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Effects of *Melissa officinalis* L. Extract on the Skin Tissues of Hyperlipidemic Rats

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In this study, the effects of *Melissa officinalis* L. on hyperlipidemic rats were investigated biochemically. The animals were fed a lipogenic diet consisting of 2 % cholesterol, 20 % sunflower oil and 0.5 % cholic acid added to normal chow and were given 3 % ethanol for 42 d. The extract was given gavage technique to rats a dose of 2 g/kg everyday for 28 d, after 14 d, experimental animals done hyperlipidemia. In hyperlipidemic groups, a reduction of the skin glutathione level (GSH), skin superoxide dismutase (SOD) activity and serum catalase (CAT), paraoxonase (PON) activity and an increase in serum cholesterol, total lipid, triglycerides and uric acid, γ-glutamyl transferase activity (GGT) and skin cholesterol, total lipid, lipid peroxidation (LPO), nonenzymatic glycosylation (NEG) and skin CAT, lactate dehydrogenase (LDH), glutathione peroxidase (GP_x) and myeloperoxidase (MPO) activity were observed. Treatment with *Melissa officinalis* L. extract reversed these effects. Present results show that *Melissa officinalis* L. extract has a protective effect against skin tissue damage as result of hyperlipidemia, in addition to hypolipidemic effect.

Key Words: Skin, Hyperlipidemia, *Melissa officinalis* L., Cholesterol, Lipid peroxidation, Glutathione, Nonenzymatic glycosylation, Antioxidant defense system.

INTRODUCTION

Hyperlipidemia, resulting from lipid metabolic changes is a major cause of cardiovascular disturbances, such as atherosclerosis and coronary heart disease[1]. Cardiovascular diseases are the most common causes of mortality and morbidity worldwide[2]. The normalization of serum levels of cholesterol and lipid through diet therapy or drug administration has been shown to decrease the incidence of coronary heart disease[3]. Medicinal plants continue to provide valuable therapeutic agents, in both modern medicine and in traditional system[4]. Many herbal medicinal products reported to have potential to reduce cholesterol and lipid in body[5]. *Melissa officinalis* L. (Lemon Balm, Labiatae) is a well-known herb used to give fragrance to different food and beverage products[6]. It has also been used as a medicinal plant for treatment of headaches, gastrointestinal disorders, nervous-ness,

rheumatism, high blood pressure, stiff neck, hysteria and melancholia[7,8]. In addition, the plant is used as antimicrobial[7], antiviral[9], antioxidative[6,10,11], antitumour[12], sedative[13] and for neurological activity[14,15]. *Melissa officinalis* L. contains volatile oil and glycosides of the alcoholic or phenolic compounds (carnosic acid) of the volatile oil (eugenol glucoside)[16], caffeic acid[8], mono and sesquiterpenes[17], triterpene acids (ursolic and oleanolic acid) and flavonoids (cynaroside, comosin, rhamnocitrin, isoquercetin and tannins). Hyperlipidemia threats the healthy of human today, causing cardiovascular system disease, renal disease and some medical problems following various diseases and organ transplantations[18,19]. Skin and other tissues is effected from hyperlipidemia[19]. No literature on the effect of *Melissa officinalis* L. extract on skin tissues could be found.

The aim of the present study to investigate the effects of *Melissa officinalis* L. on skin tissues of lipid peroxidation, glutathione, nonenzymatic glycosylation, antioxidant defense system, skin cholesterol and lipid and serum parameters in normal and hyperlipidemic rats.

EXPERIMENTAL

Dried *Melissa officinalis* L. was purchased in a herbal shop in Çemberlitas, Istanbul. Voucher specimen are deposited in the Herbarium of the Faculty of Pharmacy, Istanbul University (ISTE). Herbarium code number is ISTE-81925.

Preparation of aqueous extract: 100 g dried leaves were extracted by boiling for 0.5 h in 1000 mL distilled water. The extract was then filtered and the filtrate was evaporated to dryness under reduced pressure by using a rotary evaporator. The extract was dissolved in distilled water prior to the administration to normal and hyperlipidemic rats.

Animals: In this study, 40 male Swiss albino (*Rattus norvegicus*) rats, 8.0-8.5 months old each, were used. The experiments were reviewed and approved by local Institute's Animal Care and Used Committees. The animals were randomly divided into four groups. The animals of the group I (control) were fed normal chow and tap water *ad libitum* (n = 10). The rats of the group II were fed normal chow and treated with the *Melissa officinalis* L. extract for 28 d by gastric gavage (n = 10). The animals of the group III were fed a lipogenic diet[20] consisting of 2 % cholesterol, 20 % sunflower oil and 0.5 % cholic acid added to normal chow (n = 10). In addition, the animals in this group were given water containing 3 % ethanol. This regime was maintained for 42 d. The rats of group IV were maintained in the same food and drink regime as the animals in group III (n = 10). After two weeks, the animals showed symptoms of hyperlipidemia and they were treated with 2 g/kg daily *Melissa officinalis* L. extract by gavage for 28 d. On the 42nd day of experiment, the animals were

fasted overnight and then sacrified under ether anesthesia. Blood and skin tissue samples were taken from the animals of all groups. The serum was separated from the blood and skin tissues were immediately washed with saline and frozen until needed for study.

Biochemical assays: Blood samples from rats were collected from the tail vein at 42nd days. Serum cholesterol and total lipids sulfophosphovanilin were determined by the method of Zlatkis *et al.*[20]. Serum triglycerides and uric acid were estimated by the methods of Soloni[21] and Caraway[22], respectively. Serum CAT and skin CAT activity was determined with Aebi's method[23]. Szasz[24] and Furlong *et al.*[25] methods were used to determine the serum GGT and PON activities, respectively. Skin cholesterol and skin total lipids levels were determined by the method of Zlatkis *et al.*[20] and sulfophosphovanilin[26], respectively.

Tissue homogenization: The skin samples were homogenized to 10% (w/v) with cold 0.9 % saline using glass equipment. The homogenates were centrifuged and the clear supernatants were used for lipid peroxidation (LPO), glutathione (GSH), nonenzymatic glycosylation (NEG), protein and enzymes analyses. The lipid peroxidation levels of skin homogenates were determined by the method of Ledwozyw *et al.*[27]. Glutathione levels in tissue homogenates were determined according to the method by Beutler using Ellman's reagent[28]. NEG levels were assayed by thiobarbituric acid methods[29]. The protein content in the supernatant was estimated by the Lowry method using bovine serum albumin as standart[30]. Glutathione peroxidase (GP_x) activity was determined by the method described by Paglia and Valentine and modified by Wendel[31]. Superoxide dismutase (SOD) activity was assayed by the method described by Mylroie *et al.*[32]. MPO activity was measured in skin tissue in a procedure similar to that documented by Hillegas *et al.*[33]. LDH activity was determined by the method of Wroblewski[34].

Statistical analyses: The results were evaluated using an unpaired t-test Anova variance analysis using the NCSS statistical computer package.

RESULTS AND DISCUSSION

Serum cholesterol, total lipid and triglyceride levels are presented in Table-1. A notable difference in the serum cholesterol, total lipid and triglyceride levels of four groups was observed ($P_{Anova} = 0.0001$). Serum cholesterol, total lipid and triglyceride levels in hyperlipidemic group were significantly increased compared to control group ($^{a,b}P_{t\text{-}test} = 0.0001$, $^{c}P_{t\text{-}test} = 0.001$). *Melissa officinalis* L. extract administration caused remarkable decreases in serum cholesterol, total lipid and triglyceride levels in hyperlipidemic group ($P_{t\text{-}test} = 0.0001$, $P_{t\text{-}test} = 0.001$) (Table-1).

TABLE-1
SERUM TOTAL LIPID AND CHOLESTEROL LEVELS IN CONTROL AND EXPERIMENTAL GROUPS OF RATS

Groups	n	Cholesterol (mg/dL)*	$P_{t\text{-test}}$	Total Lipid (mg/dL)*	$P_{t\text{-test}}$	Triglyceride (mg/dL)*	$P_{t\text{-test}}$
Control	10	110.62 ± 0.62	0.0001	229.14 ± 0.79	0.4340	89.41 ± 4.88	0.370
Control + Extract	10	99.02 ± 1.70		227.05 ± 5.50		93.34 ± 6.49	
Hyperlipidemic	10	204.62 ± 10.90[a]	0.0001	683.91 ± 6.30[b]	0.0001	126.21 ± 10.15[c]	0.001
Hyperlipidemic + Extract	10	105.76 ± 4.10		321.86 ± 14.22		84.35 ± 10.42	
P_{Anova}		0.0001		0.0001		0.0001	

*Mean ± SD; n = Number of animals; [a,b]$P_{t\text{-test}}$ = 0.0001 *vs.* control group; [c]$P_{t\text{-test}}$ = 0.001 *vs.* control group.

TABLE-2
SERUM URIC ACID LEVELS AND CATALASE, γ GLUTAMYL TRANSFERASE AND PARAOXONASE ACTIVITIES IN CONTROL AND EXPERIMENTAL GROUPS OF RATS

Groups	n	Uric acid (mg/dL)*	$P_{t\text{-test}}$	Catalase activity (U/L)*	$P_{t\text{-test}}$	γ-Glutamyl transferase activity (U/L)*	$P_{t\text{-test}}$	Paraoxonase activity (U/L)*	$P_{t\text{-test}}$
Control	10	0.64 ± 0.12	0.106	3006.23 ± 101.05	0.0001	11.56 ± 1.42	0.0001	216.40 ± 4.79	0.0001
Control + Extract	10	0.51 ± 0.12		3635.32 ± 330.77		21.23 ± 1.86		152.12 ± 4.63	
Hyperlipidemic	10	0.87 ± 0.05[a]	0.0001	2724.00 ± 151.42[b]	0.0001	29.63 ± 7.03[c]	0.0001	124.46 ± 8.92[d]	0.0001
Hyperlipidemic + Extract	10	0.52 ± 0.10		3006.22 ± 107.45		0.77 ± 2.73		166.87 ± 11.12	
P_{Anova}		0.0001		0.0001		0.0001		0.0001	

*Mean ± SD; n = Number of animals; [a,b,d]$P_{t\text{-test}}$ = 0.0001 *vs.* control group; [c]$P_{t\text{-test}}$ = 0.001 *vs.* control group.

Table-2 shows the effects of *Melissa officinalis* L. extract on serum CAT, GGT and PON 1 activities and uric acid levels. There was a significant difference in serum uric acid levels of the four groups ($P_{Anova} = 0.0001$). They were markedly increased in for the hyperlipidemic rats relative to controls ($^{a}P_{t\text{-}test} = 0.0001$). *Melissa officinalis* L. extract given to the hyperlipidemic rats lowered the serum uric acid levels in a significant manner when compared to hyperlipidemic rats ($P_{t\text{-}test} = 0.0001$). The mean serum CAT activity is shown in Table-2. A significant difference in the serum CAT activity of groups was observed at the end of the 42nd day experiment ($P_{Anova} = 0.0001$). Serum CAT activity was found to be significantly decreased for hyperlipidemic groups, compared to control groups ($^{b}P_{t\text{-}test} = 0.0001$). Supplementation with *Melissa officinalis* L. extract caused significant increase in serum CAT activity in hyperlipidemic rats ($P_{t\text{-}test} = 0.0001$). In all of the four groups, serum GGT activities varied significantly ($P_{Anova} = 0.0001$). In hyperlipidemic group, these values were increased in comparison to the control group ($^{c}P_{t\text{-}test} = 0.001$). Administration of *Melissa officinalis* L. extract caused remarkable decreases in serum GGT activity in hyperlipidemic group ($P_{t\text{-}test} = 0.0001$). Another notable difference was observed in the serum PON activity ($P_{Anova} = 0.0001$). In hyperlipidemic group, PON 1 activity were found to decrease remarkably than in those in the control group ($P_{t\text{-}test} = 0.0001$) whereas after the administration of plant extract, these values were increased ($P_{t\text{-}test} = 0.0001$).

Mean skin total lipid and cholesterol levels of four groups are given in Table-3. The skin total lipid and cholesterol levels in hyperlipidemic group were found to markedly increase than in the control group ($^{a,b}P_{t\text{-}test} = 0.0001$). Extract administration notably decreased the levels of cholesterol and total lipid in skin as compared to hyperlipidemic group ($P_{t\text{-}test} = 0.0001$).

TABLE-3
SKIN TOTAL LIPID AND CHOLESTEROL LEVELS IN CONTROL AND EXPERIMENTAL GROUPS OF RATS

Groups	n	Total lipid (mg/g)*	$P_{t\text{-}test}$	Cholesterol (mg/g)*	$P_{t\text{-}test}$
Control	10	90.64 ± 1.83	0.1390	4.87 ± 0.56	0.1660
Control + Extract	10	80.85 ± 6.27		5.34 ± 0.28	
Hyperlipidemic	10	181.95 ± 6.49[a]	0.0001	23.64 ± 1.92[b]	0.0001
Hyperlipidemic + Extract	10	130.01 ± 5.17		16.80 ± 1.64	
P_{Anova}		0.0001		0.0001	

*Mean ± SD; n = Number of animals; $^{a,b}P_{t\text{-}test} = 0.0001$ *vs.* control group.

The mean skin GSH, LPO and NEG levels of the four groups are given in Table-4. According to Table-4, there was significant difference in skin GSH levels between four groups ($P_{Anova} = 0.0001$). The skin GSH levels in hyperlipidemic group were found to decrease than in the control group ($^{a}P_{t\text{-}test} = 0.0001$). Skin GSH levels increased remarkable after the administration of extract ($P_{t\text{-}test} = 0.0001$). A significant difference in the skin LPO levels of four groups was observed ($P_{Anova} = 0.003$). The skin LPO levels in hyperlipidemic rats were high as compared with those of control rats ($^{b}P_{t\text{-}test} = 0.005$). Administration of *Melissa officinalis* L. extract caused a significant decrease in skin LPO levels in hyperlipidemic group ($^{c}P_{t\text{-}test} = 0.025$). Skin NEG levels in hyperlipidemic group were significantly increased compared to control group ($^{c}P_{t\text{-}test} = 0.0001$). Skin NEG levels decreased after administration of *Melissa officinalis* L. extract ($P_{t\text{-}test} = 0.068$).

The mean skin CAT, LDH, SOD, GP_x and MPO activities are given in Table-5. In all of the four groups, a notable difference in the CAT, LDH, SOD, GP_x and MPO activities was observed ($P_{Anova} = 0.0001$). Skin CAT, LDH, GP_x and MPO activities in hyperlipidemic group were increased dramatically when compared to control group ($^{a,b,d,e}P_{t\text{-}test} = 0.0001$). Administration of *Melissa officinalis* extract caused a significant decrease in CAT, LDH, GP_x and MPO activities in hyperlipidemic group ($P_{t\text{-}test} = 0.0001$). Skin SOD activity decreased in the hyperlipidemic group ($^{c}P = 0.0001$). Again, on contrary, the administration of *Melissa officinalis* extract to rats had caused an increase in the skin SOD activity in hyperlipidemic group ($P_{t\text{-}test} = 0.0001$).

Hyperlipidemia (mainly increased level of cholesterol), usually found in elderly people, is caused by lipid metabolic changes in serum and tissues[35]. It is generally accepted that lowering of high serum cholesterol levels plays a significant role in the prevention of atherosclerosis[36]. In this study, adult Swiss albino rats were selected. A lipogenic diet administered for 42 d increased serum cholesterol, lipid and triglyceride levels. This effect was reversed by administration of *Melissa officinalis* L. extract In present study, *Melissa officinalis* L. extract decreased serum levels of total cholesterol by 48.31 %. The lipid lowering effect of *Melissa officinalis* L. extract in rats may be due to inhibition of cholesterol biosynthesis and increased faecal bile acid excretion[37]. This could stimulate the catabolism of cholesterol in the liver by feedback mechanism[38]. The aqueous extract decreased the serum triglyceride levels by 33.17 %. This decrease in serum triglyceride level could be due to increased catabolism of triglyceride and inhibition of fatty acetyl-CoA activity and glycerophosphate acetyl transferase[39]. In present study *Melissa officinalis* L. extract decreased the serum total lipid levels by 52.94 %. These effects could be due to involving inhibition of intestinal absorption of lipids.

TABLE-4
SKIN GLUTATHIONE, LIPID PEROXIDATION AND NONENZYMATIC GLYCOSYLATION LEVELS IN CONTROL AND EXPERIMENTAL GROUPS OF RATS

Groups	n	GSH (nmol GSH / mg protein)*	$P_{t\text{-test}}$	LPO (nmol MDA/ mg protein)*	$P_{t\text{-test}}$	NEG (nmol Fructose/ mg protein)*	$P_{t\text{-test}}$
Control	10	4.56 ± 0.58	0.001	1.12 ± 0.70	0.501	6.29 ± 1.86	0.0001
Control + Extract	10	11.60 ± 2.95		1.35 ± 0.25		11.03 ± 1.43	
Hyperlipidemic	10	2.54 ± 0.29[a]	0.0001	1.85 ± 0.23[b]	0.025	17.31 ± 3.28[c]	0.068
Hyperlipidemic + Extract	10	7.76 ± 1.06		1.56 ± 0.28		14.50 ± 2.62	
P_{Anova}		0.0001		0.003		0.0001	

*Mean ± SD; n = Number of animals; [a,c]$P_{t\text{-test}}$ = 0.0001 *vs.* control group; [b]$P_{t\text{-test}}$ = 0.005 *vs.* control group.

TABLE-5
SKIN CATALASE, LACTATE DEHYDROGENASE, GLUTATHIONE PEROXIDASE, SUPEROXIDE DISMUTASE AND MYELOPEROXIDASE ACTIVITIES CONTROL AND EXPERIMENTAL GROUPS OF RATS

Group	n	CAT (U/mg protein)*	$P_{t\text{-test}}$	LDH (U/mg protein)*	$P_{t\text{-test}}$	SOD (U/mg protein)*	$P_{t\text{-test}}$	GP_x (U/mg protein)*	$P_{t\text{-test}}$	MPO (U/mg protein)*	$P_{t\text{-test}}$
Control	10	0.74 ± 0.05	0.026	71.46 ± 6.90	0.0001	30.22 ± 3.48	0.0001	7.94 ± 0.76	0.001	20.39 ± 1.88	0.125
Control + Extract	10	0.60 ± 0.11		202.59 ± 7.11		10.91 ± 6.03		15.49 ± 4.17		22.56 ± 2.56	
Hyperlipidemic	10	1.99 ± 0.13[a]	0.0001	315.72 ± 12.75[b]	0.0001	8.24 ± 1.15[c]	0.0001	16.40 ± 2.05[d]	0.0001	124.14 ± 11.94[e]	0.0001
Hyperlipidemic + Extract	10	0.77 ± 0.16		252.38 ± 13.59		22.01 ± 2.41		7.67 ± 0.81		29.18 ± 6.18	
P_{Anova}		0.0001		0.0001		0.0001		0.0001		0.0001	

*Mean ± SD; n = Number of animals; [a,b,c,d,e,] $P_{t\text{-test}}$ = 0.0001 *vs.* control group.

Coronary arterial disease (CAD) is the leading cause of death in world. Serum cholesterol, which is widely used to identify individuals at high risk for atherosclerotic disease, does not directly assess the extent of cholesterol deposition in tissues[40]. Skin cholesterol has been associated with coronary arter disease (CAD) and more recently with the degree of angiographic stenosis[41]. Further, skin cholesterol has been found to be associated with inflammatory markers[42]. In present study, lipogenic diet administered for 42 d increased skin cholesterol and total lipid levels. This effect was reversed administration of *Melissa officinalis* L. extract. The results clearly demonstrate that the active principles contained in the aqueous extract have a significant hypocholesterolaemic activity and provide considerable protection against insurgence of high-fat diet induced hyperlipidemia.

Many epidemiological studies have suggested that an increased serum uric acid level is a risk factor for cardiovascular disease[43]. Several mechanism may be involved in the association between serum uric acid and atherosclerosis[44]. Serum uric aciday increase platelet adhesiveness[45] and urate crystals may be associated with increased platelet lysis[46]. Serum uric acid also may have a role in the formation of free radicals and oxidative stress[47]. In present study, serum uric acid levels were significantly higher in the hyperlipidemic groups that in control groups. Administration of *Melissa officinalis* L. significantly decreases uric acid in the serum of the hyperlipidemic group. A decrease in serum uric acid levels indicates an improving effect in cardiovascular injury. Hyperuricemia correlated positively with obesity, hypertension, hypertriglyceridemia and hypercholesterolemia[48].

Skin is a metabolically dynamic tissue that possesses the largest surface area of the body and serves as a protective layer for internal organs[49]. Skin is rich in lipids, proteins and DNA, all of which are extremely sensitive to the oxidation process. Skin is also a major candidate and target of oxidative stress[50]. Hyperlipidemia may induce oxidative stress, which is important in the pathogenesis of atherosclerosis[51]. It has been reported that during mild oxidative stress, tissues respond by the induction of antioxidant mechanisms[52]. However, enhanced oxidative stress may depress the antioxidant defenses. In the present study, it is observed that the decreased activities of antioxidant enzymes CAT in serum of rats maintained on lipogenic diet as compared to those on normal diet. Present results are in agreement with reports of previous workers which suggest that feeding a high cholesterol diet to experimental animals depresses their antioxidant system due to increased lipid peroxidation and formation of free radicals[53]. The present study showed that the administration of *Melissa officinalis* L. extract increased the activities of CAT. These effects could be due to a protective antioxidant effect of the plant constitutents present in the along with the significant lipid-lowering effect. The present study also demonstrates a significant increase in serum GGT activity after

hyperlipidemia. GGT is a key enzyme in the catabolism of GSH. Recently, it has been reported that the extracellular cleavage of GSH by GGT induces the production of ROS, suggesting that GGT plays a pro-oxidant role[54]. Therefore, the present results suggest that serum GGT might be one of enzymes related to oxidative stress after hyperlipidemia. The present study showed that the administration of *Melissa officinalis* L. extract decreased the activities of GGT. These effects could be due to a protective antioxidant effect of the plant constitutents present in the along with the significant lipid-lowering effect.

PON1 activity depends on physiological conditions or pathological states[55]. PON1 activity was reduced in several groups of patients, such as individuals with diabetes, hypercholesterolemia, cardiovascular disease[56] and hyperlipidemia[57]. Increased oxidative stress has been shown to reduce PON1 synthesis in animal and cell culture models[58]. This decrease in PON1 activity in serum was related to the degree of liver and other tissue damage. In present study, serum paraoxonase activity was significantly lower in the hyperlipidemic groups that in control groups. Administration of *Melissa officinalis* significantly increased serum PON1 activity in the hyperlipidemia group. *Melissa officinalis* extract had prevented hyperlipidemia-induced oxidative stress and normalizes PON1 activity in serum.

Glutathione is known to be a major low molecular weight scavenger of free radicals in the cytoplasm. GSH plays an important role in a variety of detoxification processes by quenching of free radicals[59]. The level of GSH significantly decreased in cases of oxidative stress[60]. Low levels of GSH are associated with a number of disease conditions known to generate high amounts of reactive oxygen species (ROS), such as observed in atherosclerosis, heart failure, diabetes and neurodegenerative disorders[17,61]. A decrease in the levels of GSH in skin during hyperlipidemia has been observed. Administration of *Melissa officinalis* L. extract increased the content of GSH in skin of hyperlipidemic rats.

Reactive oxygen species (ROS) can initiate lipid peroxidation and DNA damage leading to mutagenesis, carcinogenesis and cell death if the antioxidant system is impaired[62]. Once formed, free radicals attack cell structures within the body, causing damage to cell membrane and enzyme systems[63]. Hyperlipidemia leads to increased production of oxygen free radicals[53], which exert their cytotoxic effect by causing lipid peroxidation, resulting formation of malondialdehyde. Malondialdehyde is an end product of lipid peroxidation[64]. Several researches[65] have also reported a rise in malondialdehyde level in animals on high cholesterol diet. Elevated level of lipid peroxidation products may be responsible for some of the pathological effects in the hyperlipidemia. The present study showed that the formation of thiobarbituric acid reactive substances (TBARS) was significantly increased in skin. These results are in agreement with our previous

study[66]. Administration of *Melissa officinalis* L. extract significantly decreased the level of lipid peroxidation in hyperlipidemic rats.

Non-enzymatic collagen glycation and reaction with lipid peroxidation products bearing an aldehyde group are spontaneous chemical modifications of proteins, caused by reactions between free oxo-groups (sugar aldehydes or ketones and products of lipid metabolism) and free amino groups of peptide chains. These modifications are accumulated under normal physiological conditions (aging) or pathophysiological situations[67]. Non-enzymatic glycosylation (NEG) of skin proteins causes alteration in their structure and function. Levels of NEG were found to increase in the hypertrigly-ceridemic rats in comparison with the control rats[67]. It has been reported that, various antioxidant vitamins and compounds, flavonoids, saponins[68,69] such as vitamin C and E, trace elements like V[69], Zn[70] and Se prevent the increase of tissue NEG levels. We found a reduction of the NEG increase in the tissues of the animals to which *Melissa officinalis* extract was administered, producing a significant decrease inte hyperlipidemic group. The increase in antioxidant enzyme activity in tissue could be due to oxidative stress. CAT activity was elevated in skin. The increased observed in CAT activity could be due to higher production of H_2O_2. In the present study, we found that oral administration of *Melissa officinalis* extract to hyperlipidemic rats could reverse the elevated CAT activity in skin. Oral administration of extract decreased activity of CAT, probably indicates decreased endogenous hydrogen peroxide production. The present results demonstrated an increase in LDH activity in skin tissues, indicating cellular damage. Plant extracts treatment caused reduction in the activity of this enzyme in skin tissues. A hyperlipidemic diet brings about remarkable modifications in antioxidant defense mechanisms. Studies have shown that hypercholesterolemia diminishes the antioxidant defense system and decreases the activities of SOD in skin of hyperlipidemic group were significantly decreased compared with those of control rats[71]. Administration of *Melissa officinalis* extract significantly elevated the activity of SOD skin. These effects could be due to a protective antioxidant effect of the plant constituents present in the extract along with the significant lipid-lowering effect.

GP_x activity increased in skin. The increase could be a compensatory mechanism in response to increased oxidative stress. Administration of *Melissa officinalis* extract significantly decreased skin GP_x activity in the hyperlipidemic group.

Myeloperoxidase plays a fundamental role in oxidant production by neutrophils. Neutrophils are a potential source of oxygen free radicals and are considered to be a major affector cell in the tissue damage that occurs in several diseases[72]. In the present study, the high MPO activity in skin tissue of hyperlipidemic groups was elevated as compared with control

groups. Furthermore, the results also suggest that *Melissa officinalis* extract has a preventive effect through the inhibition of neutrophil infiltration.

Flavonoids are widely recognized as naturally occurring antioxidants that contribute to cardioprotective action[3]. Essential oils[12], rosmarinic acid, triterpenes[73] have a potential antioxidant[14,74] activity. *Melissa officinalis* L. contains large amount of flavonoids, essential oils, triterpenes and rosmarinic acid and these compounds may prevent oxidative damage[75]. *Melissa officinalis* L. has a potent antioxidative activity from flavonoids, phenolic acid, terpenes, essential oil, rosmarinic acid and caffeic acid.

Present results demonstrate that aqueous extract of the *Melissa officinalis* L. has definite antioxidant effect. Several mechanisms eliciting this effect may be present. Some of the constituents present in the extract may decrease the levels of lipid peroxidation products by scavenging free radicals like superoxide anion, hydroxyl and peroxy-free radicals. In addition, its hypolipidemic and hypocholesterolaemic effects as shown by us earlier and in the present study could also have led to decreased lipid peroxidation, by decreasing the availability of lipid substrates. Increased activity of CAT observed in this study may be due to decreased levels of lipid peroxidation products and increased availability of antioxidants from aqueous extract of *Melissa officinalis* L.

In conclusion, the findings of the current study illustrate that *Melissa officinalis* L. with its potent free radical scavenging and antioxidant properties, seems to be a highly promising agent in protecting skin tissue against oxidative damage.

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