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Effects of Thymoquinone Against D- Galactose and Aluminum Chloride Induced Testicular Dysfunction in Rats

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Abstract: Alzheimer's disease affects all tissues negatively. In this study, it was aimed to determine the effects of TQ on testicular toxicity in rats with experimental Alzheimer's disease. In the study, 33 Wistar Albino rats weighing 190-230 gr were used. Rats were 11 rats in each group; were divided into 3 equal groups as control, ALZ and ALZ+TQ. The control group was given saline by IP route for 28 days daily. The ALZ group was given 60 mg/kg D-gal + 40 mg/kg $AlCl_3$ IP for 28 days daily. The ALZ+TQ group was given 60 mg/kg D-gal + 40 mg/kg $AlCl_3$ for 28 days by IP + 20 mg/kg TQ by oral gavage for last 14 days daily. According to the findings of our study, a decrease in SOD, CAT, GPx activity and GSH levels and an increase in MDA levels were determined in the ALZ group. In the ALZ+TQ group, while SOD, GPx, CAT activities and GSH levels increased, MDA levels decreased. There was a decrease in sperm motility and sperm density in the ALZ group, and an increase in the rate of abnormal sperm and dead spermatozoa. In the ALZ+TQ group, sperm motility, abnormal sperm rate and dead spermatozon rate improved. As a result, it was determined that the decrease in semen quality and increase in oxidative stress induced by $AlCl_3$+D-Gal were suppressed by TQ, protecting the testicular tissue from oxidative damage and increasing semen quality.

Sıçanlarda D-Galaktoz ve Alüminyum Klorürün Neden Olduğu Testis Fonksiyon Bozukluğuna Karşı Timokinonun Etkileri

Anahtar Kelimeler
Alzheimer, $AlCl3$+D-Galaktoz, Oksidatif stres, Rat, Sperm, Timokinon

Öz: Alzheimer hastalığı tüm dokuları olumsuz etkilemektedir. Bu çalışmada deneysel alzheimer hastalığı modeli oluşturulan ratlarda testis toksisitesi üzerine TQ etkileri belirlenmesi amaçlanmıştır. Çalışmada ağırlıkları 190-230 gr arasında değişen 33 adet Wistar Albino cinsi rat kullanıldı. Sıçanlar, her grupta 11 sıçan olmak üzere kontrol, ALZ ve ALZ+TQ olarak 3 eşit gruba ayrıldı. Kontrol grubuna, 28 gün boyunca IP yoluyla serum fizyolojik günlük olarak verildi. ALZ grubuna 28 gün boyunca günlük olarak 60 mg/kg D-gal+40 mg/kg $AlCl_3$ IP verildi. ALZ+TQ grubuna 28 gün 60 mg/kg D-gal +40 mg/kg $AlCl_3$ IP+20 mg/kg TQ son 14 gün oral sonda ile günlük olarak verildi. Çalışma bulgularına göre ALZ grubunda SOD, CAT, GPx aktivitesi ve GSH düzeylerinde azalma, MDA düzeylerinde ise artış saptanmıştır. ALZ+TQ grubunda SOD, GPx, CAT aktiviteleri ve GSH seviyeleri artarken MDA seviyeleri azaldı. ALZ grubunda sperm motilite ve sperm yoğunluğunda azalma, anormal sperm ve ölü canlı spermatozoa oranında artış olduğunu göstermiştir. ALZ+TQ tedavi grubunda sperm hareketliliği, anormal sperm oranı ve ölü sperm oranı düzeldi. Sonuç olarak, $AlCl_3$+D-Gal'in neden olduğu semen kalitesindeki azalma ve oksidatif stres artışının TQ tarafından baskılandığı, testis dokusunu oksidatif hasardan koruduğu ve semen kalitesini arttırdığı belirlendi.

1. INTRODUCTION

Alzheimer's disease is characterized by memory loss and behavioral disorders [1]. Aluminum chloride ($AlCl_3$) and D-galactose (D-Gal) are administred to create Alzheimer's disease model [2]. Aluminum (Al) is one of the most toxic of these heavy metals [3]. Al compounds are used in many medical applications, such as antacids, phosphate binders, buffered aspirins, and vaccines [4]. Recent animal and clinical studies have reported that Al causes neuropathological changes in the central nervous system [5]. Al can accumulate in the liver and kidneys, causing hepatorenal toxicity [6]. Al also has pro-oxidative, excitotoxic, immunogenic, proinflammatory, and mutagenic effects [7]. Oral Al exposure causes a decrease in glutathione peroxidase (GPx), catalase (CAT) activities, glutathione (GSH) levels and an increase in malondialdehyde (MDA) content in rats [8]. Considered as a systemic toxic substance, $AlCl_3$ accumulates in target organs including the testicles and causes dysfunction [9]. In another study, $AlCl_3$ causes a decrease in sperm motility and density in rats, and an increase in the ratio of dead live sperm and abnormal spermatozoa [10]. D-Gal can cause aging in tissues such as the brain, kidney, and liver [11]. Moreover, D-Gal induces oxidative stress by increasing lipid peroxidation [12]. In addition $AlCl_3$ and D-Gal administration causes oxidative damage in testicular tissue of rats [13].

Medicinal plants and phytochemicals are used against neurodegenerative diseases [14]. Herbal substances are used because of their therapeutic properties against various toxicants [15-17]. One of the bioactive components of the Nigella sativa plant, from which black seed oil is obtained, is thymoquinone (TQ) [18]. TQ is known as an antioxidant, anti-neoplastic and anti-inflammatory agent [19]. In addition, TQ is one of the anti-cancer bioactive compounds [20]. TQ protects the histopathological structure of the testis by preventing oxidative damage from experimentally induced testicular damage in male rats [21]. In another study, it was reported that it improved sperm motility, density, abnormal spermatozoa rate and dead spermatozoa rate in male rats with TQ varicocele [22]. Similarly, TQ regulated the pituitary testis axis, adjusted oxidant balance, decreased apoptosis and improved sperm quality in rats with lead toxicity [23]. In line with these data, the aim of this study was to determine the effect of TQ against $AlCl_3$+ D-Gal on testicular toxicity, which was applied to create an experimental Alzheimer's model in rats.

2. MATERIAL AND METHOD

2.1. Animals and Ethical Permission

All chemicals used in the study were obtained from Sigma Chemical Co. (St. Louis, MO) unless otherwise stated. Ethical permission was obtained from the Erciyes University Animal Experiments Local Ethics Committee for the study (Protocol no:23/037). Thirty three Wistar Albino rats weighing 190-230 g were used in the study. Rats were housed in standard laboratory conditions (25±2 °C temperature, a relative humidity of %60±5 and, a 12-h light-dark rhythm) throughout the study. Rats were fed commercial pellet feed ad libitum water throughout the study. Rats were divided into 3 groups: the control group, the ALZ group, and the ALZ+TQ group, with 11 rats in each group.

The control group received saline (0.9%) intraperitoneally (IP) daily for 28 days.

ALZ group received D-galactose at 60 mg/kg dose + $AlCl_3$ at 40mg/kg by IP daily for 28 days [24].
ALZ+TQ group received 60 mg/kg D-galactose + 40 mg/kg dose $AlCl_3$ by IP daily for 28 days + 20 mg/kg TQ by oral gavage for last 14 days daily [24].

After the end of the experimental phase, the rats were sacrificed under xylazine-ketamine anesthesia. The testicular tissue of each rat was removed and separated from the epididymis. Then, the cauda epididymis was taken into a petri dish containing 5 ml of saline and trimmed at 35°C. Trimmed semen was used for semen analysis [25].

2.2. Oxidative Stress Analysis

To determine the oxidant antioxidant status in testis tissue, SOD, CAT, GPx activities, GSH levels and MDA contents were analyzed. SOD activity was measured according to the Sun et al. [26] method. CAT activity was determined using Aebi's [27] method. GPx activity was determined by the method developed by Lawrence and Burk [28]. GSH levels were measured by the method of Sedlak and Lindsay [29]. Testicular tissue MDA content was measured by the method described by Goth [30].

2.3. Reproductive Parameters Analysis

Testicular tissues separated from the epididymis were weighed using a precision balance (Radwag PS 750 R2, Poland). Results are presented in mg [31]. A light microscope with a heating plate was used to evaluate sperm motility. A small amount of semen was dropped on the slide and covered with a coverslip. Motility estimates were calculated as % with the average of three different microscope fields [32].

For the rate of dead sperm and sperm abnormalities, 10 µl of eosin and nigrosin dye were dropped on 10 µL of semen and dried on a slide, mixed with a coverslip and smeared. 200 sperm cells were examined on each slide with a light microscope. Sperm cells whose heads were stained were considered dead [33, 34]. To determine the rate of dead spermatozoa for abnormalities in sperm cells, a total of 200 sperm cells were evaluated on the prepared slide and the abnormality rates were calculated as a percentage [35].

2.4. Statistical Analysis

Biochemical and spermatological data were analyzed by one-way ANOVA using the SPSS (version 26.0; Chicago, IL) program. Differences between groups were made using the Tukey multiple comparison test. Results were

presented as mean ± standard error (S.E.M). P<0.05 values and below were considered statistically significant.

3. RESULTS

3.1. Oxidant/Antioxidant Assessment

The oxidative stress results of the experimental groups were presented in Figure 1-5. The highest MDA levels was seen in the ALZ group. ALZ+TQ treatment group partially decreased MDA level (P<0.05). While GPx, CAT, SOD activity and GSH levels were generally highest in the control group, these parameters were found to decrease in the ALZ group. TQ treatment was found to improve these parameters.

3.2. Spermatological Evaluation

The epididymal sperm parameters of the rats in the experimental groups are presented in Table 1. Accordingly, while the highest total motility value was seen in the control group, a statistical difference was found between the other experimental groups (P<0.05). There was no difference between the groups in terms of epididymal semen density. While the rate of dead and abnormal sperm rate were found to be at the highest in the ALZ group, a statistically significant difference was observed between the other experimental groups (P<0.05). There was no difference between the groups in terms of testicular weight.

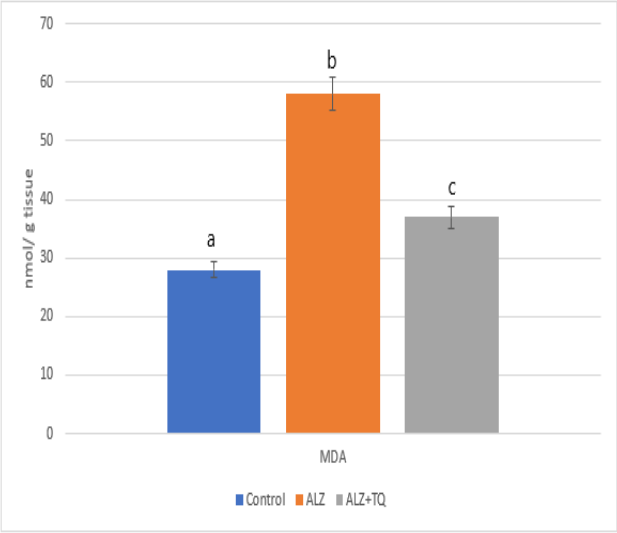

Figure 1. The malondialdehyde (MDA) levels results. Different superscript letters in the same column (a, b, c) indicate significant inter-group differences (p < 0.05)

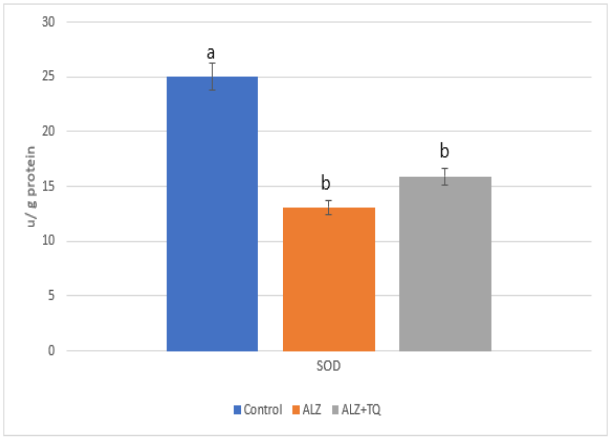

Figure 2. The superoxide dismutase (SOD) levels results. Different superscript letters in the same column (a, b) indicate significant inter-group differences (p < 0.05)

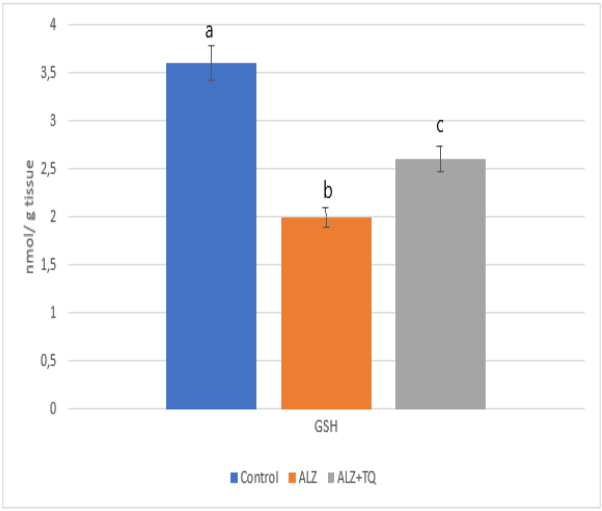

Figure 3. The glutathione (GSH) levels results. Different superscript letters in the same column (a, b) indicate significant inter-group differences (p < 0.05)

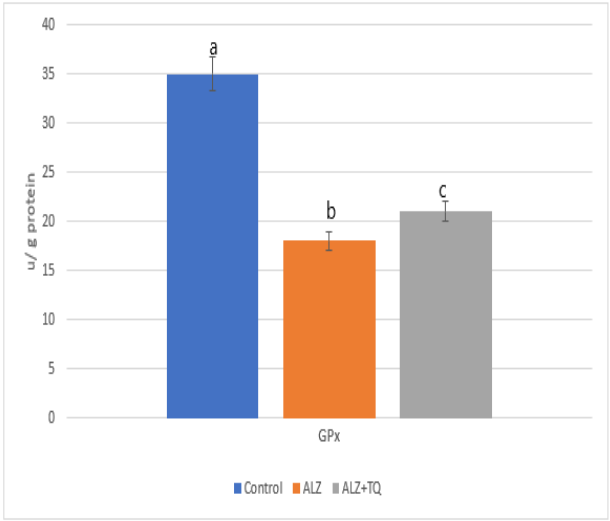

Figure 4. The glutathione peroxidase (GPx) levels results. Different superscript letters in the same column (a, b, c) indicate significant inter-group differences (p < 0.05)

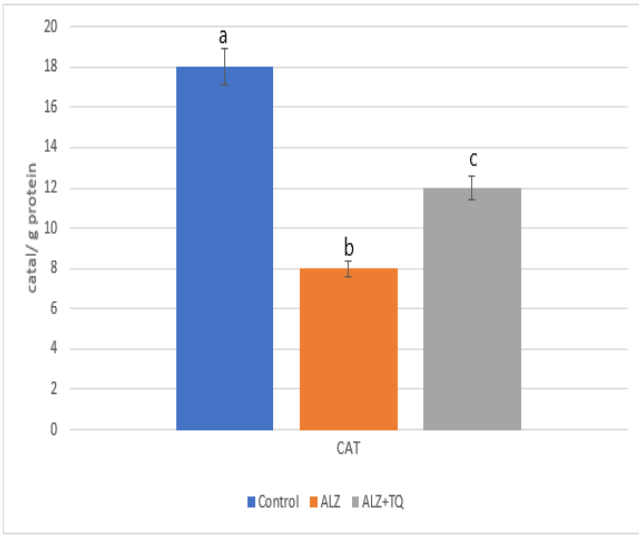

Figure 5. The catalase (CAT) levels results. Different superscript letters in the same column (a, b, c) indicate significant inter-group differences ($p< 0.05$)

Table 1. Sperm analysis results of the experimental groups

	Control	ALZ	ALZ+TQ
Total Motility (%)	81.92±3.30[a]	52.66±15.51[b]	59.41±3.13[b]
Density (%)	78,25±8,30	74,50±8,21	71,42±3,99
Dead sperm rate%	15.42±1.83[a]	22,58±2,57[b]	19,08±2,42[c]
Abnormal sperm rate (%)	7,83±1,03[a]	11,67±2,27[b]	9,33±0,65[c]
Right testis weight (mg)	1459,66±63,14	1413,75±33,75	1419,92±50,74
Left testis weight (mg)	1435,58±63,25	1394,62±39,45	1383,67±57,45

4. DISCUSSION AND CONCLUSION

Heavy metal pollution has significant adverse effects on the reproductive functions of both animals and humans [36-38]. Humans and animals are acutely or chronically exposed to heavy metals through food and water intake [39]. These toxic substances cause deterioration in endocrine mechanism and testicular functions [40]. D-Gal causes aging progression and nephropathy in the brain, kidney, and liver. It also induces oxidative stress by increasing lipid peroxidation [41]. In the present study, the effects of TQ on the changes caused by $AlCl_3$+D-Gal in the testicular tissue of rats, which were administried to create an experimental Alzheimer's model, were investigated.

Oxidative stress occurs when the balance between the antioxidant defense system and reactive oxygen species (ROS) is disrupted [42]. Oxidative stress at physiological limits is required for physiological interactions of sperm cells such as fertilization and sperm hyperactivation. However, excessive ROS induces lipid peroxidation [43]. Testis are one of the target organs for oxidative stress due to the high composition of polyunsaturated lipids in the membrane structure [44]. Antioxidant defense mechanisms protect mammalian germ cells against oxidative stress [45]. Enzymes such as SOD, CAT and GPx are the first defense mechanism against oxidative stress. GSH binds free radicals formed as a result of oxidative metabolism [46]. MDA is an important oxidant parameter for testicles [47]. In previous studies, it has been stated that $AlCl_3$ weakens the antioxidant system in rats by increasing the MDA content [48, 49]. It is stated that D-Gal induces oxidative stress by increasing lipid peroxidation [11]. Presented study, the oxidant activity induced by $AlCl_3$+D-Gal was significantly reduced in the ALZ+TQ group. Parallel to this GSH level, CAT, GPx and SOD activity decreased. This confirms the TQ antioxidant property of this condition and exhibits lipid peroxidation-reducing antioxidant properties.

Environmental pollution adversely affects the structural and physiological integrity of the testicles and may cause infertility [36, 50]. Testicular oxidative stress is one of the main factors causing infertility [51]. It is reported that oxidative damage causes spermatozoal dysfunction [52]. It has been reported that Al-induced oxidative damage and Al cross the blood-testis barrier, induce lipid peroxidation, damage the sperm membrane [53] and cause inactivation of antioxidant enzymes [54]. It has been reported that oxidative damage in spermatozoa plays an important role in the deterioration of sperm function and infertility [45]. $AlCl_3$ increases oxidative stress in testicular tissue and thus has a negative effect on testicular physiology and sperm parameters [8, 55]. It is stated that $AlCl_3$ reduces sperm motility and increases the rate of abnormal sperm [56]. Consistent with previous studies, $AlCl_3$ administration decreased spermatozoa motility, increased dead sperm ratio and abnormal spermatozoa ratio in our study. The reason for this may be that $AlCl_3$ crosses the blood testicular barrier and impairs sperm metabolism and also increases lipid peroxidation due to the increase of free radicals. It can also be shown in the deficiency in the antioxidant defense system, which is necessary for sperm functions.

As a result, $AlCl_3$+D-Gal administration in rats caused an increase in oxidative stress in testis tissue, and decrease in sperm quality. TQ treatment, strengthened the antioxidant defense system in the testis, partially prevented oxidative damage and improved sperm quality.

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