

## Biochemical Studies on the Effect of Fixed Oil Extracted from *Rosmarinus officinalis* on Blood Lipid Level in Male Albino Mice

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### Abstract

This work has been carried out to investigate the Hyperlipidemia mirrors the onset of abnormalities in lipid metabolism secondary to the manifestation and progression of the atherosclerotic disease in the patient. In addition to diet, use of medicinal plant as a pharmacologic modality in preventing alteration in lipid metabolism has received wide attention from several works. In the present study, we have therefore investigated the effect of 100 µg/ml doses of fixed oil extract from *Rosmarinus officinalis* on the levels of lipid parameters by using the protective effect of the test herbs against hyperlipidemia for two weeks. The results of experiment elicited the protective effect of 100 µg/ml doses of fixed oil extract from *Rosmarinus officinalis* were marked improvements in the level of all the test parameters were indicated.

**Keywords:** Hyperlipidemia, *Rosmarinus officinalis*, Lipid profile, Liver enzyme, Histopathological studies.

### Introduction

Many of the chronic diseases that affect human have an uneven geographic distribution. Although the general perception that several diseases, specially the various types of cancer, kidney and liver diseases as well as coronary heart disease (CHD) often result from an exposure to pollutants and toxic environmental such as chemicals, pesticides, fungicides and food additives. The high incidence of CHD is often correlated with high fat, high cholesterol and low fiber diets and the consumption of fried foods [1]. Cholesterol presents a great health hazard when its consumption is unduly increased. Hypercholesterolemia arteriopathies whether coronary or cerebral take the biggest toll of middle aged and elderly deaths. Intake of fatty diets, lack of exercise, smoking habits and mental stress participate in hypercholesterolemia and the resultant arteriopathies. Atherosclerosis, a chronic inflammatory disease which is characterized by the accumulation of plasma lipoproteins that carry cholesterol and triglycerides in the arteries, Atherosclerosis results in coronary heart disease (CHD), one of the major cause of morbidity and mortality worldwide [2]. Although the focus of research so far has been mainly on the vascular effects of hyperlipidemia, i.e. arteriosclerosis, it is now quite evident that hyperlipidemia exerts direct effects on the myocardium in addition to the development of atherosclerosis. Hyperlipidemia is often linked to oxidative/nitrosative stress in the vasculature and in the myocardium. We have previously shown an increased formation of peroxynitrite, a toxic reaction product of superoxide and nitric oxide, in the rat myocardium in cholesterol-enriched diet -induced hyperlipidemia [3]. Strong evidences have been

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put forward by various investigators for the involvement of free radicals production and lipid peroxidation in the onset of atherosclerosis [4]. One of the initial events in the development of atherosclerosis is the accumulation of cells contained excess lipids within the arterial wall. Hypercholesterolemia, especially elevated level of serum cholesterol and low-density lipoprotein (LDL), furthermore, oxidative stress is also suggested as a mechanism underlying hypercholesterolemia, which is an important factor in atherosclerosis. According to the oxidative modification hypothesis, oxidation of LDL is crucial to the cellular uptake of LDL in the first stages of atherosclerotic development [5]. Large acute doses of chronic exposure to toxic agents can overpower to the antioxidant defense system which cause hepatic cell damage. Natural or synthetic compounds having antioxidant properties can scavenge the free radicals which damage lipid, protein, DNA molecules and cell membrane. Their removals prevent the development of certain diseases [6]. Antioxidants had important role in decreasing serum lipids and retarding atherosclerosis. The observational epidemiological studies have suggested that individuals with high dietary antioxidant intake have lower risks of CHD which remains the leading cause of death in most countries [7]. Diet rich in fruits and vegetables are associated with decreased risk of CHD. Biochemical functions of naturally occurring antioxidants in biological systems such as flavonoids, polyphenols, vitamin C and E have been reported to protect the body system against reactive oxygen species. Antioxidant compounds, various efforts are now concentrated on many herbal plant extracts because of their potential to induce antioxidant effects [8]. Plant products are widely used in testing because of their low toxicity and great medicinal value. Much of research has concentrated on different plant extracts' abilities to induce antioxidant effects [9]. Rosemary essential oil is used as brain and nerve tonic, and as a remedy for mental fatigue [10] as well as antiseptic, diuretic and anti-depressant; it is also used to treat cold, influenza and rheumatic pain [11] and can enhance the performance of memory [12]. Diversity in the chemical contents of rosemary as polyphenolic compounds have been ascribed to many factors, involving the environment [13], abiotic stress [14], genetic heritance [15,16] and the phenological stages of the plants [17]. The aim of this study was to extract the fixed oil of *Rosmarinus officinalis* grow in Al-Jabal Al Akhdar, also to investigate the protective effect of the test herbs against hyperlipidemia in experiment animals.

**Table 1:** Results for the determination of lethal dose LD<sub>50</sub> of the fixed oil extracted from *Rosmarinus officinalis*. after oral ingestion in male albino mice (n = 5). LD<sub>50</sub> = 180µg/ml.

Dose(µg/ml)	(a)	Number of mice			(b)	(a×b)	% mortality
		Total	Survived	Died			
10	0	5	5	0	0	0	0%
50	40	5	5	0	0	0	0%
100	50	5	5	0	0	0	0%
150	50	5	5	1	1	50	20%
200	50	5	5	2	1	50	40%

a: The difference between the doses in two consecutive terms

b: the average number of dead animals for two consecutive terms.

## Materials and Methods

### Materials

**Plant material:** The leaves of *Rosmarinus officinalis* L, were collected from Al-Jabal Al Akhdar area in Libya 2017.

**Chemicals:** 1,1-Diphenylpicrylhydrazyl (DPPH) was supplied from Sigma and Merck company. Ascorbic acid, Folin-Ciocalteu reagent, ferric chloride, potassium ferricyanide, monobasic dihydrogen phosphate, dibasic monohydrogen phosphate, trichloro acetic acid, sodium carbonate, petroleum ether, anhydrous sodium sulfate and pyrogallol were obtained from the biochemistry laboratory of chemistry department-Benghazi University.

**Experimental animals:** Male albino mice weighing between 25 and 35gm used in this study were housed in biochemistry laboratory at Benghazi University under standard conditions of temperature (22 ± 3°C) with a dark light cycle. The animals were fed with standard diet and water ad libitum.

### Methods-sample preparation

**Extraction of fixed oil:** The fixed oil from the dry powdered leaves of *Rosmarinus officinalis* (100g) was extracted with light petroleum ether (40-60°C) in a soxhlet apparatus for about 4h and the solvent was removed by rotary vacuum evaporator. The oil sample were stored at 7°C in dark air-tight container.

**Determination of LD<sub>50</sub>:** A total Number of 80 male albino mice used in this study to determine the lethal dose of fixed oil extracted from *Rosmarinus officinalis*. In each experiment were used 40 mice, divided into five groups were given different amounts of the oil "10, 50, 100, 150, 200µg/ml" oral. The animals were monitored for 24hrs for mortality. The number of animals survived a specific dose (S) and the number of those died at that dose (D) was determined [18]. (Table 1)

The percent mortality was calculated for each dose group as in the following:

$$\% \text{ mortality} = D/(S+D) \times 100$$

Calculated lethal dose for half the number depending on the equation of [19].

$$LD_{50} = \text{Biggest dose} - \sum a \times b / n$$

a: The difference between the doses in two consecutive terms.

b: the average number of dead animals for two consecutive terms.

n: number of animals in each group.

## Experimental design

The following experiments were conducted as follows:

**The prophylactic effect of the fixed oil against hyperlipidemia:** To study the protective effect of the oil against hyperlipidemia, a total of 45 mice were used and the experiment lasted for 2 weeks. Animals were divided randomly into three groups (15 mice each) as follows:

**Group 1:** mice were fed on the standard synthetic diet (S.d) and served as negative control group (-ve).

**Group 2:** mice were daily attained to the hyperlipidemic diet (H.L.D) and served as positive control group (+ve).

**Group 3:** mice were daily administered fixed oil at a dose of 100 µg/ml oral (added to the H.L.D).

**Blood sampling:** Blood samples were collected before treatment and then after 2 weeks. Blood samples were collected from retro-orbital veins according to the procedure described by [20] by microhematocrit blood tube into the corner of the eye socket underneath the eyeball. Serum and plasma samples were carefully separated and stored frozen until using in the different biochemical analysis.

**Biochemical analysis:** The following parameters were assayed; serum total cholesterol [21], HDL-cholesterol [22], Triglycerides [23], Blood urea [24] Transaminases enzymes (ALT& AST) [25], Alkaline phosphatase (ALP) [26], Total proteins (TP) [27], Gamma-Glutamyltransferase (GGT) [28], Superoxide dismutase (SOD) [29], Malondialdehyde (MDA) [30], Glutathione Reductase (GR) [31], Glutathione Peroxidase (GPx) 1967] and Catalase (CAT) [32]. All biochemical examination is carried out in Benghazi medical center. The obtained results were statistically analyzed [33].

## Results

### Effects of fixed oil in prophylactic experiment after 2 weeks of treatment

All the plasma lipids parameters were significantly increased in induced hyperlipidemia mice (positive group), when compared to normal control values. The concentration of TC, T-chol/HDL-chol, LDL, VLDL and TG were significantly increased by (121.6%, 270.5%, 573%, 28.98%, 28.98%) respectively, but the level of HDL-cholesterol is decreased by (40.5%). Fixed oil had significant decrease in serum lipid profile levels as compared with positive group. TC, T-chol/HDL-chol, LDL, VLDL and TG were significantly decreased by (16.9%, 2.9%, 21.7%, 36.7% and 36.7%) respectively at dose of 100µg/ml of fixed oil for two weeks, while the concentration of HDL-chol is increased by (18%) at dose of 100µg/ml of fixed oil respectively. Liver function tests in hyperlipidemia mice displayed a mild to moderate increase in serum alanine aminotransferase (37.2%), aspartate aminotransferase (23.5%), G-glutamyl transferase (136.5%),

alkaline phosphatase (66%) in comparison with normal control group and significantly decrease in the serum TP by (5.75%). The sequential changes in serum ALT, AST, G-GT, ALP and TP are summarized in Figures (15,16,17,18 and 19) respectively, however in G3 which that ingested 100µg/ml of fixed oil with hyperlipidemia diet for two weeks, serum ALT, AST, G-GT and ALP was lowered by (16.1%, 8.11%, 22.4% and 21.6%) respectively, while the serum TP showed no significant difference as compared with the positive group. The mean values of blood urea in the positive control were non-significantly compared with the positive control. The average value of SOD, GR, GP<sub>x</sub> and Catalase were significantly decreased in hyperlipidemia group by (26.9%, 41.6%, 31.6% and 31.3%) respectively. The mice fed with hyperlipidemia diet showed significant increase in the plasma levels of MDA (147.7%) when compared with normal group, while the administration of fixed oil decrease the MDA level by (27.5%). (Table 2-4)

## Discussion

### Evaluation of fixed oil against hyperlipidemia

There is a wealth of scientific data coming from in vitro studies or from different animal models, supporting the validity of the oxidative hypothesis of atherosclerosis which states that the oxidative modifications of lipoproteins is a pivotal event in the evolution of atherosclerotic plaques. A corollary of this hypothesis is that antioxidant enzymes should therefore prevent LDL oxidation and protect against the development of atherosclerosis [34]. Several epidemiological studies concluded that a high intake of food rich in natural antioxidants increases the antioxidant capacity of the body and reduces the risk of several diseases. Herbal remedies or food supplements have increasingly become attractive alternatives to prevent or treat hyperlipidemia, especially for those with cholesterol at the borderline levels [35]. Antioxidant phytochemicals play an important role in human health by scavenging reactive oxygen and nitrogen species and modulating several defense systems [36-37] found that clove had the highest radical-scavenging activity followed by all spice and cinnamon. The phenolic and the flavonoids compounds are groups of secondary metabolites with broad range of biological properties such as: antioxidant, anti-atherosclerosis, cardiovascular protection and improvement of the endothelial function, it has been reported that antioxidant activity of the phenolic compounds is mainly due to their redox properties which allow them to act as reducing agents, hydrogen donors play an important role by adsorbing and neutralizing reactive free radicals, and chelating ferric ions which catalysis lipid peroxidation, and regarded as promising therapeutic agent for free radical-linked pathologies [35]. The result obtained from the GC-MS technology found that the most important components are 1,8-cineole, α-pinene, camphor, camphene, borneol. The high concentration of 1,8-cineole in fixed oil make it potentially useful in the medicines because they exhibit antibacterial, antifungal, anti-inflammatory activity and antioxidant properties according to [38].

The hypolipidemic effect of oil extracted from *Rosmarinus*

**Table 2:** Prophylactic Effects of fixed oil in prophylactic experiment on serum lipids (mg/dl  $\pm$  S.D) and % variation from the corresponding control during the induction of hyperlipidemia for 2 weeks in male mice.

Group Number	Treatment	Item	Time Intervals (Wk's)		
			0	2	% var.
1	Negative control	Trig.	48.7 $\pm$ 3.47	49.0 $\pm$ 3.70 †	0.62↑
		T.Ch.	55.5 $\pm$ 5.78	58.7 $\pm$ 4.10 †	5.77↑
		HDL.Ch.	34.6 $\pm$ 1.42	34.5 $\pm$ 1.30 †	0.28↓
		T./HDL	1.6 $\pm$ 0.09	1.7 $\pm$ 0.08†	6.25↑
		LDL	11.16 1.17±	14.4 $\pm$ 1.03	29↑
		VLDL	9.74 $\pm$ 2.03	9.8 $\pm$ 2.11	0.62↑
2	Positive control	Trig.	52.1 $\pm$ 2.11	63.2 $\pm$ 2.21*	28.98↑ a
		T. Ch	57.4 $\pm$ 4.71	130.1 $\pm$ 3.24 ***	121.6↑ a
		HDL.Ch.	32.0 $\pm$ 1.93	20.5 $\pm$ 1.81***	40.5↓ a
		T./HDL	1.8 $\pm$ 0.01	6.3 $\pm$ 0.17 ***	270.5↑ a
		LDL	14.98 $\pm$ 0.92	96.96 $\pm$ 1.00	573↑ a
		VLDL	10.42 $\pm$ 1.59	12.64 $\pm$ 2.22**	28.98↑ a
3	Fixed oil (100µg/ml)	Trig.	55.3 $\pm$ 5.03	40.0 $\pm$ 4.20 *	36.7↓ b
		T.Ch.	55.0 $\pm$ 4.06	108.1 $\pm$ 2.95**	16.9↓ b
		HDL.Ch.	33.9 $\pm$ 1.71	24.2 $\pm$ 1.73***	18↑ b
		T./HDL	1.62 $\pm$ 0.01	4.47 $\pm$ 0.22***	2.9↓ b
		LDL	10.04 $\pm$ 1.82	75.9 $\pm$ 1.90	21.7↓ b
		VLDL	11.06 $\pm$ 0.99	8.00 $\pm$ 1.73	36.71↓ b

†P &gt; 0.1. \* P &lt; 0.05. \*\* P &lt; 0.01. \*\*\* P &lt; 0.001. T.L.: total lipids. T.C.: total cholesterol.

TG.: triglycerides. HDL-C: HDL-cholesterol. T. /HDL-C: total cholesterol / HDL-cholesterol.

**Table 3:** Prophylactic effect of fixed oil on serum enzymes (U/L  $\pm$  S.D) and % variation from the corresponding control during the induction of hyperlipidemia for 2 weeks in male mice.

Group Number	Treatment	Item	Time Intervals (Wk's)		
			0	2	% var.
1	Negative control	ALT	28.8 $\pm$ 1.95	30.4 $\pm$ 2.23 †	5.55↑
		AST	54.3 $\pm$ 0.38	54.9 $\pm$ 1.65 †	1.10↑
		ALP	30.3 $\pm$ 4.03	30.9 $\pm$ 3.17 †	1.98↑
		S. GGT	8.21 $\pm$ 0.43	8.88 $\pm$ 2.11 †	8.16↑
2	Positive control	ALT	30.2 $\pm$ 1.78	41.7 $\pm$ 2.09 *	37.17↑ a
		AST	50.73 $\pm$ 2.86	67.8 $\pm$ 3.10 *	23.5↑ a
		ALP	28.9 $\pm$ 2.77	51.3 $\pm$ 2.75 †	66↑ a
		S. GGT	8.92 $\pm$ 2.00	21.0 $\pm$ 1.12*	136.5↑ a
3	Fixed oil (100µg/ml)	ALT	30.1 $\pm$ 1.09	35.0 $\pm$ 1.16 †	16.1↓ b
		AST	50.0 $\pm$ 1.89	62.3 $\pm$ 2.99 *	8.11↓ b
		ALP	31.0 $\pm$ 1.90	40.2 $\pm$ 3.14**	21.64↓ b
		S. GGT	9.11 $\pm$ 3.01	16.3 $\pm$ 2.61 *	22.4↓ b

†P &gt; 0.1. \* P &lt; 0.05. \*\* P &lt; 0.01. \*\*\* P &lt; 0.001. ALT: Alanine aminotransferase. AST: Aspartate aminotransferase. ALP: Alkaline phosphatase. S. GGT: Gamma-Glutamyl transferase.

*officinalis* may be due to its ability to combat oxidative stress by quenching free radicals generated in the body because of HFD. A significant increase in HDL-chol levels with a simultaneous lowering in serum cholesterol level definitely indicates the beneficial role of the antioxidant compounds such as 1,8-cineole,  $\alpha$ -pinene, camphor, camphene, borneol which that found in the oil. The increased levels of HDL-cholesterol concentrations also suggest that fixed oil may protect against cardiovascular diseases that result from hyperlipidemia. Nyangono et al. [35] suggests that the increase in HDL cholesterol may be due to mobilizes cholesterol from extra-hepatic tissues where it is catabolized and stimulate cholesterol 7- $\alpha$ -hydroxylase which converts cholesterol to bile acids, an important pathway of degradation of cholesterol, the earlier reports show that some of the major constituents of the rosemary oil were eucalyptol,  $\alpha$ -pinene, myrcene, p-cymene, pinene, camphor, camphene, limonene, borneol, verbenone, bornylacetate, etc. It has been found that location and seasonal change affect the variation

in composition and ultimately the biological activity of the essential oil of *R. officinalis* L. [39].

This result suggests that other phenolic compounds, beyond those found in the fixed oil, may be responsible for these responses. This effect on cholesterol reduction may be attributed to a decrease in the micellar solubilization of cholesterol in the digestive tract, to an increase in bile flow, bile cholesterol and bile acid concentration and to a subsequent increase in the fecal excretion of steroids, as previously described [40]. This protection could be attributed to rosmarinic acid, which has been reported to inhibit the expression of inducible nitric oxide synthase (iNOS) and suppress the production of superoxide and 3-nitrotyrosine in Raw 264.7 macrophages [41].

We suggest the oil rich in phenolic constituent has the highest antioxidant activity against LDL oxidation. Omer et al. [42] reported that the 1,8-cineole and  $\alpha$ - pinene have an antioxidant property and can change the affinity of the

**Table 4:** Prophylactic effect of Fixed oil on serum proteins, blood urea and antioxidant enzyme ( $\pm$  S.D) and % variation from the corresponding control during the induction of hyperlipidemia for 2 weeks in male mice.

Group Number	Treatment	Item	Time intervals (Wk's)		
			0	2	% var.
1	Negative control	T.P. (mg/dl)	6.15 $\pm$ 0.62	6.44 $\pm$ 0.19 †	4.72↑
		UREA (mg/dl)	22.9 $\pm$ 0.34	22.7 $\pm$ 0.90†	0.87↓
		SOD (U/mol)	7.11 $\pm$ 1.90	7.00 $\pm$ 1.21†	1.55↓
		MDA (nmol/ml)	10.3 $\pm$ 0.44	10.7 $\pm$ 1.25 †	3.9↑
		P. GR (U/l)	18.5 $\pm$ 0.59	18.5 $\pm$ 1.06 †	Zero
		P. GPX (mu/ml)	33.0 $\pm$ 5.01	32.3 $\pm$ 3.46 †	2.12↓
		S. CAT (U/l)	48.7 $\pm$ 2.90	47.3 $\pm$ 2.99†	2.9↓
2	Positive control	T.P. (mg/dl)	6.12 $\pm$ 0.61	6.07 $\pm$ 0.38†	5.75↓ a
		UREA (mg/dl)	21.5 $\pm$ 0.25	24.1 $\pm$ 1.11†	6.17↑ a
		SOD (U/mol)	7.52 $\pm$ 1.23	5.12 $\pm$ 0.90*	26.9↓ a
		MDA(nmol/ml)	10.1 $\pm$ 1.02	26.5 $\pm$ 1.87 **	147.7↑ a
		P. GR (U/l)	18.1 $\pm$ 1.72	10.8 $\pm$ 1.22*	41.6↓ a
		P. GPX (mu/ml)	33.7 $\pm$ 2.11	22.1 $\pm$ 2.78*	31.6↓ a
		S. CAT (U/l)	49.3 $\pm$ 1.88	32.5 $\pm$ 2.01*	31.3↓ a
3	Fixed oil (100 $\mu$ g/ml)	T.P. (mg/dl)	6.22 $\pm$ 0.20	6.34 $\pm$ 0.39 †	4.45↑ b
		UREA (mg/dl)	23.0 $\pm$ 1.92	24.6 $\pm$ 1.34†	2.07↑ b
		SOD (U/mol)	7.22 $\pm$ 1.11	5.79 $\pm$ 1.05*	13.1↑ b
		MDA (nmol/ml)	10.1 $\pm$ 1.11	*1.00 $\pm$ 19.2	27.5↓ b
		P. GR (U/l)	17.9 $\pm$ 1.38	11.9 $\pm$ 1.19*	10.2↑ b
		P. GPX (mu/ml)	32.9 $\pm$ 2.90	26.8 $\pm$ 2.05*	21.3↑ b
		S. CAT (U/l)	49.2 $\pm$ 5.21	37.6 $\pm$ 2.33*	15.7 ↑ b

†P > 0.1. \* P < 0.05. \*\* P < 0.01. \*\*\* P < 0.001. T.P.: Total protein. superoxide dismutase (SOD), Malondialdehyde (MDA), Glutathione Reductase (GR), Glutathione Peroxidase (GPx) and Catalase (CAT).

LDL particles for the LDL receptor. Also, Ramesh et al. [43] showed that dietary intake of phenolic compounds could inhibit oxidation of LDL and thereby reduce risk factors for cardiovascular disease. Flavonoids are a class of polyphenolic compounds that seem to have antioxidant properties by suppressing reactive oxygen and nitrogen species formation and protecting the antioxidant defense system, in addition flavonoids may act by making liver cells more efficient to remove LDL from blood. To do this, flavonoids increase LDL receptor densities in liver and by binding to apolipoprotein B [44].

It was observed that the enzymes such as AST, ALT, G-GT and ALP were decreased by the effect of oil when compared with positive group Olorunnisola et al. [45] reported that the co-administration of Phytochemical compounds such as flavonoids prevented liver damage resulting in the improvement of the status of liver function test. Saleh et al. [46] reported that the decrease in the serum levels of transaminases enzymes might be due to the presence of various phenolic, flavonoid compounds and sesquiterpenes that may be responsible for the protective effect of liver damage. In humans the over-production of ROS can result in tissue injury and has been implicated in disease progression and oxidative damage of nucleic acids and proteins [47]. When there is a lack of antioxidants to quench the excess reactive free radicals, cardiovascular, cancer, hyperlipidemia, diabetes, Alzheimer's and inflammatory diseases may develop in the body. Pharmaceutical products are normally supplemented with synthetic antioxidants such as butylated hydroxytoluene (BHT) and tetrabutylhydroxyquinone (TBHQ). However, natural antioxidants from plant products may be more effective in reducing ROS levels compared

to synthetic antioxidants. In addition, the dietary intake of synthetic antioxidants could be cause genotoxicity and carcinogenicity at high concentrations [48]. A possible mechanism for protection by *Rosmarinus officinalis* against liver damage could involve *Rosmarinus officinalis* components acting as free radical scavengers intercepting those free radicals [49]. The significant decreasing in the plasma MDA and increasing of antioxidant enzymes levels of hyperlipidemic mice and their tendency to return to near normal levels after administration of oil revealed their powerful antioxidant effect. Antioxidants function as rupturing the antioxidant chain that prevents the propagation of free radical reactions; this protects cells from oxidative stress. Vitamin E is particularly important, protecting cells against lipoperoxidation, during which free radical attack the fatty acids, causing structural damage to the membrane, resulting in the formation of secondary cytotoxic products such as MDA [50]. Antioxidants are believed to neutralize the free radicals in lipid chains by contributing a hydrogen atom usually from a phenolic hydroxyl group, which in turn converts phenolic groups into stable free radicals that do not initiate or propagate further oxidation of lipids [51,52] reported that, of a variety of flavonoids (rutin, dihydroquercetin, quercetin, epigallocatechin gallate, and epicatechin gallate), the catechins were most effective in inhibiting microsomal lipid peroxidation. All were able to chelate Fe<sup>2+</sup>, Fe<sup>3+</sup>, and Cu<sup>2+</sup> and effective O<sub>2</sub> scavengers at varying degrees.

Our study suggested that the health promoting capabilities effects of this oil in animal models of hyperlipidemia could be attributed to the high polyphenolic content along with the enhancement of the total antioxidant defense system of body.

As preventive antioxidants, the oil can directly intercept free radical retard or slow the oxidative processes leading to decrease MDA level. Bhosale et al. [53] reported that the lower levels of MDA and higher levels of SOD activities could be attributed to polyphenols and flavonoids. SOD catalyzes dismutation of superoxide anions into hydrogen peroxide, which was converted to water by both CAT and GPx. Nutrient antioxidants, included in the dietary antioxidants, are chain breaking antioxidants, which work with enzyme antioxidants, to regular the ROS within physiological limits [54]. Natural plant antioxidants include phenolic compounds may produce beneficial effects by scavenging free radicals [55]. Thus, phenolic compounds may help protect cells against the oxidative damage caused by free radicals, [56]. reported that the extract plant rich in phenolic compounds leads to decrease MDA and increase the SOD activity. In agreement with our study [54] indicated that the administration of *Rosmarinus officinalis* in hyperlipidemic rat decreased plasma lipid profile and enhanced the activity of the antioxidant enzymes.

## Conclusion

In conclusion, our results suggest that phenolic compounds from *Rosmarinus officinalis* protect against hyperlipidemia-induced oxidative stress, increasing the activities of antioxidant enzymes and reducing the amount of thiobarbituric acid reactive substances. The fixed oil extract was also able to improve the serum lipid profile, contributing to cardiovascular disease reduction. Future analysis involving the bioavailability of rosemary phenolic compounds and their role on modulating the signaling pathways activated under oxidative stress-related diseases may help to clarify the role of these antioxidant compounds in cholesterol homeostasis and parameters of oxidative stress.

## References

1. Betul C, Nesrin K (2012) Protective effects of vitamin E against hypercholesterolemia-induced age-related diseases. *Genes Nutr* 7: 91-98.
2. Stocker R, Keaney J (2014) Role of oxidative modifications in atherosclerosis. *Physiol Rev* 84:1381-1478.
3. Tamas C, Erika B, Peter B, Gabriella F, Aniko G et al. (2013) Hypercholesterolemia increases myocardial oxidative and nitrosative stress thereby leading to cardiac dysfunction in apoB-100 transgenic mice. *Cardiovascular Research* 76: 100-109.
4. Bansal M, Sapna J (2011) Hypercholesterolemia Induced Oxidative Stress is Reduced in Rats with Diets Enriched with Supplement from *Dunaliella salina* Algae. *Am J Biomed Sci* 1: 196-204.
5. Lifeng W, Jing S, Qida Y, Xuefeng W, Xingrong J (2012) Protective Effect of Polyphenols Extract of Adlay (*Coix lachryma-jobi* L. var. *ma-yuen* Stapf) on Hypercholesterolemia-Induced Oxidative Stress in Rats. *Molecules* 17: 8886-8897.
6. Cintra D, Costa A, Peluzio M, Matta S, Silva M (2006) Lipid profile of rats fed high fat diet based on flaxseed, peanut, trout or chicken skin. *Basic Nutrit Investig* 22: 197-205.
7. Sun P, Wang J, Yang G, Khas E, Liu Q (2010) Effects of different doses of free alpha-linolenic acid infused to the duodenum on the immune function of lactating dairy cows. *Arch Anim Nutr* 64: 504-513.
8. Olorunnisola O, Bradley G, Afolayan A (2012) Anti-hyperlipidemic and biochemical effect of extract of *Tulbaghia violacea* rhizomes on high cholesterol diet fed rats. *African Journal of Biotechnology* 11: 13498-13505.
9. Jeon K, Park E, Park H, Jeon Y, Cha S et al. (2015) Antioxidant activity of far-infrared radiated rice hull extracts on reactive oxygen species scavenging and oxidative DNA damage in human lymphocytes. *J Med Food* 9: 42-48.
10. Laybourne G, Moss M, Wesnes K, Scott S (2013) Effects of acute oral administration of rosemary and peppermint on cognition and mood in healthy adults. *Journal of Psychopharmacology* 17: A62-A62.
11. Erenmemisoglu A (2014) Effect of a *Rosmarinus officinalis* leave extract on plasma glucose levels in normoglycaemic and diabetic mice. *Pharmazie* 52: 645-646.
12. Moss M, Cook J, Wesnes K, Duckett P (2003) Aromas of rosemary and lavender essential oils differentially affect cognition and mood in healthy adults. *International Journal of Neuroscience* 113: 15-38.
13. Tigrine-Kordjani N, Chemat F, Meklati B, Tuduri L, Giraudel J et al. (2007) Relative characterization of rosemary samples according to their geographical origins using microwave-accelerated distillation, solid-phase microextraction and Kohonen self-organizing maps. *Analytical and Bioanalytical Chemistry* 389: 631-641.
14. Toumekti T, Vadel A, Onate M, Khemira H, Munne-Bosch S (2011) Salt-induced oxidative stress in rosemary plants: Damage or protection? *Environmental and Experimental Botany* 71: 298-305.
15. Yosr Z, Hnia C, Rim T, Mohamed B (2013) Changes in essential oil composition and phenolic fraction in *Rosmarinus officinalis* L. var. *typicus* Batt. organs during growth and incidence on the antioxidant activity. *Industrial Crops and Products* 43: 412- 419.
16. Jordan M, Lax, V, Rota M, Loran S and Sotomayor, J (2013) Effect of bioclimatic area on the essential oil composition and antibacterial activity of *Rosmarinus officinalis* L. *Food Control* 30: 463-468.
17. Singh M, Guleria N (2013) Influence of harvesting stage and inorganic and organic fertilizers on yield and oil composition of rosemary (*Rosmarinus officinalis* L.) in a semi-arid tropical climate. *Industrial Crops and Products* 42: 37-40.
18. AL-Ali A, Randhawa M, Shaikh N (2008) Oral and intraperitoneal LD<sub>50</sub> of thymoquinone, an active principle of *Nigella sativa*, in mice and rats. *J Ayub Med Coll Abbottabad* 20: 25-7.
19. Al-Ammar M (2001) The effect of Propolis extract on same Pathogenic bacteria.
20. Janet, Hoff (2000) Methods of Blood Collection in the Mouse. *Lab Animal* 29: 47-53.
21. Alan D, Debra L (2006) Hyperlipidemia-induced atherosclerosis. John Wiley and sons Ltd.
22. Allain C, Patrycja T, Chern J (1974) Enzymatic determination of total cholesterol in serum. *Clin chem* 20: 470.
23. Fossati P, Precipe L (1996) The determination of triglycerides using enzymatic method. *Clin Chem* 29: 733-742.
24. Tabacco A, Meiattini F, Moda E (1979) Simplified enzymatic colorimetric serum urea nitrogen determination. *Clin Chem* 25: 336-337.
25. Bergmeyer H, Horder M, Raj R (1986) Approved recommendation on IFCC methods for the measurement of catalytic concentration of enzymes. Method for Aspartate aminotransferase. *J Clin Chem Clin Biochem* 24: 497-510.
26. Bretaudiero J, Vassault A (1977) Criteria for establishing a standardized method for determining alkaline phosphatase activity in human serum. *Clin Chem* 23: 2263-2274.
27. Doumas B, Biggs H (1981) A candidate reference method for determination of total protein in serum: Development and validation. *Clin Chem* 27: 1642-1650.
28. Shaw L, Stromme J, London J, Theodorsen L (1983) IFCC methods for the measurement of catalytic concentration of enzymes. Method for gamma-glutamyltransferase. *J Clin Chem Clin Biochem* 21: 633-646.

29. Nishikimi M, Roa N, Yogi A (1972) The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochem Biophys Res Commun* 46: 849-854.
30. Ohkawa H, Ohishi W, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 95: 351.
31. Goldberg D, Spooner M (1983) In methods of Enzymatic Analysis. Deerfield Beach, Florida.
32. Paglia E, Valentine N (1967) Mechanisms of freeradical oxidation of unsaturated lipids. *J Lab Clin Med* 70: 158-169.
33. Chase C (1967) Statistical analysis of data using "t" Test according to the degree of freedom. Mc Grow Hill. India.
34. Kallol D, Biswadev B (2009) Escherichia Coli Lipopolysaccharide Administration Alters Antioxidant Profile during Hypercholesterolemia. *Indian Journal of Clinical Biochemistry* 24: 179-183.
35. Nyangono B, Chakokam N, Kuate J, Ngondi J, Enyong O (2012) Effect of Guibourtia tessmannii extracts on blood lipids and oxidative stress markers in triton WR 1339 and high fat diet induced hyperlipidemic rats. *Biology and Medicine* 4: 01-09.
36. Dhan P, Gupta K (2009) The Antioxidant Phytochemicals of Nutraceutical Importance. *The Open Nutraceuticals Journal* 2: 20-35.
37. Khatun M, Eguchi S, Yamaguchi, T, Takamura H, Matoba T (2006) Effect of thermal treatment on radical-scavenging activity of some spices. *Food Sci Technol Res* 12: 178-85.
38. Greiner J, Muller J, Zeuner M, Hauser S, Seidel, T, et al. (2014) 1,8-Cineol inhibits nuclear translocation of NF-kappa B p65 and NF-kappa B-dependent transcriptional activity. *Bba-Mol Cell Res* 1833: 2866-2878.
39. Zaouali Y, Bouzaine T, Boussaid M (2010) Essential oils composition in two Rosmarinus officinalis L. varieties and incidence for antimicrobial and antioxidant activities. *Food Chem Toxicol* 48: 3144-3152.
40. Gorinstein S, Leontowicz H, Leontowicz M, Drzewiecki J, Jastrzebski Z, et al. (2005) Red Star Ruby (Sunrise) and blond qualities of Jaffa grapefruits and their influence on plasma lipid levels and plasma antioxidant activity in rats fed with cholesterol-containing and cholesterol-free diets. *Life Sci* 77: 2384-2397.
41. Qiao S, Tsubouchi R, Takeuchi F, Murakami K, Nisimoto Y et al. (2005) Rosmarinic acid inhibits the formation of reactive oxygen and nitrogen species in RAW264.7 macrophages. *Free Radic Res* 39:995-1003.
42. Omer H, EL-Nameary R, EL-Kady A, Azza M, Badr F, et al. (2013) Improving the Utilization of Rabbit Diets Containing Vegetable Oil by Using Fennel (*Foeniculum vulgare*) And Oregano (*Origanum vulgare L*) as Feed Additives. *Life Science Journal* 10: 2625-2636.
43. Ramesh E, Elanchezhian R, Sakthivel M, Jayakumar T, Kumar R, et al. (2008) Epigallocatechin gallate improves serum lipid profile and erythrocyte and cardiac tissue antioxidant parameters in Wistar rats fed an atherogenic diet. *Fundamental and Clinical Pharmacology* 22: 275-284.
44. Pourghassem-Gargar B, Ebrahimzadeh-Attary V, Rafraf M, Gorbani A (2009) Effect of dietary supplementation with *Nigella sativa* L. on serum lipid profile, lipid peroxidation and antioxidant defense system in hyperlipidemic rabbits. *J Med Plants Res* 3: 815-821.
45. Olorunnisola S, Graeme B, Anthony J (2012) Protective Effect of *T. violacea* Rhizome Extract Against Hypercholesterolemia-Induced Oxidative Stress in Wistar Rats. *Molecules* 17: 6033-6045.
46. Saleh M, Maraia F, Mohamed H (2013) Biochemical studies on the effect of Ecballium elaterium "fruit juice as an antioxidant against hepatotoxicity induced by paracetamol. University of Benghazi Repository.
47. Middleton E, Kandaswami C, Theoharides T (2008) The effects of plant flavonoids on mammalian cells: Implication for inflammations, Heart disease and cancer. *Pharmacol Rev* 52: 673-751.
48. Ashwell R, Ndhlala M, Johannes V (2010) Natural Antioxidants: Fascinating or Mythical Biomolecule. *Molecules* 15: 6905-6930.
49. Nassar M, Ahmed H, Gaara A, El-Ghorab A, Abdel-Razik H, et al. (2007) Chemical Constituents of Clove (*Syzygium aromaticum*, Fam. Myrtaceae) and their Antioxidant Activity. *Rev Latinoamer Quím* 35: 47-57.
50. Jervis K, Robaire B (2004) The effects of long-term vitamin E treatment on gene expression and oxidative stress damage in the againg brown Norway rat epididymis. *Biology of Reproduction* 71: 1018-1095.
51. Hamid A, Aiyelagbe O, Usman L, Ameen O, Lawal A (2010) Antioxidants: Its medicinal and pharmacological Applications. *Afr J Pure Appl Chem* 4: 142-151.
52. Potapovich A, Kostyuk V (2003) Comparative study of antioxidant properties and cytoprotective activity of flavonoids. *Biochem (Moscow)* 68: 514-9.
53. Bhosale R, Jugalkishor B, Jaju C, Sudhir L, Padwal A, et al. (2012) Lipid lowering and antioxidant potential of Asparagus racemosus in hyperlipidemic rats. *Int J Basic Clin Pharmacol* 1: 168-173.
54. Singh P, Chandra A, Mahdi F, Roy A, Sharma P (2010) Reconvene and reconnect the antioxidant hypothesis in human health and disease. *IJCB* 25: 225-243.
55. Chun O, Kim D, Lee C (2003) Superoxide radical scavenging activity of the major polyphenols in fresh plums. *J Agric Food Chem* 51: 8067-8072.
56. Korakot Y, Wang Y, Wang J (2006) Effect of natural volatile compounds on antioxidant capacity and antioxidant enzymes in raspberries. *Postharvest Biology and Technology* 40: 106-115.