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Alliinin Çekal Ligasyon ve Delme (CLP) Kaynaklı Akciğer Hasarına Antioksidan ve Antiinflamatuar Etkileri

The Antioxidant and Antiinflammatory Effects of Alliin on Cecal Ligation and Puncture (CLP)-Induced Lung Injury

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Öz: Amaç: Bu araştırmanın amacı, Alliin'in çekal ligasyonu ve delme (CLP) kaynaklı akciğer hasarı üzerindeki koruyucu etkilerini incelemektir.
Gereç ve Yöntem: Deneyimizde, sıçanlar sham kontrol, CLP, CLP + Alliin 100 mg/kg ve CLP + Alliin 200 mg/kg olmak üzere 4 gruba ayrıldı. Bazı oksidan, antioksidan ve enflamatuar parametreler deney sonunda elde edilen akciğer dokularında değerlendirildi.
Bulgular: Mevcut çalışmada, CLP grubunda oksidan ve inflamatuar parametrelerin arttığını ve antioksidan parametrelerin azaldığını, ancak antioksidan parametrelerin arttığını ve tedavi gruplarında antioksidan parametrelerin azaldığını ve Alliin uygulamasının CLP'nin neden olduğu akciğer oksidatif hasarına karşı koruyucu olduğunu gözlemledik.

Anahtar Kelimeler — Çekal ligasyon ve delme, Alliin, akciğer, oksidatif stres, inflamasyon, sıçan.

Abstract: Purpose: The purpose of this research is to examine protective effects of Alliin on cecal ligation and puncture (CLP)-induced lung injury.
Material and Method: In our experiment, the rats were separated as 4 groups including sham control, CLP, CLP + Alliin 100 mg/kg and CLP + Alliin 200 mg/kg. Some oxidant, antioxidant and inflammatory parameters were evaluated in lung tissues obtained at the end of the experiment.
Findings: In current study, we observed that the oxidant and inflammatory parameters increased and antioxidant parameters decreased in the CLP group but the antioxidant parameters increased and oxidant parameters decreased in treatment groups suggesting that administration of Alliin is protective against CLP-induced lung oxidative damage.

Keywords — Cecal ligation and puncture, Alliin, lung, oxidative stress, inflammation, rat.

INTRODUCTION

Sepsis is a serious inflammatory condition that is currently stemming from pathogenic microorganisms and disrupts organ function [1]. Despite advances in critical care treatment and increased understanding of the pathophysiology of sepsis, the mortality rate of affected patients remains high (40 to 60%) even in developed countries [2]. Sepsis is a collection of disorders associated with infection arising from bacteria, viruses, or fungi. It often leads to an overwhelming response of innate inflammation [3] which results in a densely produced

inflammatory factors such as cytokines [4]. CLP-induced sepsis is one of the powerful experimental methods used in sepsis model. The studies indicates increased tumor necrosis faktör-alpha (TNF-α) and interleukin-6 (IL-6) levels in CLP-induced sepsis [5]. TNF-α is well known as one of the key cytokines mediating inflammatory responses [6]. Inflammatory cascades, aggreved by cytokines, results in increased oxidant biomarker levels [7, 8]. Myeloperoxidase (MPO), one of the inflammatory biomarkers, is released by neutrophils and plays role in these cascades [9]. Lungs are believed to be the first and mostly affected organ due to intra-abdominal sepsis [10]. Fortunately, there are several animal models demonstrating the status of reactive oxygen species (ROS) during cecal ligation and puncture (CLP)-induced sepsis in lung injury [11, 12]. An increase in the ROS level triggers apoptosis and inflammation even at the cellular level [13]. In this process, due to the cell membrane lipid oxidation, oxidant markers (MDA, etc) increase [14, 15]. While total antioxidant status (TAS) is used as an indicator of total antioxidant activity, total oxidant status (TOS) is a strong marker to determine the total oxidant activity. Oxidative stress index (OSI) is a value indicating the balance in the current oxidative status [16, 17].

Recent studies have shown that under the control of inflammation in many inflammatory diseases, including sepsis, survival and natural antioxidant agents administration is effected which are efficient against sepsis [18, 19]. Alliin (S-allyl cysteine sulfoxide, C6H11NO3S) is, one of the antioxidant agents, an important organosulfur compound derived from garlic [20]. Recent studies have shown that alliin has improving properties such as antioxidant, antiinflammatory, antidiabetic, and anti-aggregation effects [20, 21, 22, 23, 24].

In present study, we evaluated the effects of alliin, which has various biological properties such as antioxidant, antiinflammatory in lung in order to alleviate the oxidative damage in the CLP model in rats.

2.MATERIAL AND METHODS

Experimental Animals and Ethical Approval

Atatürk University Experimental Animal Ethics Committee approved (28.03.2019/64) the experiments of our study where was performed at Atatürk University Experimental Animals Research and Application Center. Male rats of Wistar albino species, obtained from Atatürk University Experimental Animals Research and Application Center were kept in polypropylene cages in standardized conditions such as 12 light/12 darkness, temperature of

22±2 °C, humidity of 55±5 %, and feed and water. 12 hours before experiment food consumption was not allowed to animals but it was free to drink water.

Experimental Animals and Experimental Design

For our experiments we used 32 healthy male rats (240-270 gr) which were randomly assigned to 4 groups in which each includes 8 subjects. The rats in group 1 (Sham control group, n=8) had 2 cm incision at the abdominal area to reach the peritoneum. Then incision was closed with a 3.0 silk suture without any procedure. The rats in group 2 (CLP group, n=8) had their cecum isolated after reaching their peritoneum through 2 cm incision. Following that, ileocecal valve was ligated up to 2 cm distally, and pierced by 18-gauge needle (4 holes). Then the cecum was put back to the abdomen and abdomen was closed with 3.0 silk suture. The rats of group 3 (100 mg/kg alliin+CLP group, n=8) had alliin administiration intraperitoneally in low dose (100 mg/kg) 30 minutes before the same CLP model in group 2. The rats of group 4 (200 mg/kg alliin+CLP group, n=8) was administered alliin intraperitoneally in high dose (200 mg/kg) 30 minutes before the same CLP model in group 2. In the CLP groups (group 2, 3, 4), the abdominal regions were washed with povidone-iodine after being shaved. Analgesic lidocaine solution was applied to the suture areas in order to prevent pain stress of the rats to remove the error margin. As postoperatively the rats had no food, but was free to reach water for 18 hours until they were sacrificed.

Biochemical Assessments

TAS and TOS are evaluated by a commercial kit (Rel Assay Diagnostics). OSI which demonstrates the TOS to TAS ratio was calculated as follows: OSI = [(TOS, μmol H_2O_2 equivalent L)/(TAS, mmol Trolox equivalent/L) × 10]. We preferred OSI as other oxidative stress indicator. The evaluation of superoxide dismutase (SOD) was predicated on superoxide radicals production. Those radicals are generated by xanthine oxidase system which performs reaction with nitroblue tetrazolium for formazan dye formation [25]. Malondialdehyde (MDA) is used to measure the amount of lipid peroxidation in lung tissue through thiobarbituric acid test [26]. The activities of MPO in the lung tissues were estimated due to the methods described by Bradley et al. [27].

Statistical Analysis

All the results have been presented as mean ± SD (standard deviation). Results heve been analyzed by One-way ANOVA and then Tukey test for pairwise comparisons of groups. The differences heve been approved significant when $p<0.05$.

3.RESULTS

It has been no morbidity or mortality in rats during experimental applications. When the CLP group compared to the sham control group, TAS (from 0.844±0.094 to 0.271±0.037, p=0.000) level decreased, whereas the TOS (from 7.099±0.718 to 11.497±0.760, p=0.000), OSI (from 0.852±0.142 to 4.316±0.747, p=0.000) levels increased. When the CLP + 100 mg/kg alliin group, compared to the CLP group, TAS (from 0.271±0.037 to 0.733±0.046, p=0.000) level increased, while TOS (from 11.497±0.760 to 8.415±0.575, p=0.000), OSI (from 4.316±0.747 to 1.151±0.101, p=0.000) levels decreased (Table 1).

When CLP + 200 mg/kg alliin group, compared to the CLP group, TAS (from 0.271±0.037 to 0.817±0.069, p=0.000) increased, while TOS (from 11.497±0.760 to 7.536±0.834, p=0.000) and OSI (from 4.316±0.747 to 0.930±0.149, p=0.000) levels decreased statistically significantly. In the CLP + 200 mg/kg alliin group, TAS levels (p=0.013) were higher but TOS (p=0.028) and OSI (p=0.004) levels were lower than the CLP + 100 mg/kg alliin group (Table 1).

Table 1: TOS, TAS and OSI levels comparisions among the experimental groups.

Experimental Groups (n=8)	TAS	TOS	OSI
Sham control (1)	0.844±0.094	7.099±0.718	0.852±0.142
CLP(2)	0.271±0.037	11.497±0.760	4.316±0.747
CLP+Alliin 100 mg/kg (3)	0.733±0.046	8.415±0.575	1.151±0.101
CLP+Alliin 200 mg/kg (4)	0.817±0.069	7.536±0.834	0.930±0.149
p value (Meaningful intergroup comparisons)	0.000 (1-2) 0.010 (1-3) 0.000 (2-3) 0.000(2-4) 0.013 (3-4)	0.000 (1-2) 0.001 (1-3) 0.000 (2-3) 0.000 (2-4) 0.028(3-4)	0.000(1-2) 0.000 (1-3) 0.000 (2-3) 0.000 (2-4) 0.004 (3-4)

TAS = Total Antioxidant Status; TOS = Total Oxidant Status; OSI = Oxidative Stress Index. Data are presented as mean ± S.D. $p<0.05$.

When the CLP group compared to the sham control group, SOD (from 284.716±17.744 to 193.710±20.898, p=0.000) level decreased, while MPO (from 155276.845±23418.744 to 382656.553±38649.132, p=0.000), MDA (from 57.191±6.647 to 92.179±11.357, p=0.000), TNF-α (from 20277.135±1624.811 to 81107.189±5346.898, p=0.002), and IL-1β (from 23799.306±1744.156 to 98889.863±6808.398, p=0.002) levels increased. When the CLP + 100 mg/kg alliin group, compared to the CLP group, while the level of SOD (from 193.710±20.898 to 245.202±35.384, p=0.003) increased, MPO (from 382656.553±38649.132 to 213298.825±24232.652, p=0.000), MDA (from 92.179±11.357 to 63.014±7.941, p=0.000), TNF-α (from 81107.189±5346.898 to 23892.692±2847.935, p=0.000), and IL-1β (from 98889.863±6808.398 to 24533.186±2329.884, p=0.000) levels decreased (Table 2).

When the CLP + 200 mg/kg alliin group, compared to the CLP group, while the level of SOD (from 193.710±20.598 to 271.120±21.588, p=0.000) increased, MPO (from 382656.553±38649.132 to 173187.873±20069.664, p=0.000), MDA (from 92.179±11.357 to 56.979±4.296 ; p=0.000), TNF-α (from 81107.189±5317.898 to 22302.043±1417.417, p=0.000), and IL-1β (from 98889.863±6808.398 to 22578.180±1111.485; p=0.000) levels decreased (Table 2).

Table 2: Comparisons of other oxidative markers and cytokines among the experimental groups.

Experimental Groups (n=8)	SOD	MPO	MDA	TNF-α	IL-1β
Sham control (1)	284.716±17.744	155276.845±23418.744	57.191±6.647	20277.135± 1624.811	23799.306±1744.156
CLP(2)	193.710±20.898	382656.553±38649.132	92.179±11.357	81107.189± 5346.898	98889.863±6808.398
CLP+Alliin 100 mg/kg (3)	245.202±35.384	213298.825±24232.652	63.014±7.941	23892.692±2847.935	24533.186±2329.884
CLP+Alliin 200 mg/kg (4)	271.120±21.588	173187.873±20069.664	56.979±4.296	22302.043±1417.417	22578.180±1111.485
p value (Meaningful intergroup comparisons)	0.000 (1-2) 0.014 (1-3) 0.003 (2-3) 0.000(2-4)	0.000 (1-2) 0.000 (1-3) 0.000 (2-3) 0.000 (2-4) 0.003(3-4)	0.000(1-2) 0.000 (2-3) 0.000 (2-4) 0.008 (3-4)	0.002(1-2) 0.008 (1-3) 0.019 (1-4) 0.000 (2-3) 0.000 (2-4)	0.002(1-2) 0.000 (2-3) 0.000 (2-4) 0.050 (3-4)

SOD=Superoxide Dismutase; MPO=Myeloperoxidase; MDA=Malondialdehyde; TNF-α=Tumor necrosis factor-α; IL-1β=Interleukin-1β. Data are presented as mean ± S.D. $p<0.05$.

When the results are evaluated, a great similarity is observed between the control group and the treatment groups.

4.DISCUSSION

Septicemia is an uncontrolled hyper-inflammatory response and and a devastating cause of mortality [2, 28]. Despite the advancements in anti-microbial drugs; sepsis and septic shock still keep being a tough issue for clinicians. The annual morbidity due to sepsis reaches 50–95 cases per 100,000 citizens in USA [29]. The diagnosis of sepsis is avalaible if there are clinical evidences of systemic inflammation. The most common body field of infection is the lungs, composing 40% of all body involvement [30, 31]. The pulmonary inflammation due to excessive production of ROS, acute lung injury (ALI) is a world-wide sepsis complication [32].

Among the several murine models of sepsis, CLP is a commonly used animal model leading to systemic inflammation in which controlled bowel perforation following cecal ligation occurs [33, 34, 35]. Infection may lead to sepsis due to bacterial translocation, mostly originated from spleen, liver or mesenteric lymph nodes. Sepsis activates inflammatory response initially via inflammatory mediators released by cytokines [36, 37]. Live body needs

inflammation, because it is the preservative response of immune system which mostly acts against microorganisms [38]. Uncontrolled or intense inflammation leads to diseases [39]. Proinflammatory cytokines such as TNF-α, IL-1β are considered as pivotal mediators for sepsis-induced lung injury [40, 41]. MDA occurs after lipid peroxidation. SOD is the only antioxidant enzyme that scavenges superoxide [42, 43]. ROS occurs as a result of aerobic metabolism and can be removed thanks to antioxidant enzymes such as SOD [44]. ROS decreases natural antioxidants, glutathione (GSH) and SOD [45].

Studies of the CLP-sepsis model in vivo showed increased ROS levels in lung tissue [46]. ROS triggers the endogenous antioxidant defense system. As a result of this cascade, as seen in our results, the concentration of SOD decreased in tissue [47, 48]. The intense release of ROS causes lipid peroxidation and the increases MDA concentration, which is the final product of unsaturated lipid oxidation. MDA also indicates oxidative damage indirectly [49]. Our results were correlate with this data [50]. MPO also increased in our study. MPO is determined intensively in neutrophils, and high concantration of MPO indicates neutrophil activation [51]. This result plays major role in the etiopathogenesis of ALI / acute respiratory distress syndrome (ARDS) [52]. Clinical and experimental studies support that antioxidant administration looks like helpful in septic states in which the lungs are the most affected remote organs [12, 53].

Many alliin-related studies are available in the literature supporting the results of our study. Alliin has shown an antiinflammatory effect by reducing TNF-α, IL-1β and MPO levels in a study [54]. Alliin reduced inflammation by reducing TNF-α and MDA levels during DENV infection [55]. Alliin decreases MDA, MPO levels and heals dextran sulfate sodium-induced ulcerative colitis and stops the inflammatory responses in lipopolysaccharide-stimulated RAW264.7 cells [56]. AHE (contains organosulfur composites like alliin, S-allylcysteine, etc.) considerably inhibited NO, cytokines and ROS production in lipopolysaccharide-induced RAW264.7 cells [23]. Alliin hindered the increment of proinflammatory gene (IL-6, TNF-α) expression in lipopolysaccharide- stimulated 3T3-L1 adipocytes [57]. Alliin attenuated nuclear factor-κB ligand (RANKL)-induced osteoclastogenesis receptor activator by scavenging ROS [24]. The study showed the protective effect of alliin on isoproterenol-induced cardiotoxicity in Wistar albino male rats by increasing antioxidants [58, 59]. Alliin exhibited antioxidant activities as protective compounds against free radical damage [60]. In parallel with these studies, in our study, antioxidant and antiinflammatory properties of alliin have been shown in CLP-induced sepsis model in rats. In the CLP group, TAS and SOD

decreased while MDA, MPO, TNF-α, IL-1β, TOS, OSI levels were increased and alliin treatment reversed these levels.

Due to our results, reduction of TNF-α, IL-1β concentrations in septic rats by alliin, suggesting that alliin alleviated CLP-induced ALI. We assessed oxidative stress in the lung tissue to investigate the improving effects of the alliin against CLP-induced lung injury and observed that oxidative stress decreased with alliin. The fact that there is no study related with the protective effects of alliin in the literature review of CLP-induced sepsis makes this study original.

Understanding of cellular damage mechanisms of sepsis is important for planning new and effective treatment methods. Sepsis studies demonstrated that inflammation and oxidative stress suppression can provide significant contributions to the sepsis treatment. In our study, inflammation, oxidative stress pathways are suppressed by alliin and this encourages hope in the treatment of sepsis.

5.CONCLUSIONS

Alliin provides a protection against lung injury arising from CLP-induced sepsis via its antioxidant and antiinflammatory properties. We have indicate that treatment with alliin at different doses reduces lung damage in experimental animals exposed to CLP-induced sepsis model. Moreover, further researches are necessarry for explain the other protective mechanism on lung tissue damage induced by sepsis.

Conflict of interest: The authors declare that there are no conflicts of interest.

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Sıçanlarda Periferal Adropin Uygulamasının Hipotalamik Hipofizer Adrenal Aks Üzerine Etkisi

The Effect of Peripheral Adropin Application on Hypothalamic Pituitary Adrenal Axis in Rats

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Öz: Amaç: Hipotalamik hipofizer adrenal (HPA) aks, strese ve inflamatuvar faktörlere yanıt oluşturmak gibi birçok görevlere sahiptir. HPA aksı, primer stres yanıt sistemidir. Adropin, enerji homeostazıyla ilişkili gen tarafından kodlanan peptit yapılı bir hormondur. Bu araştırmada adropin hormonunun HPA aksı üzerindeki histopatolojik ve immünohistokimyasal etkileri incelenmiştir.
Gereç ve Yöntem: Çalışmada otuz iki (32) Wistar Albino erkek sıçan kullanıldı. Sıçanlar 4 eşit gruba ayrıldı (n=8). Kontrol grubuna herhangi bir uygulama yapılmadı ve sham grubuna adropin çözücüsü verildi. Adropin tedavi gruplarına 4 µg/kg ve 40 µg/kg dozlarında intraperitoneal olarak uygulandı. Çalışma 10 gün sürdü. 11. günde hayvanlar sakrifiye edildi ve ilgili doku örnekleri toplandı.
Bulgular: Adropin uygulanan gruplarda kortizol, adrenalin, noradrenalin ve serotonin düzeyleri azalırken, dopamin seviyeleri arttı. Melatonin seviyelerinde önemli bir değişiklik oluşmadı. İmmünohistokimyasal boyamanın bir sonucu olarak, CRH, adropin gruplarında diğer gruplara göre artış göstermiştir.

Anahtar Kelimeler — Adropin, HPA Aksı, Hormon, İnflamatuvar, Stres.

Abstract: Purpose: Hypothalamic pituitary adrenal (HPA) axis has many missions such as responses to stress and inflammatory factors. HPA axis is the primer stress response system. Adropin is a peptid structured hormone coded by energy homeostasis related gene. In this research biochemical, histopathologic and immunohistochemical effects of adropin hormone on HPA axis were examined.
Material and Method: Thirty two (32) Wistar Albino male rats were used. The rats were separated into 4 equal groups (n=8). The control group did not receive any applications; and the sham group was given adropin-dissolvent. Adropin was administered as intraperitoneal to the treatment groups at the doses of 4 µg/kg and 40 µg/kg. The study lasted 10 days. On the 11th day, the animals were sacrified, and relevant tissue samples were collected.
Findings: While cortisole, adrenaline, noradrenaline and serotonin levels decreased, contrary dopamin levels increased in adropin administered groups. There were no important changes on melatonin levels. As a consequence of immunohistochemical staining, CRH has shown increase in adropin groups compared to other groups.

Keywords — Adropin, HPA Axis, Hormone, Inflammatory, Stress.

INTRODUCTION

Hypothalamic pituitary adrenal (HPA) axis has many missions such as responses to stress and inflammatory factors (1). It has three parts; hypothalamus, pituitary and adrenal gland. The HPA axis consists of three types of cells and hormones which are released from them: 1) Corticotropin releasing hormone (CRH) released from paraventricular nucleus (PVN), 2) Adrenocorticotropic hormone (ACTH) released from endocrine cells (corticotrophs) in the anterior pituitary and 3) Cortisol and/or corticosterone (CORT) which are glucocorticoid hormones released from endocrine cells in the zona fasciculata region of the adrenal cortex (2).

CRH is the main component of the HPA axis. ACTH stimulates the production and secretion of the steroid hormones such as glucocorticoids (3). Glucocorticoids are hormones, which are synthesized and released by the adrenal gland (4).

One feature of the activation of the HPA axis is that it provides adrenaline secretion from the adrenal medulla (5). Adrenaline stimulates central afferents, which are associated with locus coeruleus to induce the release of noradrenaline in various brain areas (6). Noradrenaline provides HPA activation by stimulating cells containing CRH in hypothalamic PVN (7).

The D1 and D2 receptors in the medial prefrontal cortex in the dopamine system modulate the HPA axis activation (8, 9). Serotonin (5-hydroxytryptomine or 5-HT) has a stimulatory effect on the HPA axis (10). Melatonin has shown an inhibitory effect on ACTH in human adrenal gland (11).

Adropin (Adr) was discovered by studies conducted in mice by Kumar et al. (12, 13). It is encoded by Enho, which is expressed in the liver and brain (12). In addition to pancreas, liver, brain and kidney tissues, it also has been shown in endocardium, myocardium and epicardium (14). The presence of Adr in the central nervous system suggests that it has a function as a neuropeptide. Furthermore, the role of autocrine/paracrine is also possible (15).

We investigated the effects of peripheral Adr on the HPA axis by applying it on rats. In the literature, we have determined that Adr is examined in studies such as energy metabolism, blood pressure, preeclampsia, polycystic ovary, breast cancer etc, yet no study about the effects on hypothalamic pituitary adrenal axis has been found.

Therefore, instead of making a stress model on HPA axis, as a starting point, we have searched what kind of effects will be seen by administering Adr hormone under normal circumstances and by examining some important hormones and tissues, which act in this axis, biochemically and histopathologically. In this respect, we believe that the results to be obtained from this research will provide both new and useful information for the literature in terms of representing a first and investigating the effects of Adr hormone on different areas.

2. MATERIALS AND METHODS

Atatürk University Experimental Animal Research and Application Center (ATADEM), Atatürk University Faculty of Medicine and Atatürk University Veterinary Faculty laboratories have been used in order to carry out the study.

The research was approved by Atatürk University Ethics Committee of Experimental Animals (19.04.2016 dated and decree no.2). All procedures in the experiment were carried out in accordance with the protocol in the ethics committee.

2.1 Animals

32 Wistar Albino male rats (Experimental Animal Research and Application Center of Ataturk University, Erzurum, Turkey) were used in the experiment. Their average weight was 300±15 g. Animals were randomized into four groups (n = 8); control group, sham group (300 µl-i.p.) pure water, low dose Adr (4 µg/kg-i.p.) group and high dose Adr (40 µg/kg-i.p.) group.

During the experiment, the animals were kept at 21±1 ° C medium. The experimental medium was prepared as 12 hours light and 12 hours dark. Animals were fed as *ad libitum* and standard rat feed was used. Tap water was preferred as drinking water.

2.2 Preparation and Application of Adr

Adr hormone obtained from Phoenix pharmaceuticals (USA) was used in this experiment. It was maintained at -20 ° C under appropriate conditions until the administration was carried out. At the beginning of the experiment, the amount to be used in each injection was dissolved in pure water.

The injection dose was 300 µl for a 300 g animal. Sham group was treated with 300 µl of Adr solvent (pure water). In low and high dose Adr groups, 4 µg/kg and 40 µg/kg, respectively, Adr hormone was administered by dissolving in pure water. Injections were administered as intraperitoneal (i.p.) for all groups at the same period intervals each day.

2.3 Completing the Experiment and Taking Samples

After 10 days of application, the animals were sacrificed. Adrenal gland, hippocampus, hypothalamus and blood samples were taken. Blood samples were centrifuged at 5000 rpm for 10 min and maintained at -80°C until the Enzyme-Linked ImmunoSorbent Assay (ELISA) kit analysis was carried out. Tissue samples taken from the hippocampus, hypothalamus and adrenal gland were kept in 10% formaldehyde solution for histopathological and immunohistochemical studies.

2.4 Hormone Analysis

Melatonin, serotonin, dopamine, cortisol, adrenaline and noradrenaline kits (Elabscience, Chinese) were used for hormone analysis. The kits were analyzed in ELISA reading device (ELISA, BioTEK powerwawe XS Winooski, U.K) by following the company protocols.

2.5 Histopathological Procedures

Adrenal tissues were detected in a 10% buffered formaldehyde solution. The tissues were washed in tap water and the formaldehyde was removed. Subsequently, routine tissue follow-up was performed (Shandon citadel 2000, Thermo). The followed tissues were embedded in paraffin and 5 μm sections were taken from these paraffin blocks with microtome (Leica RM2255/England) put to normal and polylysine slides.

Tissues taken to the normal slides were stained with hematoxylin eosin and (Olympus BX51, camera attachment DP72) was evaluated with light microscope as no (-), mild (+), moderate (++) and severe (+++) depending on the presence of the lesion.

2.6 Immunohistochemical staining

After deparaffinization of the tissues taken on the polylysine slide had been completed, it was kept for 10 min in 3% H_2O_2 to inactivate the endogenous peroxidase and was washed in Phosphate Buffer Saline (PBS). Then it was kept for 10 min at 500w in antigen retrieval solution to reveal the antigens in the tissues and washed in PBS. To prevent nonspecific binding, protein block solution was added and washed in PBS. Corticotropin releasing factor (CRF) Antibody (H-104) (Santa Cruz, Cat. No: sc-10718) was applied at 1/100 dilution ratio as primary antibody to PBS washed sections. Afterwards, the procedure specified by the Expose mouse and rabbit specific HRP / DAB detection IHC kit (abcam: ab80436) was followed. 3,3’ diaminobenzidine chromogen was used and was stained contrastly with Mayer’s hematoxylin. Positive cells were examined at 20x magnification in light microscope.

2.7 Statistical analysis

Statistical analysis of biochemical data was performed using IBM SPSS statistics 20.0 program. The normality of the obtained data was evaluated statistically. Hormone levels taken from blood plasma were evaluated by Bonferroni correction one-way variance analysis. The values were presented as mean ± standard error. $p<0.05$ was considered as statistically significant.

SPSS 16.0 program was used for statistical evaluation of histopathological data. Kruskal Wallis test was used to determine the between-groups difference of the data obtained semiquantitatively in histopathological examination, Mann Whitney U test was used to determine the groups, which form the difference. $p <0.05$ was considered as statistically significant.

3. RESULTS

3.1 Biochemical Results

The cortisol levels varying with i.p. Adr administration are shown in Figure 1. Plasma cortisol level has decreased in the high dose Adr group compared to the control group (* $p<0.05$, n=8).

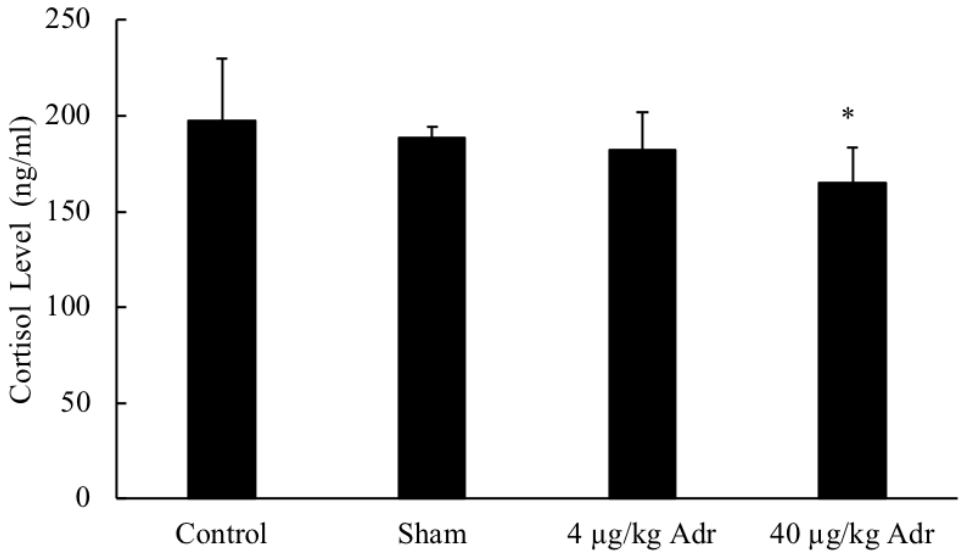

Figure 1 Effects of Adr administration on cortisol levels. Bonferroni correction one-way analysis of variance has been used to evaluate the data. Values have been presented as mean ± standard error (* $p <0.05$, n = 8).

In Figure 2, adrenaline levels, which changed with Adr administration, are shown. Plasma adrenaline levels have decreased in the high dose Adr group compared to the control group (* $p<0.05$, n=8).

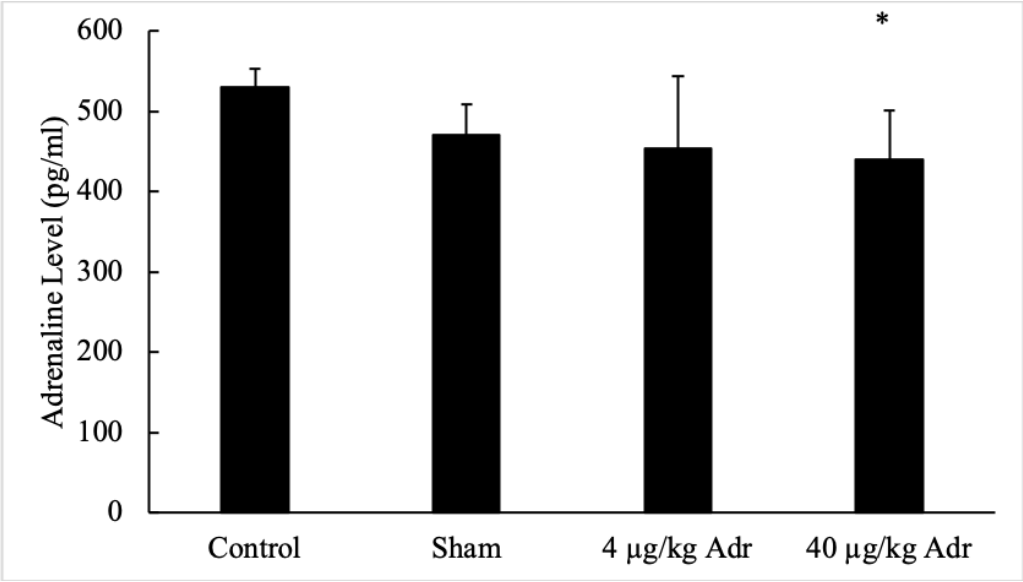

Figure 2 Effects of Adr administration on adrenaline levels. Bonferroni correction one-way analysis of variance has been used to evaluate the data. Values have been presented as mean ± standard error (* $p < 0.05$, $n = 8$).

In Figure 3, varying noradrenaline levels with Adr administration are shown. Plasma noradrenaline levels have decreased in the high and low dose Adr groups compared to the control and sham groups (* $p<0.01$, $n=8$).

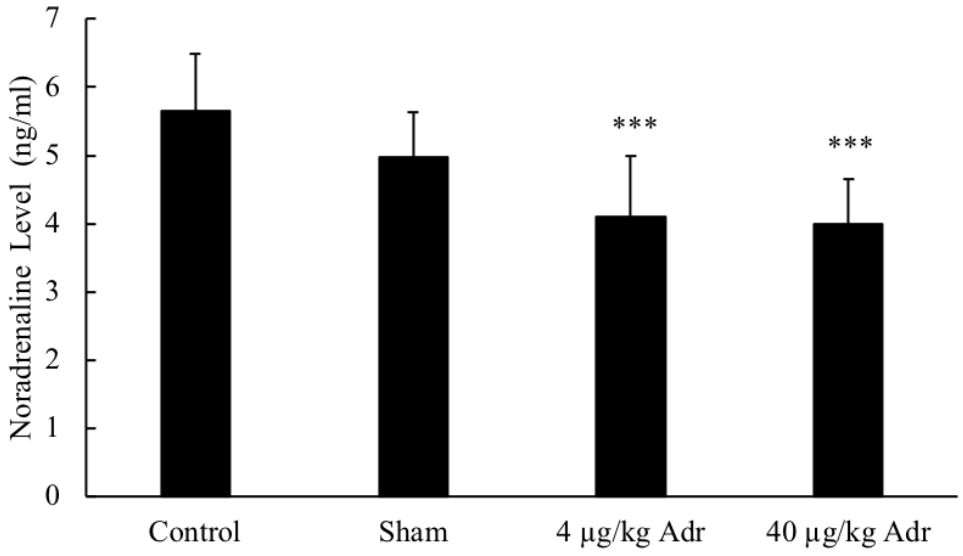

Figure 3 Effects of Adr administration on noradrenaline levels. Bonferroni correction one-way analysis of variance has been used to evaluate the data. Values have been presented as mean ± standard error (* $p < 0.001$, $n = 8$).

The dopamine levels varying with Adr administration are shown in Figure 4. Plasma dopamine levels have increased in high-dose Adr group compared to sham and low-dose groups (* $p<0.05$, n=8).

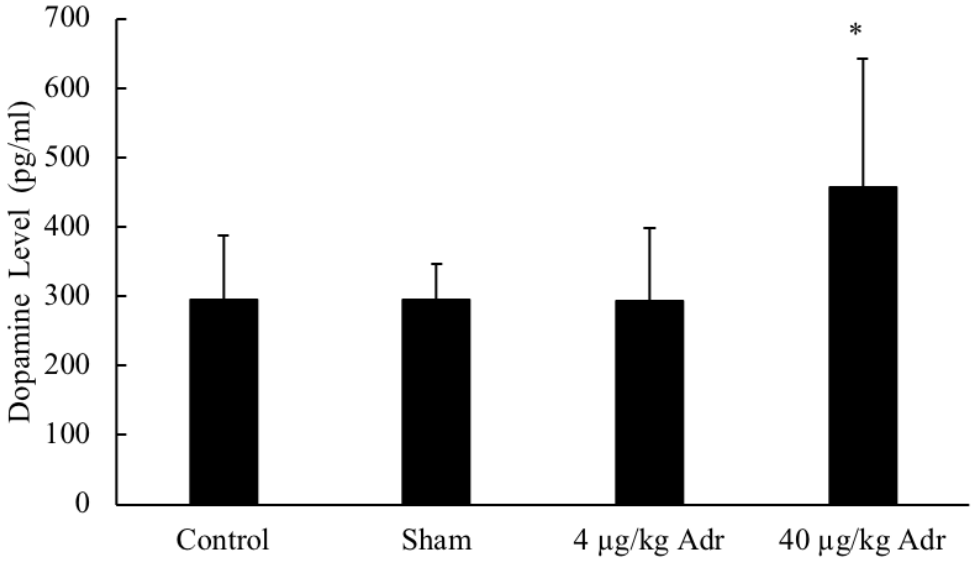

Figure 4 Effects of Adr administration on dopamine levels. Bonferroni correction one-way analysis of variance has been used to evaluate the data. Values have been presented as mean ± standard error (* $p<0.05$, n = 8).

Figure 5 includes serotonin levels changing with Adr administration. Plasma serotonin levels have decreased in high and low dose Adr groups compared to control and sham groups (* $p<0.05$, n=8).

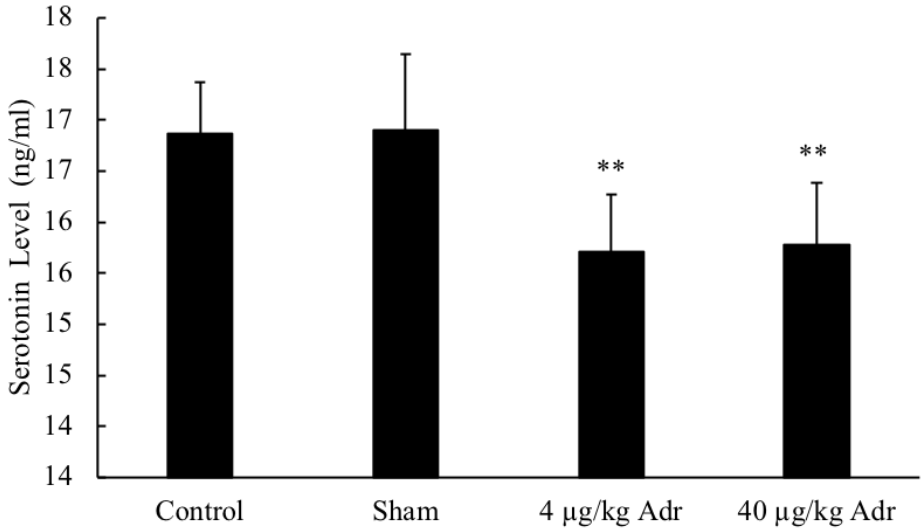

Figure 5 Effects of Adr administration on serotonin levels. Bonferroni correction one-way analysis of variance has been used to evaluate the data. Values have been presented as mean ± standard error (* $p<0.05$, n = 8).

The levels of melatonin, which are changed by Adr administration, are shown in Figure 6. No significant change has determined among the groups (n=8).

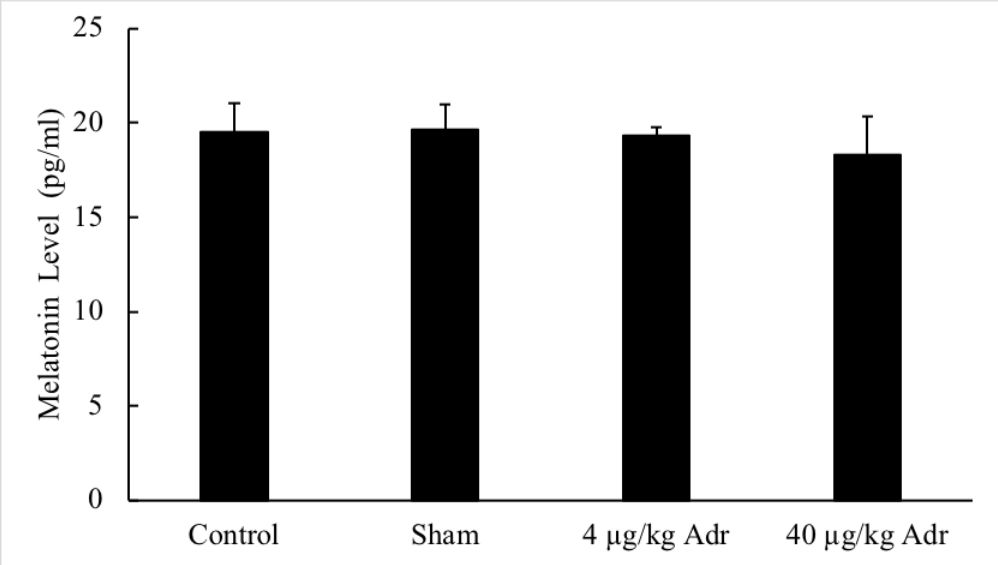

Figure 6 Effects of Adr application on melatonin levels. Bonferroni correction one-way analysis of variance has been used to evaluate the data. Values have been presented as mean ± standard error (n = 8).

3.2 Histopathological Results

Here, which the effect of Adr on adrenal gland tissue has been examined, the adrenal glands are observed to have normal histological structure in the control and sham groups (Figure 7.1., Figure 7.2). Severe hydropic degeneration has been observed in adrenal cortex cells in low-dose and high-dose Adr-treated groups (Figure 7.3., Figure 7.4.).

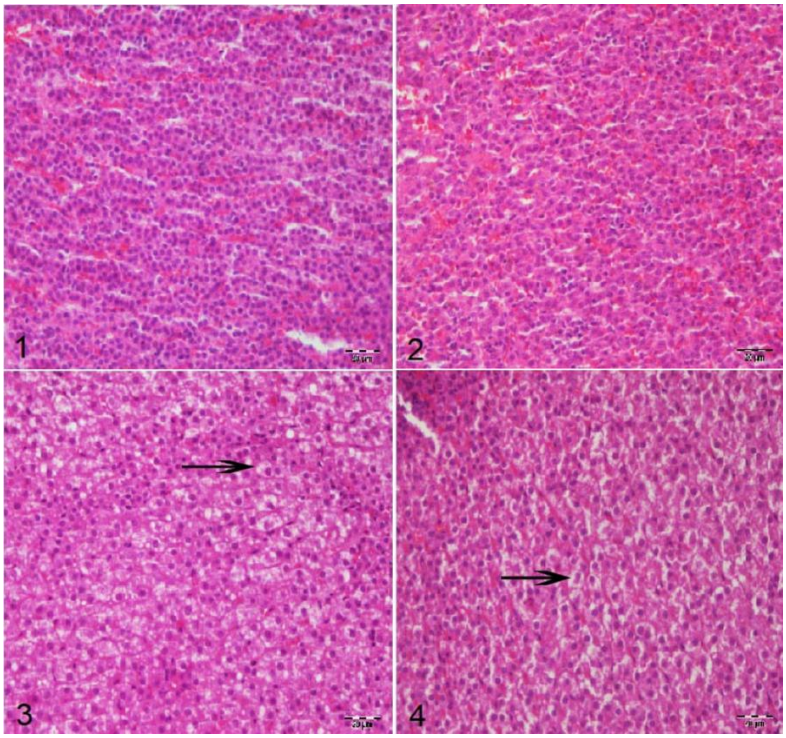

Figure 7 Effects of Adr application on adrenal gland tissues, 1. Control group. Normal histological appearance, 2. Sham group. Normal histological appearance, 3. 4 µg/kg Adr group. Severe hydropic degeneration in the adrenal cortex cells, (arrow) 4. 40 µg/kg Adr group. Severe degeneration of adrenal cortex cells (arrow)

Figure 8 shows the effects of Adr administration on the hippocampus and hypothalamus. As a result of immunohistochemical staining, an equal level of CRH immunopositivity has been found in the hippocampus region in the glial cells of all groups (Fig 8a, c, e, g). CRH has been observed moderately high in the neurons of the hypothalamus in the control group (Fig 8b). In the sham group, a mild immunopositivity has been determined (Fig 8d). Increase in CRH immunopositivity is observed in low and high doses of Adr compared to other groups (Fig 8f, h). The most intense immunopositivity has been observed in high dose administration of Adr (Fig 8h).

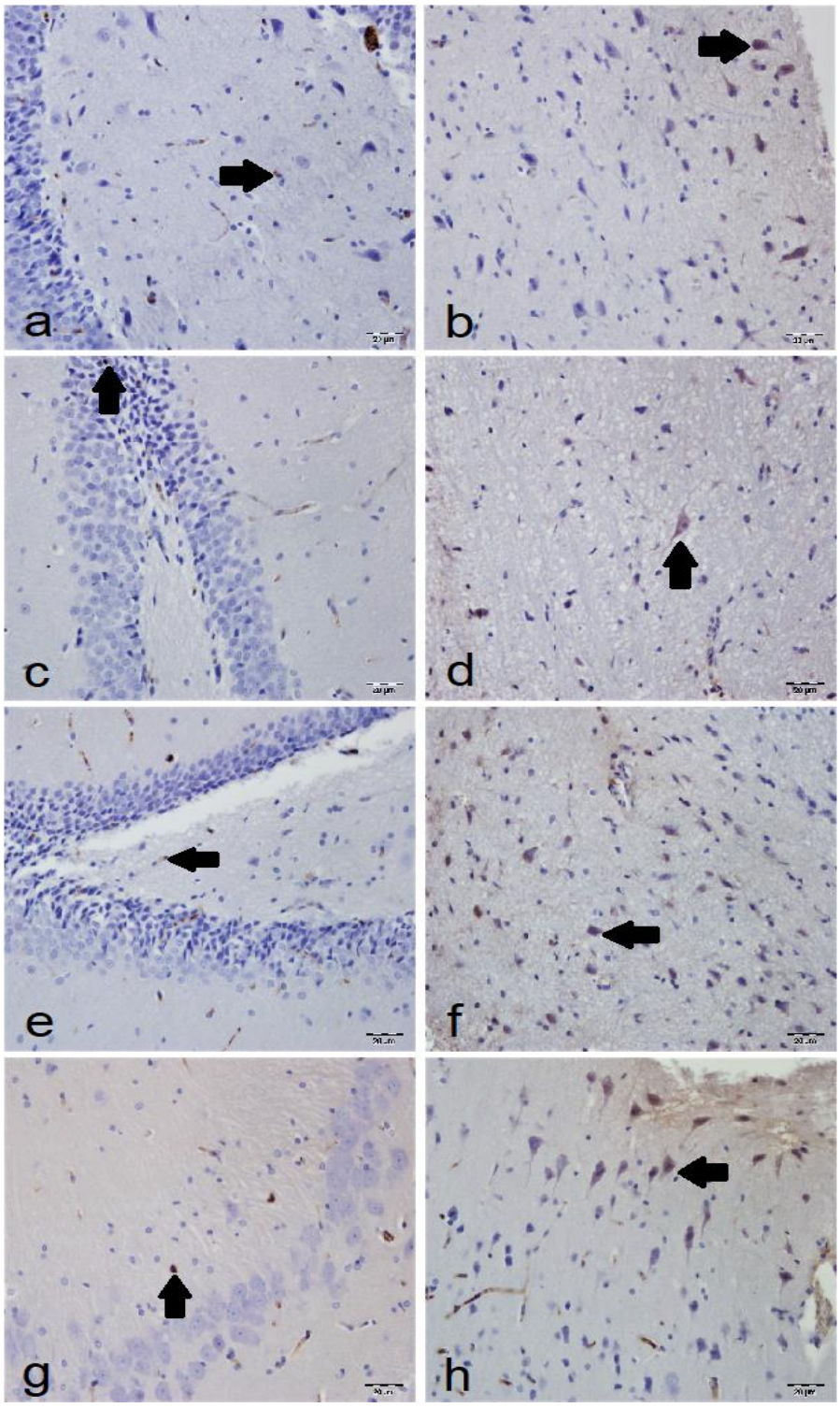

Figure 8 a) Control group, CRF immunopositivity in glial cells, arrow b) Control group, moderate CRF immunopositivity in neurons in the hypothalamus region, arrow c) Sham group, CRF immunopositivity in glia cells, arrow d) Sham group, mild CRF immunopositivity in neurons in hypothalamus region, arrow e) 4 µg/kg Adr, CRF immunopositivity in glia cells, arrow f) low dose of Adr, intense CRF immunopositivity in neurons in the hypothalamus region, arrow g) high dose of Adr, CRF immunopositivity in glia cells, arrow h) 40 µg/kg Adr, intense CRF immunopositivity in neurons in the hypothalamus region, arrow. (IHCx40)

3.3 Statistics Results

In histopathological examination, a significant difference was observed among the high and low dose group and the control and sham group in terms of degenerative changes (Table 1, $p<0.05$). No significant difference was observed between high dose and low dose groups (Table 1).

Table 1 Degenerative changes in the adrenal gland as a result of Adr application. Different letters (a, b) in the same column indicate intergroup differences ($p <0.05$).

	Hydropic Degeneration
Control	0.60 ± 0.24^{a}
Sham	0.80 ± 0.37^{a}
4 µg/kg Adr	2.20 ± 0.37^{b}
40 µg/kg Adr	2.40 ± 0.40^{b}

4. DISCUSSION

Adr, which is composed of Latin "adura'' (to throw into fire) and bir 'pinquis' (fats or oils), is a hormone which has a role in energy homeostasis (12). Adr is a newly discovered neuropeptide that is synthesized and released essentially from the brain and liver in rodents (16). Li et al. have observed that Adr regulates cardiovascular function and shows a protective effect against the development of cardiovascular diseases (17). In their study of dietary-induced obese mice, Gao et al. have found that Adr increases glucose tolerance, improves insulin resistance, and provides the primary use of carbohydrates (18). Shahjouei et al. reported that Adr is associated with many central nervous system diseases (15).

In our research, the levels of cortisol, adrenaline, noradrenaline, dopamine, serotonin and melatonin hormones were investigated in order to evaluate the effect of Adr on the HPA axis in rats. Tissue samples from hippocampus, hypothalamus and adrenal gland were examined.

Cortisol is one of the most widely used glucocorticoids as HPA axis activity biomarker in the studies (19). Cortisol, the last product of the HPA axis, is a steroid structured hormone, which is the major component of the stress response system (20). In a search of Herane-Vives et al., cortisol levels were found higher in individuals with major depressive episodes compared to healthy subjects in a 15-day period (21). In a research conducted by Barugh et al., cortisol levels were observed high in patients with stroke, for at least 7 days after stroke (22). In results, a decrease has been found in plasma cortisol level of the 40 µg / kg Adr group compared to the control group. Decrease of cortisol level as a result of Adr administration

may reduce the effectiveness of the HPA axis response by preventing the elevation of cortisol level in an HPA axis response against both physical and mental based stress.

Hypothalamic activation leads to adrenaline and noradrenaline release from the adrenal medulla (23). Adrenalin and noradrenaline released from the adrenal medulla constitute the fastest response to stress (24). Dhabhar et al. have reported a reduction not only in the production of catecholamines, but also in cortisol, in the rat study in which they performed adrenalectomy (25). We have found that plasma adrenaline level decreases in the 40 mg/kg Adr administrated study group compared to the control group, and plasma noradrenaline levels decrease in groups with 40 μg/kg Adr and 4 μg/kg Adr compared to the control and sham groups. As a result of Adr administration, a decrease in adrenaline and noradrenaline levels may lead to a reduction in the efficacy of the sympathetic response to exposure to any stress. In addition, the decrease in these hormones, which are also involved in stimulating the HPA axis, may cause a reduction in the HPA axis response level which will constitute a response to the stressor.

When a healthy person is exposed to physical stress, a saliva cortisol response forms positively associated with the level of dopamine release in the ventral striatum (26). Butts et al. have observed that glucocorticoids play a role in dopaminergic modulation in the brain (27). We found that, plasma dopamine levels were increased in 40 μg/kg Adr group compared to control, sham and low dose groups. An increase in dopamine levels as a result of Adr administration may have a positive effect on the HPA axis response.

Systemic administration of 5-HT 1A receptor agonists in rodents and humans has increased plasma ACTH and glucocorticoid production (28-31). Zhang et al. have found that while serotonin regulates the HPA axis, corticosteroids also regulate the serotonin synthesis (32). In results, plasma 5-HT levels have decreased in 40 μg/kg Adr and 4 μg/kg Adr groups compared to the control and sham groups. Decrease in 5-HT levels, as a result of Adr administration, may lead to a reduction in stimulation of the production of hormones such as ACTH and cortisol, which act in the HPA axis, and cause a reduction in the HPA axis response.

Melatonin has inhibited the glucocorticoid response to ACTH in non-human primates, sheep and rats by affecting the adrenal gland directly (33-35). Campino et al. have observed that melatonin has an inhibitory effect on ACTH in human adrenal gland (11). During our research, no significant change has been found in melatonin levels between 40 μg/kg Adr and

4 μg/kg Adr administrated groups. Since no significant change has been found in melatonin levels we think that Adr through this hormone has no effect on the HPA axis.

CRH, one of the biomarkers in stress studies (36), is one of the most necessary components in vertebrates in response to stress (37). When Yadawa et al. applied i.p. herbicide paraquat to the rats; they found that HPA axis response increased against stress due to the increase in CRH amount (38). In results, all groups CRH immunopositivity was found equal in glia cells immunohistochemically. However, CRH immunopositivity of the neurons in the hypothalamus region was found higher in 40 μg/kg and 4 μg/kg Adr administered groups compared to the control and sham groups. CRH immunopositivity in the 40 μg/kg Adr group was more intense than the 4 μg/kg Adr group. Although no significant change was found in the intensity of CRH in the hippocampus neurons, it was thought that the increase in CRH intensity in Adr-administrated groups in the hypothalamus would have a positive effect on CRH, the basic modulator of HPA axis and would affect the HPA axis response positively through this hormone.

In the tissue histopathological examination of the adrenal gland in 40 μg/kg Adr and 4 μg/kg Adr administrated group, degeneration was found in the cells compared to the control and sham groups. It was thought that this degeneration could lead to disruption in the adrenal gland step of the HPA axis response in Adr administration.

With this research, which we investigated the effects of Adr hormone on HPA axis, we have revealed that Adr, which causes decrease or increase in some of the hormones acting in the HPA axis response, may have an effect on HPA axis and we have also stated the effects on various tissues. As a result of Adr administration, while an increase has been found in CRH levels, which is the first step of HPA axis response, a decrease has been determined in cortisol, adrenaline and noradrenaline released from the adrenal gland. At the same time, there has been an increase in dopamine and a decrease in serotonin. However, no significant change has been observed in melatonin.

Although we think that Adr increases the amount of CRH, since we have not found any information in the literature whether the blood has passed the brain barrier, and that we have administered Adr as i.p, it is not clear if the increase in the amount of CRH is due to the effect of Adr on the brain. We also think that the decrease in the amount of cortisol as a result of adrenal gland degeneration contributes to an increase in the amount of CRH by having a negative feedback effect.

However, when the raise in the amount of dopamine is considered, increase in the amount of dopamine while expecting a decrease parallel to the reduction in the amount of cortisol suggests that Adr may lead an increase in dopaminergic activity by passing the blood brain barrier. In addition, this decrease in the amount of cortisol suggests that it may be due to a decrease in serotonin levels. It has been surmised that the decrease in the amount of serotonin can be directly related to the decrease in cortisol level due to Adr or the degenerative changes caused by Adr in the adrenal gland.

Even though the data we have obtained are limited to biochemical, histopathological and immunohistochemical examinations, it is important for us as it represents a first in our further studies in terms of giving idea and sheding light. Although Adr has been examined in many studies in the clinic, this research is very precious since it deals with the investigation of the effects of Adr on the HPA axis and especially the degenerative changes in the adrenal gland for the first time, and it draws attention about the possible changes in the adrenal gland in the long-term use of Adr.

5. CONCLUSION

Adr has showed a variable performance due to effect on HPA axis by increasing dopamine value while decreasing cortisol, adrenaline, noradrenaline, serotonin levels and not affecting melatonin value.

6. Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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Normotansif Obez Çocuklarda QT İntervali, QT Dispersiyonu, Düzeltilmiş QT İntervali, Düzeltilmiş QT Dispersiyonu ve Kardiyovasküler Risk Değişkenleri Arasındaki İlişki

The Relation Between QT Interval, QT Dispersion, Corrected QT Interval, Corrected QT Dispersion and Cardiovascular Risk Variables in Normotensive Obese Children

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Öz: Amaç: Bu araştırmanın amacı normotansif obez çocuklarda ventrikül aritmi ve kardiyovasküler mortalitenin erken tanısında QT intervali, QT dispersiyonu (QTd), düzeltilmiş QT intervali (QTc) ve düzeltilmiş QT dispersiyonu (QTcd) duyarlılığının olup olmadığının saptanması ve bu değerlerin başta vücut kitle indeksi (VKİ) olmak üzere kardiyovasküler komplikasyonlar açısından bakılan parametreler ile ilişkilerinin değerlendirilmesi, eğer anlamlı bir ilişki varlığı gösterilirse bu grup çocuklarda ciddi ventriküler aritmi riski olduğuna dikkat çekmektir.
Yöntem: Hasta grubuna Çocuk Endokrinoloji Bilim Dalı'nda ekzojen obezite tanısı ile takip edilen 30 obez çocuk katılırken kontrol grubuna ise Çocuk Kardiyoloji Polikliniği'ne masum üfürüm ön tanısı ile gelip normal bulunan 20 sağlıklı çocuk dahil edildi. Tüm çocukların antropometrik ve kan basıncı ölçümleri alınarak açlık serum lipidleri, glukoz ve insülin düzeyleri belirlendi. Elektrokardiyografik kayıtlar alındı ve QT intervali, QTd, QTc ve QTcd hesaplandı. İki boyutlu ve m-mode ekokardiyografi ile apikal dört boşluk pozisyonunda boşluk genişlikleri değerlendirildi.
Bulgular: Ventriküler aritmi ve kardiyovasküler mortalite erken tanısında EKG kayıtlarından elde edilen QT intervali, QTd, QTc ve QTcd hasta grubunda daha yüksekti, ancak kontrol grubuyla karşılaştırıldığında istatistiksel olarak anlamlı bir fark bulunmadı. Vücut kitle indeksi ve kardiyovasküler komplikasyonlar açısından incelendiğinde, QT intervali ile ilgili bu değerler ile kan lipidleri ve serum insülin seviyeleri arasında pozitif bir ilişki bulunmadı. Ancak artmış sol ventrikül kitlesi, vücut kitle indeksi, sol ventrikül kitle indeksi ve m-mod ekokardiyografik ölçümler beklendiği gibi pozitif korelasyon gösterdi.
Sonuç: Ekzojen obezite tanısı alan normotansif çocuklarda, erişkinlerde olduğu kadar ciddi ventriküler aritmi riski yoktur. Ancak yine de oluşabilecek komplikasyonlar açısından dikkatli olunması, bu grup çocukların ilerleyen yaşlarda oluşacak ciddi ritm bozuklukları açısından yakından izlenmesi gerekmektedir.

Anahtar Kelimeler — Obezite; QT dispersiyonu; ekokardiyografi; kardiyovasküler risk faktörleri; çocuklar.

Abstract: Objective: The goal of this search was to find out whether the sensitivity of QT interval, QT dispersion (QTd), corrected QT interval (QTc) and corrected QT dispersion (QTcd) sensitivity in the early diagnosis of ventricular arrhythmia and cardiovascular mortality in normotensive obese children exists or not and to evaluate their relation with the parameters examined in terms of cardiovascular complications, such as body mass index (BMI) and finally if there is a significant relationship between them, to draw attention to the risk of serious ventricular arrhythmia in this group of children.
Method: 30 obese children were included to patient group who were followed up with exogenous obesity at the Department of Pediatric Endocrinology and 20 healthy children were included to control group who came to the Pediatric Cardiology Polyclinic with the preliminary diagnosis of innocent murmur. Fasting serum lipids, glucose and insulin levels were examined in all children through anthropometric and blood pressure measurements. Electrocardiographic recordings were obtained and QT interval, QTd, QTc and QTcd were calculated. The apical widths in the apical four cavity positions were evaluated with two-dimensional and m-mode echocardiography.

Results: QT interval, QTd, QTc and QTcd obtained from ECG recordings in the early diagnosis of ventricular arrhythmia and cardiovascular mortality were higher in the patient group, but no statistically meaningful difference was found when compared to the control group. There was no positive correlation between these values related to QT interval and blood lipids and serum insulin levels which were examined in terms of body mass index and cardiovascular complications. However, increased left ventricular mass, body mass index, left ventricular mass index and m-mode echocardiographic measurements showed positive correlation as expected.
Conclusion: There is no risk of severe ventricular arrhythmia in normotensive children diagnosed with exogenous obesity as it is in adults. However, this group of children should be closely monitored for serious rhythm disorders that will occur later in life and care should be taken for potential complications.

Keywords — Obesity, QT dispersion, echocardiography, cardiovascular risk factors, children.

1.INTRODUCTION

Obesity is a major health problem worldwide, leading to serious economic costs and shortening of healthy lifespan [1]. The main problem in obesity is that the energy received is more than the energy consumed. This type of obesity without a defined cause is called primary obesity or exogenous obesity [2,3]. It is known that childhood obesity causes complications such as hypertension, hyperlipidemia, early atherosclerosis, psychosocial, cardiovascular, endocrinological, gastrointestinal, pulmonary, orthopedic and neurological diseases and shortens the life expectancy [4,5].

Structural and functional changes caused by obesity may lead to atrial and ventricular repolarization abnormalities. As a result of these abnormalities, various changes in P wave duration, PR distance, QT interval and QT dispersion (QTd) may occur. Many studies have performed an increase in QT interval and QTd in obese individuals [6,7].

The inter-derivation variability of the QT interval in standard 12-derivation ECG recordings is described as the QTd and it is measured via subtracting process of the minimum QT interval from the maximum QT interval (max QT-min QT). Since standard ECG probes receive signals from different myocardial regions, QTd is stated as "almost" a direct indicator of the regional heterogeneity of ventricular repolarization. It has been reported that prolonged corrected QT interval (QTc) and corrected QT dispersion (QTcd) calculated by correcting the heart rate increase the risk of ventricular arrhythmia [7-9].

The goal of this search was to find out the sensitivity of QT interval, QTd, QTc and QTcd in the early diagnosis of cardiovascular complications such as ventricular arrhythmia in normotensive obese children and to evaluate their relationship with the cardiovascular complication metrics, such as especially body mass index (BMI). Therefore, it was also aimed to draw attention to the risk of serious ventricular arrhythmias in this group of children, if a significant relationship was found.

2. MATERIALS AND METHODS

The study was carried out with the children who had applied to the Department of Pediatric Endocrinology, Faculty of Medicine, Selcuk University with the complaint of weight gain and were evaluated as exogenous obesity as a result of the clinical and laboratory data of the children. The study was started following the approval of application numbered 2013-12/62 from Selçuk University Ethics Committee.

30 obese children aged between 5-17 years without hypertension were in the search. The control group involved 20 healthy and non-obese children of similar age and sex. The parents of all children in the patient and control groups confirmed the informed consent.

Blood lipid and cholesterol levels, fasting blood glucose and basal insulin levels of all obese patients, which had been requested during routine outpatient controls by Pediatric Endocrinology Department, were taken from their files. Fasting blood glucose, blood lipid and cholesterol levels of control group were taken from the files of polyclinical control. High density lipoprotein (HDL) cholesterol levels below 35 mg/dl and low density lipoprotein (LDL) cholesterol levels above 130 mg/dl were accepted as limit values in terms of cardiovascular mortality and morbidity [10]. Triglyceride levels were evaluated according to their age and sex [11].

Anthropometric measurements and arterial blood pressure measurements were recorded in all cases. Weight measurement was performed with an empty stomach, naked body and empty bladder. Height was measured with a stadiometer. All anthropometric measurements were performed by the same method. BMI was calculated by proportioning the weight (kg) to the square of the height (m) [12]. BMI was calculated by using percentage slice curves prepared according to age and sex. 85-95 cases were considered as overweight while 95 and over cases were evaluated as obese [12,13].

Echocardiographic Examination

All echocardiographic evaluations were performed using the Aplio XV Toshiba model. The apical widths in the apical four-chamber position was evaluated by two-dimensional echocardiography. Left ventricular (LV) outflow tract and interventricular septum (IVS) were examined in the parasternal long axis position. M-mode sections were taken from the papillary muscle level and left ventricular end-diastolic thickness (LVDS), left ventricular end-systolic thickness (LVSS), and septum thickness were measured. M-mode measurements

for left atrial and aortic diameters were recorded. Left ventricular mass (LVM) was calculated [14].

Since LVM increases with height growth, LVM index (LVMI) proposed by De Simon et al. [15] were also evaluated. Heart cavity measurements of all subjects were compared with normal values reported for the weight of children.

Electrocardiographic Examination and QT Measurements

After resting for 10 minutes, all children were recorded at the Pediatric Cardiology Policlinic ECG laboratory using standard 12-derivation ECG (Nihon Kohden) recording at 25 mm/sec speed and 10 mm/mV amplitude at rest and lying position to achieve standardization. QTd, QTc interval and QTcd were calculated. The QT interval was described as the distance from the start of the QRS complex to the end of the T wave. Using the same QT waves, the QTc interval for each derivation was calculated with Bazzet formula by correcting for heart rate [16,17]. The difference among the longest QT, QTc and shortest QT, QTc duration in all derivations were calculated and QT and QTc dispersions were found. The effects of clinical characteristics on QT interval, QTd, QTc interval and QTcd were investigated. In addition, it was examined whether there was a meaningful difference in obese children and adolescents group, compared to the control group in terms of ECG findings.

Statistical Analysis

SPSS 16.0 statistical package programme was applied for the statistical analysis. Data were summarized as mean ± standard deviation (SD). Normal distribution analysis of the data was applied by Kolmogorov Smirnov test. Paired t-test was used for comparison of two groups. The differences between the frequencies of categorical variables were investigated by chi-square test. Correlations between variables were calculated using Pearson correlation coefficients. In the study, $p<0.05$ was evaluated as meaningful.

3. RESULTS

General Data

Data on heart rate, gender, age, arterial blood pressure and anthropometric parameters of the patient and control groups were given in Table 1.

Table 1: Data of age and gender, arterial blood pressure, heart rate and anthropometric parameters of the patient and control groups (mean ± SD).

	Patient (n: 30)	**Control (n: 20)**	**p**
Age (years)	11,4±2,5	12,7 ±2,6	p=0,087
Gender (M / F)	18/13	10/8	p=0,864
Body mass (kg)	58,9±15,8	41,0±13,9	**p<0,001**
Height (cm)	146,8±11,2	148,3±15,3	p=0,701
BMI (kg / m²)	26,4±4,1	18,2±3,2	**p<0,001**
Waist circumference (WC) (cm)	84,7±10,7	63,3±5,8	**p<0,001**
Hip circumference (HC) (cm)	91,4±11,3	84,9±7,4	**p=0,03**
WC / HC ratio	0,92±0,06	0,74±0,03	**p<0,001**
Systolic blood pressure (mmHg)	106±9	103±6	p>0,05
Diastolic blood pressure (mmHg)	65±7	65±5	p>0,05
Heart Rate (beats / min)	86 ±13	82±13	p>0,05

In the evaluation of demographic data, the mean age of the patient group was 11.4 ± 2.5 years and the control group was 12.74±2.6 years (p = 0.087). In the patient group, 13 (41.93%) of the patients were male and 18 (58.06%) were female, while 10 (55.5%) of the control group were female and 8 (44.4%) were male (p=0.864). In terms of mean age, gender, blood pressure and heart rate, any statistical difference was not determined (p>0.05), and the groups were homogeneous (Table 1).

When anthropometric measurements were evaluated, it was seen that no statistically meaningful difference between the average height of the two groups (146.86±11.25 cm in the

patient group and 148.33±15.31 in the control group) (p=0.701) was found. The average weight of the patient group was 58.9±15.86 kg and the control group was 41.0±13.92 kg, and there was a statistically meaningful difference beween the groups (p<0.001). BMI (26.47±4.10 kg/m² in the patient group and 18.28±3.23 kg/m² in the control group), waist circumference (84.73±10.73 cm in the patient group and 63.39±5.87 cm in the control group) and waist/hip circumference (0.92±0.06 in the patient group and 0.74±0.03 in the control group) were meaningfully higher in the patient group (p<0.001, for all). The average hip circumference (91.42±11.38 in the patient group and 84.94±7.42 in the control group) demonstrated statistically meaningful values between the groups (p=0.03) (Table 1).

The average systolic blood pressure was 106±9 mmHg, the mean diastolic blood pressure was 65±7 mmHg and the heart rate was 86±13/min in the patient group, while systolic and diastolic blood pressures and heart rate were 103±6 mmHg, 65±5 mmHg and 82±13/min in control group, respectively. No statistically meaningful difference was detected between two groups in terms of systolic and diastolic blood pressures and heart rate (p>0.05 for all) (Table 1).

When two groups were compared, as statistically, any significant difference could not be found for the mean values of fasting plasma glucose (92.74±5.74 mg/dl in the patient group and 89.83±14.1 mg/dl in the control group) and basal insulin level (15.56±8.78 µIU/ml in the patient group and 14.33±9.36 µIU/ml in the control group) (p=0.307 and p=0.648, respectively). However, mean of fasting plasma triglyceride level was 119.03±48.6 mg/dl, LDL cholesterol value was 105.82±22.7 mg/dl, HDL cholesterol value was (41.84±7.43 mg/dl) and alanine transaminase (ALT) level was 24.39±14.3 in the patient group, while in the control group, mean of fasting plasma triglyceride, LDL cholesterol, HDL cholesterol and ALT levels were 77.83±24.12 mg/dl, 84.73±14.9 mg/dl, 48.39±7.99 mg/dl and 12.78±3.49, respectively. There was a statistically meaningful difference among the groups (p=0.002, p=0.001, p=0.006 and p=0.002, respectively) (Table 2).

When fasting triglyceride and LDL limit levels were referenced as 11 and 130 mg/dl according to age and sex, 16 (51.6%) and 3 (9.6%) individuals in patient group had high values. In addition when the HDL cholesterol limit level was referenced as 35 mg/dl, 5 (16.1%) individuals in the patient group had low values.

Table 2: Laboratory data of the patient and control groups (mean ± SD).

	Patient (n: 30)	**Control (n: 20)**	**P**
Triglyceride (mg / dl)	119,03±48,6	77,8±24,1	**p=0,002**
LDL (mg / dl)	105,82±22,7	84,7±14,9	**p=0,001**
HDL (mg / dl)	41,8±7,4	48,3±7,9	**p=0,006**
Fasting blood glucose (mg / dl)	92,7±5,7	89,8±14,1	p=0,307
Basal insulin level (µIU / ml)	15,5±8,7	14,3±9,3	p=0,648
ALT	24,3±14,3	12,7±3,4	**p=0,002**

Echocardiographic Data

As a result of m-mode echocardiography, end-diastolic diameter (LVDS) was 43.4±5.62 mm in the patient group, whereas 38.94±5.26 mm in the control group with a statistically meaningful difference (p=0.008). In patient group, left ventricular end-systolic diameter (LVES) was 24.1±3.26 mm, left atrial diameter (LA) was 27±3.2 mm, interventricular septum thickness (IVS) was 8.2±0.9 mm, posterior wall thickness was 8.2±1.0 mm and shortening fraction (FS) was 43.71±6.0, while these values were 22.3±4.1, 25.06±3.4, 7.9±0.6, 7.9±0.5 and 41.94±2.8 in the control group, respectively. No statistically meaningful difference was observed between the groups (p=0.09, p=0.05, p=0.2, p=0.2, p=0.6) (Table 3). The aortic annulus (AA) was 24.2±2.3 mm in the patient group and 22.5±3.1 mm in the control group . A statistically meaningful difference was found between the groups (p=0.03).

The mean value of LVM was calculated by m-mode echocardiography and the results were 116.16 ± 35 g in the patient group and 92.0 ± 27.9 g in the control group. When compared to the control group, it was evaluated as higher in the patient group (p = 0.017).

Table 3: Results of m-mode echocardiographic evaluations of patient and control groups (mean ± SD).

	Patient (n:30)	**Control (n:20)**	**p**
LVDS (mm)	43.4±5.6	38.9±5.2	**p=0.008**
LVSS (mm)	24.1±3.2	22.3±4.1	p=0.09
LA (mm)	27±3.2	25.0±3.4	p=0.05
IVS (mm)	8.2±0.9	7.9±0.6	p=0.2
Rear wall (mm)	8.2±1.0	7.9±0.5	p=0.2
FS (mm)	43.7±6.0	41.9±2.8	p=0.6
AA (mm)	24.2±2.3	22.5±3.1	**p=0.03**

Table 4: Comparison of patient and control groups in terms of LVM (mean±SD).

	Patient (n:30)	**Control (n:20)**	**P**
LVM (gr)	116,16±35	92,0±27,9	**p=0,017**

Electrocardiographic Data

The electrocardiographic data of the control and patient groups were given in Table 5. No statistically meaningful difference was determined between the electrocardiographic data of groups (p>0.05) (Table 4).

Table 5: Relationship of electrocardiographic findings of patient and control group (mean ± SD).

	HR	QT max	QT min	QTd	QTc max	QTc min	QTcd
Patient (n= 31)	86 (±13)	0,38 (±0,03)	0,31 (±0,03)	0,072 (±0,02)	0,46 (±0,03)	0,36 (±0,03)	0,09 (±0,03)
Control (n= 18)	82 (±13)	0,38 (±0,03)	0,31 (±0,02)	0,065 (±0,01)	0,045 (±0,02)	0,37 (±0,02)	0,08 (±0,02)
P	0,37	0,63	0,75	0,27	0,18	0,64	0,08

HR: heart rate, max: maximum, min: minimum, QTd: QT dispersion, QTcd: QTc dispersion.

Positive correlation was found between BMI and LVES, IVS, LVM, and LVMI (Figure 1-4). LVDS, LVSS, IVS, LVM and LVMI were found to increase with increasing BMI.

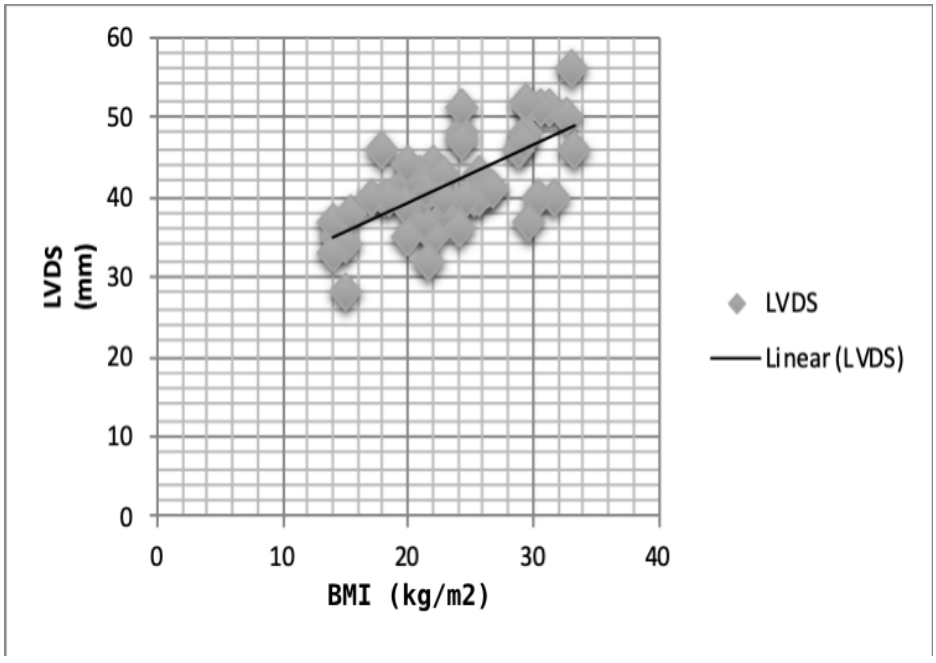

Figure 1: Relationship between LVDS and BMI ($p<0.001$).

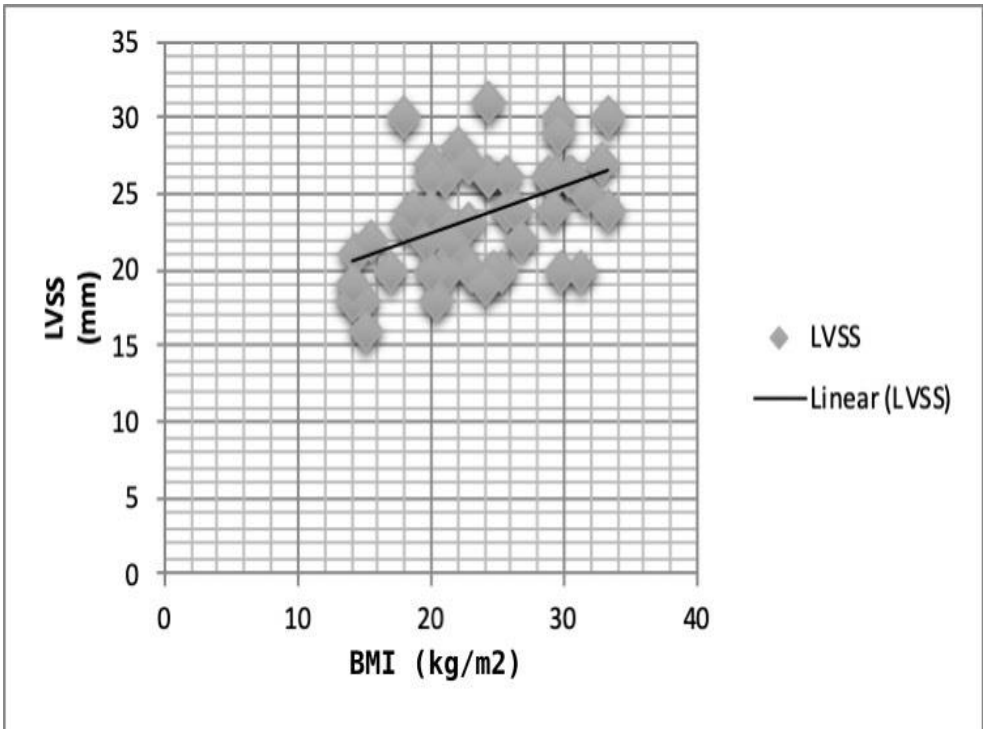

Figure 2: Relationship between LVSS and BMI (p=0.024)

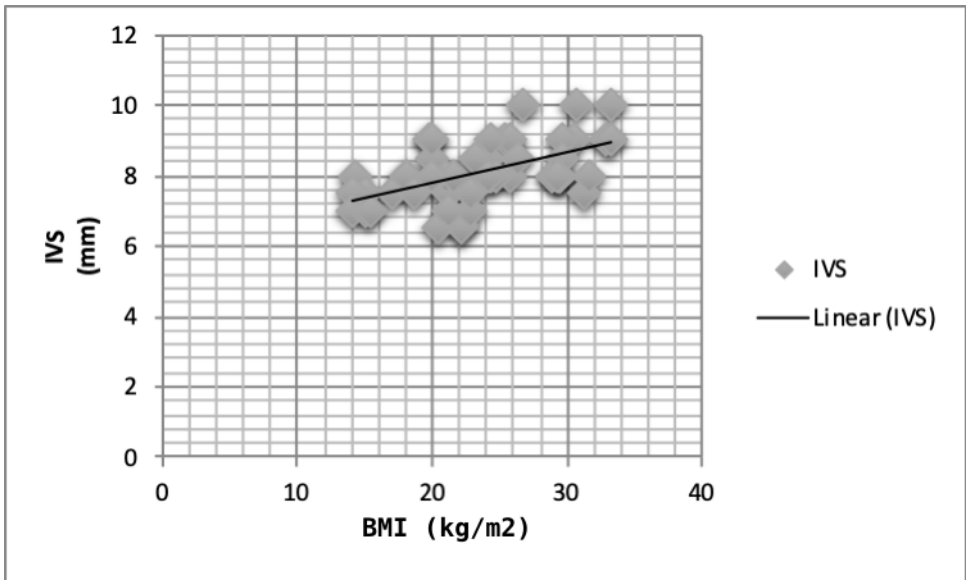

Figure 3: Relationship between IVS and BMI (p<0.001).

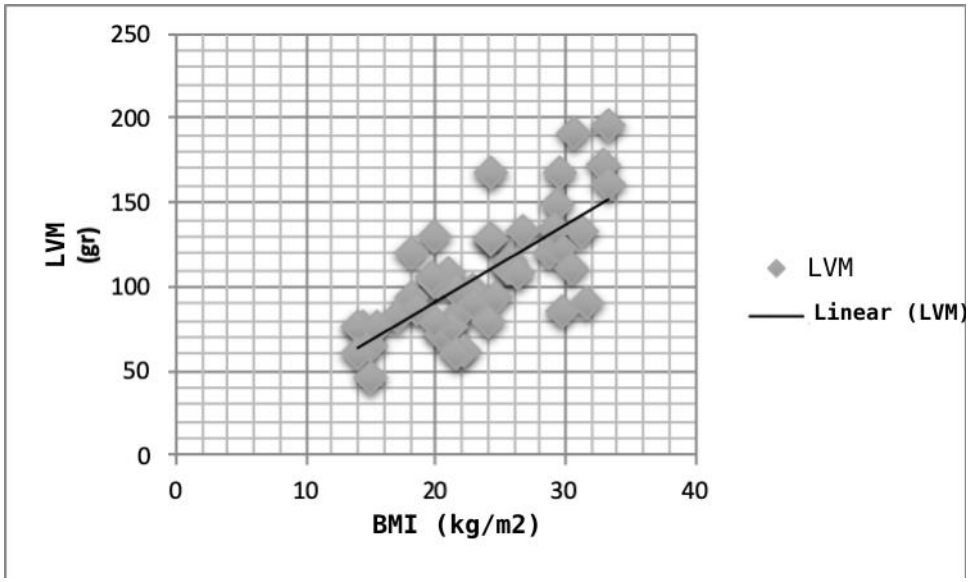

Figure 4: Relationship between LVM and BMI ($p<0.001$).

No correlation was determined between electrocardiogram results and BMI ($p>0.05$).

4.DISCUSSION

In the last decades, frequency of obesity increases logaritmically, not only in developed countries but also in developing countries such as Turkey [6]. In adults, obesity is associated with many medical complications which reduce the quality of life and increase the morbidity. Obese children are under potential risk for adult obesity [18]. Sedentary living conditions and unbalanced eating habits, genetic and socioeconomic status play a role in the etiology of obesity. In particular, adolescence obesity has been shown to cause atherosclerosis and associated cardiovascular mortality and morbidity [6,19,20]. It has been reported that the unexplained sudden death rate is 40-60 times and the frequency of ventricular extra systole beats are 30 times higher in obese individuals than the non-obese population [6]. Before the complications of obesity show up, determining the risk factors in the early period and taking the necessary precautions are very important in terms of increasing the quality of public health and reducing health expenses. In the present study, anthropometric, echocardiographic, electrocardiographic and laboratory parameters that can be used in early detection of childhood obesity and complications and their relationships with each other were investigated.

Obesity causes structural and hemodynamic changes in the heart. Excessive fat accumulation increases blood volume and cardiac stroke volume. It is thought that insulin resistance derived from metabolic changes caused by obesity also causes cardiovascular system complications [18]. Increased blood volume and hypertension in obesity, increase the anterior and posterior

load and impose a burden on the heart and cause hypertrophy. It has also been reported that obesity-induced hyperinsulinemia leads to myocardial hypertrophy via IGF-1 receptors [21]. Firstly, diastolic function and secondly, systolic function of the heart deteriorates in childhood obesity [22].

Childhood obesity is closely associated with LVI and is considered to be an important cardiovascular risk factor associated with congestive heart failure, stroke, sudden death, coronary artery disease and myocardial infarction [23,24]. Several studies have shown increased LVM in obese children [24-28]. Central fat accumulation has been shown to cause increased LVM and high cardiac output [29]. In our study, the LVM was higher significantly in the patient group than the control group.

In many studies, BMI and waist circumference measurements were associated with LVM [30-32]. Di Bonito et al. [24] found the connection between LVMI and waist/hip ratio. In another study, LVMI was positively related to height, weight, BMI, fasting insulin level [33]. In the present study, LVM was positively associated with waist/hip ratio weight, BMI, height, waist circumference, and fasting insulin level. No relationship was found between LVM with blood lipids and fasting blood glucose.

In children and adolescents with high body fat content, there are changes in sympathetic and parasympathetic activities which play role in the pathogenesis of many chronic diseases, due to the release of large amounts of inflammatory adipokines into the blood [34,35]. As a result, it is known that obese children have a higher resting heart rate [36]. On the other hand, in some studies, although there was a slight increase in heart rate in obese individuals, it was not found to be statistically significant [30,37,38]. Similarly, in the present study, although the heart rate was higher in the patient group than in the control group, the difference was not statistically significant.

Obesity has undesirable effects on children's lipid and lipoprotein values, which are accepted as important risk factors for coronary heart disease. Sedentary lifestyle, atherogenic diet and genetic factors lead to atherogenic dyslipidemia which is characterized by high triglyceride, LDL, very low density protein (VLDL) levels and HDL levels at very early ages [39]. Measurements of waist circumference or increases in waist/hip ratio positively correlates with triglyceride levels [40]. In particular, hyperinsulinemia and insulin resistance in liver during abdominal obesity cause stimulation of lipogenic enzymes in liver and occurrence of dyslipidemia [41,42]. In many studies, triglyceride and LDL levels were found to be significantly

higher in obese children compared to the non-obese control group [43,44] while HDL levels were found to be significantly lower. It was emphasized that this clinical profile was effective in the development of adult atherosclerosis [44,45]. In current study, triglyceride and LDL levels were meaningfully higher and HDL levels were lower in the patient group.

There is a risk of sudden death and ventricular arrhythmia in obese individuals [38]. In the Framingham Heart Study, the annual rate of sudden cardiac death in adult obese men and women was reported to be approximately 40 times higher than in the non-obese population [46]. Many studies have reported changes in cardiac morphology in childhood obesity, too [6,30,47]. Sudden death and ventricular arrhythmias have been reported to be associated with ventricular repolarization anomalies [48]. In many studies, prolonged QT interval is recommended to be used as a predictor of ventricular arrhythmias and potential mortality [8,9]. El- Gamal et al. reported that increased body fat and increased obesity are among the most common causes of QT interval and QTc prolongation [49].

It has been reported that prolongation of QTc and QTcd increase the risk of severe ventricular arrhythmias [7-9]. QTd causes chronic heart failure, left ventricular hypertrophy in adults and ventricular arrhythmia and sudden death in patients with long QT syndrome [38]. In a study, a positive correlation was found between QTcd and serum insulin concentration [38]. It was found that QTc and QTcd increased in obese individuals compared to control group and there was a meaningful relationship between body mass index with QTc and QTcd [50]. While some reports have shown an increase in QT interval and QTd in obese individuals [7,49], there was no correlation between QTd and BMI increase in others studies [51,52]. On the other hand, some studies have shown that short-term weight loss is associated with a decrease in QT duration [50,53].

In this study, QT interval, QTd, QTc and QTcd obtained from the ECG recordings in normotensive obese children were found to be higher than the control group, but no statistically significant difference was found. There was no positive correlation between these values related to QT interval and levels of blood lipids and serum insulin, which are metrics related to BMI and cardiovascular complications.

5. CONCLUSION

Cardiac functions are impaired in obese children. In parallel with the increase in development of obesity in children, the risk of cardiovascular diseases also increases in adulthood. Deterioration of these functions, even in the absence of clinical symptoms, is important for

the prevention of possible complications that may occur in the future. Increased BMI is associated with LVM, LVM index and m-mode echocardiographic measurements. Studies on QT interval in obese children are both limited and contradictory in their results. Therefore, further studies are needed to determine ECG findings in terms of ventricular arrhythmia and sudden death risk in obese children without clinical signs.

Conflict of Interest Statement

None declared.

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Higenaminin İskemi Reperfüzyon Tarafından İndüklenen Testis Hasarına Etkileri: Biyokimyasal Bir Çalışma

The Effects of Higenamine on Testicular Damage Injured by Ischemia Reperfusion: A Biochemical Study

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Öz: Amaç: Bu araştırmanın amacı, higenaminin iskemi reperfüzyonunun neden olduğu testis hasarındaki koruyucu etkilerini incelemektir.
Gereç ve Yöntem: Deneyimizde, sıçanlar sham kontrol, iskemi reperfüzyon ve iskemi reperfüzyon + higenamin grupları olmak üzere 3 gruba ayrıldı. Bazı oksidan, antioksidan ve inflamatuar parametreler deney sonunda elde edilen testis dokularında değerlendirildi.
Bulgular: Mevcut çalışmada, iskemi reperfüzyon grubunda oksidan ve inflamatuar parametrelerin arttığını ve antioksidan parametrelerin azaldığını, ancak tek doz higenaminin uygulanmasının, iskemi reperfüzyon tarafından indüklenen testis oksidatif hasarına karşı koruyucu olduğu öne sürülen tedavi grubunda antioksidan parametrelerin arttığını ve oksidan parametrelerinin azaldığını gözlemledik.

Anahtar Kelimeler — İskemi reperfüzyon, higenamin, testis, oksidatif stres, inflamasyon, sıçan.

Abstract: Purpose: The purpose of this research is to examine protective effects of higenamine on testis injured by the ischemia reperfusion.
Material and Method: In our experiment, the rats were separated as 3 groups including sham control, ischemia reperfusion, and ischemia reperfusion+higenamine. Some oxidant, antioxidant and inflammatory parameters were evaluated in testis tissues obtained at the end of the experiment.
Findings: In current study, we observed that the oxidant and inflammatory parameters increased and antioxidant parameters decreased in the ischemia reperfusion group but the antioxidant parameters increased and oxidant parameters decreased in treatment group suggesting that single dose administration of higenamine is protective against testicular oxidative damage resulted from ischemia reperfusion.

Keywords — Ischemia reperfusion, higenamine, testis, oxidative stress, inflammation, rat.

1.INTRODUCTION

Testicular torsion is an emergency that can occur in young fertile males and causes testicular damage. In testicular torsion, the duration and degree of torsion are important in determining the severity of the damage (1; 2). Damage after torsion occurs during both ischemia and reperfusion processes due to disruption of tissue oxygenation in ischemia and release of reactive oxygen species (ROS) by re-supply of blood flow in reperfusion (3-5). Detorsion can

therefore increase the damage to the testicles. Inflammatory cells and immature sperm cause the formation of unstable ROS molecules with unpaired electrons (6). Increased ischemia and inflammation can cause increment in ROS synthesis (7). Antioxidant endogenous enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), which increase in tissue by reperfusion, help to protect the tissue from oxidant stress (8; 9).

Higenamine (1-[(4-hydroxyphenyl) methyl]-1,2,3,4-tetrahydroisoquinoline-6,7-diol) is a phenolic alkaloid obtained in 1976 from the aconite radical (10). It is a powerful antioxidant because it prevents the formation of inflammation, apoptosis and thrombosis (11-14). Higenamine is regarded as a traditional cardiostimulant and anti-inflammatory in China. According to published reports, higenamine possesses a variety of pharmacological properties, including dilation of blood vessels and bronchi, immunomodulation and antioxidation. This study was planned to investigate the protective effect of higenamine against testicular oxidative damage caused by ischemia reperfusion (I/R).

2. MATERIALS AND METHODS

Ethical Approval and Animals

This experimental study was approved (28.03.2019/63) by Atatürk University Experimental Animal Ethics Committee before the experiment. Our experimental study was carried out at Atatürk University Experimental Animals Research and Application Center using healthy male Wistar-albino rats weighing 260-290 g, obtained from Atatürk University Experimental Animal Research and Application Center. Rats were housed in cages in laboratory conditions such as 12 hours of light, 12 hours of darkness, humidity of 55 % and a mean temperature of 25 °C. Rats were fed with standard rat feed, and provided drinking water. All animals were deprived of food 12 hours before the experiment, but were allowed to drink water.

Experimental Design and Groups

The 24 rats used in our study were weighed and divided into 3 groups, including 8 rat in each group;

Sham Control Group; in this group, each rat was fixed to the surgical setup in a supine position. Later, the abdominal midline incision was opened using sterile tecniques and the incision area closed again without performing testis I/R model.

I/R Group; in this group, the abdominal field was shaved and washed via 10 % povidone-iodine, and a 2-cm midline abdominal incision was performed using sterile techniques. It was

performed laparotomy under anesthesia. Spermatic cord was found and clamped to create ischemia for 2 hours. The clamp was removed and testis reperfusion allowed for 2 hours. At the end of the reperfusion period, testis tissue samples were rapidly taken.

I/R + higenamine 10 mg/kg; as a defined in the I/R group, ischemia was induced for 2 hours by clamping. Higenamine was administered intraperitoneally at dose of 10 mg/kg 30 minutes before reperfusion. Then the clamp was removed and the reperfusion period started. At the end of the experiment, testis tissues were taken. Rats were sacrificed by high-dose anesthesia. All the experimental steps were complated under controlled and continuous general anesthesia (ketamin/ksilazin 60/10 mg/kg bw, intraperitoneally). At the end of the reperfusion, tissue samples were taken from the section of the testis tissue exposed to I/R and purified by washing with normal cold saline.

Biochemical Analysis

Tissue samples were weighed for 100 mg and homogenized with 2 mL of phosphate buffer. Homogenized tissues were centrifuged at 5000 rpm for 20 minutes at +4 °C and the supernatants were carefully transferred to ependorfs and maintained at -80 °C. The principle of measurement of MDA, as a result of lipid peroxidation, is based on measuring the absorbance at 532 nm of the pink color compound formed as a result of the reaction of malondialdehyde (MDA) and thiobarbituric acid (TBA) (15). Total antioxidant status (TAS) value was determined with the commercially available kit (Rel Assay Diagnostics). Total oxidant status (TOS) measurement was performed with commercially available kit (Rel Assay Diagnostics). The ratio of TOS to TAS was accepted as the oxidative stress index (OSI). OSI value was calculated as follows: OSI = [(TOS, μmol/L)/(TAS, mmol/L) × 10] (16). The activity of myeloperoxidase (MPO) measurement is based on the kinetic measurement of the absorbance at 460 nm wavelength of the yellowish-orange colored complex in result the oxidation of o-dianisidine with MPO in the presence of hydrogen peroxide (17). The xanthine oxidase enzyme catalyzes the formation of uric acid from xanthine. The resulting superoxide radical forms the molecular oxygen and hydrogen peroxide with the superoxide dismutase enzyme. The resulting superoxide reacts with the tetrazolium salt to form a formazan dye in situations where the effect of the SOD enzyme is insufficient, and the SOD activity is measured with the inhibition degree of this reaction (18).

Statistical Analysis

TAS, TOS, OSI, MPO, SOD and MDA results obtained from our study were analyzed using a statistical analysis (SPSS 21, USA) program. Descriptive statistics of the values in the groups

were expressed as mean ± SD. P value was taken as 0.05. Shapiro Wilk test was used for the assumption of normality test. One-Way ANOVA test was used for normal distribution and Tukey test was used for post hoc paired comparisons. Kruskal Wallis test, which is a nonparametric test, was used for the parameters that do not conform to normal distribution. When the significance was determined, the Mann Whitney U test was performed.

3.RESULTS

Table 1 shows the concentrations of TAS, TOS, MPO, SOD, MDA and level of OSI in testis tissue in the sham control group, I/R and I/R + higenamine groups. Compared with the sham control group, the concentrations of TOS, MPO, MDA and level of OSI were significantly increased, while TAS and SOD values were decreased in the I/R group. When the sham control and treatment groups were compared, no statistical significance was found in the biochemical parameters. Compared with the I/R group, TOS, OSI, MPO, and MDA, oxidant parameters, were significantly decreased and TAS and SOD, antioxidant parameters, were significantly increased in I/R + higenamine group (Table 1, p=0.00).

Table 1: Mean values of biochemical parameters and comparison among groups.

Experimental Groups, n=8	TAS (mmol/L)	TOS (µmol/L)	OSI (arbitrary unit)	SOD (U/mg protein)	MPO (U/g protein)	MDA (µmol/g protein)
Sham control group (I)	1.337±0.094	7.943±0.677	0.597±0.079	395.743±86.700	36907.947±6754.149	213.379±28.742
I/R (II)	0.762±0.094	10.812±0.880	1.436±0.191	193.524±22.670	83630.530±8338.628	403.037±74.827
I/R + higenamine (III)	1.253±0.084	8.304±0.665	0.667±0.090	390.099±57.830	36865.317±8157.824	218.914±30.215
p value (Meaningful intergroup comparisons)	0.00 (I-II) 0.00 (II-III)	0.00 (I-II) 0.00 (II-III)	0.00 (I-II) 0.00 (II-III)	0.00 (I-II) 0.00 (II-III)	0.00 (I-II) 0.00 (II-III)	0.00 (I-II) 0.00 (II-III)

TAS = Total Antioxidant Status; TOS = Total Oxidant Status; OSI = Oxidative Stress Index; SOD=Superoxide Dismutase; MPO=Myeloperoxidase; MDA=Malondialdehyde. Data are presented as mean ± S.D. $p<0.05$.

4. DISCUSSION

One of the typical I/R injury of acute scrotal cases in urological department, namely testicular torsion, is excess rotation of the testis along with the spermatic cord causing infertility in males (19). So preserving the fertility capacity early diagnosis and treatment of testicular torsion especially surgical detortion which is believed to be gold standard treatment should be performed as soon as possible in order to provide reperfusion of testicular tissues (20; 21). Because the insufficiently oxygenated ischemic tissues may suffer conditions ranging from functional disorders to necrosis (22) re-establishment of blood flow should be the first intervention in testicular I/R injury of the torsed testis. However detorsion also leads to reperfusion injury (23) making too much ROS that the antioxidant capacity can not scavenge. Those events cause protein modification and lipid peroxidation making subsequent cellular dysfunction. The organism contains variable antioxidant enzymes that repair oxidative damage (24). Oxidative stress, apoptosis and necrosis are the mechanisms of damage in I/R (25). There is an increase in oxidative stress between ROS production and antioxidant defense mechanisms in favor of ROS. MDA increase, which is one of the best indicators of lipid peroxidation caused by ROS formation, occurs and increased in the I/R group in our study (26). After the increase in ROS with reperfusion following ischemia, MDA increases with lipid peroxidation and antioxidant preservatives such as SOD try to restore the balance (27-29). SOD is protective against the harmful effects of free radicals, such as O_2^- and H_2O_2, which are released in oxidative stress (30). TAS shows antioxidant activity and TOS reflects the intensity of oxidative stress. Any condition that exceeds the antioxidant capacity causes oxidative stress and OSI indicates this stress state (31-33). I/R study was showed that the TOS and OSI values increased, by contrast the TAS decreased in I/R group (34). In a study, the reduction of SOD caused by doxorubusin is increased by application of higenamine (35). In addition, in a collagen-induced arthritis study, the decreased GSH level was increased by administration of higenamine in the arthritis-induced group. And antioxidant properties of higenamine have been shown (36).

ROS and MDA, which we have evaluated in this study, are indicative of oxidative damage and increased with I/R in our study and decreased with higenamine administration. (26). Cell membrane damage is indicated by increased MDA elevation by peroxidation of fatty acids containing three or more double bonds (37) (38; 39). MDA causes cross-linking of molecules in the cell membrane and disrupts cell function as a result of changes in ion permeability and enzyme activity in the cell (40; 41). MPO is the main indicator of neutrophil accumulation in

I/R (42) and in our study, the increase in I/R decreased to control levels with higenamine administration. In a study, the increase in doxorubusin-induced MDA decreased by higenamine treatment (35). In the intestinal I/R study in mice, it was reported that inflammatory parameters such as MPO, TNF-α and IL-6 were increased in I/R group and higenamine administration decreased these levels and showed antiinflammatory effect (43).

The most important biochemical data of higenamine treatment can be expressed as follows: I/R injury in testes was related to dramatic increases of MDA level, TOS and OSI and MPO, and a decreases in SOD activity and TAS value in the testicular tissue. The novel result of the present study is that higenamine significantly derogated testicular tissue damage induced by I/R. Most importantly, higenamine treatment effected in the positive direction changes of the findings of MDA, TOS, OSI and MPO and stimulated an overproduction of enzymatic antioxidant SOD activity and increased TAS value.

4.1.Conclusion

These results recommend that higenamine may protect the testis by diminishing oxidative injury caused by ischemia reperfusion. We have indicate that treatment with higenamine at single dose (10 mg/kg) reduces testicular damage induced by ischemia reperfusion in testis in experimental animals exposed to a torsion for 2 hours and detorsion for 2 hours model. Part of the mechanisms of these protective effects of higenamine may be caused from supporting the antioxidant capacity by higenamine. Moreover, further researches are necessarry for explain the other protective mechanism on testicular tissue damage induced by ischemia reperfusion.

Conflict of Interest.

None declared.

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