Egypt. J. Chem. **Vol. 65**, No. 6 pp. 665- 676 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Quercetin Attenuates Testicular Dysfunction Induced By Aluminum Chloride In Male Wistar Rats

Asmaa A. Mahmoud[a*], Asmaa M. Elfiky[b], Hala A. Elreedy[b], Khadiga S. Ibrahim[b]

[a]*Department of Zoology, Faculty of Science, Ain Shams University, Cairo, Egypt*

[b]*Environmental and Occupational Medicine Department, Environmental Research Division, National Research Center, Cairo, Egypt*

Abstract

Aluminum (Al) exposure is one of the environmental factors involved in the pathogenesis of male infertility as a global health problem. Quercetin (Q) is a naturally occurring flavonol that improved testosterone (T) level and spermatogenesis against heavy metals-, diabetes-, and cancer-induced testicular intoxication in male rats. The present study aimed to investigate the therapeutic potential of Q against aluminum chloride ($AlCl_3$)–induced testicular dysfunction in rats through evaluation of hormones of the pituitary-testicular axis, sperm parameters in the epididymis, and histological alterations in rat testis. After induction of testicular dysfunction in male rats by oral administration of $AlCl_3$ (50 mg/kg) for 28 days, the therapeutic Q dose (50 mg/kg) was orally administered to rats for 28 days. The rats were divided into four groups (each group comprising eight rats): Normal control (NC), Q, $AlCl_3$, and Q+ $AlCl_3$. Q administration increased final body weight and genital organs' relative weight, and serum free testosterone (T), luteinizing hormone (LH), and follicle-stimulating hormone (FSH) in the $AlCl_3$-treated rats. Also, Q increased sperm count, motility, and progressive motility in the epididymis in $AlCl_3$-treated rats. Further, Q administration to $AlCl_3$-treated rats caused regeneration of the germinal epithelium in the seminiferous tubules and increased spermatocytes within the lumen. The current study suggests that Q administration may provide a significant therapeutic effect against $AlCl_3$-induced testicular dysfunction in rats via improving the pituitary-testicular axis hormones, leading to increased sperm count and motility in the epididymis; and amelioration of histological alterations observed in the testes of $AlCl_3$-rats.

Keywords: Male infertility; Testicular dysfunction; $AlCl_3$; Quercetin.

1. Introduction

Human male infertility is a widespread multi-factorial condition that affects up to 70 million people worldwide and is estimated to impact approximately 9% of couples globally [1].

Aluminum (Al) exposure is one of the environmental factors involved in the pathogenesis of male infertility as a global health problem [2]. The prevalent cause behind the Al toxic effect on male genital organs is the abundance of Al on Earth crust and its widespread usage in water purification, utensils, food additives, deodorants, and cosmetics [3].

Moreover, medicines such as antacids, aspirins, and first-aid antibiotics contain Al compounds [3]. Deposition of Al in testes and other reproductive organs is associated with a reduction in spermatogenesis and sperm count, testicular dysfunction, andendocrine disruption [4,5].

*Corresponding author e-mail: asmaamahmoud@sci.asu.edu.eg; asmaa_ainshams@yahoo.com.
Received date 25 October 2021, Revise Date: 12 November 2021, Accept Date: 22 November 2021
DOI: 10.21608/EJCHEM.2021.102711.4760

Leydig and Sertoli cells are the primary targets for Al-intoxication, resulting in alteration of hypothalamic-pituitary-testicular pathway and suppression of androgens production [6,7]. Also, the reduction in pituitary gonadotropin release due to Al-intoxication could diminish sperm count and viability [8].

Due to the high reactivity of Al^{+3}, it combines with chloride and circulates throughout the body[9]. Besides the induction of neurotoxicity, cognitive impairment, and Alzheimer's disease [10], the administration of aluminum chloride ($AlCl_3$) to sexually mature male Wistar rats causes reproductive organs toxicity and infertility [8,11,12]. Also, $AlCl_3$ exposure results in the destruction of testes and epididymis in rodents [12,13]. Also, oral administration of $AlCl_3$ to rabbits reduced sperm count and quality [14].

The first therapeutic line of treatment of male infertility is testosterone replacement therapy. However, the long-term treatment could result in erythrocytosis, testicular atrophy, and suppression of spermatogenesis [15]. Alternative medicine evidenced that phytochemicals extracted from plants and herbs, including phenolic acids and flavonoids could provoke the fertility health of the males. Flavonoids administration caused improvements in sperm quality, sexual functions, libido, and testosterone in experimental animal models and humans [16,17].

Quercetin (Q) (3,30,40,5,7-pentahydroxyflavone) is one of the most potent flavonols, a subclass of the naturally occurring flavonoids [10]. Q is abundant in various fruits such as white mulberry and apple [18], vegetables, such as broccoli, onion, and potato, and grains such aspeanut, soybean [17].Numerous studies have shown the diverse therapeutic properties of Q, such as anti-apoptotic, anti-carcinogenic, immune-stimulating, anti-inflammatory, and antiviral effects [19–21].

Additionally, Q stimulates antioxidant activity and inhibits oxidative damage [17]. Q also exhibits a neuroprotective effect. Q can cross the blood-brain barrier to counteract the amyloid plaques aggregation in the hippocampus and cognitive deterioration [10]. Indeed, several studies have reported the impact of Q on testicular function and spermatogenesis in rats [17,22,23]. Q co-treatment inhibited manganese-induced reduction in reproductive hormones and increased sperm quality and quantity in the treated rats [22]. Also, Q supplement alleviated testis injury in rats caused by cadmium exposure [23]. Further, the co-administration of Q with arsenic ameliorated the adverse histopathological changes in the testis of rats and improved testosterone concentration and spermatogenesis [17].The present study aimed to investigate the therapeutic potential of Q against $AlCl_3$–induced testicular dysfunction in rats through evaluation of hormones of the pituitary-testicular axis, sperm parameters in the epididymis, and histological alterations in rat testis.

2. Material and methods

2.1. Drugs and chemicals

Aluminum chloride ($AlCl_3$) hydrate (Cat. # 229393) and solid quercetin ≥ 95% (Cat. # Q4951, HPLC) were purchased from Sigma-Aldrich Chemicals Co., St. Louis, USA.

2.2. Animals

Thirty-two male Wistar rats (8 weeks) weighing 140 ± 10 g were provided from the Animal House of the National Research Centre, Giza, Egypt. The rats were housed in polypropylene cages and acclimatized to specific pathogen-free conditions for three days before the commencement of the experiments.

The rats were maintained at 24 ± 1 °C and 55%–65% humidity under a 12 h light/dark cycle. The rats received water ad libitum and a standard rodent diet (17.48% protein, 6.85% fat, 62.99% carbohydrates, 4.08% ash, 2.16% minerals and vitamins). Animal handling procedures were performed following the ethical standards of the Ethical Committee of Medical Research of National Research Centre, with approval number "18144," Giza, Egypt, and in compliance with the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

2.3. *Experimental setting*

After the acclimatization period, the rats (n = 32) were weighed and equally distributed into the normal control (NC) group, orally administered with vehicle (saline), and the $AlCl_3$ group, orally administered with 50 mg $AlCl_3$/kg bwt for 28 days [24]. After the $AlCl_3$ administration period, the NC (n = 16) and

$AlCl_3$ rats (n= 16) were allocated into four groups (8 rats in each group) to undergo treatments (with vehicle or Q50) for 28 days as follows: (1) NC group orally administered with saline by gastric intubation; (2) Q50 group orally administered with Q dissolved in saline by gastric intubation (50 mg/kg bwt) [25]; (3) $AlCl_3$ group orally administered with vehicle (saline) by gastric intubation; (4) $AlCl_3$+ Q50 group orally administered with Q dissolved in saline by gastric intubation (50 mg/kg bwt).

2.4. *Sample collection*

At the end of the experimental period, the final body weights of rats were recorded, and all rats were sacrificed by cervical decapitation after withholding food for 14 h. The blood samples were withdrawn from the retro-orbital plexus in clean dry centrifuge tubes and allowed to clot to separate the sera.

Serum samples were separated by centrifugation at 4000 r/min for 10 min at 4°C. Aliquots of serum were frozen and stored at −20 °C for further determination of hormonal assays. Testes and epididymides were excised and weighed. The right testes were fixed in10 % phosphate-buffered formalin (pH 7.4) for histological analysis, whereas the cauda regions of left epididymides were used for sperm analyses.

2.4.1. Hormonal assays

Serum free testosterone (T), FSH, and LH levels were measured by enzyme-linked immunosorbent assay (ELISA) kits for rats (CUSABIO Technology LLC, Houston, USA) following the manufacturer's instructions. The sensitivity of hormone detection per assay tube was 0.15 pg/mL for free T, 0.07 mlU/ml for FSH, and 0.15 mlU/ml for LH. These assays showed no cross-reactivity with other analogs, with inter-assay precision < 15% and intra-assay precision < 15%.

2.4.2. Sperm parameters

The cauda regions of the left epididymides were placed in a petri dish containing 2 ml of saline and dissected into small portions to release the sperm cells (spermatozoa) into the solution. Then, 10 µl of sperm solution was used to evaluate sperm concentration and motility using a hemocytometer under the light microscope using the x40 objective lens. Sperm cells were counted in five large squares of the counting chamber. Sperm concentration was calculated using this formula: Number of the counted sperm cells × dilution factor/volume × 1000 = spermcells × 10^6/ml. The percentage of sperm motility was calculated using this formula: Number of the counted motile sperm cells / total number of the counted sperm cells × 100. Motile sperm cells were classified as either non-progressive or progressive and were expressed as a percentage [26].

3.4.3.Histological procedures

The testes were fixed overnight in 10 % phosphate-buffered formalin (pH 7.4). Then, they were dehydrated using a gradient of ethyl alcohol and embedded in paraffin. Each testis was sectioned at 5 µm thickness and 50 µm intervals, and every 7^{th} section was mounted onto a microscope slide and then stained with hematoxylin and eosin (H&E). Then, the sections were examined and photographed using a camera attached to a Leica DM LS2 microscope (Leica Microsystems, Wetzlar, Germany).

3. Results

3.1. Therapeutic effect of Q on final body weight (g) and relative genital organs weight (%) in $AlCl_3$-treated rats

The $AlCl_3$-treated rats showed a significantly lower body weight than recorded in the NC group. Also, the $AlCl_3$-treated rats exhibited a significant decrease in the relative genital organs weight, including testis and epididymis, compared with the NC group ($P<0.05$). Oral administration of Q to the $AlCl_3$-treated rats significantly ($P<0.05$) improved the body weight and testis and epididymis relative weights in those rats, in comparison with the untreated-$AlCl_3$ rats (Fig 1).

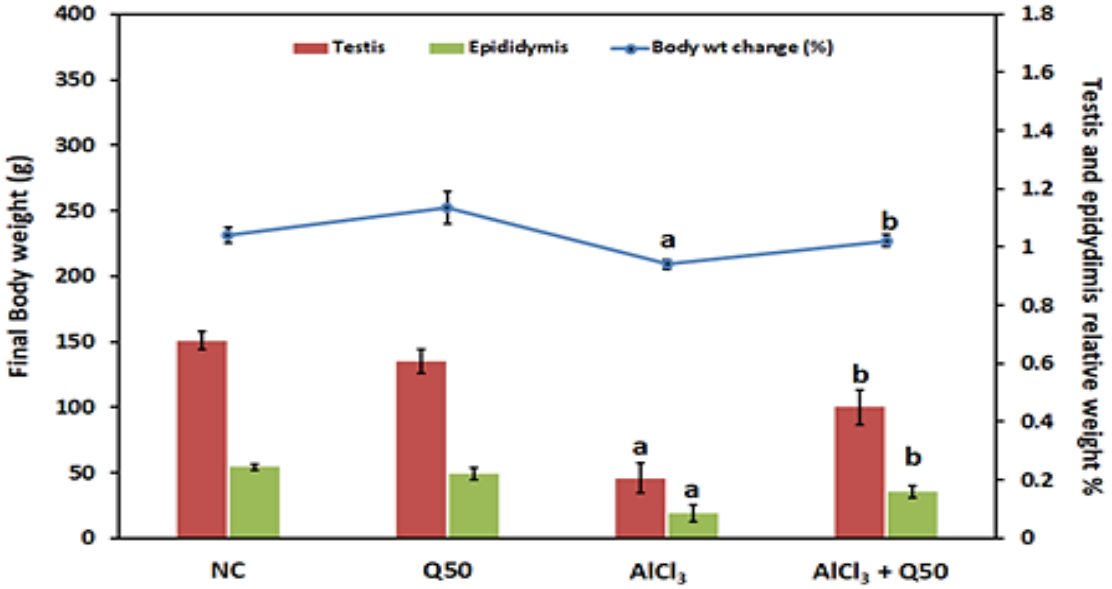

Fig 1 Effect of Q on final body weight (g) and relative genital organs weight (%) in $AlCl_3$-treated rats. Data are represented as mean ±SEM (n = 8). a: Significant change at $P < 0.05$ in comparison with the NC group; b: significant change at $P < 0.05$ in comparison with the $AlCl_3$-treated group, as determined by Tukey's test.

3.2. Therapeutic effect of Q on on serum pituitary-testicular axis hormones in $AlCl_3$-treated rats

As shown in Figs 2 & 3, serum free T, LH, and FSH levels did not show remarkable differences between NC and Q50 groups. Serum free T, LH, and FSH levels were significantly ($P<0.05$) reduced in the $AlCl_3$- treated rats, in comparison with the NC rats. Conversely, Q treatment significantly ($P<0.05$) raised the serum free T, LH, and FSH levels in the $AlCl_3$+Q50 group, in comparison with the untreated-$AlCl_3$ group.

3.3. Therapeutic effect of Q on sperm parameters in $AlCl_3$-treated rats

Fig 4 showed that the rats treated with $AlCl_3$ exhibited significant ($p< 0.05$) reductions in the sperm count and motility(%) and a lack of progressive motility in comparison with NC rats. The $AlCl_3$-treated rats then administered with Q showed significant increases in sperm count, motility (%), and progressive motility(%) compared with the untreated-$AlCl_3$ rats.

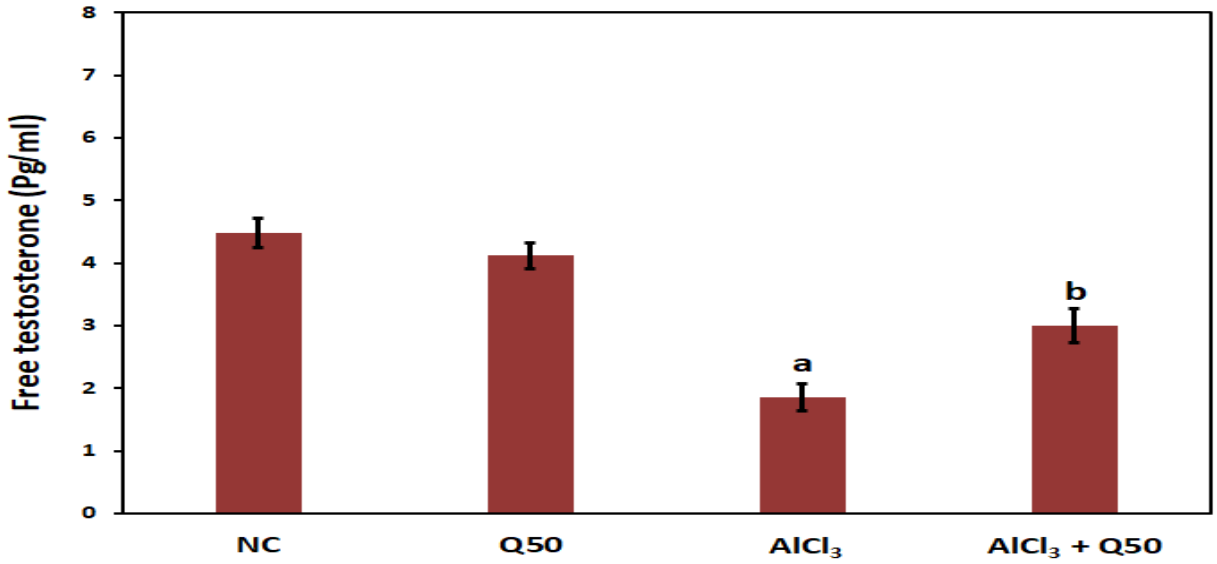

Fig 2 Effect of Q on serum free T (pg/ml) in $AlCl_3$-treated rats. Data are represented as mean ±SEM (n = 8). a: Significant change at $P < 0.05$ in comparison with the NC group; b: significant change at $P < 0.05$ in comparison with the $AlCl_3$-treated group, as determined by Tukey's test.

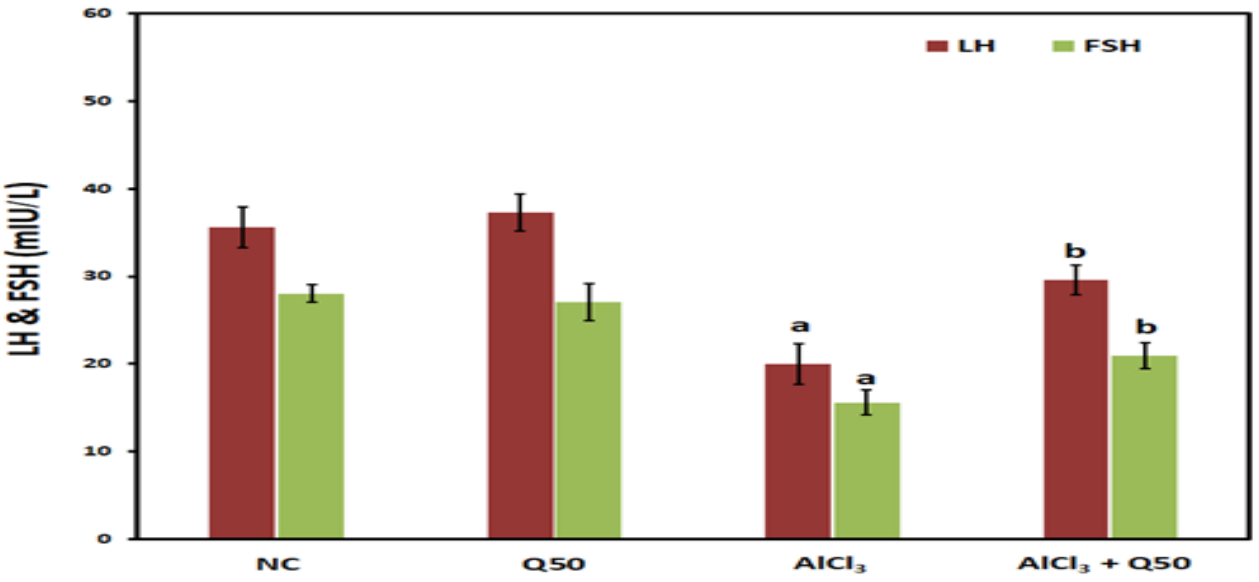

Fig 3 Effect of Q on serum serum LH (mIU/ ml) and FSH (mIU/ ml), in $AlCl_3$-treated rats. Data are represented as mean ±SEM (n = 8). a: Significant change at $P < 0.05$ in comparison with the NC group; b: significant change at $P < 0.05$ in comparison with the $AlCl_3$-treated group, as determined by Tukey's test.

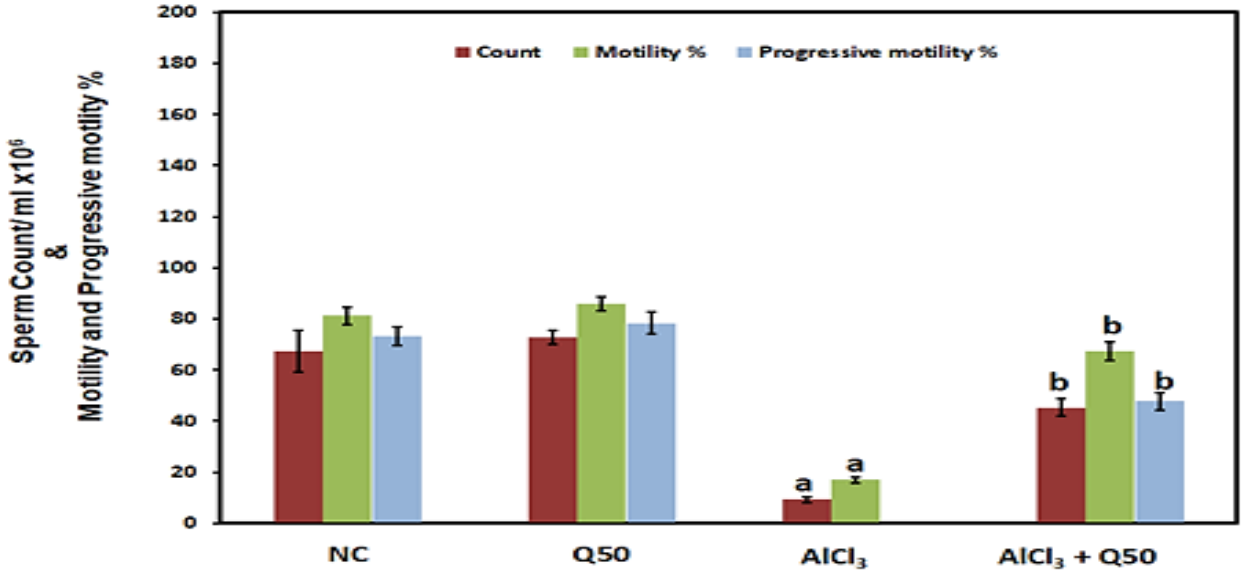

Fig 4 Effect of Q on sperm parameters in $AlCl_3$-treated rats. Data are represented as mean ±SEM (n = 8). a: Significant change at $P < 0.05$ in comparison with the NC group; b: significant change at $P < 0.05$ in comparison with the $AlCl_3$-treated group, as determined by Tukey's test.

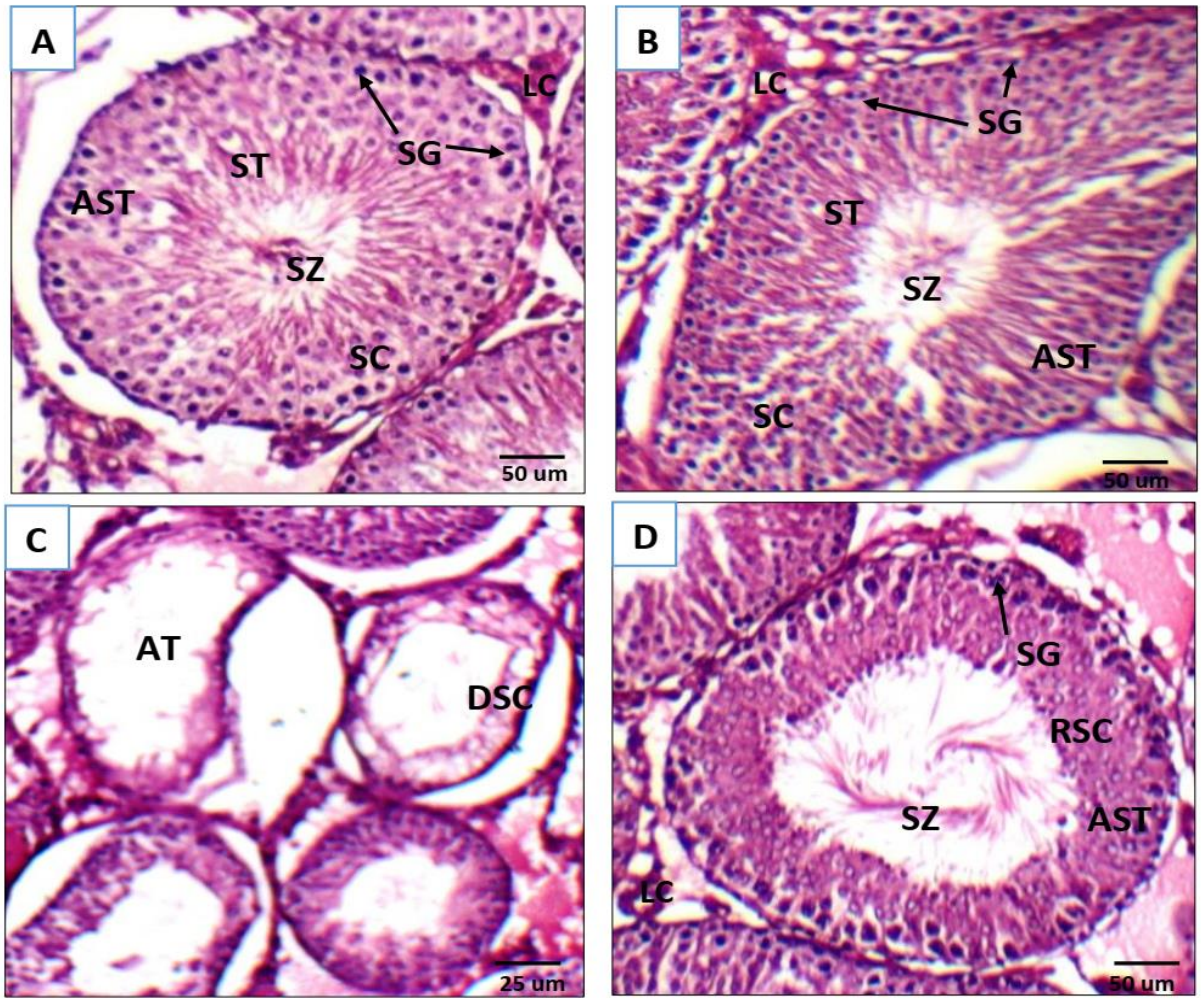

Fig 5 Representative photomicrograph showing the effect of Q on testicular histology in $AlCl_3$-treated rats using H & E staining.(A) NC; (B) Q50; (C) $AlCl_3$; (D) $AlCl_3$+Q50. AST: active seminiferous tubule; AT: atrophied seminiferous tubule; LC: Leydig cell; SG: spermatogonia; SC: spermatocytes; DSC: degenerated spermatocytes; RSC: regenerated spermatocytes; ST: spermatids; SZ: spermatozoa. Magnification= x 400.

3.4. Therapeutic effect of Q on testicular histology in $AlCl_3$-treated rats

Fig 5 shows the histological cross-sections of testes of various groups to investigate overall morphological changes. The NC and Q50 groups showed common histological features of the testes. The seminiferous tubules were active with lumens enriched with prominent layers of spermatogonia, spermatocytes, spermatids, and spermatozoa (Fig 5 A& B). $AlCl_3$ administration caused degenerative changes in spermatogonia and other spermatogenic cells, as shown by atrophied seminiferous tubules, in comparison with the NC group (Fig 5C). The treatment of $AlCl_3$ with Q resulted in regeneration of the germinal epithelium in most of the seminiferous tubules and spermatocytes within the lumen, in comparison with the $AlCl_3$ group (Fig 5D).

4. Discussion

Al is an environmental contaminant of widespread distribution that exposes humans to the risk of testicular dysfunction and adversely affects male fertility. Q is one of the hopeful flavanols against the harmful effects of environmental contaminants, particularly the heavy metals in experimental animals and humans [17,23,24]. The present study aimed to investigate the therapeutic potential of Q against $AlCl_3$-induced testicular dysfunction in rats through evaluation of hormones of the pituitary-testicular axis, sperm parameters in the epididymis, and histological alterations in rat testis.

In the current study, $AlCl_3$ administration to male Wistar rats induced disruption in the hypothalamus-pituitary-testicular axis as shown by the reduction in serum free testosterone, LH, and FSH levels, similar to previous reports on the $AlCl_3$-

intoxicated rat model [4,6,12]. Further, exposure of male rats to $AlCl_3$ caused significant loss in body weight accompanied by a pronounced reduction in the relative genital organs weight, including testis and epididymis [28,29]. In accordance with previous findings, $AlCl_3$-treated rats exhibited reductions in sperm count and motility [4,27,28,30]. Moreover, administration of AlCl3 to rats induced atrophy to the seminiferous tubules and degeneration of the spermatogenic cells, as previously demonstrated in different studies [2,4,12,13].

Both LH and FSH are gonadotropins. They are synthesized and secreted from the anterior pituitary gland (adenohypophysis) under the influence of the gonadotropin‑releasing hormone released from the hypothalamus. LH controls testosterone biosynthesis, whereas FSH regulates spermatogenesis via harmonizing the effects of testosterone on Leydigcells and activities of local paracrine on Sertoli cells of the testis [31]. Consistent with our study, previous reports documented the significant reduction in serum testosterone level in $AlCl_3$-treated rats [4,12]. Akhigbe and Ige[6] reported that oral administration of $AlCl_3$ (100 mg/kg bwt) for eight weeks to male rats resulted in a significant decrease of FSH and LH levels, suggesting that $AlCl_3$ exposure caused male rats infertility [6]. The exposure of male albino rats to aluminumsulfate (50 mg/kg bwt) for 45 days showed a pronounced reduction in serum FSH and LH levels [7]. However, Sun et al. [32]found that the concentration of LH decreased, but there were no significant changes in FSH concentration in $AlCl_3$-treated male rats. At variance with our report, Jebur et al. [4] showed that serum LH and FSH concentrations were significantly increased in rats intoxicated with $AlCl_3$ compared to the control ones.

The current study suggests that the administration of $AlCl_3$ to male rats may inhibit the secretion of gonadotropin‑releasing hormone and gonadotropins due to the Al-induced brain damage. Such damage would adversely affect the hypothalamus and pituitary gland [10], leading to perturbations in steroidogenesis and spermatogenesis. Also, Rawi and Seif Al Nassr[7] and Sun et al.[2] suggested that the down-regulation of the gene and protein expressions of FSH and LH receptors in testis after $AlCl_3$ exposure might inhibit the physiological functions of both hormones. In parallel with the previous studies on different models, Q supplementation restored the regulatory function of the hypothalamus-pituitary-testicular axis through elevation of free testosterone, LH, and FSH levels [17,23,31,33,34]. The Q co-treatment resulted in a pronounced elevation in plasma and testicular testosterone concentrations in arsenic-intoxicated rats [17] and serum of streptozotocin-induced diabetic rats [34]. In a previous study that examined the therapeutic effect of Q on testicular toxicity induced by doxorubicin in rats, the Q/sitagliptin combination significantly increased testosterone and FSH levels [33].

Furthermore, the co-administration of Q with manganese to rats significantly elevated the serum testosterone, LH, and FSH levels compared with the manganese-intoxicated rats [23]. Adedara et al. [23] indicated that the rats co-administered with Q restored the function of the pituitary-testicular axis via counteracting oxidative stress in the brain and testes and preventing injury on the Leydig cells. Moreover, Q might improve the testicular antioxidant defense system through inhibiting lipid peroxidation and increasing levels of glutathione and activities of glutathione S-transferase and glutathione peroxidase in testes and semen in rats [31].

The rats administered with $AlCl_3$ exhibit a reduction in body weight [30]. The reduction in the testes and epididymis weight of tin $AlCl_3$-treated rats was previously described in rats [28,29] and mice [35]. The epididymis is an androgen-dependent organ. Thus, low testosterone directly deteriorates epididymal epithelial cells and reduces luminal fluid and sperm concentration [36]. The role of Q in improving body weight and genital organs relative weights was previously recorded [24,34]. Co-administration of Q alongside cadmium to rats significantly improved the body weight and testes relative weight, compared with the cadmium-treated group [24]. Similarly, diabetic rats treated with Q restored body weight and relative weight of testes compared with streptozotocin-induced diabetic rats [34].

$AlCl_3$- intoxicated rats showed significantly declined sperm concentration and motility (%), accompanied by an increase in dead and abnormal sperm compared to control rats; this finding is parallel to previous studies in rats [4,27,28,30] and rabbits [14]. Jebur et al. [4] indicated the diminution of sperm count, motility, and viability in rats treated with $AlCl_3$ compared to control ones. Also, Güvenç et al. [27] observed that continuous 10 weeks of $AlCl_3$

administration to rats adversely affected the sperm concentration, motility, and the dead/alive ratio. Sperm motility is critical to attaining fertilization. Declined sperm motility and viability are correlated with increased concentrations of Al in seminal plasma and spermatozoa in humans [37]. The reduced sperm count and motility reported in the present $AlCl_3$-treated rats might be attributed to the reduction of testosterone synthesis in testes by the Leydig cells as a result of inhibition of LH production in the adenohypophysis. Numerous reports have documented reduced sperm motility and increased sperm abnormalities in mammals intoxicated with $AlCl_3$[14,29,35].

These studies associated the suppression of sperm count, viability, and motility with the diminished testosterone production and the reactive oxygen and nitrogen species that generated due to Al administration to those animals. Improving sperm count, motility, and progressive motility detected in the current study is consistent with previous studies [23,38]. Co-administration of Q with manganese to rats caused a significant amelioration of sperm morphological defects and increases in sperm progressive motility, viability.

Moreover, Qamended sperm counts, sperm motility, and sperm abnormality in cypermethrin and deltamethrin-induced reproductive deficits in male Wistar rats [38]. The increase in sperm count, motility, and progressive motility support the present hormonal observations. Q seems to counteract the adverse effect of Al exposure by reduction of the negative inhibition upon the hypothalamus and pituitary gland results in enhancing LH and FSH secretion, subsequently resulting in more testosterone production by the testes and spermatogenesis [23,35,39].

The current results revealed that $AlCl_3$ administration to male rats exerted degenerative changes in the testis. The seminiferous tubules, spermatogonia, and other spermatogenic cells have atrophied. $AlCl_3$ administration impaired the structure of testes and epididymis in the rat and mice [2,4,12,13]. Further, $AlCl_3$ treatment was shown to induce the irregular distribution of degenerative spermatids and spermatozoa in the lumina of seminiferous tubules [11,27,40].

This harmful effect may be due to the observed alterations in free T, LH, and FSH levels and inhibition of folliculogenesis in $AlCl_3$-treated rats or might be related to testicular intoxication by heavy metal. Al has the potential to cross the blood-testis barrier, inducing oxidative damage and provoking lipid peroxidation, resulting in the destruction of the biological membranes and stimulation of reproductive toxicity [27,40].

In contrast to the damage induced by $AlCl_3$ administration to male rats, the therapeutic effect of Q caused regeneration of the germinal epithelium in most of the seminiferous tubules and spermatocytes within the lumen. The observed provoked folliculogenesis is linked to the elevations in free T, LH, and FSH levels. Several studies showed similar results but in various models [17,23,24,33,34,41]. Q treatment alongside arsenic and cadmium significantly diminished the signs of testicular toxicity. Q increased the number of Leydig cells and the thickness of seminiferous epithelium with lumen filled with spermatogenic cells and the spermatozoa.

Thereby, Q enhanced steroidogenesis and spermatogenesis in arsenic [17]and cadmium-intoxicated rats [24].The reason behinds the ameliorative role of Q on the detrimental effect induced by arsenic and cadmium is boosting the testicular antioxidant defense mechanism via inhibition of testicular lipid peroxidation and provoking the activities of antioxidant enzymes such as catalase and superoxide dismutase [17,24]. Similarly, Q improved testicular architecture in manganese-intoxicated rats via maintaining testicular lactate dehydrogenase activity and the lactate metabolism, thus providing sufficient ATP to the spermatogenic cells [23].

The Q treatment stimulated the regeneration of the spermatogonial cells in the seminiferous tubules in the testis of induced testicular cancer model in rats via inhibition of TNF-α and down-regulation of caspase-3 expression [41]. Also, Kanter et al. [34] indicated that Q administration to diabetic rats increased the size of the seminiferous tubules, the number of spermatogenic cells, and expression of proliferating cell nuclear antigen (PCNA), while decreasing the number of TUNEL-positive germ cells and apoptotic index in the testis.

5. Conclusion

The current study suggests that Q administration may provide a significant therapeutic effect against AlCl3-induced testicular dysfunction in rats via

improving the pituitary-testicular axis hormones, as evidenced by elevation of free T, LH, and FSH levels, leading to increased sperm count, motility, and progressive motility in the epididymis.

Moreover, Q administration ameliorated histological alterations observed in the testes of $AlCl_3$-rats, causing the regeneration of the germinal epithelium in the seminiferous tubules. Further studies should be done to investigate the antioxidant, anti-inflammatory, and anti-apoptotic effects of Q on $AlCl_3$-induced testicular dysfunction in rats will help better understand the mechanistic activity of this promising flavanol.

Author contribution statement

Asmaa A. Mahmoud conceived the idea and experimental design, performed experimental lab work experiments, statistical analysis, and wrote the manuscript. **Asmaa M. Elfiky** conceived the idea and the experimental design, performed experimental lab work, helped in the statistical analysis and reviewed the manuscript. **Hala A. Elreedy**helped in lab work.**Khadiga S. Ibrahim** reviewed the manuscript.

Acknowledgement

The authors would like to acknowledge the National Research Center, for providing laboratory space and types of equipment for achievement of the current work.

Declarations

Ethical approval Animal handling procedures were performed in accordance with the ethical standards of Institutional Ethics Committee of the National Research Center "Animal Experimentation Sector with approval number 18144," Giza, Egypt and in compliance with the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

Competing interests

We declare that we have no conflict of interest.

Funding This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

6. References

[1]J. Boivin, L. Bunting, J.A. Collins, K.G. Nygren, International estimates of infertility prevalence and treatment-seeking: Potential need and demand for infertility medical care, Hum. Reprod. (2007). doi:10.1093/humrep/dem046.

[2]X. Sun, H. Sun, K. Yu, Z. Wang, Y. Liu, K. Liu, Y. Zhu, Y. Li, Aluminum Chloride Causes the Dysfunction of Testes Through Inhibiting the ATPase Enzyme Activities and Gonadotropin Receptor Expression in Rats, Biol. Trace Elem. Res. (2018). doi:10.1007/s12011-017-1120-1.

[3]F. Zhang, X. Sun, H. Yu, X. Yang, M. Song, Y. Han, Y. Li, Y. Zhu, Effects of aluminum trichloride on the cartilage stimulatory growth factors in rats, BioMetals. (2017). doi:10.1007/s10534-016-9982-9.

[4]A.B. Jebur, F.M. El-Demerdash, W. Kang, Bromelain from Ananas comosus stem attenuates oxidative toxicity and testicular dysfunction caused by aluminum in rats, J. Trace Elem. Med. Biol. (2020). doi:10.1016/j.jtemb.2020.126631.

[5]F. Manfo, E. Nantia, P. Mathur, Effect of Environmental Contaminants on Mammalian Testis, Curr. Mol. Pharmacol. (2015). doi:10.2174/1874467208666150126155420.

[6]R.E. Akhigbe, S.F. Ige, The role of Allium cepa on aluminum-induced reproductive dysfunction in experimental male rat models, J. Hum. Reprod. Sci. (2012). doi:10.4103/0974-1208.101022.

[7]S.M. Rawi, F.M. Seif al Nassr, Zinc sulphate and vitamin E alleviate reproductive toxicity caused by aluminium sulphate in male albino rats, Toxicol. Ind. Health. (2015). doi:10.1177/0748233712469650.

[8]E. Cheraghi, A. Golkar, K. Roshanaei, B. Alani, Aluminium-induced oxidative stress, apoptosis and alterations in testicular tissue and sperm quality in wistar rats: Ameliorative effects of curcumin, Int. J. Fertil. Steril. (2017). doi:10.22074/ijfs.2017.4859.

[9]S. V. Verstraeten, L. Aimo, P.I. Oteiza, Aluminium and lead: Molecular mechanisms of brain toxicity, Arch. Toxicol. (2008). doi:10.1007/s00204-008-0345-3.

[10]A.M. Elfiky, A.A. Mahmoud, H.A. Elreedy, K.S. Ibrahim, M.A. Ghazy, Quercetin stimulates the non-amyloidogenic pathway via activation of ADAM10 and ADAM17 gene expression in aluminum chloride-induced Alzheimer's disease rat model, Life Sci. 285 (2021) 119964. doi:10.1016/J.LFS.2021.119964.

[11]N.E.S. Mohamed, A.E. Abd El-Moneim, Ginkgo biloba extract alleviates oxidative stress and some neurotransmitters changes induced by aluminum chloride in rats, Nutrition. (2017). doi:10.1016/j.nut.2016.10.012.

[12]W.A. Moselhy, N.A. Helmy, B.R. Abdel-Halim, T.M. Nabil, M.I. Abdel-Hamid, Role of Ginger Against the Reproductive Toxicity of Aluminium Chloride in Albino Male Rats, Reprod. Domest. Anim. (2012). doi:10.1111/j.1439-0531.2011.01878.x.

[13]A.M. Abdel-Moneim, Effects of taurine against histomorphological and ultrastructural changes in the testes of mice exposed to aluminium chloride, Arh. Hig. Rada Toksikol. (2013). doi:10.2478/10004-1254-64-2013-2322.

[14]M.I. Yousef, A.M.A. El-Morsy, M.S. Hassan, Aluminium-induced deterioration in reproductive performance and seminal plasma biochemistry of male rabbits: Protective role of ascorbic acid, Toxicology. (2005). doi:10.1016/j.tox.2005.06.025.

[15]A.D. Seftel, Re: Testosterone Therapy in Men with Hypogonadism: An Endocrine Society Clinical Practice Guideline, J. Urol. (2019). doi:10.1097/01.ju.0000550117.25576.05.

[16]S.O. Abarikwu, C.L. Onuah, S.K. Singh, Plants in the management of male infertility, Andrologia. (2020). doi:10.1111/and.13509.

[17]S. Jahan, N. Iftikhar, H. Ullah, G. Rukh, I. Hussain, Alleviative effect of quercetin on rat testis against arsenic: A histological and biochemical study, Syst. Biol. Reprod. Med. (2015). doi:10.3109/19396368.2014.998350.

[18]F.M. Metwally, H. Rashad, A.A. Mahmoud, Morus alba L. Diminishes visceral adiposity, insulin resistance, behavioral alterations via regulation of gene expression of leptin, resistin and adiponectin in rats fed a high-cholesterol diet, Physiol. Behav. (2019). doi:10.1016/j.physbeh.2018.12.010.

[19]Y. Li, J. Yao, C. Han, J. Yang, M.T. Chaudhry, S. Wang, H. Liu, Y. Yin, Quercetin, inflammation and immunity, Nutrients. (2016). doi:10.3390/nu8030167.

[20]S.M. Tang, X.T. Deng, J. Zhou, Q.P. Li, X.X. Ge, L. Miao, Pharmacological basis and new insights of quercetin action in respect to its anti-cancer effects, Biomed. Pharmacother. (2020). doi:10.1016/j.biopha.2019.109604.

[21]W. Wu, R. Li, X. Li, J. He, S. Jiang, S. Liu, J. Yang, Quercetin as an antiviral agent inhibits influenza a virus (IAV) Entry, Viruses. (2015). doi:10.3390/v8010006.

[22]A.A. Mahmoud, A.M. Elfiky, F.S. Abo-Zeid, The anti-androgenic effect of quercetin on hyperandrogenism and ovarian dysfunction induced in a dehydroepiandrosterone rat model of polycystic ovary syndrome, Steroids. 177 (2022) 108936. doi:10.1016/j.steroids.2021.108936.

[23]I.A. Adedara, T.I. Subair, V.C. Ego, O. Oyediran, E.O. Farombi, Chemoprotective role of quercetin in manganese-induced toxicity along the brain-pituitary-testicular axis in rats, Chem. Biol. Interact. (2017). doi:10.1016/j.cbi.2016.12.019.

[24]J. Wang, H. Zhu, K. Wang, Z. Yang, Z. Liu, Protective effect of quercetin on rat testes against cadmium toxicity by alleviating oxidative stress and autophagy, Environ. Sci. Pollut. Res. (2020). doi:10.1007/s11356-020-08947-2.

[25]Z.I. Linardaki, M.G. Orkoula, A.G. Kokkosis, F.N. Lamari, M. Margarity, Investigation of the neuroprotective action of saffron (Crocus sativus L.) in aluminum-exposed adult mice through behavioral and neurobiochemical assessment, Food Chem. Toxicol. (2013). doi:10.1016/j.fct.2012.11.016.

[26]F.H. Abdalla, R. Schmatz, A.M. Cardoso, F.B. Carvalho, J. Baldissarelli, J.S. de Oliveira, M.M. Rosa, M.A. Gonçalves Nunes, M.A. Rubin, I.B.M. da Cruz, F. Barbisan, V.L. Dressler, L.B. Pereira, M.R.C. Schetinger, V.M. Morsch, J.F. Gonçalves, C.M. Mazzanti, Quercetin protects the impairment of memory and anxiogenic-like behavior in rats exposed to cadmium: Possible involvement of the acetylcholinesterase and Na+,K+-ATPase activities, Physiol. Behav. (2014). doi:10.1016/j.physbeh.2014.06.008.

[27]M. Güvenç, M. Cellat, İ. Gökçek, G. Arkalı, A. Uyar, İ.O. Tekeli, İ. Yavaş, Tyrosol prevents AlCl3 induced male reproductive damage by suppressing apoptosis and activating the Nrf-2/HO-1 pathway, Andrologia. (2020). doi:10.1111/and.13499.

[28]M.I. Yousef, A.F. Salama, Propolis protection from reproductive toxicity caused by aluminium chloride in male rats, Food Chem. Toxicol. (2009). doi:10.1016/j.fct.2009.02.006.

[29]V.G.S. Mouro, T.P. Menezes, G.D.A. Lima, R.R. Domingues, A.C.F. Souza, J.A. Oliveira, S.L.P. Matta, M. Machado-Neves, How Bad Is Aluminum Exposure to Reproductive Parameters in Rats?, Biol. Trace Elem. Res. (2018). doi:10.1007/s12011-017-1139-3.

[30]S. Parasuraman, B.N.Y. Qin, L.C. Hui, J.Y.K. Beng, Effect of epigallocatechin gallate on aluminum chloride-induced changes in behavior, biochemical parameters, and spermatogenesis of Sprague-Dawley rats, Beni-Suef Univ. J. Basic Appl. Sci. (2020). doi:10.1186/s43088-020-00079-3.

[31]S.O. Osawe, E.O. Farombi, Quercetin and rutin ameliorates sulphasalazine-induced spermiotoxicity, alterations in reproductive hormones and steroidogenic enzyme imbalance in rats, Andrologia. (2018). doi:10.1111/and.12981.

[32]H. Sun, C. Hu, L. Jia, Y. Zhu, H. Zhao, B. Shao, N. Wang, Z. Zhang, Y. Li, Effects of aluminum exposure on serum sex hormones and androgen receptor expression in male rats, Biol. Trace Elem. Res. (2011). doi:10.1007/s12011-011-9098-6.

[33]Z.A. Ahmed, A.N. Abtar, H.H. Othman, T.A. Aziz, Effects of quercetin, sitagliptin alone or in combination in testicular toxicity induced by doxorubicin in rats, Drug Des. Devel. Ther. (2019). doi:10.2147/DDDT.S222127.

[34]M. Kanter, C. Aktas, M. Erboga, Protective effects of quercetin against apoptosis and oxidative stress in streptozotocin-induced diabetic rat testis, Food Chem. Toxicol. (2012). doi:10.1016/j.fct.2011.11.051.

[35]C.H. Guo, Y.F. Lu, G.S.W. Hsu, The influence of aluminum exposure on male reproduction and offspring in mice, Environ. Toxicol. Pharmacol. (2005). doi:10.1016/j.etap.2004.11.007.

[36]B. Robaire, S. Seenundun, M. Hamzeh, S.A. Lamour, Androgenic regulation of novel genes in the epididymis, Asian J. Androl. (2007). doi:10.1111/j.1745-7262.2007.00316.x.

[37]E.B. Dawson, S. Ritter, W.A. Harris, D.R. Evans, L.C. Powell, Comparison of sperm viability with seminal plasma metal levels, Biol. Trace Elem. Res. (1998). doi:10.1007/BF02783337.

[38]P. Sharma, I.A. Khan, R. Singh, Curcumin and quercetin ameliorated cypermethrin and deltamethrin-induced reproductive system impairment in male Wistar rats by upregulating the activity of pituitary-gonadal hormones and steroidogenic enzymes, Int. J. Fertil. Steril. (2018). doi:10.22074/ijfs.2018.5160.

[39]E.D. Kim, L. Crosnoe, N. Bar-Chama, M. Khera, L.I. Lipshultz, The treatment of hypogonadism in men of reproductive age, Fertil. Steril. (2013). doi:10.1016/j.fertnstert.2012.10.052.

[40]Histological and Ultrastructural Studies on the Testis of Rat after Treatment with Aluminium Chloride, Aust. J. Basic Appl. Sci. (2007).

[41]E.-G.H. El-Diasty HH, El-Sayyad H, Refaat S, Cisplatin against N-Nitroso-NMethylurea Induced Testicular Carcinogenesis in Wistar Rats, Asian Pac J Cancer Prev. 22 (2021) 75–84. doi:10.31557/APJCP.2021.22.1.75.