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THE PROTECTIVE EFFECT OF SAGE (*Salvia officinalis)* OIL AGAINST HYPERCHOLESTEROLEMIA INDUCED IN FEMALE RATS

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Abstract

This present study was conducted to evaluate the hypolipidemic potential of sage oil (*Salvia officinalis*) in controlling hypercholesterolemia and their effect on liver enzymes and pancreatic lipase activitiesin female rats. Twenty four of female rats were housed in standard cages, the experimental rats were divided randomly in to three groups (8 in each group). Group 1 was fed on the standard diet considered as control group (negative), group 2 was fed on experimental diet (HFD) as a replacement for their diet for four weeks to induce hypercholesterolemia as a positive control. Group 3 (treated) was fed on experimental diet (HFD) and oral gavage of sage oil (0.05 ml/kg) as single dose daily for four weeks. Blood sample were taken from these groups after four weeks for biochemical analysis to estimate serum Malon dialdehyde (MDA), total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL-C), high density lipoprotein (HDL-C), aspartate amino transferase (AST), alanine aminotranferase (ALT) and pancreatic lipase (PL). The results showed that sage oil administration resulted in a decrease in the concentration of Malon dialdehyde, total cholesterol, triglyceride and low density lipoprotein associated with an increased high density lipoprotein (HDL-C) concentration in rats, the serum enzymes activities of aspartate amino transferase, alanine amino transferase and pancreatic lipase werealso significantly decreased. It was concluded from the present study that a protective effect against hypercholesterolemia and hypolipidemic properties of the sage oil, in addition beneficial effect in decreasing the elevated liver enzymes and has inhibitory effect against the pancreatic lipase activitymay be attributed to theantioxidant activity of one or more of its flavonoids and polyphenols.

Key words: *Salvia officinalis*, Hypercholersterolemia, Biochemical parameters and Female rats.

1. Introduction

Hypercholesterolemia is a lipoprotein metabolic disorder characterized by high serum low density lipoprotein and blood cholesterol, as one of the most important risk factors in the development and progression of atherosclerosis that lead to cardiovascular diseases (CVDs) (Rerkasan *et al*., 2008). Hypercholesterolemia poses a major problem to many societies as well as health professionals because of the close correlation between cardiovascular diseases and lipid abnormalities (Ramachandran *et al*., 2003; Ioannoue *et al*., 2006). The disorders of lipid metabolism are associated with increased oxidative stress and overproduction of oxygen free radicals (Matos *et al*., 2005). There are many factors that can raise the risk of hypercholesterolemia causing strocke or heart disease include genetic and environmental factors, genetic hypercholesterolemia result from mutations in the LDLR gene (familial

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hypercholesterolemia) while environmental result from a diet – rich in saturated fat, smoking, diabetes and high blood pressure (Hartvigsen *et al*., 2007). To overcome the problem of hyperlipidaemia day by-day several synthetic drugs of better efficacy are being introduced in the modern system of medicine, but apart from being effective most of these medications induce adverse side effects due to the highly costs for synthetic drugs, also restrictions in use of these drugs. Therefore, these potential agents could not be used for long time but hyperlipidaemia requires long term treatment (Jang and Wang, 2009).

In recent years, scientists and researchers trying to substitute herbal plant and some natural component in these plants for maintaining health and preventing diseases (Hamidour *et al*., 2014). The natural plants are generally considered to be safer than synthetic drugs, its widely prescribed today despite the fact that their biologically active compounds are unknown due to its minimal adverse effects, low costs, economical, effective and their easy availability as well as to facilitate natural product drug discovery (Bhandari *et al.*, 2008; Dallak *et al*., 2009).The aromatic plants are characterized by the presence of volatile compounds with pleasant odor known as essential oils (EOs) and various phytochemical components, especially polyphenols (such as flavonoids, phyenyl propanoids, phenolic acids and tannins) are known to be responsible for the free radical scavenging, inhibition of peroxidation, chelating transition metals and antioxidant activities of plants (Nickavar *et al*., 2007; Chein *et al*., 2011; Walch *et al*., 2011).

Traditional medicinal plants having antilipidaemic property can prove to be the useful source for the development of new oral hypolipidaemic agents or simple dietary adjuvant to existing therapies and meet the major dietary requirements in proteins, antioxidants and minerals, these are an important part of diets for hypercholesterolemic patients (Sa *et al*., 2009; Harnafi *et al*., 2009). Among these, *Salvia officinalis* L. (common sage) a member of the family of Lamiaceae. Sage (*Salvia officinalis*) the plant is mostly aromatic and perennial with flowers. It is commonly used as a spice and condiment in food preparation (Yurtseven *et al.*, 2008). Several studies suggest that the sage species can be considered for drug development because their pharmacology and therapeutic activities in many countries of Asia and Middle east especially China and India (Farhoudi *et al*., 2011; Hala *et al*., 2012; Ismail and Hammed, 2013). Sage (*Salvia officinalis* L.) has a wide range of biological activities, such as antioxidative properties (e.g., carnosic acid, rosmarinic acid and caffeic acid) and radical scavenging, antibacterial activities, hypoglycemic, anti-inflammatory properties (e.g., Ursolic acid), fungistatic, virustatic, astringent properties, eupeptic and antihydrotic effects aswell as in promoting energy expenditure and fat oxidation which may aid in body weight reduction (Lu and Yeap Foo, 2001; Oniga*et al*., 2007; Eidi and Eidi, 2009; Hussain *etal*.,2011). Sage (*Salvia officinalis)* was considered to have the highest amount of essential oil compared to the other species of *Salvia*, the essential oil of *Salvia officinalis* showed better antioxidant activities and free radical scavenging agents than some other Lamiaceae plants. In addition, sage essential oil has been used in the treatment of a wide range of diseases like cardiovascular diseases digestion problems, respiratory system metabolic and endocrine diseases and nervous disorders, essential oils are also very important sources for the screening of anticancer and antimicrobial (Kamaton *et al*., 2010; Khan *et al*., 2011; Rami and Li, 2011). The purpose of the present study was to examine the protect effect of oral administration of sage oil extract against hypercholesterolemia and oxidative stress, liver function test (AST & ALT) and pancreatic lipase activityin female rat.

2. Materials and methods

Plant preparation

The sage leaves (*Salvia officinalis*) were bought from a local market in Basra city, Iraq. The leaves were collected, washed with distilled water andthen dried under the shade at room temperature

for six days. After cleaning, the dried leaves were finely grounded into powder form by using electric mill for 3 minutes. The powdered leaves of sage (50 g) were extracted or defatted with 500 ml normal - hexan for 16 hours by using Soxhlete apparatus method at 50 °C. The filtrate was collected and cooled and then the solvent was removed under reduce pressure in a rotary evaporator in water bath of 60 °C to get 5 ml of oil. The residue obtained after evaporation kept in tight closed bottles and stored at 4 °C in a refrigerator until used for experimentation.

Experimental animals

This study was carried out on adult healthy female rats (*Rattus norvegicus*). Twenty- four female rats weighting between 180 200 gm and aged 8 weeks. The rats were allowed to acclimatize for two weeks before the start of experiment at the animal house of College of Veterinary Medicine/University of Basra. The animals were housed as four rats to each cage under optimum conditions, temperature was maintained in the range of 22 ± 3 °C with 12 hrs light and 12 hrs dark cycle before and throughout the period of the experimental work. The animals were fed with standard diet (a commercial pellet), food and water were supplied adlibitium. The feces and urine were removed daily to prevent food and water contamination. Rats were randomly distributed into three groups eight in each group as follow

Group (1): Rats of this group were fed on the standard diet and kept as a negative control.

Group (2): (Hypercholesterolemia) Rats were fed on experimental diet (HFD consisted of normal laboratory diet in powder form mixed with extra pure cholesterol (2 %) (El - Wakf *et al.*, 2015) as a replacement for their diet for four weeks to induce hypercholesterolemia as a positive control.

Group (3): (Treated) This group were fed on experimental diet (HFD) and received oral gavage of sage oil (0.05 ml/kg) as single dose daily for four weeks. The dose was chosen as described by El-Wakf *et al.* (2015).

At the end of the experimental period, the animals were fasted overnight, sacrificed under ether anaesthesia, blood samples were immediately collected from animals *via* cardiac puncture after anaesthetizing the rats by anesthetic diethyl ether inhalation, the blood samples placed in plain tubes to clot for 30 minutes at room temperature and then centrifuged with 3000 rpm for 10 minutes to obtain serum and stored at -20 °C untiluse.

Biochemical assays

Measurement of malondialdehyde as one of the main endopruduct of lipid peroxideation will be carried out in serum according to the method of Yagi (1998). Some biochemical measurement was done by using special enzymatic kits, the serum total cholesterol (TC) concentration was assayed by using an enzymatic kit depending on enzymatic method of Sidel *et al*. (1983), the serum triglycerides (TG) was determined by enzymatic kit according to Fossati and Prencipe (1982), high density lipoprotein - cholesterol (HDL-C) was done by enzymatic method according to Demacker *et al.* 1980), while low density lipoprotein - cholesterol (LDL-C) was calculated according to the equation: LDL-C = [TC-(HDL-C)] - (triglyceride/5) (Friedwald *et al.*, 1972). For the estimation of Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) activities in serum commercially enzymatic kits based on the reaction of 2, 4 dinitrophenyl hydrazine with pyruvate and/or oxaloactate to yield a brown colored complex in alkalin medium were used (Bergmeyer, 1978). The determination of pancreatic lipase activity was performed according to previously described method (Tsuzuki *et al*., 2004).

Statistical analysis

Data were statistically analyzed by One - way Analysis of Variance (ANOVA) followed by

Least Significant Difference (LSD). The p values less than 0.05 were considered statistically significant. All the results were expressed as mean ± SE. according to Snedecor and Cochron (1989).

3. Results

The Table - 1 showed that the MDA level is a good indicator for evaluating oxidative stress in the hypercholesterolemia. The results show that there is a significant increase ($P < 0.05$) in serum MDA level when the animals fed on hypercholesterolemic diet as compared with control group. While, the group treated with *Salvia officinalis* oil extract and fed on hypercholesterolemic diet showed a significant reduction ($P < 0.05$) in serum MDA level compared with hypercholesterolemia and control groups.

Table – 1: Serum Malondialdehyde (MDA)level of control, hypercholesterolemia and treated groups in female rats

Groups	Control	Hypercholesterol	Treated
Serum MDA Concentration µ mol / L	0.93 ± 0.04 C	2.3 ± 0.10 A	0.99 ± 0.03 B

The difference in the capital letters means statistical difference ($p < 0.05$) level as compared with control.

The results indicate a significant ($P < 0.05$) increased in serum total cholesterol, triglyceride, low density lipoprotein.While, there is a significant ($P < 0.05$) decreased in high lipoprotein density level in the HPC fed rats when compared to the control group fed on basic diet. The group treated with sage oil extract and fed on HPC diet showed a significant reduction ($P < 0.05$) in serum total cholesterol, triglyceride, low density lipoprotein. This reduction was proportional with a significant ($P < 0.005$) increased in high lipoprotein density level as compared with HPC group and control group.

Table – 2: The serum total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL) levels of control, hypercholesterolemia and treated groups in female rats

Groups	TC (mg/dl)	TG (mg/dl)	HLD (mg/dl)	LDL (mg/dl)
Control	95.94 ± 2.52C	85.51 ± 2.63C	67.09 ± 2.02A	38.09 ± 1.05C
Hypercholesterol	186.51 ± 2.68A	170.02 ± 3.25A	41.09 ± 1.71C	76.21 ± 6.85A
Treated	110.19 ± 6.59B	90.86 ± 1.49B	53.91 ± 1.94B	43.69 ± 3.27B

The difference in the capital letters means statistical difference ($p < 0.05$) level as compared with control.

The results show a significant ($P < 0.05$) increased in serum aspartate amino transferase (AST), alanine amino transferase (ALT) and pancreatic lipase (PL) in the group HPC fed diet as compared with control group. Whereas, in the rats treated with sage oil extract and fed on HPC diet showed a significant reduction ($P < 0.05$) in serum (AST), (ALT) and (PL) when compared to the HPC group and control group.

Table – 3: Serum aspartate amino transferase (AST) alanine amino transferase (ALT) and pancreatic lipase (PL) levels of control, hypercholesterolemia and treated groups in female rats

Groups	Control	Hypercholesterol	Treated
AST unit/l	20.82 ± 0.68C	40.11 ± 0.92A	25.05 ±1.17B
ALT unit/l	31.45 ± 0.88C	62.42 ± 2.19A	35.81 ±1.09B
PL unit/l	18.93 ± 1.3 C	40.91 ± 2.06A	20.56 ±1.12B

The difference in the capital letters means statistical difference ($p < 0.05$) level as compared with control.

4. Discussion

The current study elicited marked significantelevation (P<0.05) in the lipid peroxidation product (MDA) level in hypercholesterolemia group as compared with control group, this is an indicator of free radical generation. Within hypercholesterolemia, reactions between oxygen radicals or enzymatic oxidation and lipoproteins or more specifically phospholipids can lead to production of lipid radicals (oxLDL) or oxidized phospholipids (OxPL). These OxPL can interact with membrane receptors to accumulatewithin the cellular membrane, disrupting normal cellular function through a reduced bioavailability of NO, eliciting an immune response, leading to poor vascular function, and ultimately atherosclerosis (Stapleton *et al*., 2010). These findings agree with the results of Matos *et al*. (2005) demonstrated that the disorders of lipid metabolism are associated with increased oxidative stress and overproduction of oxygen free radicals.

Out of the results, there was a significant reduction in the average malon dialdehyde concentration in the hypercholesterolemia animals treated with the sage (*Salvia officinalis*) oil extract as compared with hypercholesterolemia rats non treated group, these results indicate that sage (*Salvia officinalis*) oil extract has antioxidant activity. This may be attributed to the active constitutes ofsage polyphenols especially phenolic and rosmarinicacid, the phenolic compounds can either stimulate endogenous antioxidant defense systems or scavenge reactive species (Nickavar *et al*., 2007; Sa *et al*., 2009; Yadav *et al*., 2011). Similar results were given by Oboh and Henle (2009) who found that the aqueous extract of *Salvia officinalis* inhibit the product of lipidperoxidation Malondialdehyde (MDA) in brain and liver of rats, also *Salviaofficinalis* extract caused significant increase in glutathione – S - transferaseand glutathione reductase in rat liver (Lima *et al*., 2005). Other workers concluded from their studies that sage modulated antioxidantpathways to minimize stress by scavenging freeradicals. thusprotecting membrane lipids of fatty acids andphospholipids from oxidative stress in sage which has potent antioxidant effect (Luvone *et al.* 2006, Elida *et al.* 2010, Nour *et al.*, 2010 and Kianbakht *et al.*, 2011).

The present results showed significant elevation (p<0.05) in serum total cholesterol, triglyceride and LDL-Clevels respectively concurrent with significant reduction in HDL-C level (p < 0.05) in the HPC fed rats as compared with control negative group. These findings agree with the studies have reported high dietary fat and cholesterol induce hypercholesterolemia in animal models (Cherng and Shih, 2005 and Martinello *et al*., 2006). Thehigh serum triglyceride level an important risk factor as it influences lipid deposition and clotting mechanisms (Harnafi *et al*., 2009). Increase in LDL-C has been pointed out as one of the risk factors for the development of atherosclerosis and related cardiovascular diseases (Getz and Reardon, 2006).The present study results showed that administration ofsage oil showed significantly (p<0.05) reduced the total serum cholesterol, triglyceride, LDL-C levels and increased the level of HDL-C of hypercholesterolemic female rats in protective group when administrated for four weeks (table 1) as compared with hypercholesterolemia rats. The hypolipidemic effect of sage oil attributed to different mechanism that act on cholesterol homeostasis by affecting on 3-hydroxy-3 methylglotryl-CoA reductase (HMG-CoA reductase), the rate limiting enzyme of cholesterol synthesis, the administration of sage oil led to decrease in HMG-CoA reductase and then retardation of cholesterol synthesis. On the other hand sage oil also had an effect on cholesterol 7α-hydroxylase activity (the rate limiting enzyme for bile acid synthesis) to increased synthesis of bile acid from cholesterol in the liver and this increase utilization of cellular cholesterol and enhanced LDL-C uptake to decrease the total serum cholesterol and other lipid profile. Moreover, the LDL receptors became available to bind with LDL-C and metabolized. Thus, an increased in LDL receptors is associated with faster turnover of LDL-C and faster removal of cholesterol from the

blood**(**Carla *et al.*, 2009). These results in agreement with the (Lu and Yeap Foo, 2000 Ninomiya *et al.*,2004 and Hamidpour *et al*., 2014)demonstrated that*Salvia officinalis* L. leaves methanolic extract have a significant inhibitory effect on serum triglyceride and cholesterol may be due to the sage is a natural source of flavonoids and polyphenolic compounds possessing strong antioxidant properties. Other workers concluded from their studies that reported that *Salvia officinalis* tea consumption is accountable for the improvement of the lipid profile inducing a decrease on the highly atherogenic LDL-C particles (which are easily oxidable and less readily cleared) and an increase in the HDL-C, this effect may be due to the ability of *Salvia officinalis* to suppress cholesterol biosynthesis (Akram and Maryam, 2009; Kianbakht *et al.*, 2011).

Plana *et al.* (2008) concluded that the reduction effect of sage on lipid profilelevel may attributed to several natural components are presence in sagethat have been shown to act on cholesterol metabolism by reducing its absorption or its synthesis, such as catechins**.** At the same time the Thujone, a major component is amonoterpene that occurs mainly as a mixture of alpha and β diastereoisomers in many plants such as *Artemisia absinthium* L. and *Salvia officinalis* L., it lowers cholesterol and triglyceride levels (Kee *et al.*, 2009; El-Kholy *et al.*, 2010). A similar finding was reported by Eidi and Eidi (2009) who showed that oral administration of 0.2 and 0.4 g/kg B.w. of the extract for 14 days exhibited a significant reduction in serum triglycerides and total cholesterol.

At the same time the hypercholesterolemic rats (positive control group) had significant ($P<0.05$) increases in serum levels of AST and ALT enzymes of hypercholesterolemic rats when compared to the normal rats. Our results were agreed with those reported by (Lu *et al*., 2007; Prasad, 2010; Saki e*t al*., 2011) who showed that a high cholesterol diet moderately elevated serum levels of ALT and AST enzymes in rats. Oral administration of sage oil to hypercholesterolemic rats significantly ($P<0.05$) reduced serum AST and ALT compared to the hypercholesterolemic rats as shown in Table - 2. The present results agreed with the results obtained by Luvone *et al.* (2006) and Elida *et al.* (2010) who showed that AST and ALT were significantly increased in diabetic group, but after treatment with sage the AST and ALT levels decreased and become near to the control level especially ALT value, this effect is due to the active constitutes of sage polyphenols, especially, phenolic and rosmarinic acid in sage which has potent antioxidant.other authors (Lima *et al.*, 2005; Kaur *et al*., 2006; Nour *et al.*, 2010; Kianbakht *et al.*, 2011) demonstrating that administration of sage lead to an inhibition in serum of AST and ALT enzymes this may be due to the sage modulated antioxidant pathways to minimize stress by scavenging free radicals, thus protecting membrane lipids of fatty acids and phospholipids from oxidative stress as well as hepatic injury (Farhoudi *et al*., 2011).

The present results at same table indicate that the levels of pancreatic lipaseare increased when the fat content of the diet Raised. Pancreatic lipase is a key enzyme in dietary triacylglycerol absorption, hydrolyzing triacylglycerol to 2-monoacylglycerol and fatty acids, when these arehydrolyzed, they are absorbed by cells and this causes increasein levels of lipids. It was well known that dietary fat is not directly absorbed from the intestine unless it has been subjected to the action of pancreatic lipase (Han *et al*., 2001). While, the administration of *Salvia officinalis* oil extract shows asignificant reduction in the enzyme level noticed in treated group when compared to normal and hypercholesterolemia groups. This indicated that *Salvia officinalis* oil extract was able to inhibit enzymatic function of pancreatic lipase which was responsible for hydrolyzing fatty acids. This may be due to the high polyphenolic content of *Salvia officinalis* leaves extract is in part responsible for the inhibitory activity onpancreatic lipase (Lu and Yeap FOO, 2001; Garza *et al*., 2011). A similar finding was reported by Sugiyama *et al*. (2011) who showed that the polyphenolic extracts from a number of plants have been shown to be effective inhibitors of the

intestinal pancreatic lipase enzyme systems. Ninomiya *et al*. (2000) demonstrated that the giving methanolic (MeOH) extract from the leaves of *Salvia officinalis* L. significantly inhibited the pancreatic lipase activity and suppressed serum TG elevation in olive oil loaded mice. Morever, carnosic acid and carnosol are two of the diterpenes isolated from the methanolic extract of *Salvia officinalis* with an inhibitory activity on pancreatic lipase.and eventually was effective in reducing body weight and obesity (Yadav *et al*., 2011).

5. Conclusion

From the present study concluded that daily oral administration of sage oil extract possess antihypercholesterolemia and hypolipidemic properties and beneficial effect in decreasing the elevated liver enzymes and has inhibitory effect against the pancreatic lipase activity may be attributed to theantioxidant activity of one or more of its flavonoids and polyphenols.

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