October 2007 Volume 1 Issue 3

Trade Science Inc.

BIOCHEMISTRY
An Indian Journal

Regular Paper

BCAIJ, 1(3), 2007 [117-121]

Salvia Officinalis Infusion Improves Liver Antioxidant Status And Protects From Hypercholesterolemia

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Received: 24th May, 2007 ; Accepted: 29nd May, 2007

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<o.o1, o.o5, 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.

KEYWORDS

Salvia officinalis;
Sage tea;
Hypercholesterolemia;
Antioxidant.

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,

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and high blood sugar..Sage was once recommended by herbalists has 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[22].

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[2,10,6,7,19,42,45]. 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[15,41].

De Chile,[13] 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[32].

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). clolesterol, 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 20^0C. Considering that sage is traditionally used as a tea, an infusion of sage was routinely prepared by pouring 150ml of boiling water onto 2g of the dried plant material and allowing to steep for 5min. 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[26].

Hydrocarbons (Phenolic compounds) were analysed by HPLC. Freeze-dried extract(0.01g) was redissolved in 1ml of ultrapure Milli Q water and aliquots of 20µl were injected in an HPLC system. Separation and identification of phenolic compounds by HPLC were performed as previously described by Santos-Gomeset al.[28].

Animals

Male Wistar rats(150-200g) were used throughout this work. Animals were maintained on a natural light/dark cycle at 20±2^0C 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 35mg/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.[40].

Group IV: Animals received sage tea (35mg/kgbw/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(Na_2HPO_4 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 4^0C and the supernatant collected..The GR activity was measured spectrophotometrically at 340nm following NADPH oxidation at 30^0C. The GST activity was measured spectrophotometrically at 340 nm following the formation of GSH conjugate with l-chloro-2,4-dinitrobenzene (CDNB) at 30^0C. The reaction mixture consisted of 1mM GSH and 1 mM 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[25]. 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 Stigmasterol, 11and 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 o LDL, and triglycerides after receiving high cholesterol diet(1%).

Lipid per oxidation 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

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

Component	Carbon no.	%
n- Dodecane	C12	2.1
n- Tetradecane	C14	2.9
n- Hevadecane	C16	3.8
n- Octadecane	C18	4.6
n- Eicosane	C20	5.1
n- Docosane	C22	5.8
n- tetracosane	C24	7.2
n- hexacosane	C26	6.9
Octacosane	C28	8.0
Tricontane	C30	8.6
Dotriacontane	C32	9.4
Cholesterol like comp		10.2
B- Sitosterol		10.9
Stigmasterol		14.5

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

Parameter	Groups			
	Control Gr.	I Sage tea	Hyperchole sterolemia	Protected
		Gr.II .	Gr.III	Gr.IV
ALT(U/L)	40±6	38±4	42±5	44±3
AST(U/L)	95±13	89±11	90±6	88±9
GR(mU/mg)	13.4±0.1	14.7±0.4*	15±0.5	13±0.7
GST(mU/mg)	107±3	119±2*	122±4**	18.3±0.6*
GSH(nmol/mg)	46.1±0.9	47.4±1.9	55±3 **	48.5±0.9 *

Values are means±S.D.M.,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

Parameters	GROUPS				
		Control GrI	Sage GrII	Hyper GrIII	Sage/ hyper GrIV
Plasma TC(mg%)	Mean±SD	82±12	75±5 NS	135±10	90±6
	P <s/control			0.001	NS
	Vs/hyper.				0.01
Triglycerides (mg%)	Mean±SD	95±11	90±6 NS	120±5	89±7
	P <vs/control			0.05	NS
	Vs/hyper.				0.05
LDL(mg%)	Mean±SD	22±1	19±0.5	30±2	21±0.5
	P <vs/control		0.05	0.01	NS
	Vs/hyper.				0.01
Lipid peroxides (n mole/L)	Mean±SD	86.5±6	85±3	117±3	85±4
	P <vs/control		NS	0.001	NS
	Vs/hyper.				0.01

Hyper: hypercholesterolemic rats, Sage/Hyper: Rats protected by sage

tea. 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[15].

From the forgoing 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.

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