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Tyrosol prevents $AlCl_3$ induced male reproductive damage by suppressing apoptosis and activating the Nrf-2/HO-1 pathway

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Abstract

Aluminium is a ubiquitous element that occurs naturally in the soil making human exposure to it is unavoidable. Tyrosol is present in olive oil and is known to have antioxidant effects. Therefore, the present study explores the toxic effects of aluminium chloride ($AlCl_3$) and evaluates the possible protection by tyrosol in male rats. Testicular injury was induced by the administration of $AlCl_3$ (34 mg kg^{-1} day^{-1}). Rats were treated with either tyrosol (20 mg kg^{-1} day^{-1}) or AlCl3 (34 mg kg^{-1} day^{-1}). The experiment lasted for 10 weeks. Biochemical, histopathological and protein expression profiles were determined to decipher the role of tyrosol in protecting the cellular damage. Further, histomorphometric analyses of testes showed deranged architecture along with other noted abnormalities. $AlCl_3$ group rats' testes showed decreased GSH levels, CAT activities, Nrf-2, HO-1, bcl-2 expressions and sperm motility whereas increased caspase-3 expressions, MDA levels, abnormal and dead/live sperm ratio. However, tyrosol treatment attenuated these changes. The present results demonstrate the beneficial role of tyrosol treatment in $AlCl_3$ induced testicular toxicity alterations of rat.

KEYWORDS

aluminium chloride, antioxidant, apoptosis and sperm quality, tyrosol

1 | INTRODUCTION

Aluminium (Al) is an element that is naturally found in soil and is the most widely distributed metal of the earth and plays a crucial role in daily life with the potential to be harmful to living things because of its characteristics (Kumar & Gill, 2009). Al is in combination with compounds such as Al sulphate and chloride, because of its reactivity (Verstraeten, Aimo, & Oteiza, 2008). Sources of human exposure to aluminium include cooking utensils, contaminated foods and leaching of aluminium from beverage cans (Hashemi-Domeneh et al., 2016; Proudfoot, 2009). Further, Al is used in pharmaceutical products such as phosphate binders and antacids. Al causes cognitive impairment in various animals, such as mice, rabbits and rats (Bilkei-Gorzo, 1993; Cao et al., 2016). Furthermore, various human and animal studies have reported that learning and memory are negatively affected after exposure to Al (Buraimoh, Ojo, Hambolu, & Adebisi, 2011; Exley, 2005). Free radicals are produced in the organism after exposure to $AlCl_3$, inhibiting the antioxidant activity in the blood and seminal plasma, liver, testicles, kidneys, lungs and brain. Moreover, several mechanisms have been reported to be involved in Al-induced infertility. Studies have reported that Al blocks voltage-gated calcium channels (Büsselberg, 1995). By means of this mechanism, Al reduces pituitary gonadotropin release, causing a decrease in the sperm count (Cheraghi, Golkar, Roshanaei, & Alani, 2017). Oxidative damage causes injuries in the testicular tissue and reduces the germ cells (Ghasemnejad-Berenji et al., 2018). Reactive oxygen compounds (ROS), emerging due to the oxidative damage, play

a central role in a series of pathological conditions in association with the environmental factors including inflammation, toxicity and endocrine disorders. ROS damages the macromolecular structures in the cell, leading to cellular dysfunction (Asadi, Bahmani, Kheradmand, & Rafieian-Kopaei, 2017). Therefore, antioxidant herbal products could help to protect the reproductive system against the untoward effects of oxidative damage (Güvenç, Cellat, Gökçek, Yavaş, & Yurdagül Özsoy, 2019).

Administration of antioxidants in the diet (such as herbal products) has recently received growing attention in oxidative stress related diseases (Zhang & Tsao, 2016). Herbal products curative effects have been associated with to being associated with the role played by free radical scavengers and the mediators involved in antioxidant defence systems (Arulselvan et al., 2016). Tyrosol is a pharmacologic compound, found especially in olive and olive oil, wine and several other herbal products (Bu et al., 2007; St-Laurent-Thibault, Arseneault, Longpre, & Ramassamy, 2011). Olive oil is one of the major sources of tyrosol (Chandramohan, Saravanan, & Pari, 2017). Several studies have proven the antioxidant, anti-inflammatory, anticancer, antistress, antiosteoporotic, cardioprotective and neuroprotective effects of tyrosol (Ahn et al., 2008; Chernyshov, Plotnikov, Smol'iakova, & Krasnov, 2007; Güvenç, Cellat, Özkan, et al., 2019; Loru et al., 2009).

Literature reviews show that studies referring to the effects of tyrosol and $AlCl_3$ on the male infertility and reproductive system are quite limited. The aim of the study was to explore the ameliorative and preventative effects of tyrosol on oxidative damage, on the antioxidant systems and spermatological parameters in $AlCl_3$-induced toxicity in rats.

2 | MATERIAL AND METHOD

2.1 | Chemicals and reagents

Chemicals were purchased from Sigma and were analytical pure grade. Seconder antibodies were purchased from Abcam and Thermo Fisher Scientific.

2.2 | Experimental protocol

In the study, 8- to 10-week-old Wistar Albino male rats weighing 220–250 g were used. Experimental protocols approved by National Animal Use and Care Committee (Decision No: 2017/11-3 Hatay, Antakya, Turkey). Rats were divided into four groups of seven. The active substance was not administered to the animals in the control group. In the other groups, the active substances were dissolved in dimethyl sulfoxide (DMSO), and a standard volume (1 ml) of the respective solution was administered to all animals in other groups via the oral route. The experimental groups were designed as follows: Control Group or Group 1 (DMSO), Group 2 or $AlCl_3$ (Al Chloride 34 mg/kg), Group 3 or Tyrosol Group (Tyrosol 20 mg/kg) and Group 4 or Tyr + $AlCl_3$ Group (Tyrosol 20 mg/kg + $AlCl_3$ 34 mg/kg). In order to allow for the completion of the spermatogenic cycle and the maturation of the spermatozoa in the epididymis, the experiment was continued for a total of 10 weeks for all groups (Sarkar, Chaudhuri, Chattopadhyay, & Biswas, 2003; Yüce et al., 2013). Tyrosol (Chandramohan, Pari, Rathinam, & Sheikh, 2015) and $AlCl_3$ (Yousef & Salama, 2009) doses were selected based on the studies in the literature. The rats were decapitated under anaesthesia end of the experiment. After euthanised the animals, their spermatozoa were examined, their testicles were taken and placed at −86°C until biochemical analyses.

2.3 | Spermatological analyses

Spermatological analyses (motility, concentration, percentage of dead/live spermatozoa and abnormal spermatozoa) were performed as described in the literature by Turk, Atessahin, Sonmez, Yuce, and Ceribasi (2007). For spermatozoa concentration, 10 µl of sperm solution was determined by haemocytometer from the epididymis of the right cauda. Left caudal epididymal tissue was used for spermatozoa motility. The percentage of spermatozoal motility was evaluated using phase contrast microscope with heating plate (37°C; Nikon E 200). Results were given as percentages. Eosin–nigrosine-stained slides were prepared to determine morphological structure, and live/dead spermatozoa, the slides stained with hancook solution, were prepared. A total of 400 spermatozoa (2,800 cells in each group) were then examined under a microscope at 400× magnification, and the percentage of abnormal spermatozoa detected.

2.4 | Biochemical analyses

After homogenising the tissue samples collected from animals, they were used for MDA, GSH, GSH.Px and CAT analyses determined spectrophotometrically. MDA was used for marker of lipid peroxidation, and its concentrations were expressed in nmol/g protein at 532 nm (Placer, Cushman, & Johnson, 1966). GSH concentrations were determined using the method described by Sedlak and Lindsay (1968). Amount of GSH were expressed in g/protein at 412 nm. The glutathione peroxidase activity was analysed by the method of Lawrence and Burk (1976). The GSH-Px activity was expressed as international units per gram protein at 340 nm. The catalase activity was measured by the decomposition of hydrogen peroxide (H202) at 240 nm, and it was stated as units/g protein (Aebi, 1983). The protein level was determined by Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951).

2.5 | Western blot analyses

Tissues were homogenised according to protocol with cold RIPA lysis buffer (sc-24948A), centrifuged at 18.405 *g* at +4°C, and

the supernatant separated. Total protein amounts in the samples were determined spectrophotometrically according to protocol using BCA (Smith et al., 1985) kit (Pierce™ BCA Protein Assay Kit). Samples were electrophoresed by loading polyacrylamide gel with equal amounts of protein (50 µg) in each well (Laemmli, 1970), and after SDS-PAGE, the proteins were transferred to polyvinylidene difluoride (PVDF) membrane by Western blotting (Towbin, Staehelin, & Gordon, 1979). PVDF membranes were then blocked with 5% milk powder and washed three times with TBS-T for 5 min to prevent nonspecific binding. Membranes 5% according to the protocol with Nrf-2 (ab137550; rabbit polyclonal antibody; 1/1,000), HO-1 (ab68477; rabbit monoclonal antibody; 1/10,000), beta actin (ab6276; Mouse monoclonal antibody; 1/10,000) antibodies After reconstitution with milk powder, it was allowed to incubate overnight, and after incubation, each membrane was washed three times with TBS-T for 5 min. Secondary antibodies (ab6721; anti rabbit; 1/10,000 and ab97240; anti rabbit; 1/100,000) were diluted with 5% milk powder, and the membranes were incubated with secondary antibodies for 1 hr. After incubation, the membranes were washed again with TBS-T three times for 5 min, and the bands obtained using chemiluminescent conjugate (ECL; Biorad 1705061) were imaged on the chemiluminescence imaging system (Biorad ChemiDoc™ XRS+; Bass et al., 2017; Sambrook, Fritsch, & Maniatis, 1989). The band densities in the obtained images were measured by the analysis program (Biorad Image Lab™ Software version 5.2.1; Biorad Laboratories, Inc.). Data were normalised to beta actin used as internal control and expressed as a percentage of control.

2.6 | Histopathological evaluation

After the experiment, systemic necropsies were performed in the sacrificed rats under anaesthesia. The testicular tissues collected in this procedure were fixed in a 10% buffered formaldehyde solution for 48 hr. A routine tissue follow-up procedure was carried out by dehydrating the tissue samples in alcohol series (70%, 80%, 90% and 100%) and by obtaining transparent tissue samples using xylol series. Then, the processed tissues were blocked in paraffin. Serial sections of 5-micron thickness were taken from the paraffin blocks using a microtome. Haematoxylin–eosin-stained sections of individual testicular tissue samples taken from each rat were examined for the appearance of spermatogenic cells in the seminiferous tubules under the research light microscope (Nikon 80i-DS-Ri2), and scoring was performed according to the Johnsen Testicular Biopsy Score method (Johnsen, 1970). The scoring criteria stated as below: 10, germinal epithelium is a multi-layer structure around the central lumen, the lumen is open and contains a large number of spermatozoon; 9, germinal epithelium is visible but insignificant amount of rash that can wash the lumen, spermatozoon and rash epithelium in occluded lumen; 8, germinal epithelium is multi-layered, but <10 spermatozoa in the lumen; 7, no spermatozoa but many spermatids present; 6, no spermatozoon and less than 10 spermatids; 5, only spermatocytes, no spermatozoon or no spermatids; 4, no spermatozoon and spermatid and <5 spermatocytes; 3, only spermatogonium is present as a germ cell; 2, only sertoli cells and no germ cells; and 1, no cells in the seminiferous tubule lumen.

2.7 | Immunohistochemical evaluation

Caspase-3 (ab13847, rabbit polyclonal antibody, 1/500) and bcl-2 (ab59348; rabbit polyclonal antibody, 1/1,000) expressions were examined by staining the tissue samples according to the streptavidin–peroxidase method (ABC) using the immunoperoxidase kit (HRP Histostain-Plus Kit; Thermo Fisher Scientific Waltham) according to manufacturer's instructions for staining procedures. After the prepared sections were taken on adhesive slides, they were processed in xylene and alcohol series. In order to eliminate the endogenous peroxidase activity, the sections were washed with PBS (phosphate buffer solution); then, they were kept in a 3% hydrogen peroxide (H_2O_2) solution for 20 min. After the sections were put in an antigen retrieval solution (citrate buffer) in a container, the lid of the container was closed and exposed to heat in an oven twice, each session lasting for 20 min. After the specimens were taken out of the oven, they were kept outside until the temperature decreased to the room temperature. The tissues were washed with PBS for the second time, and they were blocked with a blocking solution (nonimmune serum). Each individual tissue specimen was incubated at +4°C over the night after dropping caspase-3 (Thermo Fisher Scientific; 1/100 dilution), and polyclonal and bcl-2 (bcl-2 monoclonal antibody) monoclonal antibody solutions (Abcam; 1/100 dilution) on them. The sections were washed with PBS and incubated at room temperature with biotinylated secondary antibody for 20 min, and the slides were washed with PBS again. Then, the slides kept in streptavidin–peroxidase for 20 min. After this procedure, the slides were washed again with PBS. After the washing process, 3,3′-diaminobenzidine (DAB) was dropped over the specimens. Then, the specimens were left for 1–2 min. Following this procedure, all tissue specimens were kept in Mayer's haematoxylin (Bio-Optica; 05-06002 E) for 1–2 min. Then, they were washed in tap water. The sections were processed in 70% and 90% alcohol consecutively for 3 min and then kept in 100% alcohol for 10 min. Finally, the sections were kept in xylene series for 5 min for an individual concentration. After this process, the slides were mounted with Entellan. Negative controls were used in order to confirm the staining. For this purpose, the negative control slides underwent reactions with PBS instead of reacting with primary antibodies. The prepared tissue sections were inspected under the microscope (Olympus and 80i-DS-RI2), and photos were taken. The intensity of immunoreactive staining with primary antibodies was scored as mild (+), moderate (++) and severe (+++).

2.8 | Statistical analyses

Data were presented as mean ± standard error values. $p < .05$ was considered significant for statistical difference. Shapiro–Wilk normality test was used to determine whether the raw values of all measured parameters showed normal distribution. As a result of the test, it was found that the values in all parameters showed normal distribution. According to the results of this test, one-way analysis of variance (ANOVA) was used to detect differences between the groups and post hoc Duncan test was used for binary comparisons. All statistical analyses were performed using software program (SPSS 23.0).

3 | RESULTS

3.1 | Biochemical analyses

The data obtained at the end of the study for the parameters used for determining the biochemical analyses have been presented in Table 1.

At the end of the study, it was found out that MDA levels increased with $AlCl_3$ induced toxicity in Group 2, whereas, $AlCl_3$ and tyrosol administration significantly decreased the MDA levels in Group 4 ($p < .05$). The contents of GSH and activities of catalase were found out to be decreased with the administration of $AlCl_3$ in Group 2; however, they were found out to be significantly increased in $AlCl_3$ and tyrosol treated Group ($p < .001$).

3.2 | Spermatological analyses

The data about the spermatological parameters have been presented in Table 2.

The examination of the spermatological parameters revealed that $AlCl_3$ administration negatively affected the motility, the dead/alive spermatozoa ratio and the ratio of abnormal spermatozoa. In Group 2, a decrease in the motility ($p < .01$) and an increase in the dead/alive spermatozoa ratio and an increased ratio of abnormal spermatozoa were observed with $AlCl_3$ administration ($p < .001$). In Group 4, increased motility ($p < .01$), a decrease in the ratio of dead/alive spermatozoa and a decrease in the abnormal spermatozoa ratio ($p < .001$) were observed with the administration of $AlCl_3$ and tyrosol in combination.

3.3 | Results of western blot analysis

At the end of the study, the results obtained for the protein expression rates in the testicular tissue specimens have been presented in Figures 1 and 2, and the images of the bands have been presented in Figures 1 and 2. In Group 2, Nrf-2 ($p < .01$) and HO-1 ($p < .05$) expressions decreased due to $AlCl_3$ administration, and there was a significant decrease compared with the control group. In $AlCl_3$ + tyrosol group, the expression levels of Nrf-2 ($p < .01$) and HO-1 ($p < .05$) reached the same levels as those measured in the control group.

TABLE 1 The contents of MDA, GSH and the activities of GSH.Px and CAT in testicular tissue of rats

Group/Variables	MDA (nmol/g prot)	GSH (nmol/g prot)	GSH.Px (IU/g prot)	CAT (U/g prot)
Control	2.5563 ± 0.14^{a}	7.4443 ± 0.25^{a}	121.3371 ± 6.92	107.0296 ± 2.21^{a}
$AlCl_3$	3.1916 ± 0.11^{b}	4.8539 ± 0.39^{b}	100.3144 ± 4.25	82.4362 ± 3.04^{c}
Tyrosol	2.0398 ± 0.08^{c}	7.3152 ± 0.31^{a}	110.4195 ± 3.66	94.4441 ± 3.51^{b}
$AlCl_3$ + Tyrosol	2.6484 ± 0.13^{a}	7.2870 ± 0.17^{a}	107.8175 ± 2.76	101.8261 ± 1.81^{ab}
Significance	$p < .05$	$p < .001$	$p > .05$	$p < .001$

Note: Values represent mean ± *SEM* for each group. Different superscript letters (a, b, c) within the same column show statistically significant differences between the groups.

TABLE 2 Effect of Tyrosol on $AlCl_3$-induced changes in sperm quality

Sperm parameters				
Groups	Motility (%)	Concentration (million/right cauda epididymis)	Dead/Live sperm rate (%)	Abnormal sperm rate (%)
Control	79.1666 ± 1.53^{a}	205.8333 ± 6.75^{a}	27.8333 ± 0.87^{a}	17.5000 ± 0.92^{a}
$AlCl_3$	45.8333 ± 3.51^{c}	109.1666 ± 5.68^{b}	61.3333 ± 1.47^{b}	36.5000 ± 1.97^{c}
Tyrosol	66.4285 ± 2.36^{ab}	184.2857 ± 12.92^{a}	41.7142 ± 1.70^{a}	19.8571 ± 0.79^{a}
$AlCl_3$ + Tyrosol	59.1666 ± 2.38^{b}	146.6666 ± 11.22^{ab}	46.3333 ± 1.33^{a}	24.8333 ± 1.86^{b}
Significance	$p < .01$	$p < .05$	$p < .001$	$p < .001$

Note: Values represent mean ± *SEM* for each group. Different superscript letters (a, b, c) within the same column show statistically significant differences between the groups.

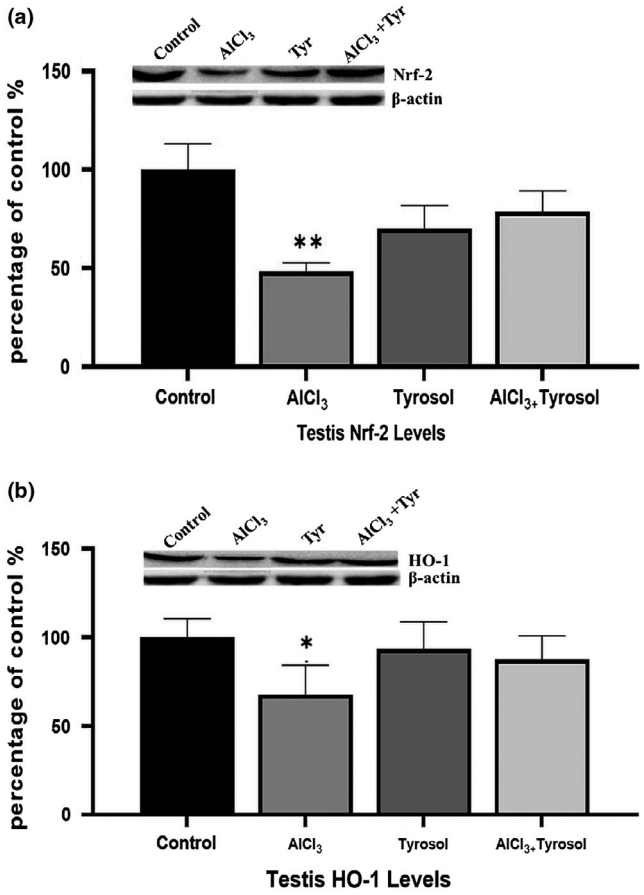

FIGURE 1 Effect of tyrosol on Western blot analysis of Nrf-2 and HO-1in the testicular tissue of control and experimental rats. Protein expression is normalised to β-actin. Data are shown as per cent of control. (a) Representative Western blot picture of Nrf-2 and beta actin in testis. Mean Nrf-2 expression levels (%). (b) Representative Western blot picture of HO-1 and beta actin in testis. Mean HO-1 expression levels (%). Data are expressed as mean ± *SEM*. $^{*}p < .05$, $^{**}p < .01$ vs. control group

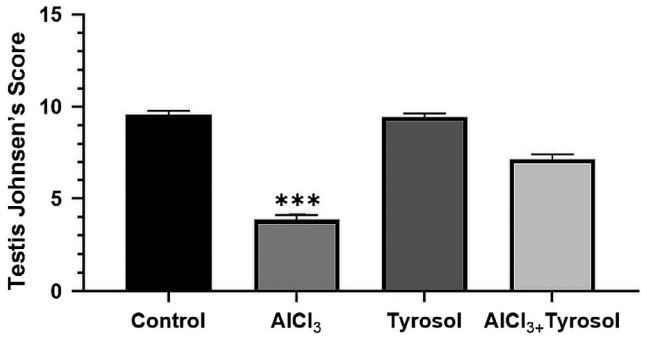

FIGURE 2 Histopathological variables in testes according to the Johnsen's score in study groups. Data are expressed as mean ± *SEM*. $^{***}p < .001$ vs. control group

3.4 | Johnsen testicular biopsy scores, and histopathological and immunohistochemical findings

The mean scores of the haematoxylin–eosin-stained testis tissue specimens determined according to Johnsen scores criteria under the light microscope have been presented in Figure 2. The images of the histopathological findings observed under the microscope have been presented in Figure 3a–f.

The Johnsen testicular biopsy scores in individual groups showed that the mean scores were the lowest in the $AlCl_3$ group. When the groups were compared, it was observed that the scores in the control group and Tyr group were almost the same, but there was a statistically significant difference between the scores of the control group and the scores in the $AlCl_3$ group ($p < .001$). The scores in the $AlCl_3$ + Tyr group were significantly different from those of the $AlCl_3$ group ($p < .001$), while they were similar to those in the control group ($p < .001$).

The microscopic examination of the testes of the rats in the control and Tyr groups revealed that the array of the seminiferous tubules, interstitial connective tissue, Leydig cells and the spermatogenic cells in tubules had a normal structure, and that the spermatogenesis was regular with the presence of germ cells in all stages of spermiogenesis, from spermatogonia to sperms (Figure 3a,f).

In the $AlCl_3$ group, it was observed under the microscope that several tubular germ cells were largely separated from the basement membrane, the tubulus membrane was thickened, the tubules came closer to each other, the interstitial space was narrowed, there was a significant cell loss or disorganisation in the tubules, the space between the Sertoli cells and spermatogonia increased, germinal cells degenerated, the nuclei of some spermatogonia showed shrinkage, and the spermatogenesis was either halted or decreased to a large extent. There were oedema and vascular congestion at spaces in the interstitial areas (Figure 3b,c).

In the $AlCl_3$ + Tyr group, the seminiferous tubules were preserved to a large extent, germinal cell loss was considerably reduced, and the spermatogenesis in the seminiferous tubules continued in the cells in the spermatogenesis series, including the spermatozoa. It was determined in this group that the pathomorphological changes in the spermatogonia did not occur at a higher rate compared with those observed in the group receiving $AlCl_3$. A small number of germinal cells were observed to have spilled into the lumen of the seminiferous tubules. The oedema and congestion in the interstitial area and the thickening in the tubulus membranes were very mild. Leydig cells were observed to have a normal histological structure (Figure 3d,e).

The microscopic images of the immunohistochemically-stained specimens with anti-caspase-3 and anti-bcl-2 primary antibodies have been presented in Figure 4a–d, and the respective immunoreactivity scores have been shown in Figure 5. No significant immunoreactivity was detected in the spermatogenic cells in the tubules in the testis specimens collected from the rats in the control and Tyr groups. The immunoreactivity of caspase-3 was observed to be moderate/severe (++/+++) in the $AlCl_3$ group, and it was mild (+) in the $AlCl_3$ + Tyr group. The immunoreactivity of bcl-2 was mild (+) in the $AlCl_3$ group, and it was moderate (++) in the $AlCl_3$ + Tyrosol group.

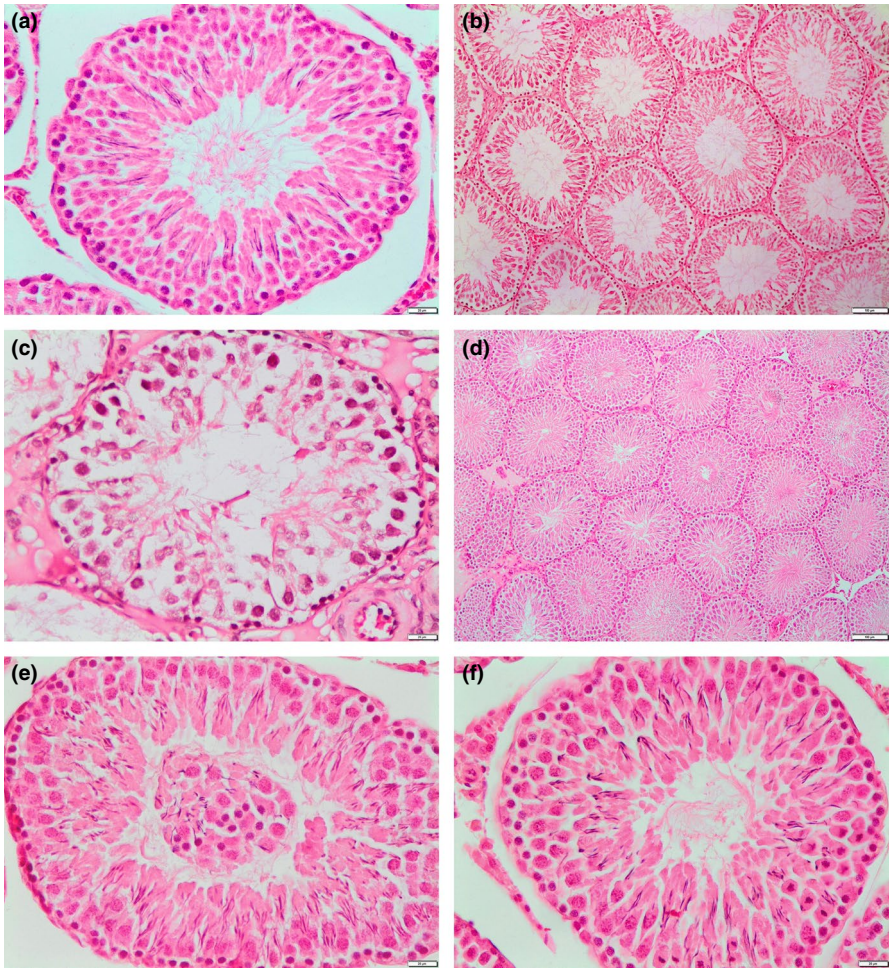

FIGURE 3 Microscopic appearance of testes. (a) Control (Bar: 20 μm). (b) $AlCl_3$ (Bar: 100 μm). (c) $AlCl_3$ (Bar: 20 μm). (d) $AlCl_3$ + Tyrosol (Bar: 100 μm). (e) $AlCl_3$ + Tyrosol (Bar: 20 μm). (f) Tyrosol (Bar: 20 μm) H&E

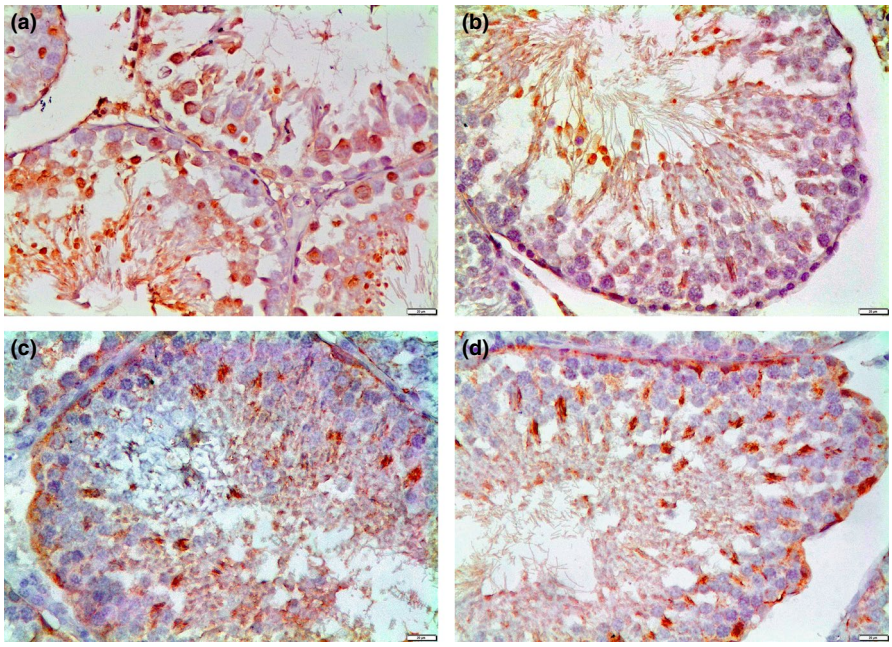

FIGURE 4 Microscopic appearance of caspase-3 and bcl-2 immunoreactivities. (a) $AlCl_3$ group—caspase-3, (b) $AlCl_3$ + Tyrosol group caspase-3. (c) $AlCl_3$ group bcl-2, (d) $AlCl_3$ + Tyrosol group bcl-2. ABC peroxidase (Bar: 20 μm)

4 | DISCUSSION AND CONCLUSION

The widespread distribution of Al consumption products expose human to the risk of Al-induced testicular injury. Although Al is considered an important factor for pathological disorders and various hypotheses have been proposed for its toxicity, its pathogenesis has not been clearly explained. The traditional Mediterranean diet's one of the major component, olive oil, as a main source of fat

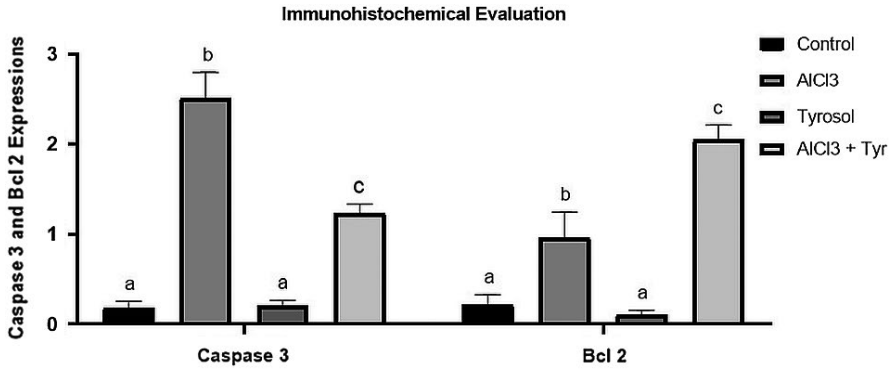

FIGURE 5 Distribution of Caspase-3 and bcl-2 immunoreactivity scores by groups. Data are expressed as mean ± *SEM*. Different superscript letters (a, b, c) within the same column show statistically significant differences between the groups. $p < 0.001$

is specific nutrient of this dietary model. Tyrosol is one of the most important antioxidant components of this olive oil (Estruch & Salas-Salvado, 2013). This study investigated the effects of tyrosol in an $AlCl_3$-induced reproductive toxicity model. The administration of $AlCl_3$ caused testicular injury, while the administration of tyrosol increased the sperm quality, induced Nrf-2/HO-1 pathway, supressed apoptosis and improved the histological picture of the testis, as well as it suppressed oxidative stress and apoptotic markers induced by the administration of $AlCl_3$. According to our knowledge, this is first study about the tyrosol's protective effects on $AlCl_3$-induced toxicity.

Environmental factors negatively affect the structural and physiological integrity of testes and demonstrate unfavourable effects on male reproductivity. It is a well-known fact that chronic exposure to environmental toxic substances including metals (such as lead, mercury, cadmium and Al) is noxious for reproductivity (Damek-Poprawa & Sawicka-Kapusta, 2003; Yousef, El-Morsy, & Hassan, 2005). Testicular oxidative stress is one of the main factors underlying male infertility (Wagner, Cheng, & Ko, 2018). Khattab and Khattab (2007) have reported that Al-induced oxidative damage and Al's capacity to cross the blood–testis barrier induced lipid peroxidation, damaging the biological membranes. Studies show that Al increases the lipid peroxidation, causing oxidative cell damage and provokes the reproductive toxicity (Cao, Zhang, Wang, & Li, 2019; Zhuang et al., 2018). It has been reported that the decrease in the antioxidant capacity in testes can occur owing to the capture of free radicals accumulated in this tissue (Morielli & O'Flaherty, 2015). It is suggested that ROS has a higher affinity for the thiol groups in the biomolecules, leading to the depletion of thiols intracellularly; consequently resulting in reduced GSH concentrations (Irvine, 1996). Mohamed and El-Moneim (2017) and Afolabi, Wusu, Ugbaja, & Fatoki (2018) showed in their study, Al administration reduced the activity level of antioxidant enzymes. In our study, $AlCl_3$ administration led to an increase in MDA levels, decreased levels of GSH and diminished CAT activity. The increase in the amount of hydrogen peroxide due to $AlCl_3$ accumulation may be suggested to reduce the antioxidant activity. Researchers have increased interest in developing new strategies for the treatment of male infertility, including the use of alternative drugs or bioactive food components. Previous studies with antioxidant agents in Al-induced testicular toxicity have reported that Gingko biloba (Mohamed & El-Moneim, 2017), Omega3 (Oda, 2016) and exogenous phosphatidylcholine (Khafaga, 2017) reduced lipid peroxidation more also, increased antioxidant activity. Recent studies have shown that tyrosol, one of the important components of olive oil, reduces oxidative damage and increases antioxidant capacity (Chandramohan et al., 2017; Kalaiselvan, Samuthirapandi, Govindaraju, Sheeja Malar, & Kasi, 2016; Sun, Fan, Yang, Shi, & Liu, 2015). In the present study, it was demonstrated that oral tyrosol treatment decreased the elevated MDA levels significantly and showed a tendency towards increasing GSH levels and CAT activities. The reduced concentration of testicular MDA observed in tyrosol treated rats is due to its anti-lipid peroxidation effect. Thus, tyrosol scavenges excessive free radicals produced by $AlCl_3$ and reduces oxidative damage, thereby protecting the testis from the deleterious effects of LPO by its antioxidant effects.

An increased concentration of reactive oxygen compounds modifies DNA and promotes local apoptosis (Sarabia, Maurer, & Bustos-Obregon, 2009). Caspases are very important mediators of apoptosis, and they are frequently activated as apoptotic proteases. They are involved in the specific cleavage of several critical cell proteins, being responsible for catalysis (Porter & Jänicke, 1999). Caspases have been one of the most appropriate targets to modulate apoptosis and to prevent apoptotic cell death. The bcl-2 protein is found in the cytoplasmic site of the mitochondrial membrane, in the endoplasmic reticulum and in the membrane of the nucleus. It is encoded by the bcl-2 gene, and it delays or inhibits apoptosis (Yilmaz, Dasdag, Akdag, & Kilinc, 2008). Studies in the literature have shown that various antioxidants improve testicular damage by regulating the apoptotic pathway. Rizk, Zaki, and Mina (2014) reported that propolis led to a reduction in doxorubicin-induced apoptosis by inhibiting the caspase-3 expression. Das, Ghosh, Manna, Sinha, and Sil (2009) reported that oral taurine administration acted by controlling arsenic-induced testicular apoptosis via bcl-2 regulation. Shi et al. (2012) reported that tyrosol showed anti-apoptotic effects by regulating the expression of bcl-2. In our study, the administration of $AlCl_3$ resulted in increased levels of caspase-3 and decreased levels of bcl-2. However, treatment of oral tyrosol reversed the above-mentioned changes, alleviating the $AlCl_3$-induced injury. It may be suggested that tyrosol showed this effect by reducing the increased amounts of free radicals in the environment and by inhibiting the activation of the apoptotic pathway.

Free radical-induced oxidative damage in spermatozoa has recently been demonstrated to play an important role in abnormal sperm functioning and infertility (Russo, Troncoso, Sanchez, Garbarino, & Vanella, 2006). The reasons affecting the sensitivity of spermatozoa to oxidative damage are antioxidant capacity of spermatozoa, the ability of spermatozoa to synthesise reactive oxygen species and unsaturated fatty acid concentration in membrane phospholipids (Aitken, 1995). Al-induced oxidative stress can lead to increased cellular permeability, inactivation of enzymes and an increase in nitric oxide (NO) levels. NO plays important role for infertility (Wagner et al., 2018). Studies in the literature have reported that $AlCl_3$ has a negative effect on sperm parameters (Cheraghi et al., 2017; Savory, Herman, & Ghribi, 2003; Yousef et al., 2005) and also decreased sperm motility and an increase in the amount of abnormal sperm in animals exposed to $AlCl_3$ orally (Alkhedaide et al., 2016; Kumari & Singh, 2015). Similarly, in our study too, $AlCl_3$ administration reduced the motility of spermatozoa, and increased the ratio of dead/live spermatozoa and increased the ratio of abnormal spermatozoa. This may be due to suppression of testosterone production as a result of increased nitric oxide production, low fructose levels in seminal plasma that reduces sperm metabolism and formation of reactive oxygen compounds that cause lipid peroxidation and DNA damage. Furthermore, low levels of antioxidant enzymes may also be related in our sperm findings, as it is required for the maintenance of spermatozoa functions.

Molecular defence mechanisms of antioxidants such as tyrosol, oleuropein and hydroxytyrosol contained in olive oil components, one of the most important sources of tyrosol, were found to resist the damaging effects of pro-oxidants and improve sperm quality (El-Kholy et al., 2015; Lancellotti et al., 2013). In our study, sperm parameters improved in the tyrosol treated group, as the sperm motility, the ratio of dead/alive spermatozoa and abnormal spermatozoa ratio were restored at the levels observed in the control group. Tyrosol is thought to show these effects by improve sperm integrity and function by reducing lipid peroxidation and altering lipid composition of sperm cells. However, given the fact that nitric oxide plays an important role in sperm motility, the decrease in cholesterol content in the layered membrane and increase the sperm quality by improving the nitric oxide signal may be due to oral tyrosol administration.

HO-1 is a molecular structure activated by Nrf-2, showing anti-inflammatory and anti-oxidative effects. Regulation of the Nrf-2/ HO-1 pathway is closely associated with oxidative stress, too (Nguyen, Sherratt, Huang, Yang, & Pickett, 2003). In the processes associated with the antioxidant activity, Nrf2, a transcription factor, is the main protective factor. Nrf-2 can be activated and can bind to the antioxidant-responsive element (ARE), consequently inducing defence mechanisms such as those involving HO-1 (Loboda, Damulewicz, Pyza, Jozkowicz, & Dulak, 2016). Several studies have reported that tyrosol stimulates the Nrf-2 pathway (Dewapriya, Himaya, Li, & Kim, 2013; Lee, Hur, Lee, Yoon, & Choi, 2018; Wang et al., 2017). In our study, the reduced levels of Nrf-2 and HO-1 with the administration of $AlCl_3$ were restored at the control group levels with tyrosol administration. The results of the present study suggested that tyrosol can regulate the Nrf-2/HO-1 pathway against $AlCl_3$-induced oxidant damage of testes in rats. The data obtained in our study suggest that tyrosol may have protective effects on testicular tissue by activating the ARE pathway.

The histopathological analyses revealed an altered testicular morphology developed in Al groups compared with normal testes. Examination of the testes revealed the following findings including degeneration of the spermatogenic cells and seminiferous tubules, thinning of the germinal epithelium, increased number of necrotic foci, degeneration of Leydig cells, vacuolar degenerative changes in the cytoplasm of the sertoli cells and decreases in the germ cell count. This situation has similarities to the results of previous studies in the literature (Khattab & Khattab, 2007; Mohamed & El-Moneim, 2017; Nuhair, 2015). However, in the rats receiving tyrosol + $AlCl_3$, it was found out that the abovementioned adverse consequences were reversed, and findings similar to the normal histological appearance of the testes were observed.

In conclusion, 10 weeks of oral tyrosol treatment provided significant protection against Al-induced testicular dysfunction and apoptosis in rats, as evidenced by significant improvement in antioxidant activity, sperm quality, and histomorphometric changes and alleviation of oxidant stress through inducing the Nrf-2/HO-1 signalling pathway. This study will provide motivation for future investigations to explore the mechanism (s) of tyrosol on $AlCl_3$-induced reproductive toxicity. The data obtained in our study suggest that tyrosol may have curative activity on the male reproductive system as an antioxidant. Furthermore, future studies examining the effects of different doses of tyrosol used for different periods of time may help better understand this active substance.

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CONFLICT OF INTEREST

Authors concluded that they have no conflict of interest.

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