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# The Effects of Potassium Bromate and Resveratrol on Cholesterol and Vitamin E Levels in Heart, Muscle and Brain of Wistar Rats

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**Abstract**

Resveratrol is a phytoalexin, highly abundant in skins of red grapes, peanuts and blueberries. The aim of this study was to examine the effects of resveratrol and kidney carcinogen potassium bromate on the levels of vitamin E and cholesterol in heart, muscle and brains of old female Wistar rats. All analyses were measured by high performance liquid chromatography.

According to our results, retinol, vitamin D3, α-tocopherol and cholesterol levels were decreased in the K and R groups when compared to C group ($p<0.01$, $p<0.05$, $p<0.05$, $p<0.05$, respectively) in the heart. Vitamin D3, α-tocopherol, α-tocopherol acetate and cholesterol levels were decreased in the R group ($p<0.05$, $p<0.001$, $p<0.001$, $p<0.001$, respectively) in the muscle. The level of cholesterol was decreased in the K and R groups when compared to C group ($p<0.05$) in the brain.

In conclusion, our results indicated that the applications of potassium bromate and resveratrol influenced cholesterol and vitamin E levels and these applications can be affected cholesterol biosynthesis in old female wistar albino rats.

**Keywords:** Resveratrol, potassium bromate, heart, muscle, cholesterol

## INTRODUCTION

Resveratrol is a naturally occurring polyphenolic compound that can be found in a variety of plants [1,2]. Resveratrol belongs to a group of substances known as phytoalexins, which are low molecular weight secondary metabolites produced by plants as a defensive response to microbial injury, fungal infection, or abiotic (i.e., environmental) stress [3]. Resveratrol is found in nature as both *cis* and *trans* isomers, however, the *trans*-isomer is believed to be the most abundant and biologically active form. Humans ingest *trans*-resveratrol by consuming foods or plants that contain it naturally or via resveratrol-containing dietary supplements. trans-Resveratrol can be found in over 300 edible plants, with significant dietary amounts found in grape skin, and it is subsequently present in red wine at concentrations up to 7.2 mg/l [4,5] and may be related to the "French Paradox" (a reduced risk of coronary heart disease and cancer, associated with the consumption of red wine) [6,2]. *trans*-Resveratrol can also be found to lesser extents in peanuts, cranberries and mulberries, as well as several inedible plants, including the roots of the Japanese knotweed, *Polygonum cuspidatum*. The knotweed has been used in Chinese herbal medicine for centuries to treat inflammation, hypertension and lipid and fungal diseases [5,7,8]. *trans*-Resveratrol has been suggested to possess cancer chemopreventive, antioxidative, antiplatelet, antifungal and cardioprotective properties; however, the mechanism(s) of these proposed effects is not fully understood. It have detected that the application of resveratrol and potassium bromate clearly reduced the amount of cholesterol in erythrocytes of old female wistar rats [9].

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 [10] 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 still concern regarding this chemical in the environment. Furthermore, bromate is generated as one of various by-products in ozonation of drinking water [11], 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 [12] that are carcinogenic in rodents [13], ozone disinfection has been proposed as an alternative method [14]. $KBrO_3$ has been classified as a genotoxic carcinogen based on positive results in the Ames test [15], and chromosome aberration and micronucleus tests [16]. It has the potential to induce 8-hydroxy-2'-deoxyguanosine (8-OHdG) formation both *in vitro* and *in vivo* [17-20]. 8-OHdG is the most

abundant oxidative DNA adduct, can induce mutations such as GC to TA transversions upon replication by DNApolymerases [21]. Moreover, Umemura et al. reported the *in vivo* mutagenic effects of $KBrO_3$ in the kidneys of *gpt* delta rats [22]. It has been postulated that this oxidative stress-induced oxidized base is responsible for the mutagenic and carcinogenic effects of $KBrO_3$ [23]. 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 [24].

The aim of this research was to examine the effects of resveratrol and the carcinogen $KBrO_3$ on the level of cholesterol and lipophylic vitamins in heart, muscle and brain of old female wistar rats.

## MATERIALS AND METHODS

### Animals

In this study, a 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 potassium bromate 80 mg/kg in physiologic saline buffer [24]. After administration of $KBrO_3$ two days, the rats in R group was injected resveratrol 33 mg/kg four times per week. In addition, in C group rat's physiological saline was injected. These treatments were continued for five weeks, after which time each experimental rat was decapitate and blood samples were collected and stored in -85 $^0$C prior to biochemical analysis.

### Determination of lipid soluble vitamins in tissue samples

300 mg heart, 500 mg muscle and 300 mg brain tissue samples were homogenized in 3 ml acetonitrile/methanol/isopropanol (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 40 $^0$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 $^0$C. Supelcosil LC 18$^{TM}$ 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 retinol acetate, and 215 nm for δ-tocopherol, vitamins D2, D3 and K1, 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.

### Total cholesterol analysis in tissue samples

300 mg heart, 500 mg muscle and 300 mg brain 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 6000×g for 10 min at 4$^0$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 [25]. Supelcosil LC 18$^0$ DB column (250 x 4.6 mm, 5 $^0$m) was used as the HPLC column. Detection was performed by UV at 202 nm and 40 $^0$C column oven [26]. 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 ± SEM. Statistical analysis was performed using SPSS

**Table 1.** The biochemical parameters in heart of rats

| Biochemical Parameters | Control (C) | $KBrO_3$ (K) | $KBrO_3$+R (R) |
|---|---|---|---|
| Retinol (µg/g) | 0.45±0.14$^a$ | 0.18±0.01$^b$ | 0.13±0.01$^c$ |
| δ-tocopherol (µg/g) | 30.70±4.70$^a$ | 113.55±44..52$^d$ | 131.93±29.64$^d$ |
| D2 (µg/g) | 22.64±2.89$^a$ | 18.16±3.02$^b$ | 24.30±7.90$^a$ |
| D3 (µg/g) | 101.15±16.51$^a$ | 44.60±5.68$^c$ | 50.62±6.68$^c$ |
| α-tocopherol (µg/g) | 74.01±7.85$^a$ | 38.78±9.40$^b$ | 50.42±9.84$^b$ |
| α-tocopherol acetate (µg/g) | 9.54±2.98$^a$ | 4.17±0.67$^c$ | 12.40±2.28$^b$ |
| Cholesterol (µg/g) | 797.86±185.50$^a$ | 574.46±111.15$^b$ | 553.04±136.98$^b$ |

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

**Table 2.** The biochemical parameters in muscle of rats

| Biochemical Parameters | Control (C) | $KBrO_3$ (K) | $KBrO_3$+R (R) |
|---|---|---|---|
| Retinol (µg/g) | 0.52±0.08[a] | 0.11±0.01[b] | 0.18±0.01[b] |
| D2 (µg/g) | 2.40±0.32[a] | 6.01±0.97[d] | 3.32±0.39[a] |
| D3 (µg/g) | 212.17±6.38[a] | 210.61±17.66[a] | 154.86±9.13[b] |
| α-tocopherol (µg/g) | 92.43±4.96[a] | 88.37±6.06[a] | 59.25±3.31[d] |
| α-tocopherol acetate (µg/g) | 4.17±0.47[a] | 4.88±0.65[a] | 1.33±0.29[d] |
| Cholesterol (µg/g) | 1017.20±69.83[a] | 1033.90±86.88[a] | 604.63±51.97[d] |

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

Software. Analysis of variance (ANOVA) and an LSD test were used to compare the experimental groups with the controls.

## RESULTS

In the heart tissue, retinol, vitamin D3, α-tocopherol and cholesterol levels were decreased in the K and R groups when compared to C group (p<0.01, p<0.01, p<0.05, p<0.05, respectively). δ-tocopherol level was increased in the K and R groups (p<0.001). The level of vitamin D2 was decreased in the K group (p<0.05). While the level of α-tocopherol acetate was decreased in the K group, its level was increased in the R group when compared to C group (p<0.01) (Table 1).

In the muscle tissue, retinol level was decreased in the K and R groups when compared to C group (p<0.05). The level of vitamin D2 was increased in the K group (p<0.001). Vitamin D3, α-tocopherol, α-tocopherol acetate and cholesterol levels were decreased in the R group (p<0.05, p<0.001, p<0.001, p<0.001, respectively) (Table 2).

In the brain tissue, the level of cholesterol was decreased in the K and R groups when compared to C group (Table 3).

## DISCUSSION

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 [27,28].

Decreasing of α-tocopherol and α-tocopherol acetate content and increasing of lipid peroxidation level were much more pronounced in the K group of heart. Decreasing of α-tocopherol in the R group has been prevented by the administration of resveratrol. These results suggest that resveratrol may counteract a decrease in α-tocopherol and α-tocopherol acetate by acting as an antioxidant *in vivo*. Furthermore, the lipid peroxidation in the heart of rats the R group significantly reduced by the administration of the resveratrol. It was found to close the level of vitamin E level in heart of the R group to the C group value. Therefore it could be said that resveratrol is effect in the protection and regeneration of antioxidant system. In addition, it has been found to associate between the elevated of lipid peroxidation and decreased of α-tocopherol and α-tocopherol acetate. When the level of α-tocopherol and α-tocopherol acetate has been found to optimal in the resveratrol treated group, lipid peroxidation level has been found to low in the same group.

In the recently studies, it has been shown that α-tocopherol is a radical scavenger and therefore, its amount reduce in the peroxide tissues [29]. α-tocopherol is inhibit free radical in the ambient and its stop reactions of lipid peroxidation. In this system, the structure of α-tocopherol is transformed into a tocopheroxyl radical [30]. One of the important antioxidants Vitamin E is moved by selenoproteins and it removes free radicals at environment [31].

In present study, in the heart and brain, the cholesterol level significantly decreased in the K and R groups when in comparison to C group (p<0.05). In resveratrol administered group, significantly reducing of the cholesterol level can be explained by reduction 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. Electrons are passed from NADPH, via cytochrome P450 reductase, to the FAD of squalene monooxygenase, where they are used to reduce one atom of molecular oxygen ($O_2$) to water, while the other oxygen atom in inserted into the substrate, squalene

**Table 3.** The biochemical parameters in brain of rats

| Biochemical Parameters | Control (C) | $KBrO_3$ (K) | $KBrO_3$+R (R) |
|---|---|---|---|
| α-tocopherol (µg/g) | 1.71±0.62[a] | 1.71±0.06[a] | 1.86±0.49[a] |
| Cholesterol (µg/g) | 1117.81±346.12[a] | 495.03±83.59[b] | 638.54±154.29[b] |

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

[32]. Inhibition of squalene monooxygenase has been shown to be effective in lowering serum cholesterol levels in dogs [33], 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 [32].

In our results, the cholesterol level in the K and R groups of heart and brain 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 [34]. They have suggested that dietary resveratrol is hypolipidemic with a tendency for anti-tumor-growth and anti-metastasis effects in hepatoma-bearing rats. Laden and Porter 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 [32].

In study of Turner et al was performed to determine whether a high dose of resveratrol (1000 μg/day) antagonizes the ability of estrogen to lower serum cholesterol. [35]. Miura et al (2003) have found that resveratrol dose-dependently suppressed both the serum triglyceride and VLDL+LDL-cholesterol levels [34].

In the muscle, α-tocopherol and α-tocopherol acetate level significantly decreased in the R group when compared to C group ($p<0.001$). In the resveratrol applied R group, it was observed that reducing of the amount of cholesterol together the amounts of α-tocopherol and α-tocopherol acetate decreased. Reduce the amount of cholesterol is caused by the hipocholesterolemic effect of resveratrol is obvious. However, we think that between molecular relationship the amount of cholesterol reduction and reducing of α-tocopherol.

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. In the recently studies, it was affirmed that SPF is effective on squalene monooxygenase that first oxidative enzyme in 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]. 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.

According to our results, in the muscle tissue, we are thinking that a relationship between reduction on the cholesterol level and α-tocopherol, these researchers suggested.

In conclusion, present results confirm that there is a relationship between the decreasing of the cholesterol and α-tocopherol levels in the muscle tissue. And it was observed that the formation of lipid peroxidation in the heart of old Wistar rats by induced a prooxidant and carcinogen chemical ($KBrO_3$) was prevented by resveratrol administration. It can be speculated that resveratrol affected cholesterol biosynthesis in old female wistar albino rats. In addition, the oxidation of exogenous antioxidant molecules such as vitamin E was prevented and thereby the antioxidant capacity of cell was protected in the heart.

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