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Effects of Resveratrol on Fatty Acid Levels in Serum and Erythrocytes of Rats Administered Potassium Bromate

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Resveratrol is a phytoalexin, highly abundant in skins of red grapes, peanuts and blue berries. The aim of this study is to examine effects of antioxidant resveratrol and carcinogen potassium bromate on the level of fatty acids in serum and erythrocytes of old female Wistar albino rats. In this study, Wistar rats were randomly divided into three groups: (1) control, (2) $KBrO_3$ (80 mg/kg, i.p. single dose) (3) resveratrol + $KBrO_3$ (80 mg/kg $KBrO_3$ i.p. single dose, 33 mg/kg resveratrol, every other day, for 35 days). In serum and erythrocytes, fatty acid levels were measured by gas chromatography. In respect of present results, while stearic and arachidonic acid levels increased in serum of $KBrO_3$ and resveratrol + $KBrO_3$ groups ($p < 0.05$), same fatty acids levels decreased in erythrocytes of same groups ($p < 0.05$) when in comparison to control. While oleic acid, linoleic acid and linolenic acid levels increased in erythrocytes of $KBrO_3$ and resveratrol + $KBrO_3$ groups ($p < 0.01$, $p < 0.05$), linoleic acid level decreased in serum of same groups ($p < 0.05$, $p < 0.001$) in comparison to control. Present results confirmed that oxidation formation in fatty acids of serum and erythrocytes of old female Wistar rats by induced the $KBrO_3$ and it's partially protected by the resveratrol. Resveratrol and $KBrO_3$ applications have influenced the amount of important fatty acids that substrates in fatty acids metabolism on duty enzymes.

Key Words: Resveratrol, Potassium bromate, Serum, Erythrocytes, Linoleic acid, Stearic acid, Arachidonic acid.

INTRODUCTION

Resveratrol (*trans*-3,4',5-trihydroxystilbene) is a stilbenoid and it is present in various fruits and vegetables, especially in most abundant grapes. It functions as a phytoalexin that protects against fungal infections in plants[1]. It has been speculated that resveratrol may act as an antioxidant, promote nitric oxide production[2], modulate vascular cell functions[3], inhibit platelet aggregation[4], alter eicosanoid metabolism[5], reduce lipoprotein oxidation[6] and increase high-density lipoprotein cholesterol[7]; thereby serving as a cardio protective agent. Resveratrol is presents most abundant in grapes, peanuts, pines and it has important biological effects almost protect from coronary heart diseases and atherosclerosis. Many studies have been indicated that natural stilbenoids, such as resveratrol, have antioxidant, antimutagenic and antiinflammatory protective effects[8].

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Potassium bromate ($KBrO_3$) is widely used as a food additive in the bread-making processes for the maturation of flour because of its oxidizing properties and as a neutralizer in cold-wave hair lotions. It is also used in food products and in the production of fish paste and fermented beverages. However, it had concluded to using in the food and cosmetic products when it was appeared that the $KBrO_3$ caused to renal cell tumors, mesotheliomas of the peritoneum and follicular cell tumors of thyroid. Renal cell tumors have been observed in male and female rats after exposure of this compound[9]. It also enhances N-ethyl, N-hydroxyethyl-nitrosamine initiated renal tumors in rats[10]. There is enhancement in cellular proliferation in kidney due to oxidative stress generated by $KBrO_3$. It has also been reported that $KBrO_3$ causes DNA strand breaks in the kidney[11]. It has been observed that lipid peroxidation was increased in the rats' kidney that exposed to $KBrO_3$. In the other study, it was investigated that the regulator effect of kolaviron, which is a biflavonoid, in the antioxidant defending system against to oxidative stress and cellular redox status in the kidneys and livers of rats administered $KBrO_3$. In that study, it was also indicated that $KBrO_3$ inhibited the activities of superoxide dismutase, glutathione peroxidase and catalase in the kidney[12]. In addition, it has been determined that level of 8-oxodeoxyguanosine (8-OH-deoxyguanosine, 8-oxodG) in the kidney DNA of treated rats[13,14]. With a single dose of $KBrO_3$ (80 mg/kg), activity in the kidney was found an increase significantly at 3 h in comparison to that at zero times[15].

Fatty acids are used as major substrates for the synthesis of various kinds of lipid including phospholipids, triacylglycerol and cholesterol esters. Oleate is the most abundant mono unsaturated fatty acid found in triacylglycerol, cholesterol esters, wax esters and phospholipids[16]. The ratio of stearic acid to oleic acid has been implicated in the regulation of cell growth and differentiation through effects on membrane fluidity and signal transduction[17]. Mono unsaturated fatty acids also influence apoptosis and may have some role in mutagenesis of some tumors[18].

Poly unsaturated and mono unsaturated fatty acids are important for normal growth, development and are suggested to play an important role in modulation of cardiovascular inflammatory diseases and cancer[19,20]. The variable health effects may be produced by n-3 and n-6 fatty acids themselves, which serve as structural components of membrane phospholipids. Their products modulate the biosynthesis of potent cellular mediators *i.e.*, eicosanoids[21].

Arachidonic acid and its metabolites play a central role in cell signaling, functioning as both vasoconstrictors and vasodilators, triggering mechanisms of angiogenesis[22], inflammation[23] and allergies[24].

The aim of this study is to examine effects of antioxidant resveratrol and carcinogen potassium bromate on the level of fatty acids in serum and erythrocytes of old female Wistar albino rats.

EXPERIMENTAL

Animals and study design: In this study, total 30-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 three groups. The first group was used as a control (C), the second group potassium bromate (K) and third group resveratrol + $KBrO_3$ (R). Rats in the K and R groups were injected intraperitoneally a single dose of $KBrO_3$ *i.e.*, 80 mg/kg in the physiologic saline buffer[15]. After administration of $KBrO_3$ for 2 days, the rats in R group were injected 33 mg/kg resveratrol for 4 times/week. In addition, in control group rat's physiological saline were injected. These treatments were continued for 5 weeks, after which time each experimental rat was decapitated and blood samples were collected and stored in -85 °C prior to biochemical analysis.

Biochemical determinations

Sample preparation for biochemical analyses: Blood samples were collected in tubes. Blood samples centrifuged at 2500 × g at 4 °C for 10 min and serum was separated. The erythrocyte pellet was washed three times by 0.9 % NaCl and centrifuged for 5 min at 2500 × g after each wash. The erythrocyte pellets and serum samples were prepared freshly for biochemical analysis.

Lipid extraction: Total lipids were extracted with hexane-isopropanol (3:2 v/v) by the method of Hara and Radin[25]. The tissue samples were homogenized. 300 µL of sera samples and 300 mg erythrocyte pellets of the homogenized tissue samples were taken and mixed with 5 mL hexane-isopropanol (3:2, v/v) in a mixer. Non-lipid contaminants in lipid extracts were extracted into 0.88 % KCl solution. The extracts were evaporated in a rotary evaporator flask and then stored at -25 °C.

Fatty acid analysis: Fatty acids in the lipid extracts were converted into methyl esters including 2 % sulphuric acid (v/v) in methanol[26]. 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 17 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 and 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.

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

RESULTS AND DISCUSSION

In the study, the effects of resveratrol on the levels of the fatty acids in serum and erythrocytes of old female Wistar rats by induced-$KBrO_3$ were examined.

Fatty acid composition of serum: The fatty acid composition of serum is shown in Table-1. In respect to present results, stearic acid (18:0) and arachidonic acid (20:4) levels were significantly higher in the K and R groups than the C group ($p < 0.05$). Linoleic acid (18:2) and poly unsaturated fatty acids (PUFA) levels were significantly lower in the K and R groups than the C group ($p < 0.05$, $p < 0.001$, $p < 0.01$, $p < 0.001$, respectively). Docosahexaenoic acid (22:6) level was significantly low in the K group ($p < 0.05$), but it was high in the R group ($p < 0.01$) when in comparison to the C group. Unsaturated fatty acids (UFA) and n-3 fatty acids levels were significantly lower in the K group than the C and R group ($p < 0.001$, $p < 0.01$, respectively). Saturated fatty acids (SFA) level was significantly higher in the K group than C and R groups ($p < 0.001$). n-6 fatty acid levels were significantly higher in the K group than the C and R groups ($p < 0.001$).

TABLE-1
FATTY ACIDS COMPOSITION OF SERUM LIPIDS (%)

Fatty acids	Control (C)	$KBrO_3$ (K)	$KBrO_3$ + R (R)
16:0	24.62 ± 0.47[a]	24.62 ± 0.37[a]	23.84 ± 0.39[a]
18:0	24.36 ± 0.32[a]	26.71 ± 0.58[b]	26.67 ± 0.46[b]
18:1 n-9	7.57 ± 0.42[a]	7.44 ± 0.46[a]	8.42 ± 0.36[a]
18:2 n-6	22.39 ± 0.45[a]	20.21 ± 0.77 [b]	17.42 ± 0.71[d]
20:4 n-6	11.29 ± 0.44[a]	12.30 ± 0.70[b]	13.40 ± 0.60[b]
22:6 n-3	9.68 ± 0.44[a]	8.69 ± 0.32[b]	10.23 ± 0.35[b]
Σ Saturated	48.98 ± 0.36[a]	51.33 ± 0.50[d]	50.51 ± 0.32[a]
Σ Unsaturated	50.93 ± 0.36[a]	48.64 ± 0.50[d]	49.47 ± 0.32[a]
Σ MUFA	7.57 ± 0.42[a]	7.44 ± 0.46[a]	8.42 ± 0.36[a]
Σ PUFA	43.36 ± 0.33[a]	41.20 ± 0.40[b]	41.05 ± 0.46[b]
Σ n-3	9.68 ± 0.44[a]	8.69 ± 0.32[c]	10.23 ± 0.35[b]
Σ n-6	33.68 ± 0.31[a]	32.51 ± 0.31[a]	30.82 ± 0.76[d]

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

Fatty acid composition of erythrocytes: The fatty acid composition of erythrocytes is shown in Table-2. In respect to present results, palmitoleic acid (16:1), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), unsaturated fatty acid and mono unsaturated fatty acids (MUFA) levels were significantly higher in the K and R groups than the C group ($p < 0.05$, $p < 0.001$, $p < 0.05$, $p < 0.05$, $p < 0.05$ and $p < 0.001$, respectively). 18:0, 20:4, docosapentaenoic acid (22:5 n-6) and saturated fatty acid levels were significantly lower in the K and R groups than the C group ($p < 0.05$, $p < 0.001$, $p < 0.05$, $p < 0.05$ and $p < 0.05$, respectively). 22:6 level was significantly higher in the R group than C and K groups ($p < 0.01$). Poly unsaturated fatty acid level was significantly lower in K group than the C and R groups ($p < 0.05$).

TABLE-2
FATTY ACIDS COMPOSITION OF ERYTHROCYTES LIPIDS (%)

Fatty acids	Control (C)	$KBrO_3$ (K)	$KBrO_3$ + Resveratrol (R)
16:0	24.84 ± 0.45[a]	24.33 ± 0.25[a]	24.90 ± 0.57[a]
16:1 n-7	0.60 ± 0.07[a]	0.81 ± 0.19[b]	1.05 ± 0.14[b]
18:0	16.14 ± 0.59[a]	14.66 ± 0.29[b]	13.81 ± 0.32[b]
18:1 n-9	9.51 ± 0.30[a]	14.71 ± 0.35[c]	13.06 ± 0.52[c]
18:2 n-6	11.58 ± 0.21[a]	12.71 ± 0.46[b]	12.05 ± 0.28[b]
18:3 n-3	0.39 ± 0.03[a]	0.43 ± 0.02[b]	0.43 ± 0.02[b]
20:4 n-6	19.56 ± 0.13[a]	17.82 ± 0.66[b]	18.61 ± 0.84[b]
22:5 n-6	1.04 ± 0.15[a]	0.75 ± 0.05[b]	0.75 ± 0.07[b]
22:5 n-3	2.71 ± 0.24[a]	1.99 ± 0.16[b]	2.17 ± 0.08[a]
22:6 n-3	4.86 ± 0.05[a]	4.62 ± 0.24[a]	5.55 ± 0.16[b]
Σ Saturated	43.02 ± 0.92[a]	40.90 ± 0.51[b]	40.34 ± 0.70[b]
Σ Unsaturated	56.25 ± 0.31[a]	59.64 ± 0.85[b]	59.82 ± 0.82[b]
Σ MUFA	13.93 ± 0.41[a]	19.06 ± 0.43[c]	17.88 ± 0.61[c]
Σ PUFA	42.32 ± 0.50[a]	40.57 ± 0.66[b]	41.93 ± 1.05[a]
Σ n-3	9.14 ± 0.34[a]	8.21 ± 0.49[a]	9.38 ± 0.27[a]
Σ n-6	33.17 ± 0.31[a]	32.36 ± 0.82[a]	32.55 ± 0.93[a]

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

Epidemiological studies have indicated that phytoestrogens, such as resveratrol, inhibit the effects of carcinogen substances and reduce cholesterol levels. It was demonstrated that resveratrol exhibits a wide range of biological effects, including antiplatelet, antiinflammatory, anticancer, antimutagenic and antifungal properties[27].

Wang *et al.*[28] has shown that resveratrol inhibited ADP-induced platelet aggregation despite no changes in serum lipid levels. In present results, the amount of 18:0 in serum was high in the K and R groups in comparison to C group. According to this result, the increasing of 18:0 in the K and R groups can be explained by the reduction in stearoyl-CoA desaturase (SCD) enzyme activity. In contrast to serum results, in the erythrocytes, it was observed that the 18:0 level decreased and 18:1 level increased in the same groups. The desaturating enzymes, Δ^9-desaturase [also referred to as stearoyl-CoA-desaturase (SCD)], Δ^6-desaturase (Δ^6D) and Δ^5-desaturase (Δ^5D), introduce *cis*-double bonds in the carbon chain of long chain fatty acids[29]. These enzymes catalyze the synthesis of the long chain mono unsaturated fatty acids (MUFA) and poly unsaturated fatty acids (PUFA), which are needed to maintain membrane structures, to participate in cellular communication and differentiation, for eicosanoid signaling and to regulate gene expression[29,17]. Stearoyl-CoA desaturase is an endoplasmic reticulum enzyme that catalyzes the biosynthesis of mono unsaturated fatty acid from saturated fatty acids and it is a rate-limiting enzyme in the biosynthesis of mono unsaturated fatty acids. Stearoyl-CoA desaturase expression affects the fatty acid composition of membrane phospholipids, triacylglycerol and cholesterol esters, resulting in changes in membrane fluidity, lipid metabolism and obesity[29]. Stearoyl-CoA desaturase can also convert the 18:0 fatty acids to the 18:1 fatty acids[30,31].

According to our estimates, the cause of the 18:1 level is higher in erythrocytes, may be due to $KBrO_3$ application. It is believed that the reason of this phenomena caused by damaging of enzymes and other molecules that the fatty acids are converts to further metabolites, because the similar results were observed in the K and R groups. This result has shown that the application of resveratrol and potassium bromate has affected the activities of enzymes in fatty acids metabolism and this result is consistent with enzyme activities. Increasing in tissues the excess of 18:0 participates to blood circulation and its level reduces in tissues. Therefore, participating to the structure of erythrocytes while the amount of 18:0 that decrease, the amount of 18:1 increases.

18:1 and 16:1 fatty acids are the most abundant fatty acids found in triacylglycerol, cholesterol esters, wax esters and phospholipids[16]. These fatty acids are synthesized by stearoyl-CoA desaturase (SCD). Cohen *et al.*[32], have reported that the mono unsaturated fatty acids increase as a result of increased SCD activity. There are the multiple roles of mono unsaturated fatty acids, variation in SCD activity in mammals would be expected to affect a variety of key physiological variables, including differentiation, insulin sensitivity, metabolic rate, adiposity, atherosclerosis, cancer and obesity[17].

In the serum, it was observed that the 18:2 level decreased in the K and R groups, the 20:4 level increased in the same groups when in comparison to C group. This event has shown that the administrations of resveratrol and potassium bromate caused increasing of the activity of Δ^6 desaturation pathway synthesis of 20:4 or increasing of the activity of arachidonyl-hydrolyzing phospholipase A_2 (PLA_2) enzyme.

This metabolic event is catalyze by $\Delta^{5,6}$ desaturase enzymes and it is investigated in essential fatty acid metabolism. The increasing of the amount of 20:4 in the K and R groups when in comparison to the values of C group can be explained by uncontrolled increasing of the activities of $\Delta^{5,6}$ desaturase enzymes. The 20:4 is an important fatty acid that found in cellular membrane phospholipids and it releases by PLA_2 enzyme. It is suggested that the amount of this fatty acid when increased, cytotoxicity increase in a cell[33-35].

In erythrocytes, it was observed that 18:2 and 18:3 levels increased in the K and R groups, the level of 20:4 decreased especially in the K group when in comparison to C group. *In vitro* experiments have shown that the more double bonds in a poly unsaturated fatty acid has, the more vulnerable to peroxidation[36]. 20:4 is present as natural component of phospholipids in membranes. It is released from phospholipids membrane by PLA_2, providing substrates for the synthesis of the potent lipid mediators of inflammation, the eicosanoids and platelet-activating factor[37-41]. The absorption and metabolism of 18:3 are similar to those of 18:2, the principal essential fatty acid of the x-6 family commonly present in Western diet. The balance required in the diet between n-3 and n-6 fatty acids are important due to their competitive nature and their different biological roles to ensure the conversion of 18:3 to 20:5 and 22:6[42]. In tissues, both 18:3 and 18:2 can be converted in fatty acids of longer

and more unsaturated chain *via* the common pathway of alternate desaturation and elongation[42]. The desaturase enzymes show preference for metabolism of different fatty acids: n-3, n-6 and n-9[43,44].

According to present results, increase of the amount of 20:4 in the K and R groups of serum and decrease of the same fatty acid level in erythrocytes especially in the K group can explain by the increase of PLA_2 enzyme activity. The increasing of the activity of this enzyme may be caused to decrease of the amount of 20:4 in the erythrocytes due to increasing of release of 20:4 from membranes and it may be caused to increase of the amount of same fatty acid in the serum.

Miura *et al.*[45] has indicated that resveratrol significantly suppressed the serum lipid peroxide levels. Fabris *et al.*[46] has demonstrated that stilbenes, such as resveratrol and piceid into the membrane with the susceptible hydroxyl group close to the double bonds of poly unsaturated fatty acid make these stilbenes particularly suitable for the prevention and control of the lipid peroxidation in membranes[46].

In the serum and erythrocytes, it was observed that while the amount of 22:6 decreased in the K group and this fatty acid significantly increased in the R group when compared with C group. Lipids, especially poly unsaturated fatty acid are preferential targets of oxidative damage[47] and the resulting defects in membrane functions may cause cell death[48]. This event has shown that the administration of potassium bromate may be caused to 22:6 fatty acid oxidations and therefore, the amount of this fatty acid decreased in this group. The increase of the amount of 22:6 indicated that resveratrol treatment prevented oxidation of this fatty acid by potassium bromate in the R group when compared with C group. Because previous studies have shown that potassium bromate is an oxidizing agent and resveratrol is a strong antioxidant[5-8,12].

Cao *et al.*[49] has suggested that the antioxidative activity of resveratrol may diminish oxidative stress and damage to cellular biomolecules such as lipids, proteins and DNA induced by platinum compounds. In this study, the increasing of the amount of 22:6 in the R group of serum and erythrocytes may be occurred by this property of resveratrol.

In the serum, while the increasing of the amount of 22:6 in the R group and decreasing of the amount of same fatty acid can arise from the effects of resveratrol on the $\Delta^{5,6}$ desaturase enzymes in the K group in comparison to C group. There are many studies about this enzyme's activity which show increasing in the carcinogenesis and tumor formation. It has been expressed that excessive unsaturated fatty acids, such as 20:4 and 22:6 are important in development of the brain, heart functions and inflammatory responses and ensuring of the balance[34]. Ajmo *et al.*[50] has indicated that resveratrol treatment led to reduced lipid synthesis and increased rates of fatty acid oxidation.

Erythrocytes are the target cells for peroxidative damage and abnormal susceptibility of erythrocyte lipids to peroxidation is believed to reflect a similar abnormality in other organs and tissues. In addition, erythrocytes are sensitive to peroxide

accumulation. They are particularly susceptible to augmenting the degree of unsaturation of membrane lipids[51]. Mocan *et al.*[52] has suggested that lipid peroxidation of plasma and erythrocyte membrane lipids may be a primary phenomenon and this should be confirmed by investigation of peroxidation of renal lipids. This phenomena is similar to present study results. Because the application of potassium bromate caused oxidation to serum and erythrocytes and thus it provided that the fatty acids of serum and erythrocytes exposed to lipid peroxidation. Yilmaz *et al.*[53] have detected that the application of resveratrol clearly reduced the amount of cholesterol in erythrocytes of rats. In the present study, while the amounts of 20:4, 22:5 (n-6), 22:5 (n-3), 22:6 and poly unsaturated fatty acids significantly decreased in the K group of erythrocytes, the amounts of same fatty acids partially decreased or increased in the R group when compared with the K and C groups. These results may be showed that the application of potassium bromate is causing to oxidation of this fatty acids and resveratrol treatment has prevented to this oxidation.

According to present results in serum and erythrocytes, the amounts of poly unsaturated fatty acids significantly decreased in the K group when in comparison to C group. This result can be explained by poly unsaturated fatty acids preferential targets of oxidative damage[47]. The application of potassium bromate caused oxidation to poly unsaturated fatty acids and these amounts significantly decreased in the K group.

We have not found any study about the effect of potassium bromate on fatty acids. Present results showed that the application of potassium bromate oxidized to some fatty acids in the serum and erythrocytes and resveratrol treatment has been partially prevented to this oxidation. Also, according to present study results, it has understood that the applications of resveratrol and potassium bromate have influenced the amount of important fatty acids that the substrates in fatty acids metabolism on some enzymes.

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