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ORIGINAL ARTICLE

Effects of Resveratrol and Potassium Bromate on Cholesterol, Vitamin E and A Level in Lung, Liver and Kidney of Wistar Rats

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ABSTRACT

In the present study, Wistar rats were randomly divided into three groups: 1. Control (C), 2. $KBrO_3$ (K), 3. Resveratrol+$KBrO_3$ (R). In tissues, cholesterol and vitamins analyses were performed by HPLC. According to our results, while the level of cholesterol increased in the K group, its level decreased in the R group when compared to C group in the lung. δ-tocopherol and cholesterol levels decreased in the K and R groups when compared to C group in the liver. Retinol and cholesterol levels decreased in the K and R groups when compared to C group in the kidney. In conclusion, our results indicated that the applications of resveratrol 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, liver, kidney, cholesterol.*

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1. INTRODUCTION

Resveratrol is a polyphenol found mainly in grapes and red wine with diverse established biological activities, such as antioxidant, anti-inflammatory, cardioprotective and anticarcinogenic roles [1,2]. Recently, a number of studies have focused on the neuroprotective effects of resveratrol, demonstrating that this compound attenuates amyloid β peptide-induced toxicity [3,4], protects against cerebral ischemic injury [5,6] and kainic acid-induced excitotoxicity [7]. Several neuroprotective properties of resveratrol have been attributed to its potent antioxidant activity that in many studies has been shown to protect the neural tissue against a variety of neurodegenerative conditions caused by oxidative stress [8-10]. As a natural phytoalexin, resveratrol is produced by a limited number of plant species such as red grapes and nuts [11,12]. Proposed benefits of resveratrol on human health include cardioprotection, neuroprotection, as well as cancer suppression [13-15].

Potassium bromate ($KBrO_3$) had been widely used as a maturing agent for flour and as a dough conditioner. However, 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 [16]. Nevertheless, there is still concern regarding this chemical in the environment. Furthermore, bromate is generated as one of various by-products in ozonation of drinking water [17], implying a potential hazard. This is important because in order to avoid the formation of trihalomethanes, major by-products in the process of drinking water chlorination [18] that are carcinogenic in rodents [19], ozone disinfection has been proposed as an alternative method [20]. $KBrO_3$ has been classified as a genotoxic carcinogen based on positive results in the Ames test [21], and chromosome aberration [22] and micronucleus tests [23]. Moreover, Umemura et al. reported the *in vivo* mutagenic effects of $KBrO_3$ in the kidneys of *gpt* delta rats [24]. It has been postulated that oxidative stress-induced oxidized base is responsible for the mutagenic and carcinogenic effects of $KBrO_3$ [25,26]. $KBrO_3$ is known to cause oxidative damage to the kidney but not to other organs. With a single dose of $KBrO_3$ (80 mg/kg), activity in the kidney was found to increase significantly at 3 h in comparison to that at zero time [27]. $KBrO_3$ is carcinogenic in the rat kidney, thyroid, and mesothelium and is a renal carcinogen in male mice [28,29].

The aim of this research is to examine the effects of resveratrol and potassium bromate on the level of cholesterol, vitamin A and E levels in lung, liver and kidney of old female Wistar rats.

2. EXPERIMENTAL

2.1. Chemicals

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

2.2. 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, a total 30 old female Wistar rats were used. 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; each group containing ten rats. The first group was used as a control (C), the second group potassium bromate ($KBrO_3$) (K), and third group Resveratrol+$KBrO_3$ (R). Rats in the K and R groups were injected intraperitoneally a single dose potassium bromate 80 mg/kg in physiologic saline buffer [27]. After administration of $KBrO_3$ two days, the rats in R group was injected intraperitoneally resveratrol 33 mg/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 decapitate and blood samples were collected and stored in -85 °C prior to biochemical analysis.

2.3. Determination of Lipid Soluble Vitamins in Tissue Samples

400 mg lung, 500 mg liver, 300 mg kidney tissue samples were 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 6000×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. 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 [30].

2.4. Total Cholesterol Analysis in Tissue Samples

400 mg lung, 500 mg liver, 300 mg kidney tissue samples were homogenized in 3 mL acetonitrile/isopropyl alcohol (70:30, v/v)-containing tubes and the mixture were vortexed for 30 s and centrifuged at 6000×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 [31]. 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 [32]. Quantification was carried out by external standardization using Class VP software. The results were expressed as µg/g wet weight tissue.

2.5. Statistical Analysis

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

3. RESULTS

In the lung tissue, α-tocopherol level decreased in the R group when compared to C group ($p<0.001$). While the level of cholesterol increased in the K group, its level decreased in the R group when compared to C group ($p<0.001$) (Table 1).

Table 1. The biochemical parameters in the lung.

Biochemical Parameters (µg/g)	Control (C)	$KBrO_3$ (K)	$KBrO_3$+R (R)
Retinol	2.16±0.38	2.77±0.38[a]	3.23±0.34[a]
α-tocopherol	5.52±1.18	6.72±0.94[a]	**2.32±0.43[d]**
Cholesterol	648.96±120.52	**1463.98±186.81[d]**	**465.19±90.84[d]**

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

In the liver tissue, retinol, α-tocopherol and α-tocopherol acetate levels decreased in the R group when compared to C group ($p<0.05$, $p<0.05$, $p<0.001$, $p<0.001$, respectively). δ-tocopherol and cholesterol levels decreased in the K and R groups when compared to C group ($p<0.001$, $p<0.05$, $p<0.001$, respectively) (Table 2).

Table 2. The biochemical parameters in the liver.

Biochemical Parameters (µg/g)	Control (C)	$KBrO_3$ (K)	$KBrO_3$+R (R)
Retinol	94.54±5.20	95.71±6.83[a]	**86.85±5.01[b]**
δ-tocopherol	10.81±1.97	**5.30±0.89[d]**	**9.14±1.43[b]**
α-tocopherol	26.91±3.40	22.36±3.91[a]	**10.28±1.47[d]**
α-tocopherol acetate	19.87±3.78	13.72±2.47[a]	**4.99±0.79[d]**
Cholesterol	124.50±14.56	**80.53±14.43[b]**	**36.86±4.20[d]**

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

In the kidney tissue, retinol and cholesterol levels decreased in the K and R groups when compared to C group ($p<0.001$, $p<0.05$, respectively). α-tocopherol and α-tocopherol acetate levels decreased in the K group ($p<0.05$) (Table 3).

Table 3. The biochemical parameters in the kidney.

Biochemical Parameters (µg/g)	Control (C)	$KBrO_3$ (K)	$KBrO_3$+R (R)
Retinol	4.52±1.14	**0.88±0.08[d]**	**1.17±0.04[d]**
δ-tocopherol	11.17±1.20	10.18±1.55[a]	9.52±0.87[a]
α-tocopherol	27.02±2.16	**18.96±2.95[b]**	24.60±3.02[a]
α-tocopherol acetate	3.02±0.80	**1.84±0.45[b]**	**1.43±0.31[b]**
Cholesterol	178.16±18.17	**134.12±21.62[b]**	**144.76±17.52[b]**

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

4. DISCUSSION

In the present study, δ-tocopherol level of the liver, α-tocopherol level of the kidney significantly decreased in the K and when in comparison to C group ($p<0.001$). Decreased in δ-tocopherol and α-tocopherol contents were much more pronounced in the K group. However, δ-tocopherol and α-tocopherol levels were not different in the R group when compared to K group may be partially prevented by the administration of resveratrol. Furthermore, the lipid peroxidation in the liver and kidney of rats R group significantly reduced by the administration of the resveratrol. It was found to close the level of vitamin E in the liver and kidney of the R group to the C group value. Therefore, it could be said that resveratrol is the effect in the protection and regeneration of the antioxidant system. In addition, it has been found to associate between the elevated of lipid peroxidation

and decreased of δ-tocopherol and α-tocopherol. When the level of δ-tocopherol and α-tocopherol has been found to optimal in the resveratrol treated group, lipid peroxidation level can be low in the same group.

In this study, cholesterol level of kidney significantly decreased in the K and R groups when compared to C group ($p<0.05$). In resveratrol applied group, reducing of cholesterol level can be explained by a decrease on the squalene monooxygenase enzyme activity. Squalene monooxygenase (SMO), a 64 kDa flavin adenine dinucleotide (FAD)-containing enzyme bound to 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 [33]. Inhibition of SMO has been shown to be effective in lowering serum cholesterol levels in dogs [34], indicating that inhibition of this enzyme can affect circulating cholesterol levels. Laden and Porter had found that activity of human squalene monooxygenase was inhibited by resveratrol. They 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 [33].

In our results, the cholesterol level in the K and R groups of kidney was lower than C group. 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., have suggested that dietary resveratrol is hypolipidemic with a tendency for anti-tumor growth and anti-metastasis effects in hepatoma-bearing rats. They have found that resveratrol dose-dependently suppressed both the serum triglyceride and VLDL+LDL-cholesterol levels [35]. In addition, Yilmaz et al. had detected that the application of resveratrol clearly reduced the amount of cholesterol in erythrocytes of old female Wistar rats [30].

In the lung and liver, α-tocopherol level significantly decreased in the R group when compared to C group. In the R group, it was observed that together cholesterol and α-tocopherol levels decreased. Reducing of cholesterol level can be caused by the hipocholesterolemic effect of resveratrol is obvious. However, we think between cholesterol reduction and reducing of α-tocopherol a molecular relationship.

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 the cholesterol biosynthesis, and over expression of cloned SPF in hepatoma cells increases cholesterol synthesis. In the recently studies, it was affirmed that SPF is effective on squalene monooxygenase that first oxidative enzyme in the cholesterol biosynthesis [36].

α-tocopherol associated protein (TAP) is a recently identified cytosolic protein thought to be involved in the intracellular distribution of α-tocopherol [37]. 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 [36]. In the present study, in the lung and liver tissue, we think that there is a relationship between decreasing of the cholesterol and α-tocopherol levels which suggested these researchers.

Regulation of sterol receptors occurs at the level of transcription, suggesting that α-tocopherol acts through specific receptors or tocopherol-responsive transcription factors [38]. α-tocopherol similarly upregulates the expression of α-TTP, and thus plays a role in its own intracellular processing [39,40]. These findings provide a link between vitamin E and the regulation of cholesterol synthesis that is independent of the antioxidant effects of vitamin E.

In conclusion, present results confirm that there is a relationship between the decreasing of the cholesterol and α-tocopherol levels in the liver and lung tissues. And it was observed that the formation of lipid peroxidation in the kidney and liver of old Wistar rats by induced a prooxidant and carcinogen chemical potassium bromate was prevented by resveratrol administration.

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