Effects of Arbutin on Fatty Acid Levels of Erythrocyte and Serum in Wistar Albino Rats Treated with Potassium Bromate

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ABSTRACT

Background: In the presented study; the Effects of Arbutin (ARB) on the Rat Erythrocyte and serum fatty acid profile which is exposed to potassium bromate (KBrO₃) were investigated. Materials and Methods: In this study, 32 Wistar albino rats weighing 250-300 g were used divided into 4 groups. Groups 1: control, group 2: KBrO₃ (single dose 100 mg / kg gavage), group 3: ARB (50 mg / kg / day (ip) for 5 days), group 4: KBrO₃ + ARB. At the end of the 5th day, alteration of fatty acid profile in erythrocyte and serum of rats in all groups was examined. Results: Rat serum essential fatty acid; palmitic acid (C16:0), myristic acid (C14:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), erythrocyte major fatty acids; palmitic acid (C16:0), myristic acid (C14:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), arachidic acid (C20:0), eicosenoic acid (C20:1), and lignoceric acid (C24:0). In addition, in our studied serum and erythrocytes; Total monounsaturated fatty acids (MUFA) varied between 8.91 ± 0.53- 11.71 ± 2.55 and 33.71 ± 2.12- 37.11 ± 2.12, respectively. It was determined that total polyunsaturated fatty acids (PUFA) varied between 5.90 ± 1.29- 9.96 ± 1.18 and 14.72 ± 3.66- 22.13 ± 4.82, respectively. Conclusion: In our study, alterations in fatty acid contents were observed, and results suggesting that arbutine affects the enzymes involved in Fatty acid metabolism and has an effect on fatty acid amounts.

Keywords: Arbutin (ARB), Potassium bromate (KBrO₃), Fatty acid (FA), Polyunsaturated fatty acids (PUFA), Monounsaturated fatty acids (MUFA), Wistar Albino Rat.

INTRODUCTION

Arbutin (ARB) is a glycosylated hydroquinone found naturally in various plant species such as bearberry (Ericaceae), pear trees (Rosaceae), and Bergenia crassifolia (Saxifragaceae),¹,² and also in wine, coffee, wheat, broccoli, and some fruits.³ ARB is a secondary metabolite in most higher plants.² Widely used as an ingredient in skin care products and traditionally in the treatment of pigment disorders in Japan.⁴ Thanks to its antioxidant, anti-microbial and anti-inflammatory activities, it is a widely used and effective agent in the field of medicine and health.⁵-⁷ In the last decade, it has attracted considerable attention due to its versatile biological activities such as lowering blood sugar.⁸ Potassium bromate (KBrO₃) is an oxidizing agent that is widely used in cosmetic products and is an important tap water contaminant, although it is used as a food additive. It is still used in many countries, including the United States, as a bread and cake improver, although it has been associated with the development of many organ damage.⁹,¹² Toxicological studies, KBrO₃; an oxidizing agent; reported
that it causes hepatotoxicity, neurotoxicity and thyroid toxicity and induces the development of mesothelioma tumors in experimental animals as well as kidney cancer in animals and humans.13 KBrO₃ is known to induce oxidative stress in tissues.14 Fatty acids (FA) are the types of lipids that have the simplest molecular structure among fat groups.15,16 It is used as the main substrates for the synthesis of various types of lipids, including FA, phospholipids, triacylglycerol and cholesterol esters.17 Polyunsaturated FA such as omega-3 and 6 have many biological properties and are biosynthetic precursors of eicosanoids. FA levels in the blood are closely related to many diseases such as cardiovascular diseases, blood pressure and arthritis. These FA act as regulators in cellular functions by participating in the structure of membrane phospholipids and other components.18 FA are biologically active molecules with a wide variety of effects.19 In this study, the effects of ARB on the FA profile in rat erythrocyte and serum tissues exposed to KBrO₃ were investigated.

MATERIALS AND METHODS

Animals and Study Design

In this study, 32 Wistar albino rats weighing 250-300 g were used. Rats were placed in cages with ad libitum rat food and water in an air-conditioned room with a 12/12hr light / dark cycle. Groups 1: Control, Group 2: KBrO₃,20 Group 3: ARB,21 Group 4: KBrO₃ + ARB; divided into 4 groups. A single dose of 100 mg / kg (gavage) was administered to the KBrO₃ group, and 50 mg / kg / day (i.p) to the ARB group for 5 days. At the end of the 5th day, the rats in all groups were taken by intra-cardiac intervention in accordance with ethical rules and kept at -80°C for comparison. The experimental study was approved by Animal Experiments Local Ethics Committee of Bingöl University (BUHA DEK:09.05.2019-2019/4-04/02).

Taking Serum and Erythrocyte Samples and Extraction of Lipids

Serum and erythrocyte sample preparation for biochemical analysis: Blood samples taken into gel biochemistry tubes were centrifuged at 2500 rpm at 4°C for 10 min and serum samples were obtained. Blood samples taken into EDTA tubes were centrifuged, and the separated erythrocyte pellet was washed with 0.9% NaCl, and then centrifuged at 2500 rpm for 5 min. This process was applied three times in a row. Erythrocyte pellets and serum samples were made ready for biochemical analysis. Lipid extraction of erythrocyte and serum samples was determined according to the method used by Hara and Radin (1978).22 300 mg erythrocyte tissue sample and 300 µL serum sample were subjected to disintegration with 5 mL n-hexane-isopropanol at a ratio of 3:2 (v / v) at 1100 rpm in Micro-D homogenizer. Non-lipid contaminants in the lipid extract were extracted using 0.88% KCl. The extracts were made ready for analysis by thinning in a rotary evaporator.

Fame Analysis from Erythrocyte and Serum

In order to perform FA analysis, lipids must first be converted to methyl esters and derivatives, which have non-polar volatile and stable structures. To prepare the methyl ester, 5mL of 2% methanolic sulfuric acid was added onto the lipid extract in n-hexane-isopropanol phase. Then they were mixed thoroughly using a vortex. This mixture was left to be methylated in a 50°C oven. At the end of the 15hr period, 5 mL of 5% NaCl was added to the mixture which was cooled down to room temperature. The FA methyl esters formed were extracted with hexane. Taking the upper hexane phase, 2% of KHCO₃ was added and the phases were separated. After 4 hr, the organic phase was evaporated under nitrogen. Samples were analyzed by adding 100 µL of hexane before analysis.23

Fatty acid analysis method in GC-MS

Simultaneous analysis with FID and MS detectors was performed using SGE brand BPX90 (100m x 0.25mm ID) column in Agilent 7890A / 597°C model Gas Chromatography-Mass Spectrometry (GC-MS) device. Chromatographic conditions; The temperature of the oven starts from 120°C, reaches 250°C with 5°C / minute, it was waited for 3 min, it reached 260°C with 2°C / minute and was waited for 8 min, it was completed in an average of 40 min. The injection volume was 1 microliter and the split ratio was 1/10. Solvent delay time was selected as 12 min, carrier gas as He and when the constant gas flow was set as 1 mL / min, H₂ flow was 35 mL / min, dry air flow 350 mL / min, N₂ 20.227 mL / min was automatically adjusted by the program. Auto sampler program: before and after each injection, the injector was set to wash itself with solvent (hexane) 5 times, then withdraw from the sample twice, leave it in the waste bottle, and give the last sample to the column at the third draw. Thus, the possibility of contamination from the previous sample was minimized.24

Statistical analysis

Statistical analysis was done using SPSS 18.0 program. Comparison analysis of variance (ANOVA) and LSD tests were used between the control group and
experimental groups. Results were given as mean ± SEM. Differences between groups (p<0.05, p<0.01 and p <0.001) were used.

RESULTS AND DISCUSSION

Fatty acid composition of serum

When the effect of KBrO₃ (K) and ARB (A) on the FA profile in serum is examined; It was observed that the amount of myristic acid (14:0) decreased at certain rates in all groups of KBrO₃ (K), ARB (A) and KBrO₃ + ARB (A) compared to the control (p<0.001, p<0.05). It was determined that the amount of palmitic acid (16:0) was decreased partially in all groups compared to the control (p>0.05). In comparison of stearic acid (18:0) and Oleic acid (18:1) FA with the control group, certain levels of increases were found in all groups (p>0.05, p<0.05). In the comparison of linoleic acid (18:2) level with respect to control, certain decreases were found in all groups (p>0.05, p<0.05). In comparison of linoleic acid (18:2) level with respect to control, certain decreases were found in all groups (p>0.05). When the total unsaturated FA levels were compared to the control, an increase was observed in ARB(A), while a decrease was observed in the other groups (p>0.05). When the total monounsaturated fatty acid (MUFA) levels were compared to the control, certain levels of increase were observed in all groups (p>0.05, p>0.05). When the total polyunsaturated fatty acid (PUFA) levels were compared to the control, certain decreases were found in all groups(p>0.05, p<0.05) (Table 1), (Figures 1-2).

FA composition of erythrocytes

When the effect of KBrO₃ (K) and ARB (A) on FA profile in erythrocyte tissue was examined; It was observed that the amount of myristic acid (14:0) and palmitic acid (16:0) FA increased in ARB (A) group and decreased partially in the other groups compared to the control (p>0.05). When the stearic acid (18:0) fatty acid amount was compared with the control, a partial decrease in the KBrO₃ (K) group and a partial increase in the other groups were observed (p>0.05). When the amount of oleic acid (18:1), fatty acid was compared with the control, a decrease was observed in the ARB group, while an increase was observed in the other groups (p>0.05). Linoleic acid (18:2) fatty acid level was decreased in all groups compared to the control (p>0.05). When the amount of arachidic acid (20:0) and lignoceric acid (24:0) fatty acids were compared with the

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Control</th>
<th>KBrO₃</th>
<th>ARB</th>
<th>KBrO₃+ARB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristic acid (C14:0)</td>
<td>29.79±0.57</td>
<td>26.76±1.61*</td>
<td>17.92±2.34*</td>
<td>19.15±2.05*</td>
</tr>
<tr>
<td>Palmitic acid (C16:0)</td>
<td>38.82±0.78</td>
<td>37.13±1.57*</td>
<td>36.24±3.5*</td>
<td>37.70±3.6*</td>
</tr>
<tr>
<td>Stearic acid (C18:0)</td>
<td>12.90±0.36</td>
<td>18.12±4.25*</td>
<td>24.95±6.37*</td>
<td>27.11±5.53*</td>
</tr>
<tr>
<td>Oleic acid (C18:1)</td>
<td>8.91±0.52</td>
<td>8.97±0.76*</td>
<td>11.72±2.55*</td>
<td>10.13±1.66*</td>
</tr>
<tr>
<td>Linoleic acid (C18:2)</td>
<td>9.96±1.18</td>
<td>9.59±1.11*</td>
<td>9.17±2.18*</td>
<td>5.90±1.29*</td>
</tr>
<tr>
<td>Σ Saturated</td>
<td>81.52±1.40</td>
<td>82.03±1.60*</td>
<td>79.11±4.20*</td>
<td>83.96±2.29*</td>
</tr>
<tr>
<td>Σ Unsaturated</td>
<td>18.48±1.40</td>
<td>18.57±1.60*</td>
<td>20.88±4.20*</td>
<td>16.04±2.29*</td>
</tr>
<tr>
<td>Σ MUFA</td>
<td>8.91±0.53</td>
<td>8.97±0.76*</td>
<td>11.71±2.55*</td>
<td>10.13±1.66*</td>
</tr>
<tr>
<td>Σ PUFA</td>
<td>9.96±1.18</td>
<td>9.59±1.11*</td>
<td>9.17±2.19*</td>
<td>5.90±1.29*</td>
</tr>
</tbody>
</table>

Figure 1: FA Composition of Serum Lipids (%).

Figure 2: FA Composition of Serum Lipids (% (Σ Saturated, Σ Unsaturated, Σ PUFA, Σ MUFA).
control, decreases were observed in all groups \((p>0.05)\). Ecosenoic acid \((20:1)\); the amount of FA increased at certain levels in all groups compared to the control. \((p>0.05, p<0.05)\). Total saturated FA levels; Compared with the control group, an increase was detected in all groups \((p>0.05, p<0.05)\). Total unsaturated fatty acid levels; Compared to the control, a decrease was observed in the Arbutin group, but an increase was found in the other groups \((p>0.05)\). Total MUFA levels; Compared to control, certain levels of increases were observed in all groups \((p>0.05)\). Total PUFA levels; When compared to the control, a certain decrease was detected in all groups \((p>0.05, p<0.05, p<0.01)\) (Table 2, Figure 3, 4, 5).

To induce oxidation in the food and cosmetic industries; \(\text{KBrO}_3\) is widely used.\(^{25}\) It is used in ovens as a food additive and flavor enhancer that gives strength and spongy character to the dough. In the bread baking process, \(\text{KBrO}_3\) is reduced to the more stable compound potassium bromide (KBr). In addition, there may be a deficiency in the reaction and it causes oxidative damage due to some amount of \(\text{KBrO}_3\) remaining. Therefore, the use of \(\text{KBrO}_3\) in bread production is banned in some countries.\(^{26}\) It is also used in cheese and beer production.\(^{27}\) It is mainly used in the maturation process of flour due to its oxidizing properties. In 1978, bioassays of \(\text{KBrO}_3\) on rats and

### Table 2: FA Composition of Erythrocytes Lipids (%).

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Control</th>
<th>(\text{KBrO}_3)</th>
<th>ARB</th>
<th>(\text{KBrO}_3+\text{ARB})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristic acid (C14:0)</td>
<td>1.89±0.38</td>
<td>1.80±0.21*</td>
<td>3.04±1.29*</td>
<td>1.57±0.17*</td>
</tr>
<tr>
<td>Palmitic acid (C16:0)</td>
<td>24.18±0.33</td>
<td>23.47±3.64*</td>
<td>25.97±3.01*</td>
<td>20.74±3.66*</td>
</tr>
<tr>
<td>Stearic acid (C18:0)</td>
<td>10.19±2.38</td>
<td>10.02±1.66*</td>
<td>14.89±4.79*</td>
<td>13.83±1.75*</td>
</tr>
<tr>
<td>Oleic acid (C18:1)</td>
<td>30.59±3.85</td>
<td>32.07±3.29*</td>
<td>24.21±5.33*</td>
<td>33.58±4.38*</td>
</tr>
<tr>
<td>Linoleic acid (C18:2)</td>
<td>22.13±4.82</td>
<td>22.09±2.89*</td>
<td>14.71±3.65*</td>
<td>19.57±2.63*</td>
</tr>
<tr>
<td>Arachidic acid (20:0)</td>
<td>3.48±2.64</td>
<td>1.32±0.24*</td>
<td>1.16±0.14*</td>
<td>1.42±0.13*</td>
</tr>
<tr>
<td>Ecosenoic acid (C20:1)</td>
<td>3.12±1.34</td>
<td>5.03±1.45*</td>
<td>12.27±2.98*</td>
<td>3.13±1.39*</td>
</tr>
<tr>
<td>Lignoceric acid (C24:0)</td>
<td>3.43±1.14</td>
<td>2.51±0.57*</td>
<td>2.06±0.65*</td>
<td>1.34±0.12*</td>
</tr>
<tr>
<td>Σ Saturated</td>
<td>43.17±7.61</td>
<td>59.99±4.92*</td>
<td>50.79±6.37*</td>
<td>43.71±5.78*</td>
</tr>
<tr>
<td>Σ Unsaturated</td>
<td>55.84±7.61</td>
<td>59.19±4.93*</td>
<td>51.20±6.37*</td>
<td>56.29±5.78*</td>
</tr>
<tr>
<td>Σ MUFA</td>
<td>33.71±2.12</td>
<td>37.11±2.12*</td>
<td>36.49±2.83*</td>
<td>36.72±3.71*</td>
</tr>
<tr>
<td>Σ PUFA</td>
<td>22.13±4.82</td>
<td>22.09±2.89*</td>
<td>14.72±3.66*</td>
<td>19.56±2.62*</td>
</tr>
</tbody>
</table>

\(d: p<0.001, c: p<0.01, b: p<0.05, a: p>0.05\)
mice for 2 years were found to be carcinogenic of this oxidizing agent. Studies have shown that KBrO₃ has detrimental effects on the nutritional qualities of bread by reducing the amount of vitamins A, B₁, B₂, E and niacin, which are the main vitamins of bread. Also in the studies, it has been reported to have the potential to cause cancer, kidney failure, deafness, redness, and eye and skin pain. In another study, it was reported that KBrO₃ caused an increase in cellular proliferation in the kidney and DNA strand breakage in the kidney due to the oxidative stress produced by it. Erythrocytes are terminally differentiated blood cells in which most of the metabolic pathways are absent, and since they act as oxygen carriers, intracellular oxygen radicals are constantly in danger. Also, an important feature of our study is that the high content of polyunsaturated lipids and transition metals makes them particularly vulnerable to oxidative damage. As a result, erythrocytes have an effective defense system against the cytotoxic effects of free radicals. Based on these features, we preferred to use erythrocytes in our study. It is known that free radicals and reactive oxygen species cause peroxidation of polyunsaturated FA and this situation can be prevented by enzymatic and non-enzymatic antioxidants. In a study, an increase was observed in the concentration of TBARS (Thiobarbituric acid reactive substances) in kidney tissues of rats treated with KBrO₃. TBARS, considered a late biomarker of oxidative stress, the final metabolite of peroxylated polyunsaturated fatty acids, TBARS not only converts reactive oxygen species into active chemicals, but also magnifies the function of reactive oxygen species through chain reaction, causes changes in cellular and functional disorders. It also shows that free radicals cause lipid peroxide formation. As a result of KBrO₃ applications, the increase in lipid peroxidation, which is evaluated by the high TBARS levels, has been documented in other studies. Khan (2003) and El-Sokkary (2006) in their study; They observed that KBrO₃ caused a significant increase in lipid peroxidation due to free radical damage in necrotic and degenerative livers of rats.

In this study, they administered KBrO₃ intraperitoneally to rats, and at the end of the study, they observed a significant deficiency of antioxidant enzymes and an increase in lipid peroxidation. With these results and findings, it was thought that lipid peroxidation played an important role in liver damage and the results obtained supported the histological changes in the liver. At the end of the study, it was reported that vitamin C effectively eliminated the oxidative damage caused by KBrO₃.

Keser et al. in a study they did; studied the effects of potassium bromate and resveratrol on FA levels in some rat tissues. They observed that the MUFA level increased in KBrO₃ and resveratrol groups in all tissues and explained this increase by the increase in SCD (stearoyl-CoA desaturase) enzyme activity. MUFA are known to have multiple roles, influencing variation in SCD activity in mammals, differentiation, insulin sensitivity, metabolic rate, adiposity, various fundamental physiological variables including atherosclerosis, cancer, and obesity. Again in the same study; They found that PUFA decreased in KBrO₃ and Resveratrol groups. They explained that the reason for this is that KBrO₃ application may cause oxidation of these FA and decrease the levels of these FA in these groups. Especially; PUFA is known to be the preferred lipid targets of oxidative damage. In our study we have presented; The fact that we obtained similar results with this study proves the effect of stearoyl-CoA desaturase (SCD) enzyme activity on the increase of MUFA values. It is also known that MUFA affect apoptosis and may play a role in the mutagenesis of some tumors. ARB is a well known tyrosinase inhibitor. It is a key enzyme involved in melanin synthesis and is widely used as a skin lightening agent for cosmetic products. In another study, ARB showed weak but long-lasting radical scavenging activity and at the same time showing exactly the same antioxidant activity as hydroquinone; In two cell-based antioxidant analysis using erythrocytes and skin fibroblasts, arbutin was found to exhibit potent antioxidant activity comparable or even superior to that of hydroquinone. In another study conducted; On the protective effects of ARB on streptozotocin (STZ) induced neurotoxicity in rats, it was concluded that ARB has a protective role against STZ-induced memory impairment in the brain and oxidative damage in the hippocampus. In a different literature study; The in vivo effects of arbutine with cyclosporine (CsA) on lipid peroxidation and antioxidant capacity in serum of treated rats were studied. It has been reported that ARB has a protective effect in CsA-induced toxicity, and that high ARB concentration has significant oxidative and lipoperoxidative effects. In another study evaluating the antioxidant capacity of arbutin; It has been demonstrated that lipid peroxidation and fibrosis observed in the liver tissue of CCl₄ injected rats, with ARB administration, decreased lipid peroxidation to normal levels and ARB administration may have a protective effect on the liver.
CONCLUSION
Since studies on the effects of KBrO₃ and ARB on FA are limited, we aimed to examine the effects of KBrO₃ and ARB on the distribution of FA profiles. At the same time, we aimed to see the antioxidant effects of arbutin against the oxidative effects of KBrO₃, which is known to cause lipid peroxidation, on erythrocytes and serum FA. Similar to the given literature studies; In our study, changes in fatty acid contents were observed, and results suggesting that ARB affects the enzymes involved in FA metabolism and has an effect on FA amounts. In the light of these results, we can say that ARB may have a protective effect against the destructive effect of KBrO₃ and this should be supported by different studies to be done.

ACKNOWLEDGEMENT
Animals were obtained from Bingöl University Experimental Research Center (BUHADYEK). Study was performed after acceptance of protocols by BUHADYEK (Date: 09.05.2019-2019/04-04/02).

CONFLICT OF INTEREST
The authors declare no conflict of interest.

ABBREVIATIONS
ARB: Arbutin; KBrO₃: Potassium bromate; FA: Fatty acids; GC-MS: Gas Chromatography-Mass Spectrometry; ANOVA: Analysis of variance; SEM: Mean±standard error of mean; MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid; TBARS: Thiobarbituric acid reactive substances; SCD: stearoyl-CoA desaturase; STZ: Streptozotocin; CsA: Cyclosporine.

REFERENCES
In the presented study, the Effects of Arbutin (ARB) on the Rat Erythrocyte and serum Fatty acid profile which is exposed to Potassium bromate (KBrO₃) were investigated. In this study, 32 Wistar albino rats weighing 250-300 g were used divided into 4 groups. Alteration of Fatty acid profile in erythrocyte and serum of rats in all groups was examined. In the light of these results, we can say that ARB may have a protective effect against the destructive effect of KBrO₃ and this should be supported by different studies to be done.
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Cite this article: Akkoyun HT, Aydın S, Akkoyun MB, Bengü AS, Ekin S, Erdem SA. Effects of Arbutin on Fatty Acid Levels of Erythrocyte and Serum in Wistar Albino Rats Treated with Potassium Bromate. Indian J of Pharmaceutical Education and Research. 2022;56(3):740-7.