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RESEARCH ARTICLE

Effects of Antioxidants Resveratrol, Catechin and Lipoic Acid and Carcinogen $KBrO_3$ on Lipophylic Vitamins and Cholesterol in Lung, Liver and Kidney of Wistar Rats [1]

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Summary

The aim of this research is to examine effects of antioxidants resveratrol, lipoic acid, catechin and carcinogen potassium bromate on the level of lipophylic vitamins and cholesterol in liver, lung and kidney tissue of Wistar albino rats. In this study, total 50 Wistar rats were randomly divided into five groups: 1. Control (C), 2. $KBrO_3$ (K) (40 mg/kg two times per week), 3. Catechin (30 mg/kg four times per week) + $KBrO_3$ (40 mg/kg two times per week) (Cat), 4. Lipoic Acid (30 mg/kg four times per week) + $KBrO_3$ (40 mg/kg two times per week) (LA) and 5. Resveratrol (30 mg/kg four times per week) + $KBrO_3$ (40 mg/kg two times per week) (R). All treatments were continued for 5 weeks, after which time each experimental rat was decapitated and tissues were collected and stored in -85°C prior to biochemical analysis. In lung, liver and kidney, lipophylic vitamins and cholesterol were measured by HPLC. According to our results, while the cholesterol level was decreased in the K, Cat, LA and R groups, the α-tocopherol level was decreased in K and R groups of the lung tissue. α-tocopherol, cholesterol and retinol levels were increased in K, Cat, LA and R groups of the liver tissue. While the δ-tocopherol, vitamin D_2, vitamin D_3 and α-tocopherol levels were decreased in K, Cat, LA and R groups, the cholesterol level was increased in Cat, LA and R groups of kidney tissue. In conclusion, our results indicated that the applications of resveratrol, lipoic acid, catechin and potassium bromate influenced cholesterol and lipophylic vitamins levels and these applications can be affected cholesterol biosynthesis in Wistar albino rats.

Keywords: *Resveratrol, Potassium bromate, Catechin, Lipoic acid, Liver, Lung, Kidney*

Wistar Sıçanların Akciğer, Karaciğer ve Böbrek Dokularında Lipofilik Vitaminler ve Kolesterol Üzerinde Antioksidanlar Resveratrol, Kateşin ve Lipoik Asit ile Kanserojen $KBrO_3$'ın Etkileri

Özet

Bu çalışmanın amacı kanserojen $KBrO_3$'e karşı resveratrol, lipoik asit ve kateşin gibi antioksidanların Wistar ratların karaciğer, akciğer ve böbrek dokularında lipofilik vitaminler ve kolesterol üzerindeki etkilerini incelemektir. Bu çalışmada, toplam 50 Wistar sıçan rasgele beş gruba dağıtıldı: 1. Kontrol (C), 2. $KBrO_3$ (K), (40 mg/kg dozunda haftada iki kez), 3. Kateşin (30 mg/kg dozunda haftada dört kez) + $KBrO_3$ (40 mg/kg dozunda haftada iki kez) (Cat), 4. Lipoik Asit (30 mg/kg dozunda haftada dört kez) + $KBrO_3$ (40 mg/kg dozunda haftada iki kez) (LA) and 5. Resveratrol (30 mg/kg dozunda haftada dört kez) + $KBrO_3$ (40 mg/kg haftada iki kez) (R). Bütün uygulamalar 5 hafta boyunca sürdürüldü ve sonra her bir deney sıçanı dekapite edildi ve dokuları alınarak biyokimyasal analizlere kadar -85°C'de saklandı. Akciğer, karaciğer ve böbrek dokularında lipofilik vitaminler ve kolesterol seviyeleri HPLC cihazıyla belirlendi. Sonuçlarımıza göre, akciğer dokusunda kolesterol seviyesi K, Cat, LA ve R gruplarında, α-tokoferol seviyesi ise K ve R gruplarında azalmıştır. Karaciğer dokusunda, α-tokoferol, retinol ve kolesterol seviyeleri K, Cat, LA ve R gruplarında artmıştır. Böbrek dokusunda, δ-tokoferol, vitamin D_2, vitamin D_3 ve α-tokoferol seviyeleri K, Cat, LA ve R gruplarında azalırken, kolesterol seviyesi Cat, LA ve R gruplarında artmıştır. Sonuç olarak, sonuçlarımız göstermiştir ki, resveratrol, lipoik asit, kateşin ve potasyum bromat uygulaması Wistar sıçanlarda lipofilik vitaminler ve kolesterol seviyelerini etkilemiştir ve bu uygulamalar sıçanlarda kolesterol biyosentezini etkilemiş olabilir.

Anahtar sözcükler: *Resveratrol, Potasyum bromat, Kateşin, Lipoik asit, Karaciğer, Akciğer, Böbrek*

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INTRODUCTION

Resveratrol is a phytoalexin, first isolated from roots of *Veratrum grandiflorum* O. Loes (white hellebore) and then from *Polygonum cuspidatum*, but remained in obscurity for almost 50 years [1,2]. It got into prominence in early nineties in the context of "French paradox"; the phenomena where in certain population of France (and Greece), in spite of regular consumption of high fat diet, gets much less heart diseases [3]. The apparent cardioprotection was attributed to the regular consumption of moderate doses of red wine rich in resveratrol [4]. Initially resveratrol was characterized by its anti-platelet aggregation properties [5] and thereafter other beneficial effects such as vasorelaxation, antioxidant functions, etc., became apparent [6,7]. In experimental animals, resveratrol is rapidly metabolized by the liver and its plasma half-life remains quite low with a concomitant decline in its concentrations in tissues like brain, lung, liver and kidney [8].

α-Lipoic acid is a disulfide compound that functions as a coenzyme in pyruvate dehydrogenase and α-ketoglutarate dehydrogenase mitochondrial reactions, leading to the production of cellular energy (ATP). α-Lipoic acid and its reduced form, dihydrolipoic acid, reduce oxidative stress by scavenging a number of free radicals in both membrane and aqueous domains, by chelating transition metals in biological systems, by preventing membrane lipid peroxidation and protein damage through the redox regeneration of other antioxidants such as vitamins C and E, and by increasing intracellular glutathione [9-12].

Catechin, commonly known as the primary polyphenolic compounds in green tea, has been of great interest for its multiple health-promoting properties, such as antioxidant, anti-obesity, hypolipidemic, and anti-carcinogeic activities [13-18].

$KBrO_3$ has been classified as a genotoxic carcinogen based on positive results in the Ames test [19], and chromosome aberration [20] and micronucleus tests [21]. It has the potential to induce 8-hydroxy-2'-deoxyguanosine (8-OHdG) formation both *in vitro* and *in vivo* [22-25]. 8-OHdG is the most abundant oxidative DNA adduct, can induce mutations such as GC to TA transversions upon replication by DNA polymerases [26].

The aim of this study was to examine effects of antioxidants resveratrol, lipoic acid, catechin and carcinogen potassium bromate on the level of lipophylic vitamins and cholesterol in liver, lung and kidney tissue of Wistar albino rats.

MATERIAL and METHODS

Chemicals

Resveratrol, lipoic acid, catechin, methanol and acetonitrile were obtained from Sigma Chemical Co. (USA). Isopropyl alcohol was obtained from Fluka BioChemica (Switzerland). Potassium bromate was obtained Merck (Germany).

Animals and Treatment

The following experiments were approved by the Ethical Committee of Firat University for the care and use of laboratory animals. In this study, total 50 old female Wistar rats were used. The animals 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 five groups; each group containing ten rats. The first group was used as a Control (C), the second group $KBrO_3$ (K), the third group Catechin + $KBrO_3$ (Cat), the fourth group Lipoic Acid + $KBrO_3$ (LA), and fifth group Resveratrol + $KBrO_3$ (R). Rats in the K, Cat, LA and R groups were injected intraperitoneally potassium bromate 40 mg/kg in the physiologic saline (0.9% NaCl) two times per week. Rats in the Cat group was injected intraperitoneally catechin 30 mg/kg, rats in the LA group was injected intraperitoneally lipoic acid 30 mg/kg, rats in the R group was injected intraperitoneally resveratrol 30 mg/kg in the physiologic saline 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 [27].

Determination of Lipid Soluble Vitamins in Tissue Samples

500 mg lung, liver and kidney tissue sample was homogenized in 3 mL acetonitrile/methanol/isopropyl alcohol (2:1:1, v/v/v) containing tubes and the samples were vortexed for 30 s and centrifuged at 6.000×g for 10 min at 4°C. Supernatants were transferred to autosampler vials of the HPLC instrument. For lipophylic vitamins, the mixture of acetonitrile/methanol (3:1, v/v) was used as the mobile phase and the elution was performed at a flow-rate of 1 mL/min. The temperature of column was kept at 40°C. Supelcosil™ LC 18 DB column (250 x 4.6 mm, 5 μm; Sigma, USA) was used as the HPLC column and detection was performed at 320 nm for retinol (vitamin A), and 215 nm for δ-tocopherol, α-tocopherol, α-tocopherol acetate, vitamin D_2, D_3, K_1. 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 [28,29].

Total Cholesterol Analysis in Tissue Samples

500 mg lung, liver and kidney tissue sample in 3 mL acetonitrile/isopropyl alcohol (70:30, v/v) containing tubes and the mixture were vortexed for 30 s and centrifuged at

6.000 g for 10 min at 4°C. Supernatants were transferred to autosampler vials of the HPLC instrument. Acetonitrile-isopropyl alcohol (70:30 v/v) was used as mobile phase at a flow rate of 1 mL/min [30]. Supelcosil LC 18™ DB column (250 x 4.6 mm, 5 μm) was used as the HPLC column. Detection was performed by UV at 202 nm and 40°C column oven [31]. Quantification was carried out by external standardization using Class VP software. The results were expressed as μg/g wet weight tissue.

Statistical Analysis

The experimental results were reported as mean ± S.E. Statistical analysis was performed using SPSS 15.0 Soft-ware. Analysis of variance (ANOVA) and an LSD test were used to compare the experimental groups with the controls [32-35].

RESULTS

In Lung Tissue

Retinol level was decreased (P<0.01) in Cat group, but its level was not different (P>0.05) between C group and the other groups. δ-tocopherol (K, Cat and R groups), α-tocopherol (all groups) levels were significantly decreased (P<0.001). Vitamin D_2 and α-tocopherol acetate (K, Cat and LA groups) levels were significantly increased (P<0.001). Vitamin D_3 level was increased (P<0.05) in K and R groups, but its level was significantly decreased (P<0.01, P<0.001, respectively) in Cat and LA groups. Vitamin K_1 level was significantly increased (P<0.001) in K group, but its level was significantly decreased in LA and R groups (P<0.001). Cholesterol level was significantly decreased (P<0.05, P<0.001, respectively) in K and R groups, but its level was not different (P>0.05) between C and other groups *(Table 1)*.

In Liver Tissue

Retinol, cholesterol (all groups), δ-tocopherol (K, LA and R groups), α-tocopherol (K, Cat, LA and R groups), α-tocopherol acetate (Cat, LA and R groups) levels were significantly increased (P<0.001). Vitamin D_2 (all groups), Vitamin D_3 (K, Cat and R groups) levels were significantly decreased (P<0.001). Vitamin K_1 level was significantly decreased (P<0.001) in R group, but its level was significantly increased (P<0.001) in K, LA and R groups *(Table 2)*.

In Kidney Tissue

Retinol level was decreased (P<0.05) in K and Cat groups, but its level was significantly increased (P<0.001, P<0.01,

Table 1. *Effects of $KBrO_3$ and antioxidants on lipophylic vitamins and cholesterol levels in lung*
Tablo 1. *Akciğer dokusunda lipofilik vitaminler ve kolesterol seviyeleri üzerinde $KBrO_3$ ve antioksidanların etkileri*

Biochemical Parameters (μg/g)	Control (C)	$KBrO_3$ (K)	Catechin+ $KBrO_3$ (Cat)	Lipoic Acid+ $KBrO_3$ (LA)	Resveratrol+ $KBrO_3$ (R)
Retinol	1.44±0.04^a	1.47±0.10^a	0.94±0.04^c	1.16±0.07^a	1.20±0.13^a
δ-tocopherol	21.25±1.17^a	13.31±0.70^d	4.46±0.09^d	10.47±0.05^d	19.49±0.73^a
Vitamin D_2	1.52±0.12^a	3.61±0.12^d	3.74±0.15^d	3.15±0.15^d	1.94±0.13^a
Vitamin D_3	13.47±0.43^a	15.08±0.64^b	10.84±0.37^c	10.54±0.06^d	15.40±0.47^b
α-tocopherol	18.81±0.60^a	16.69±0.54^c	9.97±0.23^d	6.00±0.40^d	11.53±0.47^d
α-tocopherol acetate	2.12±0.29^a	3.65±0.06^c	21.06±0.42^d	25.63±0.26^d	1.73±0.12^a
Vitamin K_1	3.48±0.27^a	9.75±0.23^d	3.49±0.10^a	1.15±0.08^d	1.02±0.08^d
Cholesterol	975.38±20.83^a	913.36±24.67^b	936.58±7.64^a	976.85±20.69^a	761.00±9.07^d

a, b, c, and d letters indicate statistical significant between groups. ***a:*** *P>0.05,* ***b:*** *P<0.05,* ***c:*** *P<0.01,* ***d:*** *P<0.001*

Table 2. *Effects of $KBrO_3$ and antioxidants on lipophylic vitamins and cholesterol levels in liver*
Tablo 2. *Karaciğer dokusunda lipofilik vitaminler ve kolesterol seviyeleri üzerinde $KBrO_3$ ve antioksidanların etkileri*

Biochemical Parameters (μg/g)	Control (C)	$KBrO_3$ (K)	Catechin+ $KBrO_3$ (Cat)	Lipoic Acid+ $KBrO_3$ (LA)	Resveratrol+ $KBrO_3$ (R)
Retinol	18.74±1.36^a	30.98±0.30^d	26.36±0.85^c	43.86±2.65^d	24.03±0.34^b
δ-tocopherol	15.40±0.21^a	24.33±0.55^d	14.23±0.47^a	45.80±0.54^d	19.45±0.15^d
Vitamin D_2	10.27±0.21^a	7.68±0.29^d	4.91±0.16^d	8.44±0.12^d	6.88±0.23^d
Vitamin D_3	99.28±1.22^a	84.09±2.17^d	78.93±1.47^d	98.52±0.57^a	73.49±0.41^d
α-tocopherol	168.23±8.13^a	567.73±12.50^d	480.48±16.51^d	466.16±0.75^d	498.79±0.56^d
α-tocopherol acetate	20.83±0.83^a	21.76±1.20^a	25.62±0.89^c	35.36±0.38^d	29.56±0.17^d
Vitamin K_1	20.47±1.21^a	120.81±1.64^d	56.86±1.34^d	213.08±0.57^d	3.98±0.25^d
Cholesterol	45.00±1.44^a	300.42±8.16^d	232.54±7.64^d	141.87±0.42^d	284.42±0.72^d

a, b, c, and d letters indicate statistical significant between groups. ***a:*** *P>0.05,* ***b:*** *P<0.05,* ***c:*** *P<0.01,* ***d:*** *P<0.001*

Table 3. *Effects of $KBrO_3$ and antioxidants on lipophylic vitamins and cholesterol levels in kidney*
Tablo 3. *Böbrek dokusunda lipofilik vitaminler ve kolesterol seviyeleri üzerinde $KBrO_3$ ve antioksidanların etkileri*

Biochemical Parameters (µg/g)	Control (C)	$KBrO_3$ (K)	Catechin+ $KBrO_3$ (Cat)	Lipoic Acid+ $KBrO_3$ (LA)	Resveratrol+ $KBrO_3$ (R)
Retinol	20.87±0.05[a]	30.47±0.05[a]	40.90±1.00[d]	106.89±0.91[d]	63.42±0.83[d]
δ-tocopherol	27.10±0.64[a]	34.98±0.89[d]	4.90±0.08[d]	20.56±0.18[d]	3.79±0.13[d]
Vitamin D_2	7.93±0.36[a]	4.03±0.31[d]	2.52±0.12[d]	6.44±0.07[d]	4.36±0.10[d]
Vitamin D_3	102.27±0.81[a]	39.71±0.69[d]	27.47±0.58[d]	88.25±0.57[d]	46.13±0.53[d]
α-tocopherol	39.91±1.13[a]	15.23±0.38[d]	15.20±0.08[d]	12.26±0.84[d]	19.62±0.10[d]
Vitamin K_1	29.46±2.04[a]	13.75±0.39[d]	9.80±0.11[d]	31.84±1.03[b]	7.90±0.14[d]
Cholesterol	634.32±18.25[a]	637.07±23.07[a]	750.42±7.34[d]	844.14±8.10[d]	771.95±8.86[d]

a, b, c, and d letters indicate statistical significant between groups. **a:** *P>0.05,* **b:** *P<0.05,* **c:** *P<0.01,* **d:** *P<0.001*

respectively) in LA and R groups. Vitamin D_2 and D_3 (all groups), α-tocopherol (all groups) levels were significantly decreased (P<0.001). Vitamin K_1 level was significantly decreased (P<0.001) in K, Cat and R groups, but its level was increased (P<0.01) in LA group. δ-tocopherol level was significantly increased (P<0.001) in K group, but its level was decreased (P<0.001) in the other groups. Cholesterol level was significantly increased (P<0.001) in Cat, LA and R groups, but its level was not different (P>0.05) between the C and K groups *(Table 3)*.

DISCUSSION

In lung tissue, α-tocopherol and cholesterol levels were significantly decreased in the K and R groups. In the R group, the reduction of cholesterol levels can be caused by the cholesterol-lowering properties of resveratrol. Keser et al.[36] showed that resveratrol administration was decreased the cholesterol level in muscle of Wistar rats. Keser et al.[37] has showed that cholesterol level was decreased in the heart and brain tissue of rats which administered resveratrol and potassium bromate. In addition these studies, we think that a molecular relationship cholesterol and α-tocopherol reduction. Laden and Porter [38] reported that the possibility that the 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 [38]. Regulation of sterol receptors occurs at the level of transcription, suggesting that α-tocopherol acts through specific receptors or tocopherol-responsive transcription factors [39]. α-tocopherol similarly up-regulates the expression of α-tocopherol transfer protein (α-TTP), and thus plays a role in its own intracellular processing [40,41]. 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 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 [42]. TAP is a recently identified cytosolic protein thought to be involved in the intracellular distribution of α-tocopherol [43].

Miura et al.[44] reported that hypocholesterolemic action of resveratrol is attributed, at least in part, to an increased excretion of neutral sterols and bile acids into feces. They have suggested that dietary resveratrol is hypolipidemic with a tendency for anti-tumor-growth and anti-metastasis effects in hepatoma-bearing rats.

In the liver and kidney tissues, the cholesterol level was significantly increased in the K, Cat, LA and R groups. Increasing of cholesterol level may be caused application of $KBrO_3$ in the all groups. Yilmaz et al.[28] showed that application of $KBrO_3$ was increased the cholesterol level in serum of Wistar rats. Retinol and α-tocopherol levels were significantly increased in the all groups in liver tissue. We think that $KBrO_3$ administration was caused increasing of these vitamin levels in the liver tissue. Because, $KBrO_3$ administration was performed in all groups which increasing of retinol and α-tocopherol levels.

It was observed that α-tocopherol level was significantly decreased in the all groups in the kidney tissue. This may be due to the application of potassium bromate. Because it was demonstrated to induce renal cell tumors in male and female F344 rats after oral administration for 2 years in the drinking water and usage of $KBrO_3$ as a food additive

is now limited, so that exposure of humans via food is very low [45].

Retinol level was increased in all groups of liver and kidney tissues. Vitamin D_2 and D_3 levels were decreased in all groups of liver and kidney tissues. The reason of these vitamin levels different may be application of $KBrO_3$ in these tissues when compared to control group.

In conclusion, present results confirm that there can be a relationship between the decreasing of the cholesterol and α-tocopherol levels in the lung tissue. It can be speculated that resveratrol affected cholesterol biosynthesis in Wistar albino rats. And it was observed that the formation of lipid peroxidation in the kidney of old Wistar rats by induced a prooxidant and carcinogen chemical ($KBrO_3$) administration.

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