***Original Research Article***

# The Effect Of Ellagic Acid On Paraoxanase -1 Activity And DNA Damage in Acute Exercise

UNDER PEER REVIEW

***ABSTRACT***

The purpose of this study was to evaluate the correlation between exhaustive and intensive exercise with changes in paraoxonase-1 enzyme activity, oxidative DNA damage and the role of ellagic acid against possible damage.

The study was carried out on 32 male and adult Spraque - Dawley rats at the Experimental Animal Research and Research Center of Afyon Kocatepe University. The experimental animals were equally divided into four groups. Swimming exercises were performed as acute exercises for once and experimental animals are made to swim in groups including two rats following the completion of the study and before the decapitation.

At the end of the experiment, obtained blood samples; Paraoxonase-1 (PON-1), Malondialdehyde (MDA) and 8-HydroxyGuanine (8-OhdG) levels were measured to determine DNA damage and DNA damage was assessed by Comer (Comet) Assay method.

As a result, PON-1 levels in rats with intense swimming training were found to be significantly lower ($p < 0.05$) than the control group. MDA and (8-OhdG) levels were significantly higher in the swimming group than in the control group ($p < 0.05$). As to the DNA damage determination by COMET analysis, DNA damage was observed in the swimming groups according to the control groups. When the ellagic acid groups were compared with the swimming groups, there was a significant increase in PON-1 levels, and the levels of MDA and (8-OhdG) were significantly lower than the swimming groups. The DNA damage was also found to be low in these groups.

***Keywords:*** exercise, ellagic acid, PON-1, DNA damage

## 1. Introduction

Exercise associated with severity and duration increases the metabolic processes and oxygen consumption and resulting in more free radicals. Increase in free radicals can trigger lipid peroxidation chain reactions, exceeding antioxidant defence capacity (1,2).

If the production of free radicals exceeds the body's antioxidant defence system, unwanted damages can occur. Free radicals can affect the cell, cell structure, proteins and nucleic acids in a negative way.

The antioxidant status varies in size and direction depending on the type of exercise and the organ. Different types of exercise are known to result in different levels of oxidative damage (3). Acute exercise affects the antioxidant system in organs such as liver, kidney and heart (4).

Antioxidants are compounds that can delay, inhibit or prevent the oxidation of compounds, trapping free radicals and reducing oxidative stress. The presence of ellagic acid in various commercial products giving antioxidant activity has also been reported. These molecules have a variety of benefits for their anti-mutagenic, antimicrobial and antioxidant properties, and inhibitors of the human immunodeficiency virus (HIV) (5).

The PON1 enzyme is involved in the antioxidant defence system because it prevents the oxidation of lipid peroxides, which are considered to play a major role in atherogenesis. At the same time, lycopene is also a potent antioxidant and anticarcinogenic.

At the same time, lycopene is a strong inflammation corrector and a vitamin with anticarcinogenic properties. It protects cells from free radical damage as well as enhances cell metabolism and strengthens the bonds between cells.

In this study, it was investigated the correlation between the antioxidant effect of ellagic acid on oxidative DNA damage in rats with acute exhaustive exercise.

UNDER PEER REVIEW

## 2. Materials and Methods

Ellagic acid was obtained from Sigma-Aldrich (Interlab, Turkey). All the other chemicals and reagents were of analytical reagent grade obtained from commercial sources.

### 2.1. Experimental Protocol

The study was performed on 32 Wistar Albino adult male rats at the experimental animal's centre of Afyon Kocatepe University. This study was approved by the ethical committee (ref no, AKU-HADYEK-450-15).

The experimental animals were divided into 4 equal groups as 5 rats in each cage. 10 days prior to the study, the experimental animals under observation were allowed to adapt to the experimental environment. Animals were kept in 12 hours of darkness and 12 hours of light. During the study, all subjects were kept under equal environmental conditions and subjects were given standard rat feed and water ad libitum throughout the study.

The feeding was scheduled twice at 09.00 A.M and 7.00 P.M.

**Group 1:** General control group with no application.

**Group 2:** Acute swimming exercise control group.

**Group 3:** The group that was applied 15 mg/kg/ day Ellagic Acid **(**EA) EA for 4 weeks and performed acute swimming exercises at the end of the application.

**Group 4:** The group that was applied 15 mg/kg/ day Ellagic Acid **(**EA) for 4 weeks and was not performed acute swimming exercises at the end of the application.

**2.2. Exercise Protocol**

Acute swimming exercise was performed in a thermostat glass swimming pool with a height of 50 cm, width 50 cm and a length of 100 cm. The pool was made of heat-resistant glass, which allows the water to remain constant at 36.5 ± 0.5 $^{o}$C. Swimming exercises were performed as one-off acute exercises and experimental animals swam in pairs at the end of the study. In case the rats were suspended in the water surface, they were directed to swim with a thin long rod. The initiation of movements without coordination and immobilization for 10 seconds underwater were considered as exhaustion criteria of rats (6).

**2.3. Biochemical Analyses**

**2.3.1. Measurement of 8-OHdG in serum**

The serum samples were analyzed for their concentration of 8-hydroxy-2' – deoxyguanosine (8-OHdG) utilizing a competitive enzyme immunoassay (ELISA) kit (Cayman Chemical Company, Ann Arbor, MI, USA) and intra- assay and inter-assay CV were determinate 5.3% and 8.2 %, respectively.

**2.3.2. Measurement of PON-1 in serum**

Serum PON1 levels were determined by a spectrophotometric method with Rel ASSAY (Rel Assay Diagnostics® Clinical Chemistry Soluons/TURKEY). The working principle is as follows.

UNDER PEER REVIEW

Fully automated paraoxonase activity measurement method consists of two different sequential reagents. The first reagent is an appropriate Tris buffer and it also contains calcium on, which is a cofactor of PON1 enzyme. The second reagent is a new developed stable substrate solution. The sample is mixed with the Reagent 1 and the substrate solution is added. Linear increase of the absorbance of p-nitrophenol, produced from paraoxon, is followed at kinetic measurement mode. Nonenzymatic hydrolysis of paraoxon was subtracted from the total rate of hydrolysis. The molar absorptivity of p-nitrophenol is 18.290 $M^{-1}$ $cm^{-1}$ and one unit of paraoxonase activity are equal to 1 mol of paraoxon hydrolyzed per litre per minute at 37°C.

### 2.3.3. Serum MDA levels

Blood samples were isolated into the serum. Whole blood (MDA) was figured by utilizing the technique for Draper and Hardley (7). This technique depends on the coupling of MDA with thiobarbituric acid.

### 2.3.4. Comet Assay in Rat Mononuclear Leukocytes

Mononuclear leukocytes were isolated to utilize income assay. For this, the technique for Kocyigit et al. (8) was taken after. In this technique; heparinized blood samples were spilt into histopaque 1077 on the test tubes and after shaping a small layer the test tubes were centrifuged at 2100 rpm for 30 min [25ºC]. From that point forward, the centre layer (contains mononuclear leukocytes) was moved into 1 mL of salinized phosphate buffer(PBS) (pH 7.4) and blended with it. At that point, this mixture was again centrifuged at 1600 rpm for 10 min (25ºC). After releasing the supernatant, the pellet was diluted as including 106 $mm^3$ by PBS (pH 7.4). After that, leukocytes were blended with 100 µL of 0.5% low-melting agarose in PBS at 37ºC. In this way, 80 µL of this mixture was layered onto a slide pre-covered with a thin layer of 1% normal melting point agarose, secured quickly with a coverslip and put away for 5 min at 4ºC to permit the agarose to harden. Subsequent to evacuating the cover-slips, the slides were drenched in newly prepared cool (4ºC) lysing solution (2.5 M NaCl, 100 mM EDTA-$Na_2$; 1%.

### 2.4. Statistical Analyses

Data got from test animals were stated as means and standard deviation of means (±SD) and analyzed using one-way analysis of variance (ANOVA), trailed by Duncan posthoc tests on

UNDER PEER REVIEW

the SPSS (18.0] software computer program. A distinction in the mean values of $p<0.05$ was thought to be significant.

## 3. Results

### 3.1. PON-1 and MDA levels

As a result of the analyzes, PON-1 levels in swimming groups were found significantly lower ($p <0.05$) and MDA levels ($p <0.05$) were found to be significantly higher ($p <0.05$) compared to the control groups. When the ellagic acid groups were compared with the swimming groups, serum PON-1 levels were significantly ($p <0.05$) increased and MDA levels decreased significantly ($p <0.05$). The results are given in Table-1.

***Table 1.*** *a,b,c In the same column values with different letters show statistically significant differences in blood MDA, PON-1 and* 8-OhdG *levels($p<0.05$).Mean±SD: Standard Deviation*

| | **8-OhdG blood (ng/ml)** | **MDA (nmol/ml)** | **PON-1** U/L |
|---|---|---|---|
| **Control** | 4,68±0,31[a] | 0.36±0.01[a] | 0.96±0.02[a] |
| **Swimming** | 9,84±0,26[b] | 0.93±0.02[b] | 0.42±0.04[b] |
| **Ellagic acid** | 4,78±0,39[a] | 0.37±0.02[a] | 0.97 ±0.02[a] |
| **Ellagic acid +swimming** | 6,12±0,27[c] | 0.51±0.04[c] | 0.67±0.03[c] |

### 3.2. 8-OHdG levels and DNA damage

Serum 8-OHdG levels were significantly higher in swimming groups than in the control group ($p <0.05$). When the ellagic acid groups were compared with the swimming groups, 8-OHdG levels were significantly lower ($p <0.05$). The results are shown in Table 1.

The DNA damage was detected in mononuclear leukocytes of rats and demonstrated in Table 2. In **Swimming** group, DNA damage levels were observed to be high level compared to the control group ($p < 0.05$). Ellagic acid has diminished DNA damage in the exhaustive exercise group.

*Table 2.* Effects of Ellagic acid DNA damage levels in blood samples

| **Group** | **DNA damage (Arbitrary Unit ±SD)*** |
|---|---|
| Control | 7.87±1.64[a] |
| Swimming | 18.12±1.8[b] |

UNDER PEER REVIEW

| | |
|---|---|
| Ellagic acid | 2.37±0.91[c] |
| Ellagic acid +swimming | 13.37±1.4[d] |

* In the same column values with different letters show statistically significant differences in blood DNA damage levels ($p<0.05$). SD: Standart Deviation

## 4. Discussıon

It is known that exercise increases the production of free oxygen radicals and it is a source of stress which causes oxidative stress. However, it is known to improve resistance against oxidative stress by affecting antioxidant enzyme activity (9). If free radicals are not neutralized, they can cause serious damage to the body by breaking down cell membrane proteins, destroying membrane lipids, hardening cell membrane and blocking cell function, passing nuclear membrane, affecting the genetic material in the nucleus and breaking the DNA and making it open to mutations, destroying immune system and forcing the immune system (10). In many studies (11), the effects of exercise time on this condition have been demonstrated. The rate of oxidant and antioxidants that will be released during exercise varies with exercise intensity (11). In acute exhausting (heavy) and severe exercises, the damaging oxidant system is more active, (12), (13) while regular and short-term non-maximal exercises further activate the antioxidant systems (14). The extent of oxidative damage during physical exercise is not only determined by free radical production but also by the defence capacity of antioxidants. Increases in exercise result have been shown in many antioxidant studies (15). When the studies investigating the effect of exercise on oxidative stress and antioxidant system, it is seen that mostly aerobic exercise forms are used (16,17). The purpose of our study is to determine the degree of oxidant and oxidant DNA damage in rats with acute exhaustive exercise and to determine the success of ellagic acid in preventing this damage. Therefore, PON-1 enzyme levels, MDA levels and oxidative stress were found in rats. 8-OHdG levels which are an indicator of oxidative DNA damage due to oxidative stress and DNA damage levels in the cells with Comet Assay were investigated.

According to our results, high levels of MDA levels and PON-1 enzyme activity, which is a marker of oxidative stress, are thought to be associated with acute swimming exercise and an oxidative stress condition. In other studies, MDA is thought to cause oxidative stress and increase lipid peroxidation reactions in proportion to the intensity and duration of the exercise.

Conflicting results with MDA have been reported. It has been reported that at least some of the differences in MDA levels may be due to the change in plasma volume of exercise (18).

UNDER PEER REVIEW

Alessio et al. (19) reported that MDA did not change in aerobic exercise. Duffaux et al. (20) reported that MDA did not show a significant increase in physical education and sports students after an intensive running test. Leaf et al. (21) reported that MDA did not change before and after maximal exercise. Grisham (22) found no significant difference in MDA in acute exercise. Dernbach et al. (23) reported that there was no change in plasma MDA levels in the rest of the athletes before and after 4 weeks of intensive rowing. Selamoğlu (24) found a statistically significant decrease in MDA in long-distance runners. Çelik et al. (25) reported a decrease in MDA levels after the acute exercise performed by football players ($p < 0.05$). When the literature is examined, there are many studies that show that short-term or acute intense exercise increases oxidative stress.

HDL-dependent PON protects LDL from oxidation. Increasing LDL oxidation by the addition of PON inhibitors depends on both PON inactivation and incorporation of HDL lipids into total lipid peroxide formation (26). Because of its dependence on HDL, the main task of in vivo serum PON is to protect HDL from the harmful effects of oxidative stress. Therefore, many studies have shown that HDL-dependent PON inhibits not only LDL oxidation but also HDL oxidation (when oxidation is induced by transition metal ions and free radicals) (27). This effect depends on the ability of PON to hydrolyze lipoprotein-mediated peroxides. In our study, we found that PON-1 serum activities of rats treated with acute swimming were significantly decreased compared to the control groups. The decrease in PON-1 activity, an important antioxidant enzyme, indicates the presence of oxidative stress.

At the same time, serum 8-OHdG levels, which are indicators of oxidative DNA damage, increased significantly in acute exercise compared to the control group and the presence of DNA damage in these groups was also determined. Our findings are consistent with previous studies (28). Fıçıcılar et al.29 reported that acute exhaustive exercise significantly reduced TAS value in a study on rats. In a study that investigated the effects of melatonin and ascorbic acid on DNA damage and oxidative stress in rats with intense exercise stress; It was determined that exercise increased TOS levels and DNA damage.

Ellagic acid is a substance with antimutagenic, anti-carcinogenic and antioxidant activity and is found naturally in the phenol structure in plants. Khanduja et al. found that GSH enzyme activity increased in rats treated with ellagic acid and explained this increase by claiming that ellagic acid could regenerate oxidized glutathione and increase GSH synthesis (30). In many similar studies, polyphenols have been reported to increase the activity of many antioxidants such as catalase, GSH-Px and SOD (31,32).

UNDER PEER REVIEW

In our study, swimming groups with ellagic acid were observed to have decreased levels of MDA and increased PON-1 levels when compared to the groups with acute swimming exercises alone, suggesting that these results showed that they had antioxidant properties and protective properties against oxidative stress. These results suggest that ellagic acid has antioxidant properties and shows protective properties against oxidative stress. In addition, it is another result of our study that the increased DNA damage markers in the swimming group decreased with the ellagic acid group.

According to our results, an oxidative stress condition occurred in rats with acute exhaustion exercise and oxidative DNA damage was detected. Protective effect of ellagic acid supplementation against damage was revealed. Further studies are needed to understand the mechanisms by which ellagic acid exerts its action to reduce oxidative DNA damage.

## CONSENT

It is not applicable.

Ethical Approval

It is not applicable.

## COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

UNDER PEER REVIEW

## REFERENCES

1. Abed KE, Rebai H, Bloomer RJ, Trabelsi K, Masmoudi L, Zbidi A, Sahnoun Z, Hakim A, Tabka Z. Antioksidant status and oksidative stres at rest and in response to acute exercise in judokas and sedantary men. *Journal of Strength & Conditioning Research*. 2011, 25(9):2400-2409.
2. Jakesevic M, Aaby K, Borge GIA, Jeppsson B, Ahrne S, Molin G. Antioxidative protection of dietary bilberry, chokeberry and Lactobacillus plantarum HEAL19 in

mice subjected to intestinal oxidative stress by ischemia-reperfusion. *BMC Complementary and Alternative Medicine*, 2011, 27: 8-11.

3. Bloomer RJ, Goldfarb AH. Anaerobic exercise and oxidative stres. *Canadian Journal of Applied Physiology*, 2004, 29: 245-63.
4. Boveris A, Navarro A. Systemic and mitochondrial adaptive responsest moderate exercise in rodents. *Free Radical Biology and Medicine*, 2008, 44: 224-229.
5. Leonardo Sepúlveda , Alberto Ascacio , Raúl Rodríguez-Herrera , Antonio Aguilera-Carbó and Cristóbal N. Aguilar. ‘’Ellagic acid: Biological properties and biotechnological development for production processes’’ African Journal of Biotechnology Vol. 10(22), pp. 4518-4523, 30 May, 2011.
6. Dawson Ca, Horvarth SM. Swimming in small laboratory animals. Medicine and Science in Sports. 1970;2:51-78.
7. Draper Hh, Hardley M. Malondialdehydedetermination as index of lipid peroxidation. Methods Enzymol. 1990; 186:421-31.
8. Kocyigit A, Keles H, Selek S, Guzel S, Celik H, Erel O. Increased DNA damage and oxidative stress in patients with Cutaneous Leishmaniasis. Mutat Res.2005;585:71-78.
9. Aguilo A, Tauler P, Pilar Guix M, Villa G, Cordova A, Tur JA, Pons A. Effect of exercise intensity and training on antioxidants and cholesterol profile in cyclists. *The Journal of Nutritional Biochemistry*, 2003, 14: 319-325.
10. Tekcan M. Oksidatif stres-antioksidan sistemler. *Infertilite, Androloji Bülteni*, 2009, 131-136.
11. Radak Z Sasvari M, Nyakas C, Pucsok J, Nakamoto H, Goto S. Exercise preconditioning against hydrogen peroxide induced oxidative damage in proteins of rat myocardium. *Archives of Biochemistry and Biophysics*, 2000, 376: 248–251.
12. Atalay M, Oksala NK, Laaksonen DE, Savita Khanna, Chitose Nakao, Jani Lappalainen, Sashwati Roy, Osmo Hanninen , Sen CK. Exercise trainingmodulates heat shock protein response in diabetic rats. *Journal of Applied Physiology*, 2004, 97: 605-611.
13. Banerjee AK, Mandal A, Chanda D, Chakraborti S. Oxidant, antioxidant and physical exercise. Molecular and Cellular Biochemistry, 2003, 253: 307-312.
14. White A, Estrada M, Walker, K, Wisnia P. Filgueira G, Valdes F, Araneda O, Behn C, Martinez R. Role of exercise and ascorbate on plasma antioxidant capacity in thoroughbred race horses. Comparative Biochemistry and Physiology, Part A Molecular and Integrative Physiology, 2001, 128: 99-104.

UNDER PEER REVIEW

15. Timothy IM, Kevin EE, Hageman KS, Poole DC. Altered regional blood flow responses to submaximal exercise in older rats. Journal of Applied Physiology, 2003, 96: 81–88.
16. Kalinowska BR, Korczala KZ, Kalinowski M, Kukla M, Birkner E, Jochem J. Activity of antioxidative enzymes and concentration of malondialdehyde as oxidative status markers in women with newly diagnosed Graves -Basedow disease and after thiamazole therapy leading to euthyroidism. *Archıwum Medycyny Wewnętrznej*, 2008, 118: 7-8.
17. Şemin I. Kayatekin BM, Gönenç S, Açıkgöz O, Uysal N, Delen Y, Güre A. Antrene farelerde bir saatlik egzersizin ince bağırsak, böbrek ve kas dokusundalipit peroksidasyonuna ve antioksidan enzimlere etkisi. *Hacettepe Journal Sport Science*, 1998, 4(9): 33-40.
18. Taş M. Futbolcularda sürat egzersizlerinin serum süperoksid dismutaz, katalaz ve malondialdehit düzeylerine etkisi. Sağlık Bilimleri Enstitüsü, Beden Eğitimi ve Spor Anabilim Dalı (Yüksek Lisans), Erzurum: Atatürk Üniversitesi, 2006.
19. Alessio HM HA, Fulkerson BK, Ambrose J, Rice RE, Wiley RL. Generation of Reactive Oxygen Species after Exhaustive Aerobic and Isometric Exercise *Medicine Science, Sports Exercise*, 2000, 32(9): 1576-1581.
20. Duffaux B, Heine O, Kothe A, Prinz U, Rost R. Blood glutation status follovving distance running. Internationel Journal Sports Medicine, 1997, 18: 89-93.
21. Leaf DA. The effect of exercise intensity on lipid peroxidation. Medicine Scients Sports Exercise, 1997, 29(8): 1106–1109.
22. Grisham MB. Reactive meabolites of oxygen and nitrogen in biology and medicine. RG Landes Company. 1992, 5–28.
23. Dernbach AR, Sherman WM, Simonsen JC, Flowers KM, Lamb DR. No evidence of oxidant stress during high ıntensity rowing training. Journal of Applied Physiology, 1993, 74(5): 2140-2145.
24. Selamoğlu S. Aerobik ve anaerbik antrenmanların sporcuların savunma sistemi üzerine etkisi. Sağlık Bilimleri Enstitüsü (Doktora), İzmir: Dokuz Eylül Üniversitesi, 2000.
25. Çelik A, Varol R, Onat T, Dağdelen Y, Tugay F. Akut egzersizin futbolcularda antioksidan sistem parametrelerine etkisi. *Beden Eğitimi ve Spor Bilimleri Dergisi*, 2007, 4: 167-172.
26. Williams, K. J., Tabas, I. (1995) The response-to-retention hypothesis of early atherogenesis. Arterioscler Thromb Vasc Biol 15:551–61.

UNDER PEER REVIEW

27. Berliner, J. A., Navab, M., Fogelman, A. M., et al. (1995) Atherosclerosis: basic mechanisms. Oxidation, inflammation, and genetics. Circulation 91:2488–96.
28. Funda Karabag Coban, Mustafa Akil, Recep Liman and İbrahim Ciğerci. ‘’Effects of Resveratrol on Oxidative DNA DamageInduced by the Acute Swimming Exercise’’ Journal of Pharmaceutical Research International 21(4): 1-10, 2018;
29. Fıçıcılar H, Zergeroğlu AM, Tekin D, Ersöz G. The effects of acute exercise on plasma antioxidant status and platelet response. *Thrombosis Research*, 2003, 111: 267-271.
30. Khanduja, K.L., Gandhi, R.K., Pathania, V. And Syal, N. (1999), “Prevention of NNitrosodiethylamine-induced Lung Tumorigenesis by Ellagic Acid and Quercetin in Mice,” Food and Chemical Toxicology, 37 , 313-318.
31. Akkuş. Đ. (1995), “Serbest radikaller ve fizyopatolojik etkileri,” Mimoza Yayınları, Konya.
32. Rodrigo, R., Rivera, G., Orellana, M., Araya, J., Bosco, C. (2002), “Rat kidney antioxidant response to long-term exposure to flavonol rich red wine,” Life Sci., 71(24), 2881- 2895.

UNDER PEER REVIEW