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RESVERATROL VE POTASYUM BROMAT SIÇANLARIN DALAK DOKUSUNDA KOLESTEROL, VİTAMİN E VE YAĞ ASİTLERİ SEVİYELERİNİ NASIL ETKİLER

ÖZET

Bu araştırmanın amacı resveratrol ve potasyum bromatın, yaşlı dişi Wistar sıçanların dalak dokusunda kolesterol, lipofilik vitaminler ve bazı yağ asidi seviyeleri üzerinde etkilerini incelemektir. Bu çalışmada Wistar sıçanlar rasgele üç gruba ayrıldı: 1. Kontrol (C), 2. $KBrO_3$ (K) 3. Resveratrol+$KBrO_3$ (R). Dalak dokusunda, yağ asitleri analizi gaz kromatografisi, kolesterol ve lipofilik vitaminlerin analizi ise HPLC cihazıyla gerçekleştirildi. Bu çalışmada, C grubuyla karşılaştırıldığında α-tokoferol seviyesi R grubunda azalmıştır ($p<0.01$). Kolesterol seviyesi C grubuyla karşılaştırıldığı zaman K grubunda artarken($p<0.05$), R grubunda azalmıştır ($p<0.001$). Stearik asit ve araşidonik asit seviyeleri C grubuyla karşılaştırıldığında K grubunda artmıştır ($p<0.05$, $p<0.005$, sırasıyla). Sonuç olarak, sıçanların dalak dokusunda α-tokoferol ve kolesterol arasında moleküler bir ilişkinin olduğu gözlenmiştir. Resveratrol ve potasyum bromat, yaşlı dişi Wistar sıçanlarda kolesterol biyosentezini ve yağ asidi metabolizmasını etkilemiş olabilir.

Anahtar Kelimeler: Resveratrol, Potasyum Bromat, Dalak, Kolesterol, A-Tokoferol

HOW RESVERATROL AND POTASSIUM BROMATE AFFECT TO CHOLESTEROL, VITAMIN E AND FATTY ACID LEVELS IN SPLEEN OF RATS

ABSTRACT

The aim of this research is to examine the effects of resveratrol and potassium bromate on the level of cholesterol, lipophylic vitamins and some fatty acids in spleen of old female Wistar rats. In this study Wistar rats were randomly divided into three groups: 1. Control (C), 2. $KBrO_3$ (K) 3. Resveratrol+$KBrO_3$ (R). In spleen, fatty acid analyses were measured by gas chromatography, cholesterol and lipophylic vitamins analyses were performed by HPLC. In this study, the α-tocopherol level was decreased in R group ($p<0.01$) when compared to C. The cholesterol level was increased in K group ($p<0.05$), but its level was decreased in R group ($p<0.001$) when compared to C. The stearic acid and arachidonic acid levels were increased in K group ($p<0.05$, $p<0.005$, respectively) when compared to C. In conclusion, it was observed that between α-tocopherol and cholesterol a molecular relationship in spleen of rats. Resveratrol and potassium bromate may be affected cholesterol biosynthesis and fatty acid metabolism in old female wistar albino rats.

Keywords: Resveratrol, Potassium Bromate, Spleen, Cholesterol, A-Tocopherol

1. INTRODUCTION (GİRİŞ)

Resveratrol is a natural phytoalexin that is present in grapes and many other plants. It was found recently that this compound possesses a variety of biological activities [1,2]. Resveratrol has shown to poss cancer chemo-preventive activity [3,4]. Therefore, the past several years have witnessed intense research devoted to the biological activity, especially the antioxidant activity, of this compound [4-11], since free radical-induced peroxidation of membrane lipids and oxidative damage of DNA have been considered to be associated with a wide variety of chronic health problems, such as cancer, atherosclerosis and aging [12,13], and gene transcription can be regulated by oxidants, antioxidants and other determinants of the intracellular redox state. The antioxidant activity of resveratrol is related to its hydroxyl (OH) groups, which can scavenge free radicals produced *in vivo* [14].

Potassium bromate ($KBrO_3$) had been widely used as a maturing agent for flour and as a dough conditioner. It was, however, demonstrated to induce renal cell tumors in male and female F344 rats after oral administration for 2 years in the drinking water [15] and usage of $KBrO_3$ as a food additive is now limited, so that exposure of humans via food is very low. Nevertheless, there is the still concern regarding this chemical in the environment. $KBrO_3$ has been classified as a genotoxic carcinogen based on positive results in the Ames test [16], and chromosome aberration and micronucleus tests [17]. Moreover, Umemura *et al.* reported the *in vivo* mutagenic effects of $KBrO_3$ in the kidneys of *gpt* delta rats [18].

2. RESEARCH SIGNIFICANCE (ÇALIŞMANIN ÖNEMİ)

The aim of this research is to examine the effects of resveratrol and potassium bromate on the level of cholesterol, some fatty acids and lipophylic vitamins in spleen of old female wistar rats. Because, resveratrol is a powerful antioxidant [12] and potassium bromate is a chemical carcinogen [18]. Present study may be confirmed that there is a molecular relationship between the decreasing of the levels cholesterol and α-tocopherol. In addition, it was observed that the levels of some fatty acids affected by the administration of potassium bromate and resveratrol in spleen of Wistar rats.

3. EXPERIMENTAL METHOD-PROCESS (DENEYSEL ÇALIŞMA)

3.1. Chemicals (Kimyasallar)

Resveratrol, n-hexane, sulphuric acid, methanol and acetonitrile were obtained from Sigma Chemical Co. (USA). Isopropyl alcohol was obtained from Fluka BioChemica (Switzerland). Potassium bromate, KCl and NaCl were obtained Merck (Germany).

3.2. Animals (Hayvanlar)

The following experiments were approved by the Ethical Committee of Firat University for the care and use of laboratory animals. Total 30 old female Wistar rats were used in this study. They were housed in cages where they had *ad libitum* rat chow and water in an air-conditioned room with a 12-h light/12-h dark cycle, and were randomly divided into three groups. The first group was used as a control (C), the second group potassium bromate ($KBrO_3$) (K) group, and third group Resveratrol+$KBrO_3$ (R). Rats in the K and R groups were injected intraperitoneally a single dose potassium bromate 0.48 mM/kg in the physiologic saline buffer [19]. After administration of $KBrO_3$ two days, the rats in the R group were injected resveratrol 0.15 mM/kg four times per week. In addition, physiological saline was injected to C group rats. These

treatments were continued for five weeks, after which time each experimental rat was decapitated and tissue samples were collected and stored in -85 °C prior to biochemical analysis.

3.3. Determination of Lipid Soluble Vitamins (Yağda Çözünebilen Vitaminlerin Analizi)

200 mg spleen samples were homogenized in 1.5 ml acetonitrile/methanol/isopropyl alcohol (2:1:1 v/v/v)-containing tubes and the samples were vortexed for 30 s and centrifuged at 6000×g for 4 min at 4 °C. Supernatants were transferred to autosampler vials of the HPLC instrument. (Shimadzu, Kyoto Japan). For lipophylic vitamins, the mixture of acetonitrile/methanol (75/25 %) was used as the mobile phase and the elution was performed at a flow-rate of 1 ml/min. The temperature of analytical column was kept at 40 °C. Supelcosil™ LC 18 DB column (250 × 4.6 mm, 5 µm; Sigma, USA) was used as the HPLC column and detection were performed at 215 nm for δ-tocopherol and α-tocopherol. Identification of the individual vitamins was performed by frequent comparison with authentic external standard mixtures analyzed under the same conditions. Quantification was carried out by external standardization using *Class VP software*. The results of analysis were expressed as µg/g wet cell pellet [20].

3.4. Cholesterol Analysis (Kolesterol Analizi)

200 mg spleen samples were extracted in 1.5 ml acetonitrile/isopropyl alcohol (70:30, v/v)-containing tubes and the mixture were vortexed for 30 s and centrifuged at 6000×g for 4 min at 4 °C. Supernatants were transferred to autosampler vials of the HPLC instrument. (Shimadzu, Kyoto Japan). Acetonitrile-isopropyl alcohol (70:30 v/v) was used as a mobile phase at a flow rate of 1 ml/min [21]. Supelcosil LC 18™ DB column (250 × 4.6 mm, 5 µm; Sigma, USA) was used as the HPLC column. Detection was performed by UV at 202 nm and 40 °C column oven [22]. Quantification was carried out by external standardization using *Class VP software*. The results were expressed as µmol/g wet cell.

3.5. Lipid Extraction (Lipid Ekstraksiyonu)

Total lipids were extracted with hexane-isopropyl alcohol (3:2 v/v) by the method of Hara and Radin [23]. The tissue samples were homogenized. 200 mg spleen of the homogenized tissue samples was taken and mixed with 5 ml hexane-isopropyl alcohol (3:2, v/v) in a homogenizator. Non-lipid contaminants in lipid extracts were extracted into 0.88% KCl solution.

3.6. Fatty Acid Analysis (Yağ Asitleri Analizi)

Fatty acids in the lipid extracts were converted into methyl esters including 2% sulfuric acid (v/v) in methanol [24]. The fatty acid methyl esters were extracted three times with n-hexane. Then the methyl esters were separated and quantified by gas chromatography and flame-ionization detection (Shimadzu GC 1.7 Ver.3) coupled to a Glass GC 1.0 software computing recorder. Chromatography was performed with a capillary column (25 m in length) and 0.25 mm in diameter, Permabound 25, Machery-Nagel, Germany using nitrogen as a carrier gas (flow rate 0.8 ml/min). The temperatures of the column, detector and injection valve were 130–220, 240, 280 °C, respectively. Identification of the individual methyl esters was performed by frequent comparison with authentic standard mixtures that were analyzed under the same conditions.

3.7. Statistical Analysis (İstatistiksel Analiz)

The experimental results were reported as mean ± S.E.M. Statistical analysis was performed using SPSS statistical software. Analysis of variance (ANOVA) and an LSD test was used to compare the experimental groups with the controls.

4. FINDINGS AND DISCUSSIONS (BULGULAR VE TARTIŞMALAR)

According to our results, the level of δ-tocopherol was lower than the C group in the R and K groups ($p<0.005$). α-tocopherol level was low in the R group ($p<0.01$) when in compared to the C group. The cholesterol level was high in the K group when in compared to the C group ($p<0.001$), but its level was lower than the C group in the R group ($p<0.001$) (Table 1).

Tablo 1. Dalak dokusunun biyokimyasal parametreleri
(Table 1. The biochemical parameters of spleen)

Biochemical Parameters	Control (C)	$KBrO_3$ (K)	$KBrO_3$+R (R)
δ-tocopherol (µg/g)	10.02 ± 1.61^a	3.75 ± 0.10^c	5.06 ± 1.06^c
α-tocopherol (µg/g)	16.68 ± 1.81^a	13.30 ± 2.47^a	9.81 ± 1.09^c
cholesterol (µmol/g)	2.03 ± 0.18^a	3.75 ± 0.52^d	1.21 ± 0.33^d

a: $p>0.05$ **b:** $p<0.05$ **c:** $p<0.01$ **d:** $p<0.001$

The level of palmitoleic acid (16:1 n-7) in the K group was lower than R and C groups ($p<0.05$). The level of stearic acid (18:0) was high in the R group ($p<0.05$). While the level of oleic acid (18:1 n-9) decreased in the K group ($p<0.05$), its level in the R group increased when compared to C group ($p<0.01$). The level of linoleic acid (18:2 n-6) in the K group was lower than the C group ($p<0.005$), the level of arachidonic acid (20:4) was higher than the C group in the K group ($p<0.001$). The level of total saturated fatty acids in the K group were higher than C group ($p<0.05$). The level of total unsaturated fatty acids in the K group were lower than C group ($p<0.05$). The level of monounsaturated fatty acids (MUFA) decreased in the K group ($p<0.005$), its level increased in the R group when compared to C group ($p<0.05$). The level of polyunsaturated fatty acids (PUFA) were lower than the C group in the R group ($p<0.001$). The levels of n3 and n6 fatty acids were in the K group higher than the R group ($p<0.005$, $p<0.05$, respectively) (Table 2).

Tablo 2. Dalak dokusunun yağ asidi kompozisyonu (%)
(Table 2. The fatty acid composition of spleen)(%)

Fatty Acids	Control (C)	$KBrO_3$ (K)	$KBrO_3$+R (R)
16:0	25.14 ± 0.33^a	25.37 ± 0.51^a	25.21 ± 0.39^a
16:1 n-7	4.34 ± 0.38^a	3.23 ± 0.30^b	5.18 ± 0.25^a
18:0	10.89 ± 0.45^a	12.78 ± 0.62^b	10.61 ± 0.66^a
18:1 n-9	17.18 ± 0.70^a	14.80 ± 0.76^b	19.85 ± 0.45^b
18:2 n-6	16.44 ± 0.50^a	13.90 ± 0.54^b	15.32 ± 0.50^a
20:3 n-6	0.54 ± 0.10^a	0.77 ± 0.11^b	0.58 ± 0.09^a
20:4 n-6	10.87 ± 0.38^a	13.40 ± 0.09^b	10.06 ± 0.42^a
22:4 n-6	0.91 ± 0.08^a	0.37 ± 0.03^b	0.34 ± 0.01^b
Others	10.77 ± 0.38^a	11.48 ± 0.42^a	10.10 ± 0.50^a
∑Saturated	38.02 ± 0.55^a	40.34 ± 0.30^b	37.65 ± 0.79^a
∑Unsaturated	63.71 ± 0.74^a	60.85 ± 1.50^b	64.92 ± 0.25^a
∑MUFA	27.53 ± 0.82^a	22.73 ± 1.53^c	31.63 ± 0.81^d
∑PUFA	36.93 ± 0.58^a	38.11 ± 0.29^b	33.55 ± 0.73^b
∑ n-3	7.53 ± 0.44^a	8.45 ± 0.35^a	6.70 ± 0.35^a
∑ n-6	29.65 ± 0.43^a	28.78 ± 0.63^a	26.53 ± 0.70^a

a: $p>0.05$ **b:** $p<0.05$ **c:** $p<0.01$ **d:** $p<0.001$

Epidemiological studies have been indicated that phytoestrogens inhibit cancer formation and growth, reduce cholesterol levels [25]. Resveratrol is a phytoestrogen and, it exhibits a wide range of biological effects, including antiplatelet, anti-inflammatory, anticancer, antimutagenic and antifungal properties. It is also a potent antioxidant, reactive oxygen species scavenger and metal chelators [26,27].

In the present study, it was found that the levels of α-tocopherol decreased in the R group. However, the cholesterol level significantly increased in the K group, its level same rate decreased in the R group when compared to C group.

In the R group, the reduction of cholesterol levels can be caused by the cholesterol-lowering properties of resveratrol. In addition, we think that between cholesterol reduction and reducing of α-tocopherol a molecular relationship. The hypocholesterolemic action of resveratrol has been attributed *in vivo* [28]. These authors have suggested that dietary resveratrol is hypolipidemic with a tendency for anti-tumor-growth and anti-metastasis effects in hepatomabearing rats. Inhibition of squalene monooxygenase has been shown to be effective in lowering serum cholesterol levels in dogs [29]. Laden and Porter had found that activity of human squalene monooxygenase was inhibited by resveratrol. They reported that the possibility protective effect of resveratrol on the development of cardiovascular disease may be explained in part by the inhibition of endogenous cholesterol biosynthesis. Decreasing of cholesterol level in the R group may be explained by a decline the squalene monooxygenase enzyme activity. Squalene monooxygenase is an enzyme in the endoplasmic reticulum of eukaryotic cells, catalyzes the epoxidation of squalene across a C=C double bond to yield 2, 3-oxidosqualene in the first oxidative step of cholesterol biosynthesis [30].

Regulation of sterol receptors occurs at the level of transcription, suggesting that α-tocopherol acts through specific receptors or tocopherol-responsive transcription factors [31]. α-tocopherol similarly up-regulates the expression of α-TTP, and thus plays a role in its own intracellular processing [32,33]. These findings provide a link between vitamin E and the regulation of cholesterol synthesis that is independent of the antioxidant effects of vitamin E.

Supernatant protein factor (SPF) is a recently cloned member of a family of cytosolic lipid-binding proteins that includes Sec14p, α-tocopherol transfer protein, and cellular retinal-binding protein. SPF stimulates the conversion of squalene to lanosterol in the downstream pathway for cholesterol biosynthesis, and overexpression of cloned SPF in hepatoma cells increases cholesterol synthesis. The recent identification of the supernatant protein factor (SPF) as α-tocopherol-associated protein (TAP) has called into question its long-standing association with the cholesterol biosynthesis. Unexpectedly, the sequence of TAP is identical to that SPF. TAP binds α-tocopherol, but not other isomers of tocopherol, with high affinity; in the presence of α-tocopherol TAP translocates to the nucleus and activates reporter gene transcription [34]. TAP is a recently identified cytosolic protein thought to be involved in the intracellular distribution of α-tocopherol [35].

Shibata *et al.* showed that to address the role of SPF in cholesterol synthesis, lipids were isolated from McARH7777 cells incubated with ^{14}C-acetate. SPF over expression stimulated cholesterol synthesis by more than two-fold in these cells, and also increased the synthesis of squalene and lanosterol. As only an estimated 10% of the cells contained the SPF cDNA in this transient transfection assay, this represents a 20-fold increase in the cholesterol biosynthesis in these cells. Moreover, the increase in squalene

and lanosterol synthesis suggests a generalized up-regulation of the cholesterol biosynthetic pathway with SPF overexpression [36].

Reducing in the level cholesterol of the R group can be caused by the hipocholesterolemic effect of resveratrol is obvious. However, we think that between cholesterol reduction and reducing of α-tocopherol a molecular relationship. α-tocopherol is well known as a radical scavenger that prevents peroxidation of the lipids in cell membranes. The antioxidant activity of α-tocopherol can help to prevent cardiovascular disease, atherosclerosis and cancer [37]. α-tocopherol reduces low-density lipoprotein (LDL) oxidation [38] and prevents endothelial cell injury from oxidized lipids [39]. A few actions of vitamin E have been suggested to be independent of its antioxidant effects. α-tocopherol, but not β-tocopherol or other vitamin E isomers, inhibits protein kinase C and thereby decreases arterial smooth muscle cell proliferation [40].

Resveratrol inhibits platelet aggregation both *in vitro* and *in vivo* and this effect may be one of the mechanisms by which resveratrol prevents atherosclerosis [41]. Zern *et al.* have found that hepatic acyl CoA: cholesteryl acyltransferase activity was 27 % lower in the grape diet-fed group compared with controls [42]. In addition, concentrations of cholesterol in the aorta were 33 % lower in guinea pigs fed the grape diet. The hypocholesterolemic action of resveratrol is attributed, at least in part, to an increased excretion of neutral sterols and bile acids into feces Miura *et al.* [28]. In addition, Yilmaz *et al.* have shown that the application of resveratrol significantly reduced the amount of cholesterol in erythrocytes [20].

In fatty acid composition of spleen tissue, the level of 18:0 was low in the K group when in compared to C group ($p<0.05$). The decreasing of 18:0 in the K group can be explained by the increasing of Stearoyl-CoA desaturase (SCD) enzyme activity. SCD is an endoplasmic reticulum enzyme that catalyzes the biosynthesis of monounsaturated fatty acid from saturated fatty acids, and it is a rate-limiting enzyme in the biosynthesis of monounsaturated fatty acids. SCD converts the 18:0 fatty acids to the 18:1 fatty acids [43,44].

The level of 20:4 decreased in the K group when in compared to C group ($p<0.05$). This event has shown that the administration of potassium bromate may be affected Δ^6 desaturation pathway synthesis. This metabolic event is catalyze by $\Delta^{5,6}$ desaturase enzymes and it investigate in essential fatty acid metabolism. Because, decreasing of the level of 20:4 can be explained by decreasing of the activities of desaturase enzymes. The 20:4 is an important fatty acid that found in cellular membrane phospholipids, and it is the release by arachidonyl-hydrolyzing phospholipase A_2 (PLA_2) enzyme [45-47].

5. CONCLUSION AND RECOMMENDATIONS (SONUÇ VE ÖNERİLER)

We have not found any study about the effect of potassium bromate on fatty acids. In conclusion, present study may be confirmed that there is a molecular relationship between the decreasing of the levels cholesterol and α-tocopherol. In addition, it was observed that the levels of some fatty acids affected by the administration of potassium bromate and resveratrol.

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