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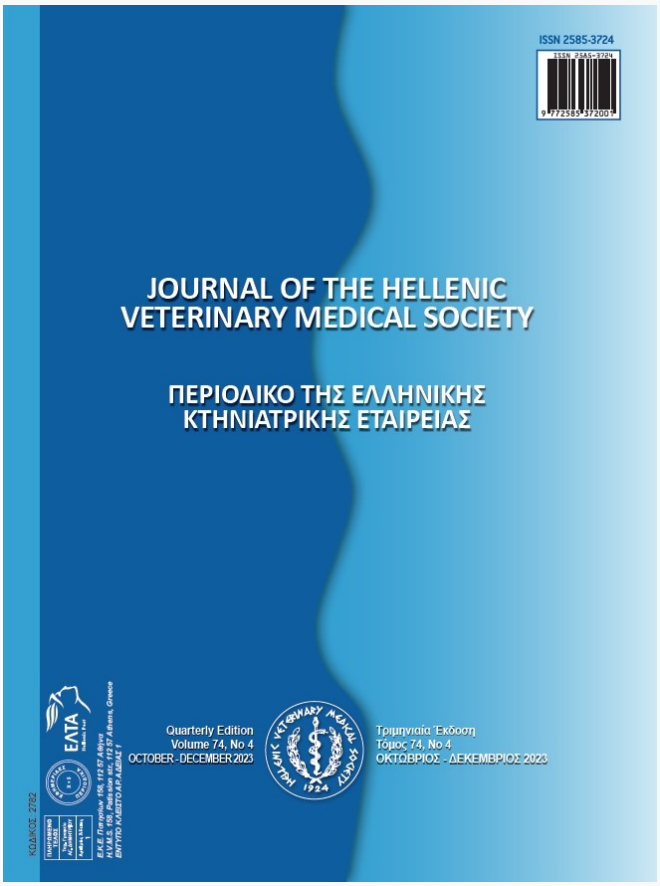

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Research article
Ερευνητικό άρθρο

The effect of *Melissa officinalis* on diet-induced hyperlipidemia, hypercholesterolemia, oxidative stress and inflammation

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ABSTRACT: The Lamiaceae family is an essential medicinal plant family. *Melissa officinalis* L. is a well-known medicinal plant of Lamiaceae. The main objective of the study was to investigate the effects of *Melissa officinalis* infusion given to the drinking water of the rats with hyperlipidemia and hypercholesterolemia in this study. It is seen that the high-fat diet (HFD) group causes weight gain from the third week. In case of VLDL, LDL, HDL and cholesterol levels were found statistically significant differences ($p<0.05$), fed a cholesterol-enriched high-fat diet group (HFD) compared to the control. It is seen that the cholesterol level of the high-fat diet+*Melissa officinalis* infusion (HFD+MOI) group was statistically significantly ($p<0.05$). lower than the HFD group. In addition, the group in which *Melissa officinalis* was applied had positive effects on the antioxidant system and reduced the total oxidant system. The fact that the IFN-γ level of the HFD group was higher than the control indicates that the diet may have an inflammatory effect. As a result, HFD application with cholesterol caused hyperlipidemia and hypercholesterolemia in rats. The cholesterol-lowering effect of *Melissa officinalis* is observed. Remarkably, *Melissa officinalis* did not alter serum calcium levels while lowering cholesterol. In addition, it can said that HFD has a strengthening effect on the antioxidant system on oxidative stress.

***Keywords*:** *Melissa officinalis* L.; hyperlipidemia; hypercholesterolemia; high-fat diet; antioxidant

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INTRODUCTION

Traditional medicine uses plants for both their healing and preventative properties. Herbs are known as functional foods and/or nutraceuticals when used for preventive purposes to maintain general health. The Lamiaceae family is an important medicinal plant family. Species of this family are easily recognized by their square stems and opposite leaves. species belonging to the family are aromatic and have essential oils (Nieto 2017). Aromatic essential oils used in the cosmetics, flavoring, fragrance, perfumery, pesticide and pharmaceutical industries are primarily found in leaves as well as in all above-ground parts of plants (Carović-Stanko et al. 2016)

Melissa officinalis L. is a perennial herb that lives at least three years. It is about 1 m tall, with soft, hairy leaves 2 to 8 cm long and heart-shaped. The leaf surface is rough and deeply veined. The leaf margin is a comb or toothed. It produces white or pale pink flowers in small clusters of 4-12 in summer. It is often referred to as Lemon Balm due to its lemon-like aroma and scent (Bağdat and Coşge 2006). It grows up to 1800 m from sea level in forest clearings, bushes, maquis, stream edges, vacant lands, and roadsides. *Melissa officinalis* has a characteristic odor due to the essential oil it contains. The essential oil rate varies between 0.01-0.25%. In the chemical composition of the essential oil, there are citronellal (2-40%) and citral (10-30%) as the main components. In addition, β-caryophyllene, germacrene D, o-cimene and citronellol are essential oils with high concentrations (Kızıl 2009; Shakeria et al. 2016).

M. officinalis is one of the species used as a medicinal plant. Although there have been developments regarding its culture in recent years, mainly drug material is collected from natural areas. *Melissa* genus has three subspecies (ssp. *officinalis*, ssp. *altissima*, ssp. *inodora*) and only ssp. *officinalis* is used for medicinal purposes (Gürbüz et al. 2008). There are different applications, such as the use of infusion prepared with the above-ground partsafter adding lemon into the infusion, its use by mixing the above-ground parts with honey after drying and powdering, or the use of this plant leaves with *Thymus longicaulis* (Başkal et al. 2017).

Many medicinal plants have conducted many bioactivity studies with infusions (or water extracts). These studies have shown that the phenolic content of different plants has antioxidant activity and the capacity to modulate xenobiotic metabolizing enzymes involved in drug detoxification (Ferguson, 2001). This study investigated the effect of *Melissa officinalis* L. infusion on hypercholesterolemia and hyperlipidemia induced by a cholesterol-containing high-fat diet (HFD).

MATERIALS AND METHODS

Infusion of Plant Material

Identification of the *Melissa officinalis* L. was made by Prof. Dr. Mustafa Kargioglu. The above-ground part of the plant, consisting of branches, leaves, flowers and stems, was dried, and ground in a sun-free environment. The infusion process was the method described byAhmed et al. (2010). Powdered plant material (20 g) was added to boiling water (1000 mL) and infused for 15 minutes. The infusion was filtered, and the filtrate was used fresh. The *Melissa officinalis* L. infusion (MOI) was given to the rats in the treatment group in drinking water (1:1) for three weeks.

Experimental Animals, Preparation of High Fat Diet and Experimental Application

A total of 24 male Wistar-Albino rats were used in this study. Rats were obtained from Afyon Kocatepe University Experimental Animals Research and Application Center. Animals were housed at the center, maintaining 55-60% humidity and a 12:12 hour light-dark cycle. All interventions to animals took place in this center. The study was carried out by permissions obtained from AfyonKocatepe University Experimental Animals Local Ethics Committee (Approval Date/No: 12.12.2013/424).

The working groups were designed as follows:

Group 1: Control group: The rats in this group were fed with standard rat chow and drinking water.

Group 2: HFD group: The rats in this group were fed with HFD throughout the study.

Group 3: HFD-MOI group: The rats in this group were fed with HFD for two weeks. *Melissa officinalis* L. infusion was added to the drinking water of the rats in a 1:1 ratio for the next three weeks.

The HFD application was designed to promote weight gain and hyperlipidemia. The high-fat diet was prepared by standard rat feed taken from the factory and adding 5% fatty soybean and 5% chicken meal as protein source, 48% tallow fat as an energy source and 2% cholesterol to provide hypercholesterolemia.

The feed was pelleted and stored in a deep freeze. The metabolic energy value of the prepared HFD was measured as 4930 cal/kg in AfyonFood Control Laboratories (Afyonkarahisar, Türkiye). HFD was prepared fresh every week and given to the animals 1 hour after removal from the deep freezer (Hazman et al. 2016). At the end of the 5-week study, rats were administered ketamine (65 mg/kg) and xylazine (7 mg/kg). Blood samples were taken and prepared for biochemical analysis.

Biochemical Analysis

Lipid profile parameters; high density lipoprotein (HDL), low density lipoprotein (LDL), very low-density lipoprotein (VLDL), triglyceride (TG), total cholesterol, liver function tests; aspartate aminotransferase (AST),alanine aminotransferase (ALT) andlactate dehydrogenase (LDH) enzymesand serum calcium (Ca) levels were determined with an autoanalyzer in Afyonkarahisar Health Sciences University, Medicine Faculty, Biochemistry laboratory. Inflammatory parameters; tumor necrosis factor-alpha (TNF-α), interleukin 1-Beta (IL-1β), interleukin-6 (IL-6) and interferon-gamma (IFN-y) levels with e-Bioscience (Vienna, Austria) commercial kits determined. Leptin and insulin levels were measured with kits (BioVendor, Bratislava, Slovakia and DRG-Diagnostic, Marburg, Germany) using an ELISA device (ELx800, BioTek Inc., Winooski, VT, USA). Total antioxidant status (TAS) and total oxidant status (TOS) were measured using kits(Rel Assay, Gaziantep, Turkey).Oxidative stress index (OSI) was calculated by dividing the TOS values of the samples by the TAS values in percent.

Statistical Analysis

Statistical calculations of the findings were made using the SPSS 18.0 software package. Obtained data are given as "mean ± standard deviation" (Mean ± SD). A normality test was applied to the groups. Statistical analysis was performed by using analysis of variance (ANOVA) and Duncan's post-test among parametric tests to the data determined to be normally distributed. Kruskal-Wallis and Mann-Whitney U from non-parametric tests were applied to the data that did not show normal distribution to determine statistical differences.

RESULTS AND DISCUSSION

Obesity is an independent risk factor for cardiovascular diseases. In addition, it causes other risk factors such as hypertension, hyperlipidemia and diabetes. In recent years, obesity and related metabolic complications have reached epidemic proportions. Many experimental animal models have been developed to understand these pathologies better and evaluate potential treatments(Marques et al. 2016). Despite the multifactorial etiology of obesity, the increase in its incidence indicates that environmental and behavioral factors (such as dietary factors) are more effective than genetic changes in the obesity epidemic (Malik et al. 2013). Therefore, animal models of polygenetic diet-induced obesity have been used preferentially. It has been determined that a semi-purified diet containing animal fats mimics the pathophysiology of human obesity and metabolic syndrome by causing obesity, hyperglycemia, hypertriglyceridemia and hyperleptinemia in rats (Marques et al. 2016; Nilsson et al. 2012; Malik et al. 2013).

Hypercholesterolemia is one of the main risk factors fordeveloping of cardiovascular diseases such as atherosclerosis and its complications, acute myocardial infarction or hypertension. There is a close relationship between these diseases and lipid abnormalities, exceptionally high plasma cholesterol level, and blood pressure(Matos et al. 2005). A high-fat diet is directly related to hyperlipidemia in humans (Matos et al. 2005). An attempt was made to provoke hyperlipidemia in laboratory animals to better understand the relationship between disturbances in cholesterol metabolism and atherogenesis and to test possible treatments for lowering circulating cholesterol levels. High-fat diets containing cholesterol have been used to induce hypercholesterolemia in rats. This study aimed to create dietary models that cause hypercholesterolemia in rats using a fatty diet containing cholesterol. Rat weights were measured weekly throughout the study and are given in Table 1. There was no statistically significant weight gain in control group rats throughout the study. After three weeks, rat weights in the HFD group were statistically significantly different from the initial weights ($p<0.05$). This shows that the fatty diet with added cholesterol causes weight gain in HFD group rats. The rat weights in the HFD-MOI group at the 4th and 5th weeks were statistically different from the initial weights ($p<0.05$). In this group, it was observed that the rat weights did not change at the end of the 2nd week when the MOI application was started, but in the 4th and 5th weeks when the application continued, the rat weights were higher than the control.

Table 1. Changes in rat weights (g) during the study

	Control	HFD	HFD-MOI
Beginning	229.29±23.08	191.33±18.99[a]	194.67±19.97[a]
First week	240.00±22.54	215.22±17.63	208.89±16.18
Second week	240.29±21.45	222.89±20.51	218.67±14.28
Third week	239.86±20.88	229.44±22.72[b]	218.89±13.63
Fourth week	244.29±19.39	240.33±25.59[b]	230.89±17.35[b]
Fifth week	241.57±12.53	234.44±27.25[b]	227.00±18.22[b]

Values are mean±standard deviation; n=8. a, b: Different letters in the same column represent statistically significant differences ($p<0.05$). HFD; High-Fat Diet, MOI; *Melissa officinalis* Infusion

When energy consumption exceeds the energy expended, excessive fat accumulation occurs in adipose tissue and other tissues, including the liver. Accumulated fat can disrupt the normal cellular and physiological function of tissues. The liver plays a critical role in lipid metabolism by uptake of serum-free fatty acids (FFA), which are involved in synthesizing, storing and transporting lipid metabolites (Das and Choudhuri, 2020). Fatty liver (also known as hepatic steatosis) is widely accepted to occur when lipid accumulation exceeds the rate of lipid excretion. This formation can be caused by many reasons such as alcoholism, chemotherapy, non-alcoholic fatty liver disease, abnormal metabolism, and toxic damage, as well as infections. Disruption of bile acid synthesis with irregular cholesterol flow results in cholesterol accumulation in the liver, and hepatic lipid metabolism.As a result, the process of steatosis results in lipid accumulation in the liver (Zhang et al. 2021)

LDL cholesterol is the primary target of lipid-lowering therapy in cardiovascular prevention (Bonovas et al. 2011). Statins prevent the production of cholesterol in the liver cell by causing hydroxymethylglutaryl coenzyme A reductase enzyme inhibition and increase the clearance of apoB-containing lipoproteins by inducing the expression of apoB/E (LDL receptor) receptors on the cell surface, thus reducing the level of circulating LDL cholesterol. Statins, the most potent LDL-lowering drugs among existing lipid-lowering drugs, reduce the LDL cholesterol level by 25-45% at standard doses. Apart from their LDL cholesterol-lowering effects, they also cause a 5-15% increase in HDL cholesterol and a 7-30% decrease in triglycerides (Jones et al. 1998)

Statins carry a low risk of hepatotoxicity and myopathy (Bhardwaj and Chalasani2007). For these rare side effects, follow-up is usually necessary following the initiation of therapy for liver transaminases and myocyte reaction. Hepatic reaction (elevated transaminases) is generally observed at the beginning of treatment. If transaminases increase three times the upper limit of normal, the drug should be discontinued (Ertaş 2009). Many pathophysiological mechanisms have been proposed to explain statin myopathy. Decrease in non-cholesterol products of the mevalonate pathway, decrease in sarcoplasmic cholesterol, increase in cellular fat, decrease in GTPase production, decrease in creatinine in the cell, disorders in calcium balance, immune disorders and mitochondrial dysfunctions are some of these. In particular, a reduction in mitochondrial energy production and a reduction in ubiquinone (Q10) production have been blamed in this regard (Rosenson et al. 2017)

Table 2 shows the biochemical parameters change in rat serum throughout the study. It is seen that HFD application does not effect liver enzymes (AST, ALT, and LDH). It was seen that the VLDL and HDL levels of the HFD and HFD+MOI groups were significantly lower than the control ($p<0.05$), while the LDL levels were statistically significantly higher ($p<0.05$). The unique situation in Table 2 is that the cholesterol level of the HFD-MOI group was statistically significantly lower than the HFD group ($p<0.05$). *Melissa officinalis* application significantly reduced the cholesterol level. There is also imperative to no difference between the calcium concentrations of all three groups. It can be said that MOI application reduces cholesterol without affecting calcium levels. There was no statistically significant difference in TG, insulin, and leptin levels in the HFD and HFD-MOI groups compared to the control throughout the study.

Inflammation is a comprehensive physiological response to a foreign organism. Depending on the processes and cellular mechanisms, it can be seen as acute and chronic inflammation. It is known that inflammation is an essential factor in the progression of various chronic diseases/disorders, including diabetes, cancer, cardiovascular diseases, eye disorders,

Table 2. Biochemical characteristics of high-fat diet and high-fat diet+*Melissa officinalis* infusion application

	Control	HFD	HFD-MOI	p value
AST (U/L)	193.50±67.80[a]	201.00±46.00[a]	149.11±47.56[b]	0.134
ALT (U/L)	45.50±9.44[a]	64.29±25.10[a]	65.89±39.13[a]	0.393
LDH (U/L)	814.00±160.58[b]	683.71±11.00[a, b]	486.67±187.34[a]	0.015
TG (mg/dL)	51.83±18.26[a]	45.29±16.32[a]	38.11±3.37[a]	0.206
VLDL (mg/dL)	10.67±3.67[a]	9.00±3.21[b]	7.56±1.67[b]	0.139
LDL (mg/dL)	8.67±0.66[a]	196.29±19.18[b]	145.11±61.43[b]	0.001
HDL (mg/dL)	46.50±7.50[b]	30.00±9.68[a]	33.89±7.15[a]	0.05
Cholesterol (mg/dL)	65.83±17.15[a]	235.29±19.85[b]	186.56±57.65[c]	0.002
Calcium (mg/dL)	10.28±0.29[a]	10.41±0.33[a]	10.31±0.40[a]	0.776
Insulin (ng/mL)	0.25±0.07[a]	0.39±0.28[a]	0.31±0.1[a]	0.371
Leptin (ng/mL)	126.45±3.36[a, b]	132.93±11.27[b]	124.17±1.27[a]	0.069

Values are mean ± standard deviation; n=8. a, b, c: Different letters in the same line represent statistically significant differences ($p<0.05$). AST: aspartateaminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, HFD; High-Fat Diet, MOI; *Melissa officinalis* Infusion

arthritis, obesity, autoimmune diseases, and inflammatory bowel disease(Arulselvan et al. 2016). The production of free radicals from different biological and environmental sources is also due to the imbalance of natural antioxidants that cause various inflammatory-related diseases (Arulselvan et al. 2016). A free radical is a molecule or atom that carries one or more unpaired electrons and can exist independently. Meanwhile, free radicals have an odd number of electrons; this makes them short-lived, highly reactive and unstable. As a result, it can react quickly with other substances trying to capture the necessary electron to achieve stability. The free radical can be stabilized by attacking the nearest stable molecule and "stealing" its electron. The attacked molecule can lose its electron and become a free radical, initiating a chain reaction cascade that damages the living cell. Reactive oxygen species (ROS) are radical derivatives such as singlet oxygen and hydrogen peroxide. In the inflammatory response, leukocytes and mast cells are present at the damage sites, leading to a "respiratory burst" due to increased oxygen uptake, thereby increasing the production and release of ROS at the damaged site. However, inflammatory cells produce more soluble inflammatory mediators such as cytokines, arachidonic acid and chemokines, which act through active inflammatory cells at the site of infection and release more reactive species. Initiation of cyclooxygenase-2 (COX-2), inducibility of nitric oxide synthase (iNOS), high expression of inflammatory cytokines including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, and chemokines (CXC, chemokine receptor 4), changes in the expression of specific microRNAs has been shown to be involved in oxidative stress-induced inflammation. This inflammatory/oxidative environment triggers an unhealthy cycle that can damage healthy stromal cells and adjacent epithelial cells (Coussens and Werb 2002; Federico et al. 2007; Poyton et al. 2009; Reuter et al. 2010)

Numerous studies have shown that plants' flavonoid and phenolic content contribute to their antioxidant activities. Natural antioxidants are abundant in many plant sources, have no side effects, and are less expensive. Natural compound-based antioxidants play a protective role against the formation of free radicals. Therefore natural-based antioxidants are the most valuable therapeutic agents for reducing diseases

triggered by oxidative stress. Flavonoids and phenolic compounds play an active role as anti-inflammatory factors and their antioxidant activities. Anti-inflammatory activities of natural compounds have been reported in many studies and are seen in many preclinical studies (Ravipati et al. 2012). Some studies examining the in vivo and in vitro antioxidant properties of *Melissa officinalis* . Hohmann et al. (1999) stated that the extract prepared from the aerial parts of *Melissa officinalis* with 50% methanol showed antioxidant activity and that there were rosmarinic acid, caffeic acid, luteolol and luteolol 7-O-glucoside in this extract, which showed antioxidant activity. Mulkens and Kapetanidis (1987) isolated 7 glucosides, the aglycone of which are nerole, geraniol, neric acid, geranic acid, eugenol, benzyl alcohol, and β-phenylethyl alcohol, from the leaves of *Melissa officinalis* . In a study evaluating the fresh and dry leaves of *Melissa officinalis*, *Mentha piperita* and *Origanum vulgare* in terms of antioxidant activity, the free radical scavenging effect of all three plants was high. However, it was determined that the linoleic acid peroxidation inhibitory effect of dried *Melissa officinalis* leaves decreased significantly compared to fresh leaves (Capecka et al. 2004). Tagashira and Ohtake (1998) in their study to determine the components with antioxidant properties in *Melissa officinalis* leaves, criticized that the antioxidant property of *Melissa officinalis* is only related to the rosmarinic acid content. They stated that they isolated six main components with antioxidant properties, such as 2-(3',4'-dihydroxyphenyl)-1,3-benzodioxol-5-aldehyde in the structure of 1,3-benzodioxol isolated for the first time, showing the most potentantioxidant effect. Antioxidative/oxidative stress and inflammation marker levels are given in Table 3. It is seen that the total antioxidant level of the HFD-MOI group was statistically significantly higher ($p<0.05$) than the HFD group, and *Melissa officinalis* supported the antioxidant status. In addition, it is seen that the TOS levels of this group were statistically significantly lower ($p<0.05$) than the control and HFD group and suppressed the oxidant status. Therefore, the Oxidative stress index (OSI) of the HFD-MOI group is statistically significantly lower than the other groups ($p<0.05$). Other studies with *Melissa officinalis* have determined that apart from rosmarinic acid, it contains caffeic acid, cafteric acid, hydroxyjasmonic acid glucoside, caftaric acid glucoside and sagerinic acid (Aicha et al. 2020; Barros et al. 2013; Ozarowski et al. 2016). Rosmarinic acid and caffeic acid are phytochemicals that stand out with their antioxidative properties. The antioxidant property of the plant may be due to these structures. When the inflammation levels were examined, no difference was observed between TNF-α, IL-1β and IL-6 levels of all groups. TNF-α is a potent proinflammatory cytokine secreted from myeloid cells by activation of MAPK and NFkB signaling pathways. It activates the release of TNF-α, IL-1β and IL-6 (Locksley et al. 2001). Since there was no difference in TNF-α levels between the groups, no difference could be observed in IL-1β and IL-6 levels. Interferon-γ (IFN-γ) levels of the HFD and HFD-MOI groups were higher than incontrol. IFN-γ is a cytokine secreted by natural killer (NK) and natural killer T cells and involved in innate and adaptive immune responses. This type of interferon

Table 3. Antioxidant/Oxidant and Cytokine Levels

	Control	HFD	HFD-MOI	p
TAS (μmol Trolox Equiv./L)	0.79±0.13$^{a, b}$	0.66±0.32^{a}	1.03±0.35^{b}	0.071
TOS (μmol H_2O_2 Equiv./L)	10.96±3.92^{b}	11.09±3.97^{b}	5.40±0.77^{a}	0.006
OSI (Arbitrary Unit)	1809.96±471.16^{a}	2052.40±108.95^{a}	593.70±250.38^{b}	0.003
TNF-α (pg/mL)	45.25±1.51^{a}	45.98±1.83^{a}	44.12±5.59^{a}	0.614
IL-1β (pg/mL)	50.82±9.73^{a}	69.37±30.71^{a}	52.37±6.31^{a}	0.266
IFN-γ (pg/mL)	130.80±17.44^{a}	182.29±39.44^{b}	180.07±58.43^{b}	0.057
IL-6 (pg/mL)	32.35±1.95^{a}	35.01±1.20^{a}	35.01±3.25^{a}	0.068

Values are mean ± standard deviation; n=8. a, b, c: Different letters in the same line represent statistically significant differences ($P<0.05$). HFD; High-Fat Diet, MOI; *Melissa officinalis* Infusion, TAS: Total antioxidant status, TOS: Total Oxidant Status, OSI; Oxidative StressIndex; TNF-α: Tumor Necrosis Factor-α, IL-1β; Interleukin-1β, IFN-γ:Interferon-γ, IL-6; Interleukin 6

suppresses the differentiation of adipocytes and decreases insulin sensitivity by mediating the activation of the JAK-STAT1 pathway. IFN-γ plays a role in regulating of the inflammatory response in obesity (Rocha et al., 2008). Researchers stated that IFN-γ plays a vital role in regulatingglucose metabolism and weight gain. Obese IFN-γ-knockout mice exhibited milder nsülin resistance, reduced adipocyte size, and M2-related cytokine expression in adipose tissue compared to healthy mice. It has been stated that IFN-γ is associated with glucose homeostasis, adipogenesis and cytokine expression in adipose tissue (O'Rourke et al. 2012). In addition, the percentage of CD4+ T cells that secrete IFN-γ was higher in obese children than in normal-weight healthy children (Pacifico et al. 2006). In Table 3, it can be said that the IFN-γ levels of the HFD group were statistically significantly higher than the control, and the high-fat diet caused inflammation. However, the IFN-γ levels of the HFD-MOI group did not differ from the HFD group.

CONCLUSION

As a result, it is seen that the application of a high-fat diet together with cholesterol causes weight gain in rats from the third week. It is seen that the levels of VLDL, LDL, HDL and cholesterol in rats treated with HFD are different from the control and induce hyperlipidemia and hypercholesterolemia. It was found that the cholesterol level of the HFD-MOI group was different from the HFD group and showed a cholesterol-lowering effect. Besides all these effects, serum Ca levels were the same in all groups. It is seen that HFD application suppresses the antioxidant system, while *Melissa officinalis* supports the antioxidant system. The high IFN-γ level in the HFD-treated groups indicates that the applied diet has an inflammatory effect. It exhibits the biological properties of bioactive extracts and their natural compounds by blocking two major signaling pathways, NF-κB and mitogen-activated protein kinases (MAPKs), which have a significant role in the production of various proinflammatory mediators. Further studies can reveal this mechanism.

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CONFLICT OF INTEREST

None declared.

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