Abstract
The aim of this study was to examine the effects of anabolic steroids on eccentric exercise-induced kidney tissue, using biochemical and histological methods. Sprague Dawley male rats (n=36) were equally divided into the estrogen (17-β Estradiol, 10 mg/kg per day)-induced and non estrogen-induced groups. Both groups were subdivided into rest groups (n=6), and as exposed to the eccentric exercise groups (n=12) that were also subdivided into decapitated one hour (n=6) and 48 hour (n=6) groups after eccentric exercise. Histopathological effects of estrogen treatment on eccentric exercise-induced rat kidney tissues were seen. The histopathological results revealed that hemorrhage, endothelium-dependent vasodilatations and degenerative changes in proximal tubules, glomeruli, and connective tissue in the estrogen-given and estrogen-given plus eccentric exercise groups. Distal tubule degenerations and dilatations with peritubular capillary dilatations and polymorphonuclear leucocyte (PMNL) infiltrations to connective tissue in eccentric exercise and eccentric exercise plus estrogen-given groups were detected but also it was noticeable decreases in PMNL infiltrations in estrogen-given groups. Biochemical results showed oxidative stress in experimental groups except Control group. It has been concluded that estrogen could not have been effective for preventing eccentric exercise-induced kidney damage and might have toxic effects on kidney tissues on clinical practice however anti-inflammatory effect on kidney tissue detected. It has been requested further investigation.

Keywords: Eccentric exercise, Estrogen, Kidney, Rat

The Effect of Estrogen Treatment on Eccentric Exercise-Induced Damage in Rat Kidneys

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Östrojen Tedavisinin Eksantrik Egzersiz Uygulanan Sıçan Böbrekleri Üzerinde Oluşturduğu Hasara Etkisi

Özet

Anahtar sözcükler: Böbrek, Eksantrik egzersiz, Östrojen, Sıçan

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INTRODUCTION

Physical exercise is an activity that improves and maintains physical fitness, overall health and wellness. Regular moderate exercise has positive effects on the immune system, cardiovascular, nerve, endocrine and renal systems [1-5]. Beside this, doing too much exercise may be harmful. Without enough rest, striated or heart muscle tissue damages may occur that can cause dynamic upper respiratory tract or heart rhythm abnormalities and etc [6,7]. Otherwise, many negative effects like proteinuria, hematuria and changes on renal hemodynamics that may cause acute renal failure may occur [8,9].

Estrogens, a group of compounds, which are female sex hormones, have a major role for both menstrual and ovarian cycles. Estrogens bind to and activate the estrogen receptors (ER) which moderate the expression of many genes when they enter the cell [10-12]. The presence of the ER determines the actions of estrogens in the cell [13]. Estrogens play several important roles in protein synthesis, coagulation, lipid metabolism and structural changes like promoting development of female secondary sex characteristics, accelerating metabolism, increasing fat stores or stimulating endometrial growth [10,11,14,15]. There are also many benefits of estrogens such as preventing role in atherosclerosis via vascular protective action [14,15] and immunological role with anti-inflammatory properties providing migration of polymorphonuclear leucocytes (PMNLs) [15]. Beside this, some researches revealed that estrogen administration can cause various types of DNA damage in estrogen-responsive tissues via free radicals and lipid hydroperoxide-mediated DNA modifications [16].

Researches on the role of estradiol on eccentric exercise-induced rat kidney tissues are limited. The aim of the present study was to assess the role estrogen usage on eccentric exercise-induced rat kidney tissues.

MATERIAL and METHODS

Animals

Thirty six male, 12 weeks old Sprague Dawley rats, weighing 245±22.99 g were kept in facilities accredited by international guidelines. All experimental designs and trials were conducted with the approval of the Instutional Animal Care and Use Committee of Ataturk University (ATADEM-Approval No: B.30.2.ATA.0.23.71-514; 22.04.2011). The rats in groups of six per cage were housed in environmentally controlled room in the conditions of constant temperature/humidity with reversed a 12-hour light/dark cycles. They were allowed free access to food (AIN-93 puried rodent diet) and water. After one day of aclimatization, the animals were divided randomly into six groups, as shown in Fig. 1.

Drug Administration

Animals were injected daily subcutaneously with β-estradiol 3-benzoate (10 μg 0.1 ml of sunflower oil-1.100 g body wt-1) [17] for 30 consecutive days and following the final injection after 24 h the animals were acutely exercised [18,19].

Eccentric Exercise Protocol

A motorized rodent treadmill having an electric shock...
grid was used for modeling the acute exercise. Animals were forced to run on a 15% downhill grade at 20 m·min⁻¹ speed for 90 min [20]. After 1 h or 48 h exercise management all rats were decapitated and dissected. Then their kidney tissues were removed and kept in proper conditions for histological procedures.

Biochemical Analyses

Rat kidney tissues were collected and homogenized with liquid nitrogen in a mortar and were kept at -80°C for the biochemical investigation. Measuring malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione S-transferase (GST) activities were done weighing 15 mg kidney tissue for each group of rats and 1.5 ml of an appropriate buffer for treating. An Qiagen tissue lyser LT homogeniser was used to homogenize the mixtures on ice in eppendorf tubes with steel balls for 15 min. Then homogenates were filtered and centrifuged respectively in a refrigerated centrifuge at 4°C. The supernatants were used to evaluate MDA, SOD, CAT, GPx and GST activities. All analyses were carried out at room temperature.

MDA Level: MDA as an end outcome of lipid per-oxidation that reacts with thiobarbituric acid manufacturing thiobarbituric acid reactive substance (TBARS) was measured. After the occurring of a pink chromogen reaction, MDA level can be measured at 532 nm spectrophotometrically, an MDA standard to create a standard curve against which the sample readings were plotted was used. MDA level was expressed as nmol/min/mg tissue.

SOD Activity: The SOD activity measurement was achieved according to the method proposed by Misra and Fridovich [22] that depends on the generation of superoxide radicals. Superoxide radicals react with nitroblue tetrazolium (NBT) to form formazan dye, produced by the xanthine and xanthine oxidase system. The SOD activities of kidney tissues were identified at 560 nm by the degree of inhibition of NBT reaction. The activity of SOD was expressed as mmol/min/mg tissue.

CAT Activity: CAT activity was defined as the amount of CAT required to decompose 1 nmole of H₂O₂ per minute, at 25°C and pH 7.8 and The kinetic analysis of the activity of CAT was started after the addition of H₂O₂ and at 240 nm the color reaction was measured [23]. CAT activity was expressed as mmol/min/mg tissue.

GPx Activity: GPx activity was measured according to the reaction between glutathione that remains after the action of 5, 5-dithiobis-(2-nitrobenzoic acid) and GPx to form a complex as IU/g wet tissue that absorbs maximally at 412 nm [24]. The activity of GPx of 1 U/mg was defined as 1 μg of GSH consumed μmol/min/g.

GST Activity: The GST activity was assayed according to the method of Habig detected by at 340 nm spectrophotometrically by using 1-chloro-2,4-dinitrobenzene with reduced glutathione which produces a dinitrophenyl thioether and GSH level was expressed as IU/min/g [25].

Histological Analysis

The specimens were fixed in 10% buffered formalin solution for 72 h, tissue processing for the conventional light microscopic technique examination was applied. Tissues were get from water to paraffin wax and sectioned 5 μm thicknesses using a microtome (Leica RM2125RT, Germany). Sections were stained with Hematoxylin & Eosin and photographed under a light microscope with a camera attachment (Nikon Eclipse E600, Japan).

Statistical Analysis

Biochemical parameter results (MDA, SOD, and etc.) were analyzed statistically using SPSS (IBM SPSS Statistics 18.0, IBM Corporation, Somers, NY, USA). Using one-way analysis of variance (abbreviated as one-way ANOVA) followed by Duncan test (P value <0.05 was determined as significant) the numerical data of groups were analyzed. The values were stated as mean ± standard deviation.

RESULTS

Biochemical Results

Biochemical results revealed that there were significant differences between the control and the other experimental groups in the MDA levels of all groups (P<0.05) and the experimental groups did not have significant differences with each other. Control group had the lowest MDA value when compared to other experimental groups. The SOD values were found higher in Control group and lower in Estrg group than in eccentric exercise and eccentric exercise plus in estrogen-given groups. The values of CAT were found higher in Control group and lower in Exc 1 group than estrogen-given and estrogen-given plus eccentric exercise groups. There was not any significant difference in terms of GPx and GST parameters in the experimental groups (Table 1).

Histopathological Results

Control Group: The medulla and cortex parenchyma and stroma were seen normal. The nephrons with Bowman’s capsules and spaces, proximal and distal convoluted tubules, post tubular capillaries, Henle’s loops, and collecting and papillary duct systems were seen in normal architecture. The connective tissue with its properties and the vessels’ epithelium and their tunicas were determined normal (Fig. 2).

Estrg Group: There were scattered hemorrhagic foci in this group. The congested veins and peritubular capillaries were conspicuous. Spaces were detected near the some hemorrhagic foci. Vasoconstrictions in some arteries
were distinguished and the walls of some veins were not conspicuous in which fibrinated blood was seen. Because of increasing in mesangium and dilatations and congestion in the capillaries of glomeruli, it was hard to see renal corpuscles and Bowman's spaces. Degenerative podocytes and proximal convoluted tubule cells were discriminated. The lumens of tubules were very narrow (Fig. 2).

**Exc 1 Group**: It was very distinctive to see the increasing congestions in veins and peritubular capillaries and

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### Table 1. Statistical comparison between all studied biochemical parameters in the kidney tissues of all groups

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SOD (mmol/min/mg)</th>
<th>CAT (mmol/min/mg)</th>
<th>GST (μmol/min/g)</th>
<th>GPx (IU/min/g)</th>
<th>MDA (nmol/min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>881.65±305.37a</td>
<td>373.58±114.50a</td>
<td>0.30±0.09a</td>
<td>24.62±45.40a</td>
<td>193.52±31.96c</td>
</tr>
<tr>
<td>Estrg</td>
<td>522.13±182.80b</td>
<td>282.95±60.48b</td>
<td>0.16±0.05a</td>
<td>53.38±21.60a</td>
<td>243.52±57.25b</td>
</tr>
<tr>
<td>Exc 1</td>
<td>583.11±198.70b</td>
<td>249.13±73.72b</td>
<td>0.20±0.07a</td>
<td>48.79±25.66a</td>
<td>238.50±48.10b</td>
</tr>
<tr>
<td>Estrg + Exc 1</td>
<td>563.52±306.78b</td>
<td>312.11±104.93b</td>
<td>0.28±0.12a</td>
<td>52.63±25.24a</td>
<td>482.64±127.22b</td>
</tr>
<tr>
<td>Exc 48</td>
<td>608.60±205.85b</td>
<td>293.21±95.45b</td>
<td>0.24±0.17a</td>
<td>80.61±54.46a</td>
<td>421.88±110.99b</td>
</tr>
<tr>
<td>Estrg + Exc 48</td>
<td>570.31±210.54b</td>
<td>295.49±51.79b</td>
<td>0.23±0.18a</td>
<td>119.04±63.92a</td>
<td>327.89±90.73b</td>
</tr>
</tbody>
</table>

Means in the same column by the same superscript letter are not statistically significantly different under the LSD test (P<0.05). Results are mean±standard deviation of the mean.

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Fig 2. Micrograph of kidney tissue in lower magnification for all groups, black star- hemorrhage with fibrinated blood, yellow star- dilated peritubular capillaries, thin green arrow- dilated distal convoluted tubules, thin black arrow- degenerated proximal convoluted tubules, thick black arrow- narrowed bowman space that was hard to see, thick green arrow- degenerative areas in parenchyma, a- a necrotic glomerulus, b- a necrotic and hemorrhagic glomerulus, H&E staining.

Şekil 2. Tüm gruplarda böbrek dokulunun mikroskopik görünümü; siyah yıldız- fibrinleşme ile beraber hemoraji, sarı yıldız- dilate olmuş peritüpler kilcal damarlar, ince yeşil ok- dilate olmuş distal kıvrımlı tüberler, ince siyah ok- dejenere olmuş proksimal kıvrımlı tüberler, kalın siyah ok- daalımsız, zor görülebilen Bowman boyluğu, kalın yeşil ok- parankımda dejenertatif alanlar, a- necrotik bir glomerül, b- necrotik ve hemorajik bir glomerül, H&E boyama
degeneration areas in parenchyma. Renal corpuscles were seen with Bowman spaces. There were hemorrhagic foci like in the Estr group, in that area, fibrinoid blood was seen and there were increased fibrosis in peritubular area. There were necrotic podocytes, proximal convoluted cells and especially distal convoluted tubule cells with condensed nuclei. Distal convoluted tubule dilatations were detected, too. The most increased dilatations and disruptions in the epitheliums of peritubular capillaries (they seem like clusters of spaces with dilated distal convoluted tubules) were distinguished in this group. PMNL infiltrations in the area of glomeruli and peritubular capillary dilatations were seen. The congestion and dilatations in the capillaries of glomeruli and increased mesangium were detected in this group, but also that was less than Group Estr. There were degenerations in parietal layer cells and increasing in the connective tissue cells, too (Fig. 2).

**Estr + Exc 1 Group:** There were some hemorrhagic foci like in the Estr and Exc groups. The dilatations in distal convoluted tubule were distinctive like in the Exc 1 group but it was less. There were scattered post capillary tubules' dilatations. Renal corpuscles were seen with Bowman spaces. Degenerative cells with eosinophilic cytoplasms and condensed nuclei in proximal convoluted tubule cells were clearly seen in this group than in other experimental groups. Degenerative cells in glomeruli were discriminated. The hemorrhage in glomeruli and peritubular capillaries were less than Estr and Exc 1 groups. Degenerative podocytes were noticeable. The congestion in and fibrosis around of vessels were seen. There was PMNL infiltration and it was less than the Exc 1 group (Fig. 2).

**Exc 48 Group:** The Bowman spaces were seen. Many of the distal convoluted tubules were dispersed and post capillary tubules were dilated. A few necrotic glomeruli were detected differently (Fig. 2a) The hemorrhagic foci in this group were less and smaller than in Estr and Exc 1 groups but also congestions and fibrinated blood were seen in some post capillary tubules. Disruptions in the epithelium of distal convoluted tubules and post capillary tubules were determined. Some degenerative areas were distinguished in parenchyma. The PMNL infiltrations in the area of glomeruli and peritubular capillary dilatations were seen like Exc 1 group (Fig. 2).

**Estrg + Exc 48 Group:** There were increased glomerular hemorrhage and necrotic glomeruli in this group (Fig. 2b). Dispersed hemorrhagic foci were seen, too. Degenerative areas in parenchyma like Exc 48 group were detected, but also decreased degenerations in tubular cells were distinguished. In addition, dilatations and fibrinated bloods in veins and post tubular capillaries were seen (Fig. 2).

**DISCUSSION**

Regular exercise has been confirmed as a countermeasure to defend and control against cardiac injury, diabetes, mental health and etc [26-29]. On the other hand, acute, intense or prolonged exercises have been stated as a risk factor for infertility, damaging bone structure or others [30,31]. There are also many stated harmful effects of these types of exercises such as abruptly rise in blood plasma calcium concentrations, developing of fatal hypotension, reducing in the number of lymphocytes and suppressing the natural killer cells’ functions in that immunodepression time micro-organisms can pass the first line of defence [31-33]. Variety of studies have revealed that acute physical exercise can trigger a stress response and significant pathological changes, comprising cell apoptosis in the tissues of the distant organs, such as kidney, liver and intestine as well as in working skeletal muscles. During intense exercise, some organs including kidney undergo partial ischemia that causes reducing in the blood supply, after that, tissue reoxygenation and excessive production of ROS occur [34,35]. Through the oxidative stress, apoptosis of various cell types can occur [36]. In previous studies it has been concluded that the apoptosis of the distal tubular and collecting duct cells occur in rat kidney tissues as a response to the acute exercise [37] and intense exercise cause proteinuria and biochemical changes in proximal convoluted tubules [38]. Several defence mechanisms have been demonstrated to decrease the ROS concentration, including both antioxidant enzymes (SOD, CAT, and GPx) and non-enzymatic agents (GSH) in intense exercise- induced kidney tissues [37]. Otherwise endothelium-dependent vasodilatations can occur via increasing production of oxidative stress in humans [39-41].

Estrogen is an important agent for the anti-inflammatory and antithrombotic roles. In addition, estrogen may cause vasomotor dysfunction that is a possible cause for triggering of plaque rupture [42]. It has been observed in as little as 15 min an acute improvement endothelium-dependent vasodilatation occurred in vascular tissues after estrogen treatment that could be related with interaction of the classic steroid-hormone receptor [43]. Estrogen treatment has not only effects the endothelium but also involves extracellular matrix, vascular smooth muscle, and the establishment of collaterals [34,45]. Stumpf et al. [46] have detected estrogen receptors are in interstitial cells, glomeruli and proximal tubule cells in kidney tissues. Estrogen binds to specific cytoplasmic and nuclear receptors [16,47]. Faroqui et al. [48] reported that the management of estrogen to postmenopausal women is related with hypophosphatemia which is associated with a reducing in phosphate reabsorption in the proximal tubules.

In this study, the biochemical results revealed that decreases in antioxidant enzymes' activities in Exc 1 or Estr groups. These results could be associated with acute effects of intense exercise that cause reducing in blood supply, partial ischemia, that give rise to the more production of ROS in Exc 1 group and degenerations in responsive tissues to estrogen like interstitial cells, glomeruli and proximal tubule cells via cytoplasmic or...
nuclear receptors’ interactions in Estrg group. The MDA levels in all experimental groups were significantly higher than in control group, and Estrg+Exc 1 group had the highest value. That could be related to the acute effect of estrogen and exercise together. In histological results in experimental groups except control group hemorrhage occurred but it was more in estrogen-given and estrogen-given plus eccentric exercise group. Hemorrhage could occur due to the antithrombotic effects of estrogen or an acute development of endothelium-dependent vasodilation and alterations in connective tissue due to the response to estrogen. The most dilatation in distal convoluted tubule neighboring peritubular capillaries dilatations that were seen like clusters of spaces and significantly degenerations in distal tubule cells occurred in the Exc 1 group. These results could be related with to the apoptosis of distal tubular cells rejection to acute exercise and the endothelium-dependent vasodilatations through the increased oxidative stress in peritubular capillaries in Exc 1. The most degenerative proximal tubules in Estrg + Exc 1 were discriminated that could be associated with estrogen receptors in proximal tubules triggering molecular alterations via oxidative stress. The PMNL infiltrations were less in Estrg + Exc 1 and Estrg + Exc 48 groups than in the Exc 1 and Exc 48 groups, respectively, and these results had accordance with SOD and CAT activities of Estrg and Exc 1 groups, respectively. Decrease in antioxidant enzymes’ activities indicate oxidative stress process in which PMNL infiltration occur. These results had also revealed that the role of anti-inflammatory effect of estrogen. The other distinctive results; there were increased degenerative areas in parenchyma and necrotic renal corpuses in Exc 48 and especially in Estrg + Exc 48 groups that showed prolonged effects of estrogen and exercise together on kidney tissues could cause damages in parenchyma tissue.

In conclusion, this current study was carried out to examine the role of estrogen on eccentric exercise-induced rat kidney tissue. The researches on this issue are limited. It has been requested further investigation to examine with other histological methods such as immunohistochemical and stereological methods and other biochemical methods such as studying related oxidative stress parameters.

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