Salvia Officinalis Infusion Improves Liver Antioxidant Status And Protects From Hypercholesterolemia

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ABSTRACT

This study was aimed to determine the phytosterol components of Salvia officinalis infusion (sage tea). Also, its antioxidant and hypocholesterolemic function. This study was performed on Swiss rats divided into 4 groups; normal control group I. Group II, in which animals were fed on normal diet and received sage tea in a dose of 35mg/kg bw./day. Group III, where rats were maintained on high cholesterol diet for 4 weeks. Group IV, in which rats were maintained on high cholesterol diet while receiving sage tea in a dose of 35mg/kg bw./day for 4 weeks. Plasma transaminases, cholesterol, triglycerides, LDL, and lipidperoxides, liver GSH, and its related enzymes, glutathione-S-transferase(GST) and glutathione reductase(GR) activities were determined to study biosafety of sage and its protective effect against hypercholesterolemia. Sage tea exerted a significant decrease in total cholesterol, triglycerides, LDL, and lipid peroxides of rats maintained on high cholesterol diet. (Group IV) compared to group III. \( p<0.01 \), \( 0.05 \), \( 0.01 \) and \( 0.001 \) respectively. The present study shows that sage tea drinking had no toxicity to the liver and no adverse effects on growth parameters in rats. It also shows that sage tea drinking positively affected the antioxidant status of the liver, mainly the GSH, GST and GR activities of the rat livers. It can be concluded that, phytosterol, (B-Sitosterol and Stigmasterol) in sage tea acts as an antioxidant and exerts protective effect against hypercholesterolemia. © 2007 Trade Science Inc. - INDIA

INTRODUCTION

Salvia officinalis (sage), is a common aromatic and medicinal plant native from mediterranean countries that is in widespread use. It is also known as garden meadow, has a long tradition of culinary and medicinal use. Sage was once used to help preserve meat and over the past 2,000 years or so. It has been recommended by herbalists to treat just about every known condition, from snakebite to mental illness. In fact, in medieval times the French called the herb toute bonne, which means, “all is well”. Modern research has shown that sage, can help reduce excessive perspiration, digestive problems, sore throats, premenstrual cramps,
and high blood sugar. Sage was once recommended by herbalists to treat fever, a usage that probably arose from sage’s ability to reduce perspiration. Modern research has demonstrated that sage reduces perspiration by as much as 50 percent, and Commission E, the group that evaluates the safety and efficacy of herbs for the German government, approves the use of sage infusions to treat excessive perspiration.

Experimental evidence already exists for a variety of bioactivities for different types of extracts of *Salvia officinalis* such as antioxidant, anti-inflammatory, hypoglycemic and anti-mutagenic activities. 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.

De Chile documented that, oxidation of low density lipoprotein (LDL) is a major risk factor for atherosclerosis and hence search for potent natural antioxidants, such as flavonoids, against LDL oxidation is the focus of extensive research. Dietary intervention studies incorporating phytosterol-enriched margarine spreads have reported significant decreases in total and low-density lipoprotein (LDL) cholesterol in populations with both normal lipid levels and those with hypercholesterolemia.

The present study was aimed to determine the phenolic components of sage, and study its biosafety in vivo with rats. Toxic effects to the liver of sage tea drinking are tested in vivo on rats monitoring the plasma transaminase activities. The liver glutathione content and glutathione reductase (GR) and glutathione-S-transferase (GST) activities in rats liver were evaluated to study the antioxidant protection conferred by sage tea drinking. Also, its protection against hypercholesterolemia induced by high cholesterol diet containing sodium cholate.

**MATERIALS AND METHODS**

**Chemicals**

Reduced glutathione GSH, glutathione reductase (EC 1.6.4.2) GR and glutathione peroxidase GPX kits were purchased from sigma (St. Louis, MO, USA). Cholesterol, triglycerides, LDL, and lipidperoxide kits were purchased from bio-Merieux (France). All others reagents were of analytical grade.

**Plant material, preparation of sage tea and analysis of its phenolic compounds**

*Salvia officinalis*

The aerial parts of plants were lyophilized and kept at 20°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 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.

Hydrocarbons (Phenolic compounds) were analysed by HPLC. Freeze-dried extract (0.01 g) was redissolved in 1 ml of ultrapure Milli Q water and aliquots of 20 µl were injected in an HPLC system. Separation and identification of phenolic compounds by HPLC were performed as previously described by Santos-Gomes et al.

**Animals**

Male Wistar rats (150-200 g) were used throughout this work. Animals were maintained on a natural light/dark cycle at 20 ± 2°C and given food and tap water ad libitum. Animals were divided into the following groups 15 rats each:

**Group I** : Considered as control group, in which animals were maintained on normal diet.

**Group II** : Animals were received sage tea in a concentration of 35 mg/kg bw/day by oral tube and normal diet for 4 weeks.

**Group III** : Animals of this group were maintained on high cholesterol diet, containing 1% cholesterol and 0.25% sodium cholate for 4 weeks to induce hypercholesterolemia according to Seki, et al.

**Group IV** : Animals received sage tea (35 mg/kg bw/day) and maintained on high cholesterol diet for 4 weeks.

Animals were sacrificed after 4 weeks and plasma were collected. Livers were dissected for immediate determination of glutathione levels as well as the activi-
ties of glutathione-related enzymes, to evaluate in vivo the liver toxicity of sage tea drinking and its antioxidative effect.

Total cholesterol, triglycerides, LDL, and lipid-peroxides were determined in animals of group I, III and IV to evaluate the protective effect of sage tea against hypercholesterolemia.

**Methods**

The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured spectrophotometrically in plasma of rats using Randox kits. The activities are expressed as μmol of substrate oxidized per minute per liter of plasma (U/L).

**GR and GST**

For measurement of rat liver glutathione reductase (GR) and glutathione-S-transferase (GST) activities, the livers were homogenised individually in a phosphate/glycerol buffer pH 7.4 (Na₂HPO₄ 20mM; β-mercaptoethanol 5mM; EDTA 0.5mM; BSA 0.2%(w/v); aprotinine 10μg/ml and glycerol 50%, v/v) and centrifuged at 10,000×g for 10min at 4°C and the supernatant collected. The GR activity was measured spectrophotometrically at 340nm following NADPH oxidation at 30°C. The GST activity was measured spectrophotometrically at 340nm following the formation of GSH conjugate with l-chloro-2,4-dinitrobenzene (CDNB) at 30°C. The reaction mixture consisted of 1mM GSH and 1mM CDNB (dissolved in ethanol) in 50mM Hepes(pH 7.4). The activity was calculated using an extinction coefficient of 9.6mM⁻¹ cm⁻¹ and expressed as nmol of conjugate per minute per milligram protein (mU/mg).

**Lipid peroxidation**

The extent of lipid peroxidation was estimated by the levels of malondialdehyde. The thiobarbituric acid reactive substances (TBARS) assay at 535 run was used as described, previously.[16]

**Glutathione content**

The glutathione content of rats livers, assay as previously described,[2] with some modifications.[28] The results are expressed as nmol GSH per milligram of protein.

**Statistical analysis**

Data are expressed as mean ±S.E.M. The comparison between the means of treatment (sage tea) and control group was performed using student’s t-test. P-values ≤0.05 were considered statistically significant.

**DISCUSSION**

The herb sage has a long history of use in food and medicine. The present study shows that sage tea contain high concentration of phytosterol, B. Sitosterol and Stigma masterol, 11 and 14.5% respectively. These structurally related steroids has the ability of free radical scavenging[23,43]. It was reported that flavonoids components present in sage tea can be responsible for its antioxidant effects[8,35,36,39]. Our results showed that, sage tea has on toxicity in rat liver. However, comprehensive safety studies have not been performed. Amin, et al.[4] established that, sage essential oil contains the neurotoxic substance thujone. Maximum safe doses in young children, pregnant or nursing women, or people with severe liver or kidney disease have not been established. Lima, et al.[27] reported that, sage tea did not have toxic effects of its own, herb-drug interactions are possible and may affect the efficacy and safety of concurrent medical therapy with drugs that are metabolized by phase I enzymes.

Animals fed high cholesterol diet and sodium cholate showed high plasma cholesterol, triglycerides, LDL and highly significant increase in lipid peroxides. Also, oxidative stress was reported as significant increase of liver GSH and GSR. Our results are in agreement with those of[20], where they reported that, total cholesterol in the rats were elevated consistently after 3 weeks of receiving high cholesterol diet contains 0.3% sodium cholate, also hepatic necrosis was reported. Also[32], reported high levels of LDL, and triglycerides after receiving high cholesterol diet(1%).

Lipid peroxidation is a process where by cellular membranes are damaged due to the oxidative deterioration of polyunsaturated lipids, which may lead to cell death and disease in living organisms[33].

In the present study sage caused a highly significant decrease in plasma total cholesterol, triglycerides, LDL and lipid peroxides, in rats fed high cholesterol diet during receiving sage tea for 4 weeks (Group IV) compared to hypercholesterolemic rats (Group III). This may be due to the high concentration of phytosterols present in sage
Salvia officinalis infusion improves liver antioxidant status

Yoshida[43] reported that phytosterol contained in vegetable oils is known to exert a hypocholesterolemic effect and act as an antioxidant, modest radical scavenger, and physically as a stabilizer in the membranes. A higher content of glutathione as well as increased activity of GST and GR reported in rat liver of sage tea drinking animals indicating a better recovery from hypercholesterolemia. Glutathione is the major cellular nucleophile and provides an efficient detoxification pathway for a variety of electrophilic reactive metabolites[21,28,34]. The higher activity of GR could contribute to the maintenance of glutathione in the reduced form when challenged with hypercholesterolemia. 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 up-regulating the corresponding genes by interaction with antioxidant response elements (AREs) that transcriptionally regulate these genes[18].

From the foregoing results, it can be concluded that, Salvia officinalis water extract as used in the work can be consumed as the plant’s herbal tea positively affects the antioxidant status of the liver and may have hepatoprotective potential against hypercholesterolemia.

### REFERENCES


### TABLE 1: Percentage of phenolic and hydrocarbons components of sage tea

<table>
<thead>
<tr>
<th>Component</th>
<th>Carbon no.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Dodecane C12</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>n-Tetradecane C14</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>n-Heptadecane C16</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>n-Octadecane C18</td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td>n-Eicosane C20</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>n-Tetracosane C24</td>
<td>7.2</td>
<td></td>
</tr>
<tr>
<td>n-Hexacosane C26</td>
<td>6.9</td>
<td></td>
</tr>
<tr>
<td>Octacosane C28</td>
<td>8.0</td>
<td></td>
</tr>
<tr>
<td>Tricontane C30</td>
<td>8.6</td>
<td></td>
</tr>
<tr>
<td>Dotriacontane C32</td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td>Cholesterol like comp</td>
<td>10.2</td>
<td></td>
</tr>
<tr>
<td>B- Sitosterol</td>
<td>10.9</td>
<td></td>
</tr>
<tr>
<td>Stigmasterol</td>
<td>14.5</td>
<td></td>
</tr>
</tbody>
</table>

Free Phenols = 0.5 mg %; Total Phenol = 0.55 mg %; N.B: Phenols determined as tannic acid.

### TABLE 2: Effect of sage tea on plasma transaminases activities, liver glutathione levels and liver glutathione-related enzyme activities after 4 weeks of receiving high cholesterol diet in rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Gr.</th>
<th>I Sage tea</th>
<th>Hypercholesterolemia</th>
<th>Protected</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/L)</td>
<td>40±6</td>
<td>38±4</td>
<td>42±5</td>
<td>44±3</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>95±13</td>
<td>89±11</td>
<td>90±6</td>
<td>88±9</td>
</tr>
<tr>
<td>GR (mU/mg)</td>
<td>107±3</td>
<td>119±2*</td>
<td>122±4**</td>
<td>18±3±0.6*</td>
</tr>
<tr>
<td>GST (mU/mg)</td>
<td>46.1±0.9</td>
<td>47.4±1.9*</td>
<td>55±3**</td>
<td>48.5±0.9*</td>
</tr>
</tbody>
</table>

Values are means±S.D., n=8. *p<0.05, **p<0.01 when compared with the respective control

### TABLE 3: Effect of sage tea on total cholesterol, triglycerides, LDL, and lipid peroxides (as malonaldehyde), in hypercholesterolemic rats and those protected by sage

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control Gr.</th>
<th>Sage GrI</th>
<th>Hyper GrII</th>
<th>Sage/ hyper GrIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma TC (mg%)</td>
<td>Mean±SD</td>
<td>82±12</td>
<td>75±5 NS</td>
<td>135±10 NS</td>
</tr>
<tr>
<td>Triglycerides (mg%)</td>
<td>Mean±SD</td>
<td>95±11</td>
<td>90±6 NS</td>
<td>120±5 NS</td>
</tr>
<tr>
<td>LDL (mg%)</td>
<td>Mean±SD</td>
<td>22±1</td>
<td>19±0.5 0.05</td>
<td>30±2 0.01</td>
</tr>
<tr>
<td>Lipid peroxides (n mole/L)</td>
<td>Mean±SD</td>
<td>86.5±6</td>
<td>85±3 NS</td>
<td>117±3 NS</td>
</tr>
</tbody>
</table>

Hyper: hypercholesterolemic rats, Sage/Hyper: Rats protected by sage tea.
[34] A.Reed; Annual Review of Pharmacology and Toxicology, 30, 603-611 (1990).
[38] Santos-Gomes, R.M.Seabra, P.B.Andrade, M.Fernandes Ferreira; Plant Science, 162, 981-987 (2002).