Siess et al., 1996; Birt et al., 2001;
290 Ross and Kasum, 2002; Ren et al., 2003). There is also a possibility that components of
291 the essential oil fraction present in sage tea could contribute to the increase in the GST
292 activity, since monoterpenes (including camphor) have been reported to induce phase II
293 enzymes such as GST and UGT (Elegbede et al., 1993; Banerjee et al., 1995).
294 Unidentified compounds present in this water extract belonging to other classes of
295 compounds, such as aminoacids, organic acids, sugars and other polar compounds could
296 also contribute to the observed effects. It should also be kept in mind that due to the
297 complexity of the mixture that plant extracts are, a synergistic interaction between the
298 compounds could be the ultimate cause for the observed effects.

299 A higher content of glutathione as well as increased activity of GST and GR
300 were present in the cells from sage tea drinking animals indicating a better recovery

301 from collagenase treatment. Glutathione is the major cellular nucleophile and provides
302 an efficient detoxification pathway for a variety of electrophilic reactive metabolites
303 (Reed, 1990; Kedderis, 1996; Lu, 1999). The higher activity of GR could contribute to
304 the maintenance of glutathione in the reduced form when challenged with *t*-BHP. In
305 addition, an enhancement of *de novo* glutathione synthesis by the hepatocytes of sage
306 drinking animals induced by a possible bioactive compound present in the sage water
307 extract can not be ruled out. Some studies suggest that the enhancement of phase II
308 enzymes by antioxidants, such as polyphenols present in plant water extracts, is
309 achieved by upregulating the corresponding genes by interaction with antioxidant
310 response elements (AREs) that trancriptionally regulate these genes (Ferguson, 2001). It
311 has also been shown that the γ-glutamylcysteine synthetase (γ-GCS), a key enzyme in
312 *de novo* glutathione synthesis, is also transcriptional regulated by AREs (Lu, 1999;
313 Griffith, 1999; Myhrstad et al., 2002), and it is known that several treatments that
314 induce expression of phase II detoxifying enzymes also result in elevated γ-GCS
315 activity as well as increased intracellular GSH levels (Mulcahy et al., 1997). So,
316 although not studied, there is the possibility that also in this case, the interaction of
317 some compounds present in the water extract of sage with AREs *in vivo*, would result in
318 a higher GST and γ-GCS activities and explain the significant increased GSH recovery
319 after 4 hours in culture of hepatocytes of sage tea drinking rats.
320 Concluding, this study shows that the *S. officinalis* water extract obtained and
321 consumed as the plant's herbal tea positively affects the antioxidant status of the liver
322 and may have hepatoprotective potential that justify further studies. Because failure to
323 cope with oxidative stress is a common factor in the aetiology of many diseases salvia's
324 effects on the improvement of the antioxidant response could provide an explanation for
325 the wide ranging medicinal properties attributed to salvia.

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## Results (tables)

Table 1 – Phenolic and volatile compounds of sage tea.

| Component | | % | µg/ml sage infusion |
|---|---|---|---|
| Water | | 99.65 | |
| Phenolic acids | | | |
| | Rosmarinic acid | 0.04 | 362.0 |
| Flavonoids | | | 163.7 |
| | Luteolin 7-glucoside | 0.01 | 115.3 |
| | Others luteolin glycosides (3) | < 0.01 | 48.5 |
| Volatile components | | << 0.01 | 4.8 |
| | 1,8-Cineole | | 0.9 |
| | *cis*-Thujone [= (-)-thujone] | | 1.7 |
| | *trans*-Thujone [=(+)-thujone] | | 0.3 |
| | Camphor | | 0.5 |
| | Borneol | | 0.7 |
| | Others (20) | | 0.7 |
| Unknown | | 0.30 | 2972.0 |

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.

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

Values are means ± SEM, n=5. * $P \leq 0.05$ 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.

| Parameter | *In vivo* beverage | Rat hepatocytes (after isolation) | Primary cultures of rat hepatocytes - *t*-BHP (mM) | | |
|---|---|---|---|---|---|
| | | | 0 | 0.75 | 1 |
| LDHextr (U/mg) | water | - | 0.06 ± 0.01 | 0.40 ± 0.03 ** | 0.72 ± 0.09 *** |
| | sage tea | - | 0.09 ± 0.03 | 0.40 ± 0.07 * | 0.78 ± 0.14 *** |
| TBARS (nmol/mg) | water | - | 0.10 ± 0.06 | 1.89 ± 0.09 *** | 3.38 ± 0.45 *** |
| | sage tea | - | 0.03 ± 0.02 | 1.30 ± 0.27 * | 2.62 ± 0.45 *** |
| GSH (nmol/mg) | water | 21.9 ± 1.3 | 38.1 ± 2.7 | 25.0 ± 0.6 *** | 12.5 ± 1.2 *** |
| | sage tea | 20.4 ± 3.1 | 51.4 ± 3.6 ## | 36.3 ± 1.4 *** ## | 23.3 ± 2.1 *** ## |
| GSSG (nmol GSHequiv/mg) | water | tr | 0.9 ± 0.5 | 7.9 ± 0.5 ** | 8.4 ± 1.2 *** |
| | sage tea | tr | 0.7 ± 0.2 | 9.3 ± 2.3 *** | 9.7 ± 1.2 *** |
| GR (mU/mg) | water | 21.4 ± 1.6 | 22.0 ± 0.9 | 19.9 ± 2.2 | 20.4 ± 2.5 |
| | sage tea | 21.5 ± 1.2 | 25.5 ± 2.9 | 24.0 ± 1.7 | 19.9 ± 0.3 |
| GST (mU/mg) | water | 209 ± 4 | 168 ± 9 | 162 ± 12 | 135 ± 8 |
| | sage tea | 260 ± 18 # | 210 ± 9 # | 184 ± 7 | 153 ± 4 * |

Values are means ± SEM, n=4 (except rat hepatocytes after isolation, n=3). * $P \leq 0.05$, ** $P \leq 0.01$ and *** $P \leq 0.001$ when compared with the respective control. # $P \leq 0.05$, ## $P \leq 0.01$ and ### $P \leq 0.001$ 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)