# The Possible Protective Effect of Sage (Salvia Officinalis L.) Water Extract Against Testes and Heart Tissue Damages of Hypercholesterolemic Rats

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## ABSTRACT

Sage (Salvia officinalis) is one of the aromatics and medicinals having biological activities and pharmacological properties. The possible protective role and antioxidant activity of sage water extract (SWE) against metabolic disorders induced by hypercholesterolemic diet (HCD) in the heart and testis tissues of rats were evaluated in this study. The results of gas chromatography/mass spectrometry (GC/MS) analysis revealed that Camphor, 1,8-cineole and α-thujone represent the main compounds of volatile oil extracted from S. officinalis. The findings of the biological study showed that the dietary intake of HCD significantly increased serum lipid contents, activities of cardiac marker enzymes and the level of malondialdehyde (MDA) in testes and heart tissues, and significantly reduced the level of high-density lipoprotein-cholesterol (HDL-C), testosterone (T), follicle stimulating hormone (FSH) and leutinizing hormone (LH) with suppression of antioxidant status compared to the controlled rats. Whereas, supplementation of rats with HCD along with SWE (1 ml/ Kg B. wt) reduced the damaging effects induced by HCD with a significant decrease in hypercholesterolemic state, reduction of lipid peroxidation, induction of heart and testes functions and elevation of the activity of antioxidant enzymes and glutathione contents. In conclusion, this study showed that SWE could be used as natural anti-oxidants supplements due to the presence of essential oil, phenolic contents and other antioxidant components in Sage.

**Key Words:** Salvia Officinalis, Hypercholesterolemic Diet, Essential Oil, Antioxidant Activity

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## INTRODUCTION

Hypercholesterolemia (HC) is one the major risky diseases that has been increased in developing countries in recent years [1]. The occurrence of hypercholesterolemia induced over production of reactive oxygen species with elevation of lipid peroxide content and inhibition of antioxidant defense system [2]. Therefore, HC is closely associated with developing of many chronic diseases including cardiovascular diseases, progression of renal failure and certain metabolic and reproductive disorders [3]. It has been estimated by the World Health Organization (WHO) that approximately 26 million deaths occur annually because of hypercholesterolemia-related cardiovascular diseases (CVDs) [4]. Several lipids lowering drugs such as fibrates, statins, and bile acid sequestrants can be used to regulate the lipid metabolism by different mechanisms but these drugs have many side effects [5].

There has been an interest in an alternative medicine in recent decades, because there are thousands of potential medicinal plants that contain many bioactive substances and antioxidants [6]. Sage (Salvia officinalis L., Lamiaceae) is a common aromatic plant that has been used as a medicinal plant in treating different diseases such as cancer, diabetes, heart diseases, reproductive disorders and

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hypercholesterolemia [6]. The beneficial effects of Sage have been related to its bioactive components including volatile fatty acids, diterpens, saponins, flavonoids, phenolic acids, resin and oestrogenic substances. With regard to the possible antioxidant activity of sage, the aim of this study was to evaluate the protective effects of Salvia officinalis against metabolic disorders induced by hypercholesterolemic diet in the heart and testes tissues of rats.

## MATERIALS AND METHODS

Dried leaves of Sage were purchased from a local herb market. Chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

### Analysis of sage essential oil by GC/MS:

The essential oil was isolated from sage (100g) according to the method of [7]. The volatile oil analysis was carried out using gas chromatography-mass spectrometry instrument GC/MS.

### Preparation of sage water extract (SWE):

Sage water extract (sage tea): It was prepared by pouring 150 ml boiling water onto 2 g of dried grounded leaves and allowing it to steep for 5 min [8].

### Experimental Protocol:

### Animals and biochemical Assay

Male albino rats (150 ± 20 g) were acclimated to controlled laboratory conditions for two weeks. Rats were maintained on stock rodent diet and tap water that were allowed ad libitum. The rodent controlled diet was composed of 15% casein, 10% corn oil, 5% cellulose, 4% salt mixture, 1% vitamins mixture and starch 65% [9].

All the animal procedures were accomplished based on the Ethics Committee of the National Research Centre conformed to the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH publication No. 85 – 23, 1996).

### Experimental design

The animals were randomly divided into four groups, each comprised of 7 rats.

**Group C:** rats were fed on normal diet for 10 weeks, served as a control,

**Group HCD:** rats were fed on hypercholesterolemic diet (basal diet + 1% cholesterol+ 16% fat and 0.2% Cholic acid) [10] for 10 weeks.

**Group SWE:** rats were fed on normal diet and supplemented orally with sage water extract (1 ml/ Kg body wt) [11] for 10 weeks.

**Group HCD + SWE:** rats were fed on HCD supplemented orally with sage water extract (1 ml/ Kg B. wt) [11] for 10 weeks.

Each rat was weighed at the initial and end of the experiment. At the end of the experiment (10 weeks), rats were fasted for 24 hours and anaesthetized with diethyl ether. For obtaining serum for biochemical analysis, blood samples were collected through heart puncture and allowed to coagulate, and then centrifuged. Also, for biochemical investigation, cardiac and testes tissues were discarded.

### Biochemical Analysis

Total cholesterol (TC), triglycerides (TG) and high-density lipoprotein-cholesterol (HDL-C) were indicatd based on the procedure described by [12], [13], and [14], respectively. Low-density lipoprotein-cholesterol, very Low-density lipoprotein-cholesterol and risk ratio were evaluated according to [15] by the following equations: LDL-C (mg/dl) = TC-(TG/5+HDL-C), vLDL (mg/dl) = TG/5. The levels of lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) were determined by the method of [16], aspartate transaminase (AST) and alanine transaminase (ALT) were determined according to [17]. Estimation of testosterone hormone was performed based on the method of [18], Follicle stimulating hormone (FSH) and leutinizing hormone (LH) according to [19].

Cardiac and testes tissues were dissected, thoroughly washed with ice-cold 0.9% NaCl, weighed, minced and homogenized (10% w/v) using 66 mmol/L chilled phosphate buffer (pH 7.0). The tissue homogenates were centrifuged at 6000 rpm for 15 min and the supernatants were used to estimate malondialdehyde (MDA) [20], GSH [21], superoxide dismutase activity (SOD) [22] and Catalase activity (CAT) [23].

### Statistical analysis

Results were presented as mean ± SE (n = 7). Experimental data were analyzed using one-way analysis of variance (ANOVA). Duncan's multiple range test was used to determine significant differences between means. The statistical analyses were performed using computer program Statistical Packages for Social Science (SPSS) [24]. Differences between means were considered significant at $P < 0.05$.

## RESULTS

The results of GC/MS analysis showed that S. officinalis is composed of high amount of essential oils, most of them are aromatic. Camphor (24.55%), 1,8-cineole (17.86%) and α-thujone (14.07%) are represented as the major essential oils of sage (Table 1).

**Table 1. Percentage of essential oil constituents of Salvia officinalis (%)**

| Compound | Percentage (%) | Compound | Percentage (%) |
|---|---|---|---|
| limonene | 3.61 | α-terpinyl acetate | 2.17 |
| 1,8-cineole | 17.86 | γ-selinene | 3.54 |
| linalool | 0.19 | caryophyllene oxide | 1.12 |
| α-thujone | 14.07 | humulene epoxide | 1.09 |
| β- thujone | 6.03 | isoaromadendrene epoxide | 0.35 |
| β-pinene | 0.23 | trans-Z- α- bisabolene epoxide | 0.21 |
| caryophyllene | 3.92 | Caryophyleine (I3) | 1.16 |
| camphor | 24.55 | manoyl oxide | 5.57 |
| humulene | 4.73 | β-selinene | 0.39 |
| α-terpineol | 1.37 | borneol | 3.13 |
| **Total identified compounds** | | **95.29** | |

Data showed that HCD-group had a significant increase in the serum level of lipid contents (TC, TG, LDL-C and vLDL-C) and a remarkable decrease in HDL-C relative to the control group. On contrast, supplementation of rats with HCD along with SWE induced a significant increase in HDL-C and reduction in the serum level of TC, TG, LDL-C and vLDL-C when compared to the rats fed on HCD only (Table 2).

**Table 2. Effect of administration of sage water extract on serum lipid profile in diet induced hypercholesterolemic rats**

| Animal groups | TC (mg/dl) | TG (mg/dl) | HDL-C (mg/dl) | LDL-C (mg/dl) | v-LDL (mg/dl) |
|---|---|---|---|---|---|
| Control | 129.46±9.85$^{c}$ | 164.16±11.82$^{c}$ | 70.52±5.46$^{a}$ | 26.11±2.55$^{c}$ | 32.83±2.64$^{c}$ |
| HCD | 412.15±24.14$^{a}$ | 517.20±41.25$^{a}$ | 13.48±1.60$^{c}$ | 295.23±23.49$^{a}$ | 103.44±7.82$^{a}$ |
| SWE | 118.22±8.68$^{c}$ | 153.72±12.10$^{c}$ | 73.58±6.27$^{a}$ | 13.90±2.11$^{d}$ | 30.74±2.33$^{c}$ |
| HCD+ SWE | 181.46±11.45$^{b}$ | 220.50±14.65$^{b}$ | 59.94±4.36$^{b}$ | 77.42±9.72$^{b}$ | 44.10±3.64$^{b}$ |

Means in the same column with different superscripts are significantly different at (P<0.05), Values are expressed as mean ± S.E. (n=7)
**HCD:** Hypercholesterolemic diet,
**SWE:** Sage water extract.



The results indicated that HCD induced a significant increase in the activity of serum LDH, CPK, AST and ALT compared with the control group. The activity of these enzymes significantly reduced in the group of rats which received HCD and SWE when compared to the rats received HCD (Table 3).

**Table 3. The Effect of administration of sage water extract on serum LDH, CPK, ALT and AST activities in diet induced hypercholesterolemic rats**

| Animal groups | LDH (nmol/ml) | CPK (nmol/ml) | ALT (U/L) | AST (U/L) |
|---|---|---|---|---|
| Control | 712.27±35.64$^{c}$ | 237.74±24.11$^{c}$ | 15.26±0.68$^{c}$ | 26.14±1.57$^{c}$ |
| HCD | 1055.28±41.28$^{a}$ | 432.66±27.42$^{a}$ | 51.11±1.46$^{a}$ | 54.66±2.92$^{a}$ |
| SWE | 707.05±32.37$^{c}$ | 228.95±22.23$^{c}$ | 14.83±0.72$^{c}$ | 25.82±1.66$^{c}$ |
| HCD+ SWE | 816.29±34.12$^{b}$ | 303.12±25.50$^{b}$ | 22.68±1.11$^{b}$ | 34.12±2.15$^{b}$ |

Means in the same column with different superscripts are significantly different at (P<0.05), Values are expressed as mean ± S.E. (n=7)
**HCD:** Hypercholesterolemic diet
**SWE:** Sage water extract

In this work, the induction of hypercholesterolemia in rats resulted in remarkable reduction in the level of testosterone, LH and FSH compared to the controlled rats. On the other hand, feeding rats HCD with SWE induced a significant increase in the level of these hormones compared to HCD group (Table 4).

**Table 4. The effect of administration sage water extract on the level of T, LH and FSH in diet induced hypercholesterolemic rats**

| Animal groups | T (n mol/L) | LH (IU/L) | FSH (IU/L) |
|---|---|---|---|
| Control | 4.80± 0.27$^{a}$ | 0.84± 0.09$^{a}$ | 0.77± 0.05$^{a}$ |
| HCD | 2.42± 0.15$^{c}$ | 0.55± 0.07$^{c}$ | 0.56± 0.04$^{c}$ |
| SWE | 4.91± 0.30$^{a}$ | 0.86± 0.08$^{a}$ | 0.78± 0.06$^{a}$ |
| HCD+ SWE | 3.96± 0.22$^{b}$ | 0.76± 0.07$^{b}$ | 0.69± 0.05$^{b}$ |

Means in the same column with different superscripts are significantly different at (P<0.05), Values are expressed as mean ± S.E. (n=7)
**HCD:** Hypercholesterolemic diet
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The cardiac and testicular tissues of HC-rats exhibited a significant increase in the level of MDA and a significant decrease in the level of GSH and activity of the enzymatic antioxidants (CAT and SOD) compared to those from the control group. By contrast, in the HCD and SWE group, MDA significantly reduced and GSH and the antioxidant enzymes significantly improved (Table 5).

**Table 5. The Effect of administration of sage water extract on the cardiac and testicular antioxidant status in diet induced hypercholesterolemic rats**

| Parameters | | Control | HCD | SWE | HCD+SWE |
|---|---|---|---|---|---|
| **MDA** (n mol/g tissue) | Heart | 177.54 ±6.28$^{c}$ | 364.37 ±7.84$^{a}$ | 169.55±6.67$^{c}$ | 230.88±5.86$^{b}$ |
| | Testes | 134.52± 5.11$^{c}$ | 222.30±5.66$^{a}$ | 127.92± 4.85$^{c}$ | 168.57±4.73$^{b}$ |
| **GSH** (mg/g tissue) | Heart | 27.32 ±2.55$^{a}$ | 17.12 ±1.84$^{c}$ | 27.78 ±2.70$^{a}$ | 23.92±2.46$^{b}$ |
| | Testes | 22.46± 1.53$^{a}$ | 12.18±1.48$^{c}$ | 22.94± 1.48$^{a}$ | 18.59±1.62$^{b}$ |
| **SOD** (U/mg protein) | Heart | 46.58 ±3.47$^{a}$ | 34.25 ±3.18$^{c}$ | 47.06 ±3.35$^{a}$ | 43.42 ±3.42$^{b}$ |
| | Testes | 21.14± 1.37$^{a}$ | 13.15±0.76$^{c}$ | 21.50± 1.65$^{a}$ | 19.17±1.12$^{b}$ |
| **CAT** (U/g protein) | Heart | 3.72±0.18$^{a}$ | 1.64±0.09$^{c}$ | 3.84±0.16$^{a}$ | 3.02±0.15$^{b}$ |
| | Testes | 3.52± 0.33$^{a}$ | 1.58±0.14$^{c}$ | 3.58± 0.36$^{a}$ | 2.85±0.12$^{b}$ |

Means in the same row with different superscripts are significantly different at (P<0.05), Values are expressed as mean ± S.E. (n=7).
**HCD:** Hypercholesterolemic diet.
**SWE:** Sage water extract

## DISCUSSION

Consumption of HCD could be linked to the metabolic disorders and chronic diseases and can lead to weight gain and elevated blood lipid levels [25]. In this research, the protective role of the SWE on the heart and testes tissues of hypercholesterolemic rats has been emphasized.
The results of GC/MS analysis of the essential oil of sage in this study were in agreement with the results presented in the study of [26] and [27] but with variable concentrations depending on location and stages of development, environmental, physiological and morphological factors [28]. The oil composition was found to meet the standards given by ISO 9909 for use of S. officinalis for medicinal purposes [29]. [27] obtained that the oil composition of S. officinalis was found to be rich in oxygenated monoterpenes including α-thujone, β-thujone, 1,8-cineole, camphor and borneol.
In this work, hypercholesterolemia in rats was induced by feeding rats HCD for 10 weeks. The levels of serum TC, TG, LDL-C and vLDL-C significantly increased above the normal upper limits and the level of HDL-C reduced significantly under the effect of HCD relative to the normal group. HCD was concerned with the increasing concentrations of serum and hepatic total TC, especially the level of vLDL-C and LDL-C in serum, which is considered to be a primary risk factor of cardiovascular disease [30]. Also, [30] demonstrated that the cholesterol administration reduced the fatty acid catabolism, which may account for the enhancement of hepatic TG level. A significant rise in the activity of cardiac enzymes LDH,

CPK, AST and ALT was observed in the group HCD when compared to the controlled rats. The elevation in the activity of these enzymes could be attributed to the tissue damage induced by HC and the leakage of these markers in the plasma [31]. Furthermore, HCD could induce suppression in hepatic and cardiac functions evidenced by augmentation of serum levels of AST, ALT, LDH and CK-MB activities [32]. The data in this work revealed that HCD causes reproduction problems by decreasing the level of testosterone, LH and FSH compared to the controlled rats. [33] indicated that the high fat diet induced an increase in total cholesterol and triglycerides levels and the poor development of testicles in pubertal rats. Hypercholesterolemia causes a disturbance in hypothalamus pituitary axis activity. [34] reported that the increase of oxidative stress and decrease of endogenous antioxidant activity lead to the spermatogenesis disturbance. The induction of HC in this study resulted in a significant increase in oxidative stress in heart and testes tissues, evidenced by high level of MDA and a significant decrease in the level of GSH and activity of the enzymatic antioxidants (CAT and SOD) compared to those from the control group. [35] suggested that hypercholesterolemia leads to the increased lipid peroxidation which is in consistent with several clinical and experimental studies. Oxidative stress has emerged as an important pathogenic factor in the development of hypertension and also most of the complications related to the hypertension are associated with oxidative stress, induced by the generation of free radicals [36]. [37] indicated that free radical and suppression of antioxidant enzymes are the principal factors involved in the pathogenesis of hypercholesterolemia. [38] concluded that hyperlipidemia induced by diet reduces the SOD, CAT, GPx and GST activities, and parallely elevate the lipid peroxide levels, thereby leading to inhibition of antioxidant defense system.

On the other side, feeding rats HCD with SWE resulted in an obvious protection of SWE against the disturbance induced by HCD evidenced by remarkable reduction in the lipid profile contents, activity of serum LDH, CPK, AST and ALT and lipid peroxidation accompanied by a significant elevation in the HDL-C, testosterone, LH and FSH and activation of antioxidant defense system. The study of [39] revealed that Salvia officinalis tea consumption is accountable for the improvement of the lipid profile inducing a decrease on the highly atherogenic LDL-C particles and an increase in the HDL-C which may be due to the ability of SWE to reduce cholesterol biosynthesis. The hypocholesterolemic effect of SWE may be attributed to several sage natural components that have been shown to act on cholesterol metabolism by reducing its absorption or its synthesis, such as catechins [40]. Also, thujone, one of monoterpenes that was found as a mixture of alpha and β diastereoisomers in Salvia officinalis L., lowers cholesterol and triglyceride levels [41]. The effect of SWE on cardiac enzymes in this study is in agreement with the results of [42] who indicated that the treatment of methomyl-administered rats with Salvia officinalis and Ruta graveolens ethanolic extracts produced a potential amelioration of the impaired CK-MB, AST and LDH activities. The authors revealed that the antioxidant activity of S. officinalis can protect against cellular damage induced by degenerative diseases and thereby prevent leakage of cardiac enzymes in the plasma [42]. [43] concluded that the aqueous extract of Salvia officinalis caused a positive effect on some fertility parameters and pituitary-testicular hormone axis by increasing the level of Testosterone, LH and FSH. The improving effects of SWE on male reproductive system may be related to the effects of Sage components including vitamins C and E, flavonoids and antioxidant [43]. [44] reported that Salvia officinalis extract has the ability to stimulate the growth of testes and enhance the proliferation, maturation and differentiation of spermatozoa due to the presence of saponine, alkaloids in the salvia extract. It was found that the antioxidant properties of SWE could be attributed to the presence of a lot of active components that have high antioxidant activity such as carnosol, carnosic and rosmarinic acids, rosmadial, rosmanol, epirosmanol, methyl carnosate, and luteolin-7-O-β-glucopyranoside. These antioxidant compounds have the ability to stimulate endogenous antioxidant defense systems and scavenge reactive species [45]. [46] suggested that the presence of vitamins C and E, flavonoids and phenolic compounds in SWE might lead to the regulation of signal transduction pathway of cell growth and proliferation, modulation of enzyme activity related to the detoxification, stimulation of the immune system and DNA repair and regulation of hormone metabolism.



## CONCLUSIONS

From the results of this study, it could be postulated that SWE plays a vital role against HC and induces the repair of the damaged heart and testes. SWE reduces the level of lipid contents, increases cellular anti-oxidant activities and protects cardiac and testicular tissues by scavenging free radicals. This research concluded that the biological activity of SWE could be attributed to the presence of the essential oil, phenolic contents and other antioxidant components. Therefore, SWE could form promising resources to the supplement for natural anti-oxidants.

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